Chapter

Application of Raman Spectroscopy in Biomedical Diagnostics

Nikiwe Mhlanga, Phumlani Tetyana, Sanele Nyembe and Lucky Sikhwivhilu

Abstract

In vivo cellular imaging and *in vitro* assays or sensors are fundamentally used to study the spatiotemporal interaction of molecules at biological interfaces. The study of these interfaces informs various applications such as diagnostics/detection of foreign materials or processes in the biological system. Raman spectroscopy, an optical, non-destructive, label-free fingerprinting tool offers a wide array of applications in both *in vitro* and *in vivo* diagnostics owing to its relatively short acquisition time, non-invasiveness and ability to provide biochemical molecular information. It has been explored in tissue imaging, *in vitro* diagnosis, DNA/RNA analysis, metabolic accretions, single cell analysis photodynamic therapy, etc. The chapter details the application of the optical Raman platform in the detection and imaging of diseases/tissues. The challenges associated with SERS applications and the future outlook as a biomedical diagnostic tool are also discussed.

Keywords: Medical diagnostics, nanoparticles, Surface Enhanced Raman Spectroscopy, Surface Plasmon Resonance, Enhancement Factor

1. Introduction

The rapid and sensitive detection, identification, and quantitative analysis of bio-species at very low concentrations for pre-symptomatic and symptomatic diagnosis represent a new frontier in biomedical research, enabled by nanomedicine [1]. The surge to achieve such diagnostics has ushered in the exploration of various tools such as Raman optical spectroscopy. The emerging Raman spectroscopy tool has the potential to provide fingerprints of bio-species, quantify and differentiate biomarkers [2].

The vibrational modes of the bio-species are measured from inelastic Raman scattering and the analysis of these modes gives a molecular picture of the bio-species [2]. However, conventional Raman spectroscopy presents a weak signal due to a small scattering cross-section. Plasmonic metal surfaces mitigate the issue of the low signal and this phenomenon is known as surface-enhanced Raman spectroscopy (SERS) [2].

Several variants of Raman besides SERS have been developed to mitigate biomedical diagnostic issues presented by the conventional Raman. Resonance Raman spectroscopy improves the low signal by the use of a laser excitation wavelength that corresponds to the electron absorption maximum of the bio-species [3].

For *in vivo* applications the penetration depth of the Raman laser to the targeted tissues is usually a bottleneck. Nonetheless, spatially offset Raman spectroscopy (SORS) measures diffuse Raman scattering from regions away from the laser excitation and thus collects vibrational modes from deeper tissues. The SORS and SERS technique are integrated into the SESORS variant, a tool that will benefit biomedical *in vivo* diagnostics [3]. The Raman spectroscopy also falls short in resolving nanostructures. To enable sub-nanometer spatial resolution and signal enhancement a Tip-enhanced Raman (TERS) that combines SERS with apertureless near-field scanning optical microscopy using a metallic tip is used [4]. This chapter presents recent developments on some of the Raman variants in biomedical diagnostics, materials developments for the application, challenges, and forecasts into the future of Raman biomedical diagnostics.

2. Materials used in Raman diagnostics

2.1 Gold and silver colloidal nanoparticles: bottom-up

It is commonplace that many condensed-matter systems exhibit collective excitation modes involving coherent oscillations of the medium. As such surface Plasmon's are known to be the collective excitation of free conduction electrons excited by electromagnetic radiation at the metal-dielectric interface [5]. Over the years the use of noble metal thin films or nanoparticles (NPs) surfaces has underpinned the success in this area. Despite all this, the study of the interaction between light and metallic nanostructures holds promise for this emerging research area of Plasmonic which is rapidly gaining traction [6–9]. Well-established targeted technologies to engineer Plasmonic nanostructures allows for better control and manipulation of visible light at the nanometer scale leading to new possible application areas with real life impact [10–12].

Although most noble metals exhibit Plasmonic properties and can potentially be used as SERS substrate gold and silver are the most widely used owing to their superior enhancement factors (EFs) and Plasmonic resonance in the visible and NIR regions [13–15]. Typically, these are used in their metallic form but also as composite with other materials. However, in order to attain the highest sensitivity and specificity these NPs materials must have the Plasmonic properties that include the resonance frequency of the surface Plasmon's and the magnitude of the electromagnetic field that is generated at the surface. Inevitably, these properties are, in the main, influenced by the type, size, shape, composition, and dielectric environment [16–19]. Despite all this, Ag has proven to have higher EF than Au making it more attractive for various applications.

Typically SERS substrates are configured in two forms and these are colloidal suspensions (NPs) and solid substrates. NPs are the most commonly used form primarily because of simplicity of preparation and relatively high EFs. Interestingly, the most significant SERS enhancement is achieved when molecular structures are bound to noble metal nanostructures with a size range of 5–15 nm (Au and Ag NPs are shown in **Figure 1**). Furthermore, it must be noted that the nature of interaction between the molecules and nanostructured surfaces, as well as the charge properties of the molecular structure play a pivotal role in terms of performance of SERS-based Raman measurements. For example, when colloidal noble metal NPs are put to use the surface charge of the NPs and that of the molecules become fundamentally important [20, 21]. This implies that maximum SERS activity is attained when the detected molecule has the opposite charge to interacting colloidal NPs. This phenomenon is ascribed to the induced aggregation resulting from reduced zeta

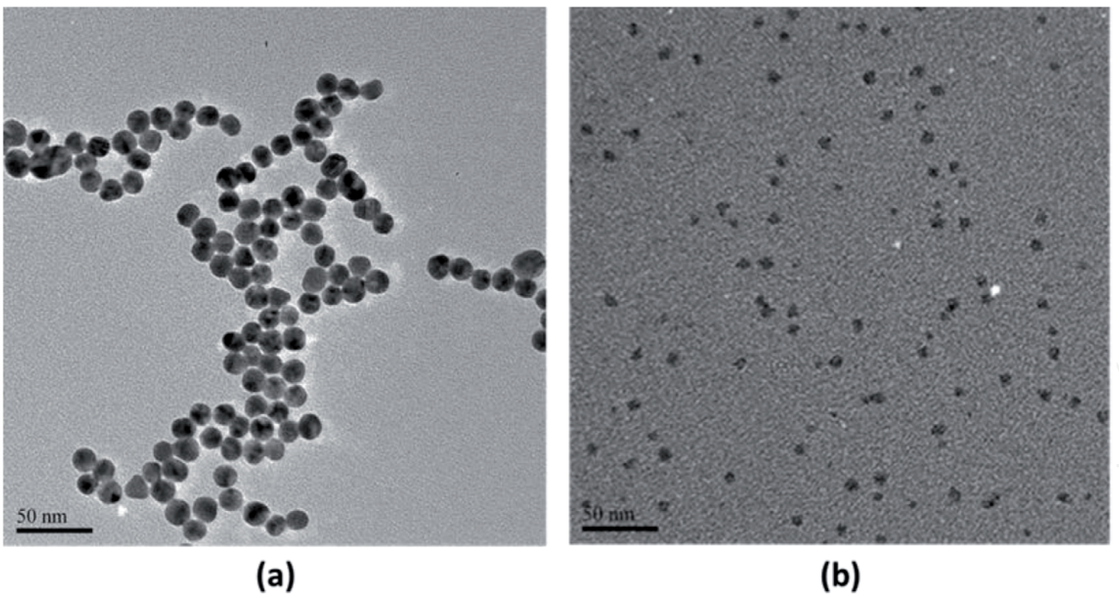

Figure 1.
TEM images of colloidal (a) Au and (b) Ag NPs.

potential of NPs [20]. Kahraman, *et al*. reported that the highest EF in the case of Ag NPs is achieved when the NPs have a pyramid-like shape [22]. On the other hand meticulously, controlling aggregation is useful for improving SERS enhancement as this could lead to a higher possibility of "hot spot" formation [23, 24]. In view of this, important consideration must be given to the fact that very large aggregates could abate the effective formation of surface Plasmons as a result of the deformations and dampening of the electron cloud within the aggregate leading to poor SERS activity. Data has shown that small-sized aggregates tend to greatly improve the EFs [25]. Furthermore, the methods of preparation are known to influence the SERS activity of Au and Ag colloidal NPs. This is because the structure of a substrate has an important role on the properties of these metal NPs and subsequently influences the applications of the substrate. Au and Ag colloidal NPs are sufficiently produced using chemical reduction method such as Citrate method.

2.2 Colloidal gold nanowires: bottom-up

Besides, the use of Au and Ag as gold standard for superior EF and possibly creating a stable platform for these metals by anchoring them on a support like graphene it is clear that the effect of changing the shape of the material on the EF is significantly large. Elongating the Au structures to very thin nanowires leads to improved enhancement owing to their large surface area and excellent molecule adsorption ability. The molecule adsorption is attributed to the fact that Au nanowires adsorb Raman active compounds via two mechanisms, namely, surface adsorption due to the surface reactivity of Au nanowires and network entrapping through their "web" network [26, 27]. The Au nanowires, as shown in **Figure 2**, reveal that ultrathin wires tend to form a spider web-like network with interstices smaller than 1 nm. These interstices have been reported to be responsible for further entrapment of adsorption molecules [27]. The spider web-like network are exclusively found in Au nanowires and not in the spherical Au NPs. Hence, Au nanowires are reported to have a relatively higher EF value compared to spherical NPs. Depending on the method of preparation for the NPs, geometrical parameters such as size distribution are not often well controlled as a result of production limitation such as random deposition or irreproducibility of size distributions leading to disordered samples.

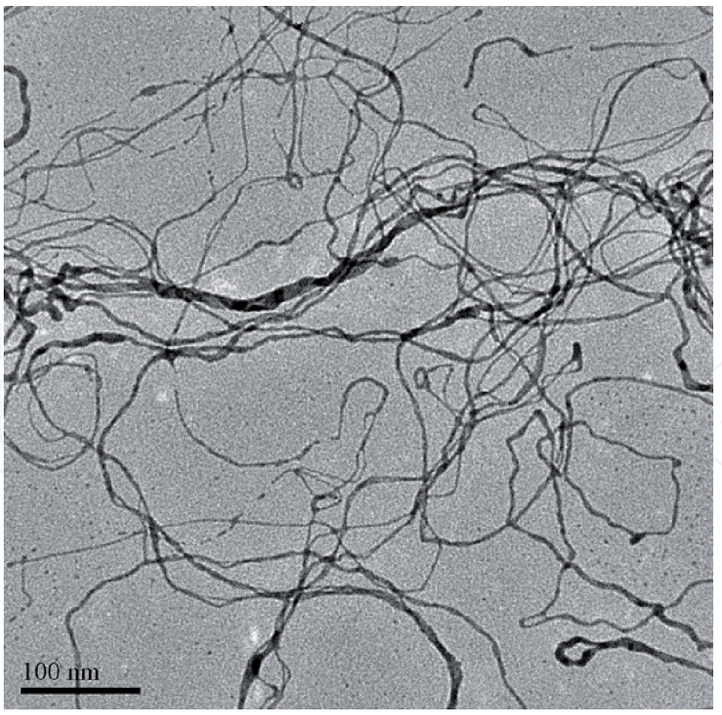

Figure 2.
The TEM image of ultrathin Au nanowires.

However, in the case of nanowires reproducibility seems to be easily controlled leading to the presence of hot spots (highly localized area of intense electric field) resulting in significant enhancement and homogeneous SERS signal (reproducibility). This phenomenon is particularly important in terms of provision of unambiguous analyte quantification as the enhancement should be highly reproducible while it is necessary to optimize SERS efficiency in relation to Plasmon excitation conditions [28]. For example, the SERS signal is directly related to the excitation wavelength and Raman scattering wavenumber [28–36] or the coupling between nanostructures [37–39]. Furthermore, aggregation of Au nanowires is less likely than in the case of colloidal Au NPs.

