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REVIEW

SERS: a versatile tool in chemical and biochemical diagnostics

Katharina Hering · Dana Cialla · Katrin Ackermann · Thomas Dörfer · Robert Möller · Henrik Schneidewind · Roland Mattheis · Wolfgang Fritzsche · Petra Rösch · Jürgen Popp

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Abstract Raman spectroscopy is a valuable tool in various research fields. The technique yields structural information from all kind of samples often without the need for extensive sample preparation. Since the Raman signals are inherently weak and therefore do not allow one to investigate substances in low concentrations, one possible approach is surface-enhanced (resonance) Raman spectroscopy. Here, rough coin metal surfaces enhance the Raman signal by a factor of 10^4–10^{15}, depending on the applied method. In this review we discuss recent developments in SERS spectroscopy and their impact on different research fields.

Keywords Surface-enhanced Raman spectroscopy · SERS substrates · Single-molecule detection · Tip-enhanced Raman spectroscopy

Introduction

Since the first observation of an enhanced Raman signal of pyridine adsorbed on a roughened silver electrode by Fleischmann et al. [1] in 1974, this effect—surface-enhanced Raman scattering (SERS)—has been a field of great scientific interest. The SERS technique has gained particular importance in recent years in many different research fields like surface science, electrochemistry, biology, and material science. An even larger enhancement is observed when the SERS technique is coupled with the resonance Raman enhancement. Indeed, single-molecule detection has even been reported with surface-enhanced resonance Raman scattering (SERRS).

Although SERS spectroscopy is widely applied, the mechanism leading to the surface enhancement is not completely understood yet. In general there are two main contributions: the electromagnetic and the charge transfer (CT) mechanism.

The electromagnetic contribution of the SERS enhancement is due to an electromagnetic field enhancement caused by a plasmon excitation through the incident laser light on the metal surface. Electrons in the metal are excited to an oscillation against the metal cores, called a surface plasmon. In nanostructured surfaces the exited surface plasmons lead to an electromagnetic field, which reaches out of the metal surface, where the analyte is located [2].

Since surface plasmons have to be excited by the incident laser light, the excitation wavelength for a SERS experiment must be adapted to the plasmon wavelength of the respective metal and also to the nanostructure of the metal surface, which also has an effect on the plasmon resonance wavelength. For this reason SERS excitation lines cover mainly the visible spectral region up to the near infrared (NIR) between 450 and 1,064 nm. With these laser wavelengths the surface plasmons of coinage metals like silver and gold, which are widely used as SERS-active substrates, can be excited. Excitation with UV light has also been reported in the literature [3].

The CT mechanism on the other hand is associated with a charge transfer process between the adsorbed analyte and

K. Hering · D. Cialla · K. Ackermann · T. Dörfer · R. Möller · P. Rösch · J. Popp (✉)
Institut für Physikalische Chemie,
Friedrich-Schiller-Universität Jena,
Helmholtzweg 4,
07743 Jena, Germany
e-mail: juergen.popp@uni-jena.de

H. Schneidewind · R. Mattheis · W. Fritzsche · J. Popp
Institut für Photonische Technologien,
Albert-Einstein-Str. 9,
07745 Jena, Germany

the metal surface. Therefore, vibrations involved in the charge transfer process are enhanced, which is similar to the resonance enhancement [4].

The SERS effect is used and applied in several ways, for example, tip-enhanced Raman spectroscopy (TERS) in which an atomic force microscopy (AFM) tip is coated with a SERS-active metal [5]. This tip, through which the laser light is guided, is brought close to the analyte. This technique can afford a very high lateral resolution. In biology SERS is used as an analytical tool for DNA and bacteria which can be investigated in the living state. Processes in living cells can also be detected with the technique. For quantitative SERS measurements the technique can be combined with fluidic devices or with isotopically labeled standards. This review gives an overview of the latest developments in such SERS topics.

SERS-active substrates

SERS requires metallic structures with feature sizes clearly below the wavelength of light. The ability of a metal to show the SERS effect is determined through the frequency-dependent complex dielectric function, namely the ratio between real and imaginary parts and their relation to the dielectric function of the environment. The enhancement effect increases with the electrical field gradient experienced by the adsorbed molecule. Thus, any surface protrusions or particles acting as SERS-active features should be as sharp as possible due to the so-called lightning rod or antenna effect. Furthermore, the enhancement can be increased between two or more neighboring sharp structures, i.e., arrays made of opposite features are preferable. Further requirements for SERS-active substrates are: (1) chemical and biological compatibility with the analytes or cells which have to be measured, (2) chemical and temporal stability, and (3) reproducible and economical preparation.