The size of the nanowires and its potential influence on SERS signal is considered important. It is understood that the surface of Au nanowires increases with an increase in the diameter of the wires thereby inducing an increase in the number of adsorbed molecules and consequently leading to a larger Raman signal. In order to ensure a controlled enhancement as contribution by the nanostructures orientation of the wires is important [40, 41]. Although nanowires tend to self-assemble into dense bundles resulting from the electrostatic interaction the effect of SERS activity remains insignificant. Au nanowires with a well-controlled geometry provide well controlled and reproducible SERS signal. The high aspect ratio combined with the bundling yield structures with impressive SERS activity. Ultrathin colloidal Au nanowires are produced using facile modified chemical reduction method (citrate method) making them very affordable [42, 43].

2.3 Au/Au nanowires: top-down

Whilst the use of nanowires as SERS substrate seems to be gaining momentum on all fronts Sivashanmugana *et al.* reported on the fabrication of well-oriented Au/Ag multi-layered nanorod arrays using a focused ion beam techniques [44]. The study revealed that the shape, thickness, space between nanorods and size dimensions have influence on the performance of SERS. With good experimental

conditions these factors can be optimized before fabrication of the substrate and this is often done by varying the thickness of Au and Ag layers for optimum performance. Ag layer plays a pivotal role in respect to the improvement of SERS mechanism as it induces the electromagnetic effect at the Au surface [36–38, 44].

While SERS-active substrates are usually obtained either by the solvent-cast deposition of colloidal NPs [45, 46] or by lithography techniques such as electron beam lithography [46, 47], nanoimprint [48], and nano-indentation [49, 50]. These fabrication methods are based on high precision regular patterns. Consequently, most of these methods place emphasis on significant improvement on enhancement factors. However, they tend to yield low reproducibility and high cost, and they lack the essential characteristics required to make SERS a platform-enabling technology. Interestingly, the use of nanowires of a single entity or multiple has led to the construction of very reproducible SERS-active substrate, especially with gold nanorods arrays. The Localized Surface Plasmon Resonance (LSPR) from the nanorods and the lighting-rod effect of the Plasmonic field enhancement strongly depend on the shape and the spacing between the nanorods [33, 51–56].

The SERS method has great potential for the detection of Raman- active species, ranging from single molecules to biomolecules. The past five years have seen various approaches being developed for fabrication of SERS-active substrates with high sensitivity using noble metal nanostructures via bottom-up, top-down, or template-assisted routes. Nano-based substrates with relatively high SERS enhancement factors can be easily produced, with the EF largely dependent on the size and shape of the nanostructures that give rise to the effect. In order for SERS substrates to be used as a platform for applications such as bio-sensing or diagnostics various issues including sensitivity, selectivity, and intensity-concentration dependency must be addressed. The potential application of nano-based and shape-dependent (nanowires and NPs) SERS substrates in diagnostics will be discussed in the next section.

3. Application

3.1 Application of Raman spectroscopy in *In-vitro* diagnostics

In-vitro diagnostics (IVDs) are tests done on the external of the human body, on biological samples (blood or tissues) that are extracted from the human body or expressed to mimic the human body samples. IVDs serve healthcare through detection, cure, treatment and prevention of diseases. The test tube IVDs are crucial in healthcare and proffers the following merits: non-invasiveness; possible prevention of patient side-effect; a rapid diagnosis that facilitates earlier treatment even in remote areas [57]. The unique IVDs are characterized into 3 groups based on their applications: 1. Clinical laboratory IVDs – for samples that require a clinical laboratory with advanced instrumentations and specialized/trained personnel. A good example is the polymerase chain reaction (PCR) test. 2. Near-patient IVDs – advanced instrumentation is not required for these samples but a trained person is still needed to administer e.g. physician or a nurse. 3. In-home IVDs/Point of Care Testing (POCT) - test is simple, easy to use, does not require trained personnel or instrumentations. Examples of POCT devices include popular pregnancy test kits and glucose meters [57]. The last decade has propelled and translated the clinical and near-patient IVDs into the in-home (POCTs) IVDs to control and manage diseases. This translation is advantageous for early, rapid diagnosis even in remote World Health Organization (WHO) regions.

The fabrication or architecture of the IVDs devices mobilizes different scientific platforms and instruments. The optical Raman spectroscopy merited by its high

chemical specificity, minimal to zero sample preparation, wide visible/near-infrared spectral range is inherently used in medical diagnostics. The capability of Raman spectroscopy to detect and quantify changes in cells, tissues and biofluids is an impetus for its application in IVDs [58]. The conventional Raman spectroscopy is however insufficient due to low inelastic Raman photons. SERS mitigates the low inelastic Raman photons. SERS uses roughened Plasmonic noble metal surfaces to amplify or enhance the Raman signal [57, 59]. Application of SERS in IVDs is driven by its high sensitivity, fingerprinting, large dynamic range, and multiplexing competencies [57, 60], molecular specificity, non-invasiveness, potential to resolve the composition of complex molecular bio-analytes [61].

SERS IVDs immunoassays started with the classical ELISA mimicking sandwich assay on a solid support or free floating and recently advanced to automated systems such as lateral flows ((LFAs) and lab on chip (microfluidics) [60]. The choice of the SERS substrate discussed in the materials section is vast and offers sufficient choices for the detection of bio-analytes [61] which in turn serves in clinical practices for the prognosis and diagnosis of diseases [5, 61].

SERS IVDs	**Bioanalyte/disease**	**SERS substrate**	**Reference**
Label-free SERS IVDs	Cancer (blood plasma protein	Ag NPs	Lin *et al*. [62]
	Quantification of hepatitis B DNA	Ag NPs	Batool *et al*. [63]
	HIV-1	Ag nanorods	Yada *et al*. [64]
	Nasopharyngeal cancer DNA	Negatively charged Ag NPs	Lin *et al.* [65]
	Breast cancer tissue	Ag Nps	Shen *et al*. [66]
	Sjogren's syndrome from saliva	Cl-Ag NPs	Moisoiu *et al*. [67]
	Track bi'ological injuries caused by radiation from serum and urine samples of mice	Au NPs spurted on the nano tip of a canonical anodic aluminum oxide template	Muhammad *et al.* [68]
	Huma tear uric acid	Polydimethylsiloxane film layered with polystyrene, SiO_2 and Au	Narasimhan *et al.* [69]
	Ovarian and endometrial cancer extracellular vesicles	biosilica/Ag NPs composite substrates	Rojalin *et al.* [70]
	creatinine	Nano-Au on Ag film nanostructures	Su *et al.* [71]
Labeled-SERS IVDs	DNA and microRNA	Au core/stellate shell	Wang *et al*. [72]
	Plasmodium falciparum DNA	Magnetic beads	Ngo *et al.* [73]
	mouse IgG	Au NPs	Frimpong *et al.* [74]
	SARS-coV-2 IgM/IgG	SiO_2@Ag	Liu *et al.* [75]
	Myocardial infarction biomarker	Ag@Au NPs	Zhang *et al.* [76]
	HIV-1 DNA	Au NPs	Fu *et al.* [77]
	Single prostate cancer cells	Au NPs	Willner *et al.* [78]
	Estrogen receptor alpha	Au NPs	Kapara *et al.* [79]

Table 1.
SERS IVDs.

SERS immunoassays inherit a labeled/indirect or label-free configuration. In Label-free SERS, the Raman reading comes from the fingerprint of the bio-analyte while with labeled SERS, the characteristic spectra is that of a Raman tag [61]. The label–free is simple as opposed to the labeled system which incorporates the tag on the metallic nanostructures. Both systems have been used in the detection of proteins, nucleotides, and fatty acid/lipids. Changes or alterations in these bio-samples inform the diagnosis of communicable and non-communicable diseases [61]. **Table 1** [62–79], list a few examples of bio-analytes or diseases detected using SERS IVDs.

3.1.1 Traditional sandwich SERS IVDs

The traditional sandwich SERS immunoassay is characterized by a SERS substrate and a SERS immunoprobe and inherits the ELISA principle. It is crafted with SERS surfaces (Plasmonic metals structures), which could be supported on a platform i.e., solid support (metal, non-metallic) or the new flexible supports, or free-floating in colloidal form. Several research papers have reported on the solid substrates [80–85]. We have fabricated immune SERS substrates on solid supports (glass and silicon wafer) [86] and a schematic of the preparation of the SERS substrates is shown in **Figure 3**. Noble metal NPs (Au/Ag) are self-assembled onto solid support using wet chemistry methods [86]. These SERS substrates are applied in the detection and quantification of malaria *plasmodium falciparum* antigens. A capture and detection *p. falciparum* antibodies sandwiches the *pf* antigen and it could be confirmed indirectly via a 4-mercaptobenzoic acid SERS tag [87]. **Figure 4** schematically details the SERS

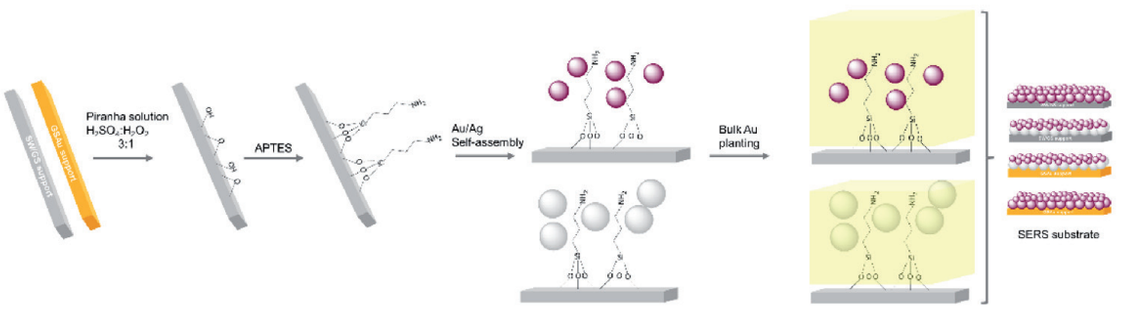

Figure 3.
Schematic representation of the preparation of SERS immune substrates on solid support. Reprinted with permission from ref. [86].

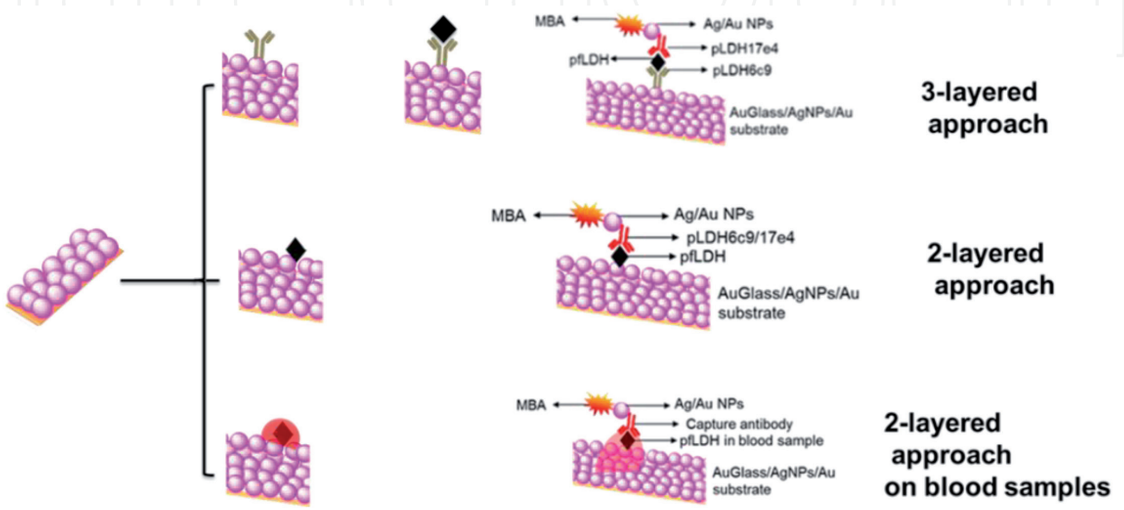

Figure 4.
SERS immunoassay schematic. Reprinted with permission from ref. [87].

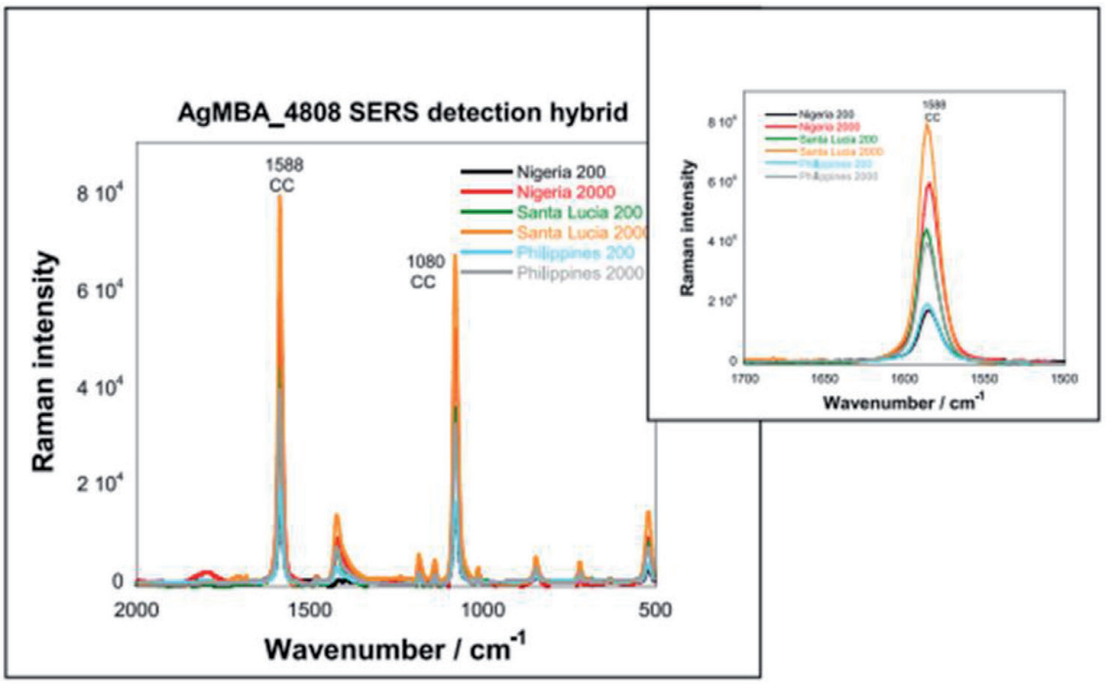

Figure 5.
Labeled-SERS spectra showing quantitative and qualitative detection of pf antigen from WHO malaria blood specimens. Reprinted with permission from ref. [87].

probe. The labeled SERS sandwich assay proves the capability of the SERS IVDs for the quantitative and qualitative detection of bio-analytes; **Figure 5** shows the SERS spectra of the SERS IVDs tested on WHO malaria specimens from different regions with known bacterial loadings.

The next section will highlight a few examples of the upcoming flexible SERS substrate. The flexible SERS substrates include polymers, graphene, graphene oxide and nanowires [88]. The lack of biodegradability and non-uniformity presented by the conventional solid SERS substrates and lithography approaches, respectively, is an impetus for the flexible substrates [89].

Fan *et al.* [90] reports on a reproducible label-free SERS hybrid of graphene oxide conjugated to popcorn shaped Au NPs. The ultrasensitive graphene oxide-Au SERS probe is used for testing of HIV DNA and MRSA bacteria and has a detection limit of 10 DFU/mL for the latter. Korkmaz *et al.* [91] demonstrated an affordable simple development of a porous biosilica Plasmonic composite flexible SERS substrates. The technique involved deposition of the materials on a regular office-grade adhesive tape. The characterization of the novel material reveals unique properties: pore size, Plasmon resonance, Raman enhancement suitable for biosensing. The usability of the platform was tested on bioanalytes; proteins and bacteria. However, due to the porous nature of the nanocomposites, smaller proteins and nanostructures disperse within the substrate and afford a reduced particle density for optical detection. Hence, low analyte concentrations cannot be detected with the exclusion of particles larger than 100 nm. The platform is useful in the detection of bacteria and other bio-analytes of nano- to micro-meter sizes.

He *et al.* [92] explored the use of graphene in SERS immunoassays. A SERS-active substrate of Au NPs grown on graphene using chemical vapor deposition is developed and used for multiplexing detection of DNA. The presence of both the Au metallic substrate and graphene leads to an enhanced signal. The Au NPs enables assemble of DNA capture probes which is normally problematic on the graphene. The SERS Au-graphene SERS platform showed extraordinarily high sensitivity and

specificity for DNA detection with a detection limit of 10 pM. The multiplexing capability is tested with the simultaneous detection of two DNA targets.

3.1.2 Automated SERS IVDs

Automated SERS IVDs include LFAs and microfluidics synergized with the SERS phenomenon. LFAs are cemented for POCT due to their simplicity, user-friendliness, long-term stability, rapid detection which makes them a suitable choice for remote WHO regions. Incorporation of the SERS component into the LFAs results in SERS/LFAs IVDs. The SERS-LFAs principle is still the same as that of the classical LFAs with an addition of a Raman tag. The SERS tag is conjugated to the Plasmonic metal nanostructures, which are mostly Au NPs [93].

The SERS-LFAs improves sensitivity and enable both qualitative and quantitative detection of bio-analytes, an improvement of the traditional LFAs [93].

Ma et al. [94] capitalizing on the flexibility, portability and simplicity of polydimethylsiloxane (PDMS) used it as a matrix/support of SERS Plasmonic metallic substrates for SERS-LFAs. A novel PDMS-based SERS-LFAs for ultrasensitive and quantitative detection of ferritin (FER), a liver cancer biomarker, is developed. The hydrophilic-hydrophobic Ag/PDMS strip is prepared and anti-FER is mobilized on the Ag strip (test line, control line 1 and control line 2). The FER bio-analyte flowed smoothly into the hydrophobic PDMS substrate coupled with SERS immonoprobes of raspberry-shaped Au NPs conjugated to 4-MBA SERS tag and anti-FER results in the detection of FER with a detection limit of 0.41 pg./ml.

Microfluidics pertains to the science and technology of handling fluids and the micro/nanofluidics devices are an ensemble of miniaturized components such as pumps, channels, valves, mixers and separators to facilitate the movement of the fluids [95].

The application of microfluidics in diagnostics proffers the following advantages: high-to-volume ratio, precise fluid control, low sample consumption and high integration with functional components [96].

For the detection of bio-analytes, microfluidics is coupled with optical, electron chemical, or electrical techniques [96]. Raman spectroscopy is one such optical technique and the synergy results in SERS-microfluidics devices [96]. The SERS/microfluidics are used for the analysis of various biospecies.

Pallaoro *et al*. [97] proved the capability of the SERS/microfluidics platform in the detection of cancer from flowing cells. A mixture of cells, cancerous (prostrate) and non-cancerous from bodily fluids are incubated with label-mediated SERS probes. The SERS probes are based on Ag NPs dimer core labeled with SERS tag and paired with an affinity-biomolecule. They are circulated continuously in the microfluidics channel and exposed to Raman laser which differentiates the cells based on their characteristic fingerprints.

3.2 Application of Raman spectroscopy in *In-vivo* diagnostics

Raman spectroscopy has gained much popularity over the years in applications that determine the biochemical composition of cells and tissues. This technique has advanced significantly and has found widespread use outside the laboratory in applications such as materials analysis, process control and environmental monitoring amongst others. Recently, Raman spectroscopy has found use in clinical applications such as in vivo diagnostics and monitoring, through coupling with other diagnostic systems. Initially, most applications of Raman spectroscopy that relate to biochemical analysis are mostly based on *ex-vivo* or

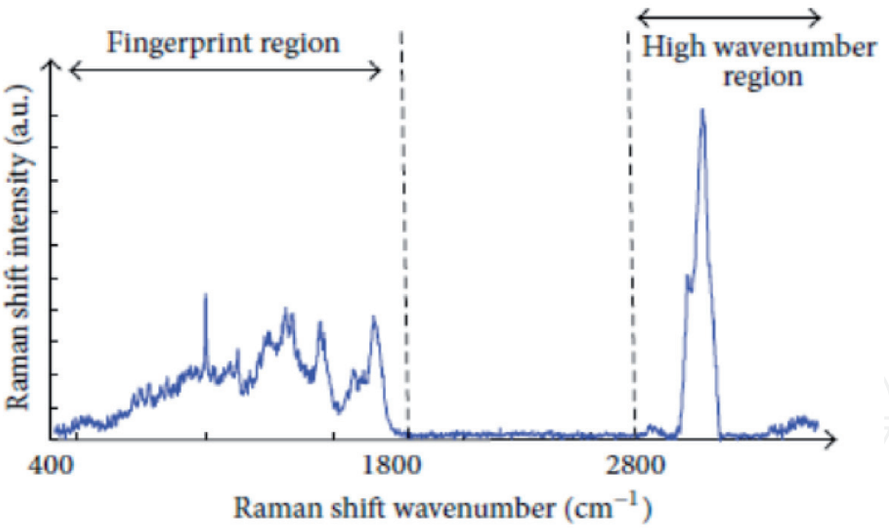

Figure 6.
Raman spectra showing the fingerprint region which depicts the area of the spectra where biological molecules and other associated molecules appear [104].

in vitro assays, but until recently, there has been a great migration from ex-vivo to in-vivo applications [98, 99].

Raman scattering occurs as a result of a change in the polarization of molecules due to light. This technique provides quantitative assessment of the biochemical composition of biological tissues. When a spectra is obtained during assessment, the peaks are highly specific to the molecular chemistry of the specimen under investigation, with intensities directly proportional to the molecular content. This allows for the investigation of concentrations and ratios of constituents within the specimen under investigation [100, 101].

Thus, Raman spectroscopy provides chemical fingerprints of biological materials such as cells, tissues or biological fluids through inelastic scattering of light by vibrating molecules [58, 102, 103]. This fingerprint represents molecular vibrations brought about by chemical bonds, thus deciphering the samples chemical or biochemical composition. The spectra is collected in the fingerprint region from 400 and 1800 cm^{-1}, as depicted in **Figure 6**. Collection of the Raman spectra does not disrupt the cellular environment. This is considered one of the advantages that have made Raman spectroscopy superior amongst other optical diagnostic tools [100, 104].

Some of the properties that have made Raman spectroscopy suitable for use in in-vivo applications include its excellent chemical specificity which result in the formation of a fingerprint like spectrum without interference from water, minimal or lack of sample preparation and its ability to employ advanced optical technologies in both the visible and near infrared spectral ranges [58, 98, 105, 106]. Water is known to be a weak Raman scatterer and shows no interference with the spectra of solutes in aqueous solution. Water absorbs in the region between 2000 and 4000 cm^{-1} in the Raman spectra, which is in the infrared (IR) and near infrared (NIR) regions, and thus falls beyond the fingerprint region in which the molecules of interest absorb [106, 107].