Rough surfaces and island films

Electrochemically roughened metal electrodes were the first substrates showing surface-enhanced Raman scattering [1] and enhancement factors for pyridine of about 10^5–10^6 were achieved [6]. Silver nanoparticles, grown on glass substrates, show the best average enhancement factors of about 10^7 [7]. SERS-active surfaces can also be prepared by deposition onto inexpensive rough substrates, e.g., zeolite crystals [8] or porous glass ceramics. Rough surfaces may offer high sensitivity because their larger surface area may provide more target molecules. Gold dendrite nanostructures were prepared by using a galvanic exchange reaction achieving distances in the range of the effective plasmon resonance [9]. Nanoporous gold SERS-active substrates prepared by corroding a silver–gold alloy gave an enhancement factor of 10^9–10^{11} [10]. A similar approach using random hole arrays in gold films leads to hole-enhanced Raman spectroscopy (HERS) with an enhancement of 10^{11} [11].

Metal colloids and nanoparticles

The preparation of colloid dispersions of metal nanoparticles by an easy reduction of the respective metal salts with various reduction agents requires only simple chemical laboratory equipment [12]. Spheres show naturally a smaller enhancement compared to ellipsoidal or spiky particles. Particles in colloidal dispersions often show a size and shape distribution, leading to an undesired broadening of the resonance.

Beside pure silver or gold nanoparticles, core-shell bimetal nanoparticles of silver and gold allow a shift of the resonance frequency as a function of the shell thickness [13]. Jackson and Halas obtained enhancement factors above 10^{10} using silver nanoshells [14]. For the in situ investigation of catalytic reactions with SERS, mixed colloids of silver or gold with the catalytically important palladium were prepared [15]. Not only spherical particles can be prepared, but a variety of shapes including cubes [16, 17], prisms [18], rods [19], and octahedra [20] depending on reaction conditions and surface-active agents.

Nanosphere lithography (NSL) and films over nanospheres (FON)

Nanosphere lithography (NSL) was proposed by Fischer and Zingsheim in 1981 [21]. In principle, a solvent containing nanospheres of homogeneous diameter (mostly polystyrene latex spheres) is arranged to dry in a dense monolayer on a substrate serving as a mask for a directed deposition of a metal layer (10–100 nm), giving an hexagonal array of triangles. Figure 1 (adapted from [22]) shows the principle and an example of an NSL array. In 1995 Hulteen and van Duyne prepared periodic particle arrays showing nanometer features using single layer and double layer self-assembled sphere masks. They reported the preparation of defect-free silver arrays with areas of 4–25 μm^2 and proposed the term nanosphere lithography (NSL) [23]. Enhancement factors above 10^7 were measured on benzenethiol adsorbed on NSL-fabricated silver arrays [24].

The films over nanospheres (FON) approach uses the NSL process without removing the spheres, resulting in moth-eye-like metal surfaces as a possible SERS-active substrate [25, 26]. With FON structures enhancement factors of about 10^6 were obtained compared to 10^8 with NSL structures [27]. Multilayer FON substrates with gold

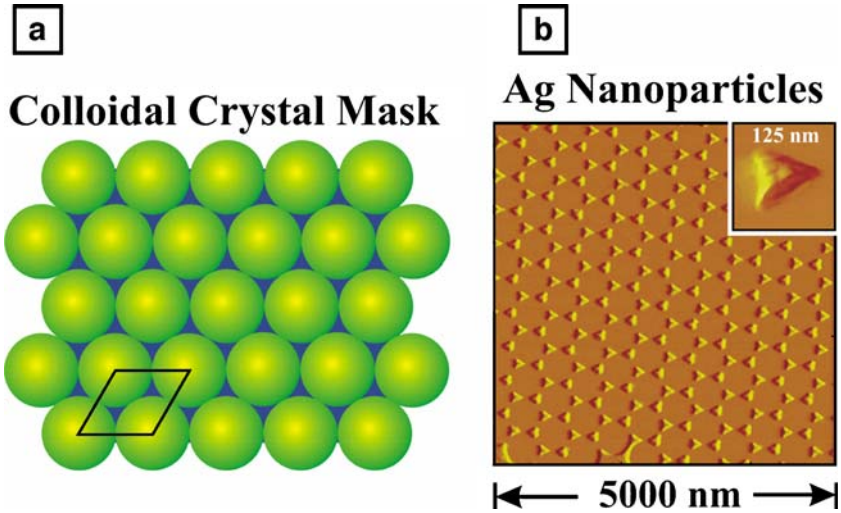

Fig. 1 Schematic principle (**a**) and representative AFM image (**b**) of a single layer NSL pattern. The AFM image was taken from an array prepared using nanospheres of 542-nm diameter and 48 nm thermally evaporated silver (adapted from [22])

over silver show the high enhancement of 10^6 of silver, while providing the long-term stability from gold [28].