3.2.1 Instrumentation requirements for In vivo *applications*

Although Raman spectroscopy has been found to yield exceptional results in in-vivo applications, certain adjustments and additional components are required for optimum performance. According to Ramirez-Elias and Gonzalez [108], in vivo measurements require an integrated system capable of providing spectral

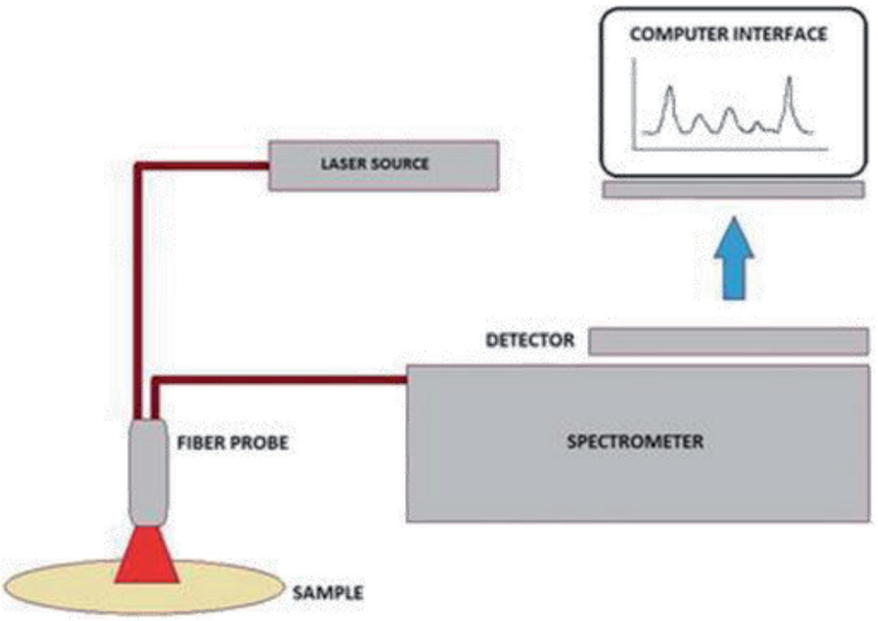

Figure 7.
A typical Raman spectroscopy setup showing the components required to perform an in vivo analysis [109].

acquisition and analysis in real time. Such a system includes a light source (lasers), light delivery system, Raman probe, signal delivery, and a signal detection system, as shown in **Figure 7** [110]. For in vivo applications, it has been reported that a spectra of sufficient signal-to-noise ratio (SNR) should be obtained within a few seconds on the signal collection time, or even faster. Thus, the instrumentation for in vivo applications should have an improved signal detection system and also be able to minimize noise contributions [111].

Raman spectroscopy has gained much popularity over the years in applications that determine the biochemical composition of cells and tissues. This technique has advanced significantly and has found widespread use outside the laboratory in applications such as materials analysis, process control and environmental monitoring amongst others. Recently, Raman spectroscopy has found use in clinical applications such as in vivo diagnostics and monitoring, through coupling with other diagnostic systems. Initially, most applications of Raman spectroscopy that relate to biochemical analysis were mostly based on ex-vivo or in vitro assays, but until recently, there has been a great migration from ex-vivo to in-vivo applications [98, 99].

3.2.2 Excitation or light source

A light source is used to deliver power or energy to the sample to generate Raman scattered photons. Raman scattering is known to be a relatively weak phenomenon and thus it is vital to ensure that sufficient power is directed towards the test sample. Laser is the most commonly used light source in the Raman spectroscopy system due to their narrow bandwidth and high power output [110]. Raman scattering is dependent on a variety of factors including signal to noise ratio, maximum permissible exposure and an increase in temperature [112]. Various lasers have been used in optical systems and only lasers offering the following characteristics are desired for use in in vivo applications; the first characteristic to consider is the line width of the emission. According to Sato et al., the line width of the emission must be narrow [99]. This requirement ensures that laser line broadening does not propagate into Raman bands through convolution, which results in irrevocable adjacent Raman peaks [113, 114].

Secondly, the wavelength of the laser light and its intensity should be stable or carefully selected. For in vivo applications, excitation with ultraviolet or visible light is not recommended. When UV light is used for excitation, there is a risk of photochemical damage to the tissue being investigated. Also, when visible is light is used for excitation, there is a strong auto-fluorescence that is generated in the biological sample under investigation. Thus, near infrared (NIR) excitation sources are commonly used. This has been chosen since only a few biological fluorophores are known to have peak emission in this region of the spectrum thereby reducing background autofluorescence and absorbance and further simplifying signal processing needed for extracting the Raman bands [107, 112].

3.2.3 Fiber optic probes

During tissue examination, excitation or illumination light needs to be delivered to the tissue and Raman signals emitted from the tissue should be transmitted back to the detectors. This is done through the use of optical fibers which enhance signal collection and lessen interfering signals. Interfering signals include signal-to-noise ratio and also includes both the Raman signal and photoluminescence generated within the optical fibers [115]. The design of the probe is mostly dependent upon the envisaged use. The following parameters are considered: Raman configuration, location of organ under investigation, microanatomy of the tissue, and the pathophysiology of the disease [112]. A probe suitable for in vivo applications should have the following characteristics; The probe should be inexpensive and sterilizable if its reusable, output of the laser delivery fiber should be filtered to prevent the Raman signal induced from the fiber reaching the sample, high signal collection efficiency, light emitted from the sample should be filtered to avoid Raman signal contributions from the fiber material [98, 111, 116].

3.2.4 Signal detection or spectrograph

A Raman detection system that is used for clinical applications consist of an imaging spectrograph linked with a charge-couple device (CCD). For in vivo Raman applications, a spectral acquisition of no more than a few seconds is required, and can be achieved through the use of a fast spectrograph and a highly sensitive detector. A suitable detection system for in vivo applications requires an appropriate imaging spectrograph that is linked to the sample interface, such as optic fibers, on one end and the CCD on the other end. Other components that form part of the detection system include rejection filters that are responsible for eliminating any laser light or elastically scattered light from the signal [112].

3.2.5 Raman spectra in In vivo *diagnosis and monitoring*

This section captures some of the biomedical applications that have employed Raman spectroscopy as an *in vivo* diagnostic tool, in conjunction with other techniques.

3.2.5.1 Breast cancer diagnosis and monitoring

Saha *et al*. demonstrated the diagnosis of early stage breast cancer by the real time detection of microcalcifications during stereotactic breast core needle biopsies. They performed their study on 159 tissue sites in 33 patients in order to detect microcalcifications in breast tissue biopsies. The authors used ordinary least squares fitting to approximate spectra that had been acquired with a breast model that

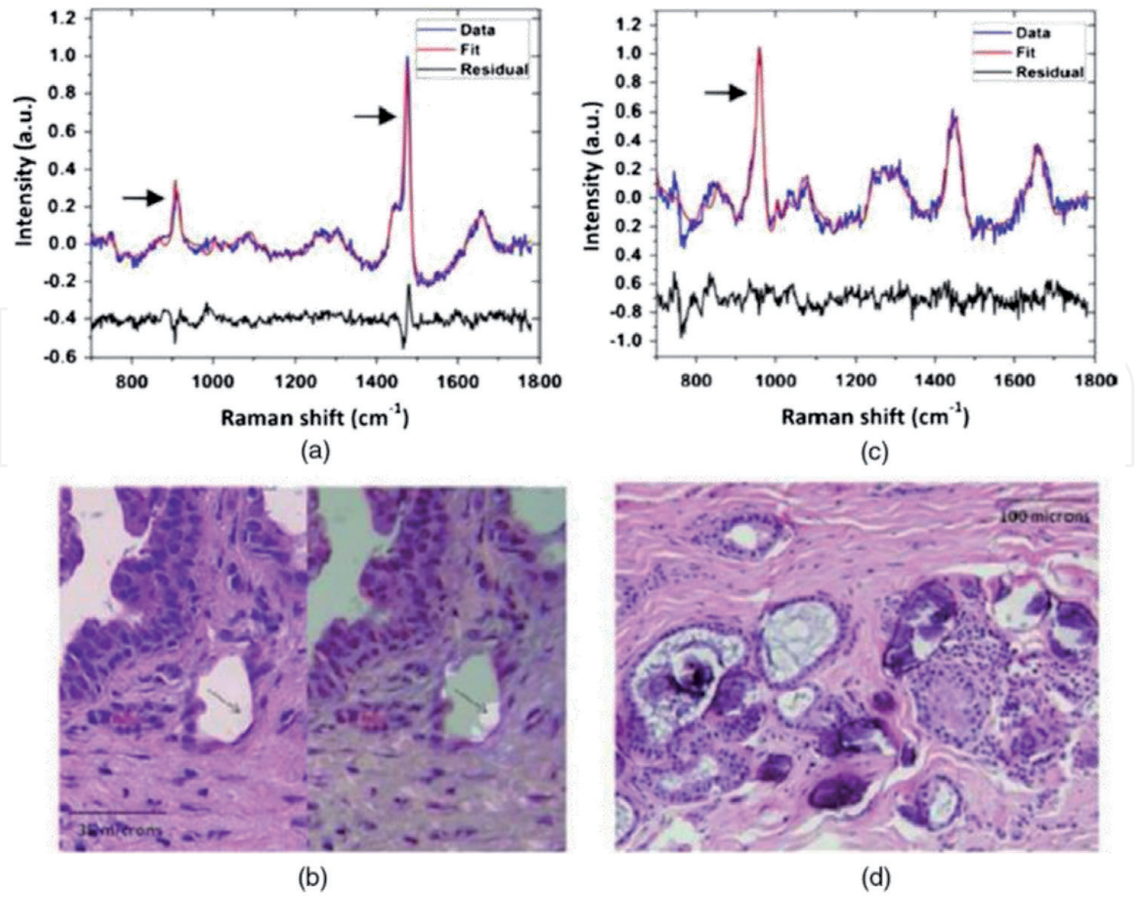

Figure 8.
Raman spectra and histopathology of breast lesion with types I and types II microcalcifications [117]. (a) and (b) depicts the Raman spectrum of type I microcalcifications characterized by calcium oxalate peaks and the spot on the biopsy where the fingerprint was collected. (c) and (d) characteristic peaks of type II microcalcifications calcium hydroxyapatite and data collection location on the biopsy, respectively.

had been developed previously. They demonstrated the possibility of distinguishing between various microcalcifications based on the appearance of vibrational bands that represent calcium oxalate in the fingerprint region at positions 912 and 1477 cm-1, as depicted in **Figure 8(a)**. The location where the spectrum was collected in the biopsy is shown in **Figure 8(b)**. **Figure 8(c)** shows the appearance of the peak or band at 960 cm-1 which represents the presence of calcium hydroxyapatite (microcalcification type II), with **Figure 8(d)** showing the exact position on the biopsy [98, 117].

3.2.5.2 Diagnosis of skin cancer

The use of Raman spectroscopy in the in vivo diagnosis of skin cancers has been sought after due to the easy optical access to skin. Lieber and colleagues demonstrated the possibility of using Raman in the diagnosis of skin cancers through the use of a fiber optic Raman probe which recorder a sensitivity of 100% and a specificity of 91% [118]. Recently, Raman spectroscopy is coupled with autofluorescence (AF) imaging and used to diagnose basal cell carcinoma of the skin using multimodal spectral imaging, as shown in **Figure 9**. AF is used to investigate the main spatial features of skin resections for use in selecting and prioritizing sampling points in Raman spectroscopy. Skin resections are collected during Mohs surgery, and proper investigation of resection margins is obtained using 500–1500 Raman spectra and without sectioning, staining or any other form of sample or tissue preparation step [58].

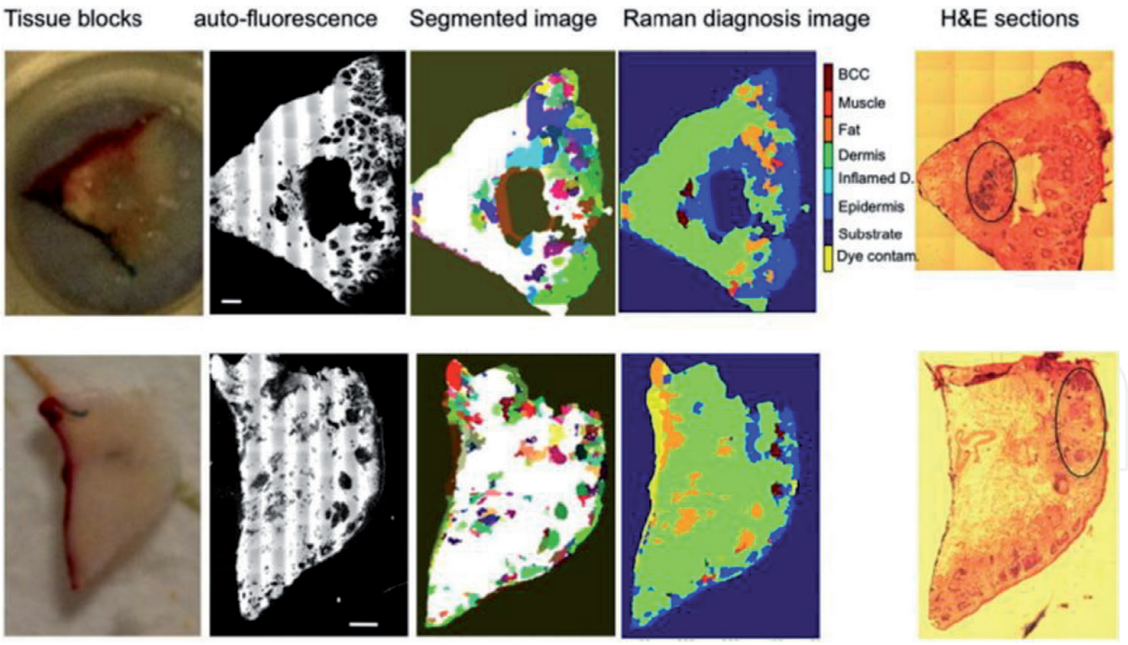

Figure 9.
Use of Raman Spectroscopy coupled with auto-fluorescence imaging in the in vivo detection of BCC in skin resections obtained during Mohs surgery [58].

4. Limitations of SERS in biomedical diagnostics

An effective SERS application for biomedical diagnostic requires a Plasmonic substrate. The metal substrates are the most superior as they produce relatively higher EFs. Plasmonic substrate play dual role in SERS technique, firstly, it interacts directly or indirectly with the targeted molecules, secondly, it amplifies the Raman signal [119]. One of the limitations often reported for SERS technique is irreproducibility of its results mostly associated with the substrate being irreproducible, quasi-ununiformed and unstable [120]. In recent decades with nanotechnology taking center stage in various areas of science, the SERS surface have shifted from bulk solid metals to colloidal nanoparticles [120]. The nanoparticles offer higher surface area and tailored optical properties over bulk solid metals leading to higher reaction "hotspots" for better enhancement of SERS signal [121].

However, the use of colloidal metal NPs does not come without its own disadvantages. Metal NPs synthesized without any stabilizing agent often have unacceptably wide particle size distribution and a mixture of undesired morphologies. Hence compromising the reproducibility of SERS application [122]. Contrary, stabilized NPs offer controlled particle size and uniformity. However, it can block the targeted molecule from adsorbing to the metal surface through steric hindrance leading to suppressed SERS signal [123].

In general the success of SERS activity is dependent on the interaction between the adsorbed molecules and the surface of Plasmonic nanostructures. This means that if the two are mismatched activity is compromised leading to poor or no enhancement factor. It is a requirement that the analyte must adsorb on the surface effectively. Thus, it should have a higher SERS cross-section than any possible interference of contaminants. It must be noted that the influence of the metal substrate on both the physical and chemical properties of the metals and the stability including the reversibility or reusability of the same material remains a major drawback in respect to applicability [122]. On the other hand many methods used to produce nanostructured materials fail to produce materials with narrow size distribution leading to poor reproducibility of the SERS substrate.

Even though stabilized metal nanoparticles offers a better Plasmonic surface to bulk metals but it compromises the interaction with the targeted analyte. Therefore, to further advance this technology commercially, the retention of Plasmonic properties in the presence of stabilizing agents of colloidal nanoparticles will be key [124].

5. Future prospects of surface enhances Raman Spectroscopy in biomedical diagnostics

SERS interest has catapulted in the research community in the past couple of decades owing to its capability of quantitative analysis of very low concentrations and fingerprints of bio-species [125–130]. Over the past 4 decades SERS technique has blossomed into various areas of research and technology due to reasons discussed in details in the previous sections. However, further efforts are still required to further advance this technique commercially [131, 132]. In this section the highlights of future prospects of SERS real world applications in selected areas of research, application and technology will be discussed. General issues hindering this technique would also be briefly highlighted in order to understand its true potential.

The new and novel areas of application of SERS in the near future include ultraviolet-SERS, tip enhanced-SERS and biological sensing [132–136].

5.1 Ultraviolet surface enhanced Raman Spectroscopy (UV-SERS)

SERS applications are largely based on the enhancement of Raman scattering molecules that are either physically or chemically adsorbed onto a Plasmonic surface. The most effective surfaces for Raman reporters has been metallic surfaces such as Ag, Au and Cu because they produce superior enhancement factors [137–139]. However, they are limited to the visible and near infrared region of the spectrum, which limits the use of SERS in the same region. The use of SERS technique in the ultraviolet region is highly desirable and remains an unexplored terrain [132, 140–143]. UV SERS would enable resonance detection of molecules in the UV wavelength range such as protein residues, DNA bases etc. Analysis of these molecules are currently analyzed with other techniques which are often not as sensitive as SERS technique. The challenge of achieving UV SERS is finding a Plasmonic material that support surface enhancement in the UV region. Research has been on going in this area, however, there has been no success in finding a material that absorbs in the UV region that outperforms or close to the enhancement factors offered by Au, Ag and Cu metals [133, 142–144]. The potential of UV SERS outweighs the current challenges and with the rapid advancement in material nanotechnology, There is hope that the ideal material will be found in the near future.

5.2 Tip enhanced Raman Spectroscopy (TERS)

Merging of SERS technique with one of the microscopic techniques such as atomic force microscopy or scanning tunneling microscopy has led to the discovery of TERS, which is an advanced and powerful imaging tool. TERS is one of a very promising spectroscopic method that can analyze a variety of samples with sub diffraction limit imaging capabilities [145–147]. In TERS technique, the electromagnetic field enhancement is located at the sharp metallic/semiconducting cantilever tip that is eradicated with a laser beam. A localized region of SERS enhancement is created when the cantilever tip is brought in close proximity to the sample of interest; this enables structural and compositional analysis of the sample [145]. Spatial resolution achieved through this hybrid technique is far superior to any other

microscopic techniques commercially available. This method has been used for over a decade, initially for analysis of strong Raman scatterers such as buckyballs and dye molecules. However, the TERS technique has rapidly grown into characterization of more sophisticated and diverse samples such as single stranded RNA, individual single walled carbon nanotubes, hydrogen bonding in DNA [147], single particle dye sensitized solar cells, amongst others [146]. Few issues are still holding this technique from being routinely used commercially such as the difficulty of calculating the enhancing region for single molecules, hence the resolution and the enhancement factor. In the near future this issue would be solved since analysis of individual biomolecules is crucial for understanding and managing deadly diseases.

5.3 Biological diagnostic and sensing

SERS application has catapulted biosensing into new heights in the past decade. However, the prospects of becoming a commercial clinical diagnostic technology has stagnated. This is due to its low tissue penetration depth achievable [148, 149]. However, there are clinical areas where SERS have shown superiority and advantages over other techniques such as fluorescence imaging, MRI etc. The future of imaging single cells is likely to be via SERS based technology because of better brightness, high sensitivity and lack of photo bleaching [149]. Furthermore, SERS offers far better resolution in the micrometer range compared to commercially used MRI which offers resolution in a range of a few millimeters. The risk of using the low resolution MRI, for example, in detection of cancer cells would require 100 000 cancers cells in a tumor to be detectable via MRI technique [150]. This might render this detection too late for some aggressive tumors. Advantages offered by SERS in biosensing are likely to be leveraged in the future to make this technique a diagnostic technique with true clinical reach.

SERS is one of the most sensitive analytical methods which offers detection of molecules in low concentrations (ppb) and provides rich structural information. In this section some of the SERS applications that are likely to be commercially used outside of the laboratory while also demonstrating the versatility of this technique have been discussed. We envisage great extension of SERS technique and an advent use of UV SERS and TERS in biological diagnostic, sensing and imaging.

6. Conclusions

In this chapter recent advances relating to the use of SERS in biomedical applications were reviewed and reported. The point of departure was to concisely introduce the fundamentals of Plasmonics and SERS in respect to the enhancement of the Raman signal of molecules that are closest to the metallic nanostructures. For this reason, various metallic nanostructures that could be used to achieve the highest possible SERS enhancement factors were discussed. Discussion on the SERS efficiency of Plasmonic substrates made of gold nanowires prepared using a chemical route (bottom-up) was made. This was followed by the influence of the geometrical parameters (diameter and length of the wires or aspect ratio) on the SERS signal as a way to optimize it.

Emphasis was placed on the development of SERS as potentially dynamic technique for point-of-care diagnostics taking from its high sensitivity and multiplexing capabilities. The approach was to compare different methodologies that can be translated across various types of biomarkers. Techniques such as sandwich SERS and automated SERS for in vitro diagnostics have been elucidated. Moreover, progress made in respect to strong capture ligands such as DNA that are also specific

has been unpacked and holds promise for a much needed solution. These techniques have been used in both the detection and quantification of various diseases including malaria, cancer, hepatitis B, etc. using different bioanalytes such as antigens, protein, DNA, respectively. On the other hand developments on diseases diagnosis based on label-free sensing techniques that are sensitive to low analyte concentrations found in the physiological environment were elucidated as they offer hope for therapeutic intervention. It is relatively acceptable that most disease states usually start with small changes in cellular processes that ultimately augmented as the disease progresses without medical intervention. Although SERS sensitivity can be excellent in vivo measurements require an integrated system that is capable of providing spectral acquisition and analysis in real time.

Although SERS is undoubtedly a promising technique for diagnostic purposes and for uses as POC devices the success rests on overcoming the barriers through further advancements in the fabrication of SERS substrate, assays, platforms, and making them cost-effective.

The discussion has shown that SERS allows for the continuous and highly sensitive detection and quantification of various biomarkers and end-products of disease states, making it an excellent option in the diagnosis and treatment of various diseases that are cause health concerns. However, with advances in the development of different types of novel techniques such as UV-SERS and TERS, SERS will remain an indispensable technique showing a great promise for in vitro and in vivo disease detection.

Acknowledgements

The authors wish to acknowledge the financial support of Mintek, the Department of Science and Innovation (DSI) of South Africa, and the National Intellectual Property Management Office (NIPMO) of South Africa.

Author details

Nikiwe Mhlanga[1,2*], Phumlani Tetyana[1,2], Sanele Nyembe[1,2] and Lucky Sikhwivhilu[1,2]

1 DSI/Mintek Nanotechnology Innovation Centre, Randburg, South Africa

2 Advanced Materials Division, Mintek, Randburg, South Africa

*Address all correspondence to: nikiwem@mintek.co.za

References

[1] Henry AI, Sharma B, Cardinal MF, Kurouski D, Van Duyne RP. Surface-enhanced Raman spectroscopy biosensing: in vivo diagnostics and multimodal imaging. Analytical chemistry. 2016; 88(13):6638-6647.

[2] Chalapathi D, Padmanabhan S, Manjithaya R, Narayana C. Surface-enhanced Raman spectroscopy as a tool for distinguishing extracellular vesicles under autophagic conditions: a marker for disease diagnostics. The Journal of Physical Chemistry B. 2020; 124(48):10952-10960.

[3] Ember KJ, Hoeve MA, McAughtrie SL, Bergholt MS, Dwyer BJ, Stevens MM, Faulds K, Forbes SJ, Campbell CJ. Raman spectroscopy and regenerative medicine: a review. NPJ Regenerative medicine. 2017; 2(1):1-10.

[4] He Z, Qiu W, Kizer ME, Wang J, Chen W, Sokolov AV, Wang X, Hu J, Scully MO. Resolving the Sequence of RNA Strands by Tip-Enhanced Raman Spectroscopy. ACS Photonics. 2020; 8(2):424-430.

[5] Chen C, Zhou X, Ding T, Zhang J, Wang S, Xu J, Chen J, Dai J, Chen C. Preparation and characterization of ZnO/SiO_2/Ag nanoparticles as highly sensitive substrates for surface-enhanced Raman scattering. Mater. Lett. 2016;165: 55-58.

[6] Ding SY, Wu DY, Yang ZL, Ren B, Xu X, Tian ZQ. Some progresses in mechanistic studies on surface-enhanced Raman scattering, Chem. J. Chin. Univ. Chin. 2015; 29:2569-2581.

[7] Fu Q, Zhan Z, Dou J, Zheng X, Xu X, Wu M, Lei Y. Highly reproducible and sensitive SERS substrates with Ag inter-nanoparticle gaps of 5 nm fabricated by ultrathin aluminum mask technique. ACS Appl. Mat. Interfaces. 2015; 7:13322-13328.

[8] Chao Z, Song L, Zhou Y, Nie W, Chen P. Impact of PS/SiO_2 morphologies on the SERS activity of PS/SiO_2/Ag nanocomposite particles. Colloid Polym. Sci. 2014; 292:2841-2848.

[9] McLellan JM, Li Z, Siekkinen AR, Xia Y. The SERS activity of a supported Ag nanocube strongly depends on its orientation relative to laser polarization. Nano Lett. 2007; 7:1013-1017.

[10] Caridad JM, Winters S, McCloskey D, Duesberg GS, Donegan JF, Krstic V. Hot-volumes as uniform and reproducible SERS-detection enhancers in weakly-coupled metallic nanohelices. Sci. Rep. UK. 2017; 7:1-7.

[11] Araujo A, Caro C, Mendes MJ, Nunes D, Fortunato DE, Franco R, Aguas H, Martins R. Highly efficient nanoplasmonic SERS on cardboard packaging substrates. Nanotechnol. 2014; 25:415202.

[12] Mikac L, Ivanda M, Gotic M, Mihelj T, Horvat L. Synthesis and characterization of silver colloidal nanoparticles with different coatings for SERS application. J. Nanopart. Res. 2014; 16:2748.

[13] Lu X, Rycenga M, Skrabalak SE, Wiley B, Xia Y. Chemical synthesis of novel plasmonic nanoparticles. Annu Rev Phys Chem 2009; 60:167-192.

[14] Sharma B, Frontiera RR, Henry A-I, Ringe E, Duyne RPV. SERS: materials, applications, and the future. Materials Today. 2012; 15:16-25.

[15] Ding T, Sigle DO, Herrmann LO, Wolverson D, Baumberg JJ. Nanoimprint lithography of Al nanovoids for deep-UV SERS. ACS Appl Mater Interfaces. 2014; 6: 17358-17363.

[16] Jain PK, Lee KS, El-Sayed IH, El-Sayed MA. Calculated absorption

and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. J Phys Chem B. 2006; 110:7238-7248.

[17] Kelly KL, Coronado E, Zhao LL, Schatz GC. The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. J Phy. Chem. B. 2003; 107:668-677.

[18] Hutter E, Fendler JH. Exploitation of localized surface plasmon resonance. Adv Mater 2004; 16:1685-1706.

[19] Ly N, Foley K, Tao N. Integrated label-free protein detection and separation in real time using confined surface plasmon resonance imaging. Anal Chem 2007; 79: 2546-2551.

[20] Alvarez-Puebla RA, Arceo E, Goulet PJ, Garrido JJ, Aroca RF. Role of nanoparticle surface charge in surface-enhanced Raman scattering. J Phys Chem B 2005; 109:3787-3792.

[21] Faulds K, Littleford RE, Graham D, Dent G, Smith WE. Comparison of surface-enhanced resonance Raman scattering from unaggregated and aggregated nanoparticles. Anal Chem 2004; 76:592-598

[22] Mehmet Kahraman, Emma R. Mullen, Aysun Korkmaz, Sebastian Wachsmann-Hogiu. Fundamentals and applications of SERS-based bioanalytical sensing. Nanophotonics 2017; 6(5):831-852

[23] Kahraman M, Tokman N, Culha M. Silver nanoparticle thin films with nanocavities for surface-enhanced Raman scattering. Chem Phys Chem 2008; 9:902-910.

[24] Kahraman M, Tokman N, Türkoğlu G. Surface-enhanced Raman scattering on aggregates of silver nanoparticles with definite size. J Phys Chem C 2008; 112:10338-10343.

[25] Kahraman M, Aydin Ö, Culha M. Size effect of 3D aggregates assembled from silver nanoparticles on surface-enhanced Raman scattering. Chem Phys Chem 2009; 10: 537-542

[26] Lee SJ, Morrill AR, Moskovits M. Hot spots in silver nanowire bundles for surface-enhanced Raman spectroscopy. Journal of the American Chemical Society. 2006 Feb 22;128(7):2200-2210.

[27] Feng H, Yang Y, You Y, Li G, Guo J, Yu T, Shen Z, Wu T, Xing B. Simple and rapid synthesis of ultrathin gold nanowires, their self-assembly and application in surface-enhanced Raman scattering. Chemical Communications. 2009(15):1984-1986.

[28] Yilmaz A.; Yilmaz M. Bimetallic Core–Shell Nanoparticles of Gold and Silver via Bioinspired Polydopamine Layer as Surface-Enhanced Raman Spectroscopy (SERS) Platform. Nanomaterials. 2020; 10:688.

[29] Félidj N, Aubard, J, Lévi G, Krenn, JR., Hohenau A, Schider G, Leitner A, Aussenegg FR. Optimized surface-enhanced Raman scattering on gold nanoparticle arrays. Appl. Phys. Lett. 2003; 82:3095-3097.

[30] Haynes C.L, Van Duyne R.P. Plasmon-Sampled Surface-Enhanced Raman Excitation Spectroscopy. J. Phys. Chem. B 2003; 107:7426-7433.

[31] McFarland AD, Young MA, Dieringer JA, Van Duyne RP. Wavelength-Scanned Surface-Enhanced Raman Excitation Spectroscopy. J. Phys. Chem. B 2005; 109: 11279-11285.

[32] Laurent G, Félidj N, Aubard J, Lévi G. Krenn JR, Hohenau A, Aussenegg FR. Surface enhanced Raman scattering arising from multipolar plasmon excitation. J. Chem. Phys. 2005; 122:011102.

[33] Grand J, de la Chapelle ML, Bijeon J-L, Adam P-M, Vial A. Royer. Role of localized surface plasmons in surface-enhanced Raman scattering of shape-controlled metallic particles in regular arrays. P. Phys. Rev. B 2005; 72:033407.

[34] Billot L, Lamy de la Chapelle M, Grimault A-S, Vial A, Barchiesi D, Bijeon J-L, Adam PM, Royer P. Surface enhanced Raman scattering on gold nanowire arrays: Evidence of strong multipolar surface plasmon resonance enhancement. Chem. Phys. Lett. 2006; 422:303-307.

[35] Colas FJ, Cottat M, Gillibert R, Guillot N, Djaker N, Lidgi-Guigui N, Toury T, Barchiesi D, Toma A, Di Fabrizio E Red-Shift Effects in Surface Enhanced Raman Spectroscopy: Spectral or Intensity Dependence of the Near-Field? J. Phys. Chem. C 2016; 120:13675-13683.

[36] Guillot N, Shen H, Frémaux B, Péron O, Rinnert E, Toury T, Lamy de la Chapelle M. Surface enhanced Raman scattering optimization of gold nanocylinder arrays: Influence of the localized surface plasmon resonance and excitation wavelength. Appl. Phys. Lett. 2010; 97:023113.

[37] Gunnarsson L, Bjerneld E.J, Xu H, Petronis S, Kasemo B, Käll M. Interparticle coupling effects in nanofabricated substrates for surface enhanced Raman scattering. Appl. Phys. Lett. 2001; 78:802-804.

[38] Kessentini S, Barchiesi D, D'Andrea C, Toma A, Guillot N, Di Fabrizio E, Fzio B, Marago MO, Gucciardi GP, Lamy de la Chapelle MJ. Gold Dimer Nanoantenna with Slanted Gap for Tunable LSPR and Improved SERS. Phys. Chem. C 2014; 118: 3209-3219.

[39] D'Orlando A, Bayle M, Louarn G, Humbert B. AFM-Nano Manipulation of Plasmonic Molecules Used as "Nano-Lens" to Enhance Raman of Individual Nano-Objects. Materials 2019; 12:1372.

[40] Gillibert, R.; Sarkar, M.; Bryche, J.-F.; Yasukuni, R.; Moreau, J.; Besbes, M.; Barbillon, G.; Bartelian, B.; Canva, M.; de la Chapelle, M.L. Directional surface enhanced Raman scattering on gold nano-gratings. Nanotechnology. 2016; 27:115202.

[41] Gillibert R, Sarkar M, Moreau J, Besbes M, Canva M, de la Chapelle M.L. Near Field Enhancement Localization on Plasmonic Gratings. J. Phys. Chem. C 2016; 120: 27562-27570.

[42] Sarkar M, Bryche J-F, Moreau J, Besbes M, Barbillon G, Bartenlian B, Canva M. Generalized analytical model based on harmonic coupling for hybrid plasmonic modes: Comparison with numerical and experimental results. Opt. Express 2015; 23: 27376-27390.

[43] Sarkar M, Besbes M, Moreau J, Bryche J-F, Olivéro A, Barbillon G, Coutrot AL, Bartenlien B, Canva M. Hybrid plasmonic mode by resonant coupling of localized plasmons to propagating plasmons in a Kretschmann configuration. ACS Photonics 2015; 2:237-245.

[44] Sivashanmugan K, Liao J-D, You J-W, Wu C-L. Focused-ion-beam-fabricated Au/Ag multilayered nanorod array as SERS-active substrate for virus strain detection. Sensors and Actuators B 2013; 181:361– 367

[45] Tian ZQ, Ren B, Li JF, Yang ZL. Expanding generality of surface enhanced Raman spectroscopy with borrowing SERS activity strategy. Chemical Communications 2007; 34:3514-3534.

[46] Le F, Brandl DW, Urzhumov YA, Wang H, Kundu J, Halas NJ, Aizpurua J, Nordlander P. Metallic nanoparticle arrays: a common substrate for both

surface-enhanced Raman scattering and surface-enhanced infrared absorption. American Chemical Society Nano 2008; 2 (4):707-718.

[47] Le Ru EC, EtchegoinGrand J, Felidj N, Aubard J, Levi G, Hohenau A, Krenn JR. Surface enhanced Raman spectroscopy on nanolithography-prepared substrates. Current Applied Physics2008; 8:467-470.

[48] Das G, Mecarini F, Gentile F, De Angelis F, Kumar MHG, Candeloro P, Liberale C, Cuda G, Di Fabrizio E. Nano-patterned SERS substrate: application for protein analysis vs. temperature, Biosensor and Bioelectronics. 2009; 24:1693-1699.

[49] Chang CW, Liao JD, Lin YY, Weng C. Fabrication of nano-indented cavities on Au for the detection of chemically-adsorbed DTNB molecular probes through SERS effect. Journal of Colloid and Interface Science. 2011; 358;384-391.

[50] Bao PD, Huang TQ, Liu XM, Wu TQ. Surface-enhanced Raman spectroscopy of insect nuclear polyhedrosis virus. Journal Raman Spectroscopy. 32001; 2 (4): 227-230.

[51] Chang CW, Liao JD, Shiau AL, Yao CK. Non-labeled virus detection using inverted triangular Au nano-cavities arrayed as SERS-active substrate. Sensor and Actuators B: Chemical 2011; 156 (1):471-478.

[52] Chang CW, Liao JD, Lin YY, Weng CC. Detecting very small quantity of molecular probes in solution using nano-mechanically made Au-cavities array with SERS-active effect. Sensor and Actuators B: Chemical 2011; 153 (1):271-276.

[53] Yao CK, Liao JD, Chang CW, Lin JR. Spatially reinforced nano-cavity array as the SERS-active substrate for detecting hepatitis virus core antigen at low concentrations. Sensors and Actuators B. 2012; 174:478-484.

[54] Lin YY, Liao JD, Ju YH, Chang CW, Shiau AL. Focused ion beam-fabricated Au micro/nanostructures used as a surface enhanced Raman scattering-active substrate for trace detection of molecules and influenza virus. Nanotechnology. 2011; 22: 185308-185316.

[55] Lin YY, Liao JD, Yang ML, Wu CL. Target-size embracing dimension for sensitive detection of viruses with various sizes and influenza virus strains. Biosensors and Bioelectronics. 2012; 35:447-451.

[56] Xie Z, Tao J, Lu Y, Lin K, Yan J, Wang P, Ming H. Polymer optical fiber SERS sensor with gold nanorods. Optical Communications. 2009; 282 (3): 439-442.

[57] Zhou W, Gao X, Liu D, Chen X. Gold nanoparticles for *in vitro* diagnostics. Chemical Reviews. 2015;115(19):10575-10636.

[58] Kong K, Kendall C, Stone N, Notingher I. Raman spectroscopy for medical diagnostics—From in-vitro biofluid assays to in-vivo cancer detection. Advanced drug delivery reviews. 2015;89:121-134.

[59] Abramczyk H, Brozek-Pluska B. Raman imaging in biochemical and biomedical applications. Diagnosis and treatment of breast cancer. Chemical reviews. 2013;113(8):5766-5781.

[60] Wang Z, Zong S, Wu L, Zhu D, Cui Y. SERS-activated platforms for immunoassay: probes, encoding methods, and applications. Chemical reviews. 2017;117(12):7910-7963.

[61] Joseph MM, Narayanan N, Nair JB, Karunakaran V, Ramya AN, Sujai PT, et al. Exploring the margins of SERS in practical domain: An emerging

diagnostic modality for modern biomedical applications. Biomaterials. 2018;181:140-181.

[62] Lin J, Chen R, Feng S, Pan J, Li Y, Chen G, et al. A novel blood plasma analysis technique combining membrane electrophoresis with silver nanoparticle-based SERS spectroscopy for potential applications in noninvasive cancer detection. Nanomedicine: Nanotechnology, Biology and Medicine. 2011;7(5):655-663.

[63] Batool F, Nawaz H, Majeed MI, Rashid N, Bashir S, Akbar S, et al. SERS-based viral load quantification of hepatitis B virus from PCR products. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2021:119722.

[64] Yadav S, Senapati S, Desai D, Gahlaut S, Kulkarni S, Singh J. Portable and sensitive Ag nanorods based SERS platform for rapid HIV-1 detection and tropism determination. Colloids and Surfaces B: Biointerfaces. 2021;198:111477.

[65] Lin D, Wu Q, Qiu S, Chen G, Feng S, Chen R, et al. Label-free liquid biopsy based on blood circulating DNA detection using SERS-based nanotechnology for nasopharyngeal cancer screening. Nanomedicine: Nanotechnology, Biology and Medicine. 2019;22:102100.

[66] Shen L, Du Y, Wei N, Li Q, Li S, Sun T, et al. SERS studies on normal epithelial and cancer cells derived from clinical breast cancer specimens. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020;237:118364.

[67] Moisoiu V, Badarinza M, Stefancu A, Iancu SD, Serban O, Leopold N, et al. Combining surface-enhanced Raman scattering (SERS) of saliva and two-dimensional shear wave elastography (2D-SWE) of the parotid glands in the diagnosis of Sjögren's syndrome. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020;235:118267.

[68] Muhammad M, Shao C, Huang Q. Label-free SERS diagnostics of radiation-induced injury via detecting the biomarker Raman signal in the serum and urine bio-samples based on Au-NPs array substrates. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2019;223:117282.

[69] Narasimhan V, Siddique RH, Park H, Choo H. Bioinspired disordered flexible metasurfaces for human tear analysis using broadband surface-enhanced Raman scattering. ACS omega. 2020;5(22):12915-22.

[70] Rojalin T, Koster HJ, Liu J, Mizenko RR, Tran D, Wachsmann-Hogiu S, et al. Hybrid nanoplasmonic porous biomaterial scaffold for liquid biopsy diagnostics using extracellular vesicles. ACS sensors. 2020;5(9):2820-2833.

[71] Su X, Xu Y, Zhao H, Li S, Chen L. Design and preparation of centrifugal microfluidic chip integrated with SERS detection for rapid diagnostics. Talanta. 2019;194:903-909.

[72] Wang X, Xiao M, Zou Y, Lai W, Pei H, Alam MF, et al. Fractal SERS nanoprobes for multiplexed quantitative gene profiling. Biosensors and Bioelectronics. 2020;156:112130.

[73] Ngo HT, Gandra N, Fales AM, Taylor SM, Vo-Dinh T. Sensitive DNA detection and SNP discrimination using ultrabright SERS nanorattles and magnetic beads for malaria diagnostics. Biosensors and Bioelectronics. 2016;81:8-14.

[74] Frimpong R, Jang W, Kim J-H, Driskell JD. Rapid vertical flow immunoassay on AuNP plasmonic paper

for SERS-based point of need diagnostics. Talanta. 2021;223:121739.

[75] Liu H, Dai E, Xiao R, Zhou Z, Zhang M, Bai Z, et al. Development of a SERS-based lateral flow immunoassay for rapid and ultra-sensitive detection of anti-SARS-CoV-2 IgM/IgG in clinical samples. Sensors and Actuators B: Chemical. 2021;329:129196.

[76] Zhang D, Huang L, Liu B, Ni H, Sun L, Su E, et al. Quantitative and ultrasensitive detection of multiplex cardiac biomarkers in lateral flow assay with core-shell SERS nanotags. Biosensors and Bioelectronics. 2018;106:204-211.

[77] Fu X, Cheng Z, Yu J, Choo P, Chen L, Choo J. A SERS-based lateral flow assay biosensor for highly sensitive detection of HIV-1 DNA. Biosensors and Bioelectronics. 2016;78:530-537.

[78] Willner MR, McMillan KS, Graham D, Vikesland PJ, Zagnoni M. Surface-enhanced Raman scattering based microfluidics for single-cell analysis. Analytical chemistry. 2018;90(20):12004-12010.

[79] Kapara A, Findlay Paterson KA, Brunton VG, Graham D, Zagnoni M, Faulds K. Detection of Estrogen Receptor Alpha and Assessment of Fulvestrant Activity in MCF-7 Tumor Spheroids Using Microfluidics and SERS. Analytical Chemistry. 2021.

[80] Kunushpayeva Z, Rapikov A, Akhmetova A, Sultangaziyev A, Dossym D, Bukasov R. Sandwich SERS immunoassay of human immunoglobulin on silicon wafer compared to traditional SERS substrate, gold film. Sensing and Bio-Sensing Research. 2020;29:100355.

[81] Li M, Cushing SK, Zhang J, Suri S, Evans R, Petros WP, et al. Three-dimensional hierarchical plasmonic nano-architecture enhanced surface-enhanced Raman scattering immunosensor for cancer biomarker detection in blood plasma. ACS nano. 2013;7(6):4967-4976.

[82] Granger JH, Porter MD. The case for human serum as a highly preferable sample matrix for detection of anthrax toxins. ACS sensors. 2018;3(11):2303-2310.

[83] Știufiuc GF, Toma V, Buse M, Mărginean R, Morar-Bolba G, Culic B, et al. Solid plasmonic substrates for breast cancer detection by means of SERS analysis of blood plasma. Nanomaterials. 2020;10(6):1212.

[84] Králová ZO, Oriňak A, Oriňaková R, Petruš O, Macko J, Radoňak J, et al. Electrochemically deposited silver detection substrate for surface-enhanced Raman spectroscopy cancer diagnostics. Journal of biomedical optics. 2018;23(7):075002.

[85] Choi M, Kim S, Choi SH, Park H-H, Byun KM. Highly reliable SERS substrate based on plasmonic hybrid coupling between gold nanoislands and periodic nanopillar arrays. Optics express. 2020;28(3):3598-3606.

[86] Mhlanga N, Domfe T, Skepu A. Fabrication of surface enhanced Raman spectroscopy substrates on solid supports. Applied Surface Science. 2019;476:1108-1117.

[87] Mhlanga N, Domfe T, Skepu A, Ntho TA. Sandwich-based surface-enhanced Raman scattering probes for detection and quantification of malaria. Journal of Raman Spectroscopy. 2020;51(12):2416-2424.

[88] Xu K, Zhou R, Takei K, Hong M. Toward flexible surface-enhanced raman scattering (SERS) sensors for point-of-care diagnostics. Advanced Science. 2019;6(16):1900925.

[89] Xu K, Wang Z, Tan CF, Kang N, Chen L, Ren L, et al. Uniaxially

stretched flexible surface plasmon resonance film for versatile surface enhanced Raman scattering diagnostics. ACS applied materials & interfaces. 2017;9(31):26341-26349.

[90] Fan Z, Kanchanapally R, Ray PC. Hybrid graphene oxide based ultrasensitive SERS probe for label-free biosensing. The Journal of Physical Chemistry Letters. 2013;4(21):3813-3818.

[91] Korkmaz A, Kenton M, Aksin G, Kahraman M, Wachsmann-Hogiu S. Inexpensive and flexible SERS substrates on adhesive tape based on biosilica plasmonic nanocomposites. ACS Applied Nano Materials. 2018;1(9):5316-5326.

[92] He, S, Liu K.-K, Su S, Yan J, Mao X, Wang D, He Y, Li L.-J, Song S, Fan C. Graphene-based high-efficiency surface-enhanced Raman scattering-active platform for sensitive and multiplex DNA detection. Analytical chemistry, 2012:84(10): 4622-4627.

[93] Chen H, Das A, Bi L, Choi N, Moon J-I, Wu Y, et al. Recent advances in surface-enhanced Raman scattering-based microdevices for point-of-care diagnosis of viruses and bacteria. Nanoscale. 2020;12(42):21560-21570.

[94] Ma Y, Liu H, Chen Y, Gu C, Wei G, Jiang T. Improved lateral flow strip based on hydrophilic– hydrophobic SERS substrate for ultra– sensitive and quantitative immunoassay. Applied Surface Science. 2020;529:147121.

[95] Fallahi H, Zhang J, Phan H-P, Nguyen N-T. Flexible microfluidics: Fundamentals, recent developments, and applications. Micromachines. 2019;10(12):830.

[96] Xing Y, Zhao L, Cheng Z, Lv C, Yu F, Yu F. Microfluidics-Based Sensing of Biospecies. ACS Applied Bio Materials. 2020.

[97] Pallaoro A, Hoonejani MR, Braun GB, Meinhart CD, Moskovits M. Rapid identification by surface-enhanced Raman spectroscopy of cancer cells at low concentrations flowing in a microfluidic channel. Acs Nano. 2015;9(4):4328-4336.

[98] Cordero E, Latka I, Matthäus C, Schie IW, Popp J. In-vivo Raman spectroscopy: from basics to applications. Journal of biomedical optics. 2018;23(7):071210.

[99] Sato H, Tanaka T, Ikeda T, Wada S, Tashiro H, Ozaki Y. Biomedical applications of a new portable Raman imaging probe. Journal of Molecular Structure. 2001;598(1):93-96.

[100] Bergholt MS, Serio A, Albro MB. Raman spectroscopy: Guiding light for the extracellular matrix. Frontiers in bioengineering and biotechnology. 2019;7:303.

[101] Wachsmann-Hogiu S, Weeks T, Huser T. Chemical analysis in vivo and in vitro by Raman spectroscopy—from single cells to humans. Current opinion in biotechnology. 2009;20(1):63-73.

[102] Baraldi P, Tinti A. Raman spectroscopy in art and archaeology. Journal of Raman Spectroscopy: An International Journal for Original Work in all Aspects of Raman Spectroscopy, Including Higher Order Processes, and also Brillouin and Rayleigh Scattering. 2008;39(8):963-965.

[103] Kim JA, Wales DJ, Yang G-Z. Optical spectroscopy for in vivo medical diagnosis—a review of the state of the art and future perspectives. Progress in Biomedical Engineering. 2020;2(4):042001.

[104] Ramos IRM, Malkin A, Lyng FM. Current advances in the application of Raman spectroscopy for molecular diagnosis of cervical cancer. BioMed research international. 2015;2015.

[105] Moore TJ, Moody AS, Payne TD, Sarabia GM, Daniel AR, Sharma B. In vitro and in vivo SERS biosensing for disease diagnosis. Biosensors. 2018;8(2):46.

[106] Auner GW, Koya SK, Huang C, Broadbent B, Trexler M, Auner Z, et al. Applications of Raman spectroscopy in cancer diagnosis. Cancer and Metastasis Reviews. 2018;37(4):691-717.

[107] Fenn MB, Xanthopoulos P, Pyrgiotakis G, Grobmyer SR, Pardalos PM, Hench LL. Raman spectroscopy for clinical oncology. Advances in Optical Technologies. 2011;2011.

[108] Ramírez-Elías MG, González FJ. Raman spectroscopy for in vivo medical diagnosis. Raman Spectroscopy (April). 2018.

[109] Ramírez-Elías MG, González FJ. Raman spectroscopy for in vivo medical diagnosis. Raman Spectroscopy (April). 2018 Feb 28.

[110] Zhao J, Lui H, McLean DI, Zeng H. Real-time Raman spectroscopy for noninvasive in vivo skin analysis and diagnosis. New developments in biomedical engineering. 2010;24:455-474.

[111] Wolthuis R, Schut TB, Caspers P, Buschman H, Römer T, Bruining H, et al. Raman spectroscopic methods for in vitro and in vivo tissue characterization. Fluorescent and luminescent probes for biological activity: Elsevier; 1999. p. 433-455.

[112] Pence I, Mahadevan-Jansen A. Clinical instrumentation and applications of Raman spectroscopy. Chemical Society Reviews. 2016;45(7):1958-1979.

[113] Rzhevskii A. The recent advances in Raman microscopy and imaging techniques for biosensors. Biosensors. 2019;9(1):25.

[114] Wang W, Zhao J, Short M, Zeng H. Real-time in vivo cancer diagnosis using raman spectroscopy. Journal of biophotonics. 2015;8(7):527-545.

[115] Wei D, Chen S, Liu Q. Review of fluorescence suppression techniques in Raman spectroscopy. Applied Spectroscopy Reviews. 2015;50(5):387-406.

[116] Krafft C, Sergo V. Biomedical applications of Raman and infrared spectroscopy to diagnose tissues. Spectroscopy. 2006;20(5-6):195-218.

[117] Saha A, Barman I, Dingari N, McGee S, Volynskaya Z, Galindo L, et al. Raman spectroscopy: a real-time tool for identifying microcalcifications during stereotactic breast core needle biopsies. Biomedical optics express. 2011;2(10):2792-2803.

[118] Lieber CA, Majumder SK, Ellis DL, Billheimer DD, Mahadevan-Jansen A. In vivo nonmelanoma skin cancer diagnosis using Raman microspectroscopy. Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery. 2008; 40(7):461-467.

[119] Zhengkun, W. Ning, S. Yong, Z. Jie, Z. AgNPs decorated volcano-like Ag arrays for ultra-sensitive Raman detection. Optical Materials Express, 2020, 10 (12), 3393-3399.

[120] Morton, S. M.; Jensen, L. Understanding the Molecule-Surface Chemical Coupling in SERS. J. Am. Chem. Soc. 2009, 131, 4090– 4098.

[121] Kleinman, S. L.; Sharma, B.; Blaber, M. G.; Henry, A.-I.; Valley, N.; Freeman, R. G.; Natan, M. J.; Schatz, G. C.; Van Duyne, R. P. Structure Enhancement Factor Relationships in Single Gold Nano antennas by Surface-Enhanced Raman Excitation Spectroscopy. J. Am. Chem. Soc. 2013, 135, 301–308.

[122] C. Zapata-Urzua, M. P ´ ´erez-Ortiz, G. A. Acosta, J. Mendoza, L. Yedra, S. Estrad´e, A. Alvarez-Lueje, L. J. N ´ u´nez-Vergara, ˜ F. Albericio, R. Lavilla and M. J. Kogan, J. Colloid Interface Sci., 2015, 453, 260-269

[123] T. L. Moore, L. Rodriguez-Lorenzo, V. Hirsch, S. Balog, D. Urban, C. Jud, B. Rothen-Rutishauser, M. Lattuada and A. Petri-Fink, Chem. Soc. Rev., 2015, 44, 6287-6305.

[124] P. G. Etchegoin and E. C. Le Ru, Phys. Chem. Chem. Phys., 2008, 10, 6079-6089.

[125] Guillot, N. and Lamy de la Chapelle, M. The electromagnetic effect in surface enhanced Raman scattering: Enhancement optimization using precisely controlled nanostructures, Journal of Quantitative Spectroscopy and Radiative Transfer, 2012, 113(18), 2321-2333,

[126] Langer, J., Jimenez de Aberasturi, D., Aizpurua, J., Alvarez-Puebla, R. A., Auguié, B., Baumberg, J. J., Liz-Marzán, L. M. Present and future of surface-enhanced Raman scattering. 2020:ACS Nano, 14(1), 28-117.

[127] Dieringer, J. A., McFarland, A. D., Shah, N. C., Stuart, D. A., Whitney, A. V., Yonzon, C. R., Van Duyne, R. P. Introductory Lecture : Surface enhanced Raman spectroscopy: new materials, concepts, characterization tools, and applications. 2006: Faraday Discussions, 132, 9-26.

[128] Sivashanmugan, K., Liao, J., Liu, B.H., Yao, C., Focused-ion-beam-fabricated Au nanorods coupled with Ag nanoparticles used as surface-enhanced Raman scattering-active substrate for analyzing trace melamine constituents in solution, Analytica Chimica Acta, 2013, 800, 56-64.

[129] Stiles, P. L., Surface-enhanced Raman Spectroscopy. 2008: Annu Rev Anal Chem, 1, 601.

[130] Langer, J, Jimenez de Aberasturi, D., Aizpurua, J., Present and Future of Surface-Enhanced Raman Scattering, ACS Nano, 2020, 14 (1), 28-117

[131] Gagnon, P. Technology trends in antibody purification. 2012: Journal of Chromatography A, 1221, 57-70.

[132] Grilo, A. L., & Mantalaris, A. The increasingly human and profitable monoclonal antibody market. 2019: Trends in Biotechnology, 37(1), 9-16.

[133] Dörfer, T. Deep-UV surface-enhanced Raman scattering. 2007: J Raman Spectrosc, 38 (1),1379-1381.

[134] Abalde-Cela Sara, Aldeanueva-Potel Paula, Mateo-Mateo Cintia, Rodríguez-Lorenzo Laura, Alvarez-Puebla Ramón A. and Liz-Marzán Luis M. Surface-enhanced Raman scattering biomedical applications of plasmonic colloidal particles J. R. Soc. Interface. 2010, 7, 435-450

[135] Gao L, Zhao, H., Li, T, Huo, P., Chen D and Liu, B, Atomic Force Microscopy Based Tip-Enhanced Raman Spectroscopy in Biology, International journal of molecular sciences, 2018, 19(4), 1193-1195.

[136] Alvarez-Puebla, R. A., Liz-Marzan, L. M., & Abajo, F. J.´ Light Concentration at the Nanometer Scale. 2010:J. Phys. Chem. Lett, 1, 2428 2434.

[137] Solís, D. M., Taboada, J. M., Obelleiro, F., Liz-Marzán, L. M., & García de Abajo, F. J. Optimization of nanoparticle-based SERS substrates through large-scale realistic simulations. 2017: ACS Photonics, 4(2), 329-337.

[138] Li, K., Stockman, M. I., & Bergman, D. J. Self-similar chain of metal nanospheres as an efficient nanolens. 2003: Physical Review Letters, 91(22), 227402.

[139] Liu, Y., Tian, X., Guo, W. et al. Real-time Raman detection by the cavity

mode enhanced Raman scattering. Nano Research, 2019, 12 (7), 1643-1649.

[140] Taguchi, A. Deep-UV tip-enhanced Raman scattering 2009: J Raman Spectrosc, 40 (1), 1324-1330.

[141] Tian, Z.Q. SERS from Transition Metals and Excited by Ultraviolet Light Top. 2006: Appl Phys, 103 (1) 125-128.

[142] Sharma, B., Cardinal, M. F., Ross, M. B., Zrimsek, A. B., Bykov, S. V., Punihaole, D., Van Duyne, R.P. Aluminum Film-Over-Nanosphere Substrates for Deep-UV Surface Enhanced Resonance Raman Spectroscopy. 2016: Nano Lett, 16, 7968 7973.

[143] Ren, B., Surface-Enhanced Raman Scattering in the Ultraviolet Spectral Region: UV-SERS on Rhodium and Ruthenium Electrodes. 2003: J Am Chem Soc, 125, 9598.

[144] Stockle, R. M., Nanoscale chemical analysis by tip-enhanced Raman spectroscopy. 2000: Chem Phys Lett, 318, 131.

[145] Domke, K. F., & Pettinger, B. Studying surface chemistry beyond the diffraction limit: 10 years of TERS. 2010: Chemphyschem: A European Journal of Chemical Physics and Physical Chemistry, 11(7), 1365-1373.

[146] Bailo, E., & Deckert, V., Tip-enhanced Raman scattering. 2008: Chemical Society Reviews, 37(5), 921-930.

[147] Bailo, E., & Deckert, V., Tip-enhanced Raman spectroscopy of single RNA strands: towards a novel direct-sequencing method. 2008: Angewandte Chemie (International Ed. in English), 47(9), 1658-1661.

[148] Fabris, L., SERS tags: The next promising tool for personalized cancer detection?. 2016: ChemNanoMat: Chemistry of Nanomaterials for Energy, Biology and More, 2(4), 249-258.

[149] Indrasekara, A. S. D. S., & Fabris, L., SERS-Based Approaches toward Genetic Profiling. 2015: Bioanalysis, 7, 263 278.

[150] Ngo, H. T., Freedman, E., Odion, R. A., Strobbia, P., De Silva Indrasekara, A. S., Vohra, P., ... Vo-Dinh, T., Direct detection of unamplified pathogen RNA in blood lysate using an integrated lab-in-a-stick device and ultrabright SERS nanorattles. 2018: Scientific Reports, 8(1). doi:10.1038/s41598-018-21615-3