Electron beam lithography (EBL)

Electron beam lithography (EBL) as a standard microelectronics technology is an excellent method to prepare highly reproducible patterns of variable shape down to some tens of nanometers in size. Etching as well as lift-off were already tested to produce SERS-active substrates with different materials, e.g., silver, gold, or copper deposited by sputtering or evaporation [29]. Felidj and co-workers [30] measured SERS spectra on oblate gold spheroid arrays prepared by EBL. The enhancement factor was in the order of 10^5, assuming one monolayer to be active. The highest enhancement factors achieved on gold arrays of 2×10^6 were reported in [31]. Experiments on gold dot arrays revealed that repeated usage after substrate cleaning yielded reproducible results [32].

With EBL a number of different shaped structures and gratings were formed including cylinders and trigonal prisms in square and hexagonal arrays (see for example Fig. 2 adapted from [33]) or so-called nanowires, i.e., elongated structures with a high aspect ratio, in order to

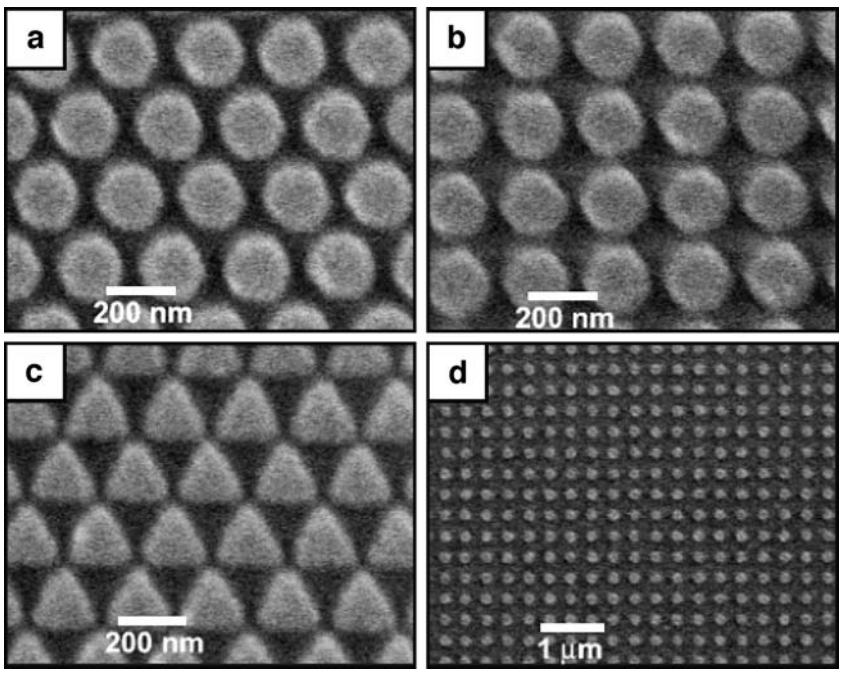

Fig. 2 Scanning electron micrographs of EBL-prepared nanoparticle arrays (all 35 nm in height): **a** hexagonal array of cylindrical structures with a diameter of 200 nm, period 260 nm; **b** square array of cylindrical structures with a diameter of 200 nm, period 250 nm; **c** hexagonal array of trigonal prisms with a perpendicular bisector of 170 nm, period 230 nm; **d** square array of cylindrical structures with a diameter of 200 nm, period 350 nm (adapted from [33])

study effects of higher multipole order [34]. De Jesus and co-workers [35] used a polymer pillar array from the exposed e-beam resist and metallised it. Arrays of cubic, hexagonal, and elliptical nanoparticles were created with the pillars being SERS-active and not the metal film in the gaps between (see Fig. 3).

Application

The SERS substrates mentioned in the previous section were used for various applications. This section provides a short overview of the latest SERS research, e.g., in DNA detection and multiplexing using SER(R)S, which additionally even allows single-molecule detection. A totally new field of SERS investigation is the excitation in the ultraviolet region with non-coin metal substrates.

Since SERS allows for the detection of very low concentrations, this method can also be used for quantitative analysis, e.g., of drugs. The application can also be applied in biospectroscopy for the investigation of bacteria, eukaryotic cells, or tissues. Combining the SERS technique with an atomic force microscope affords information with a spatial resolution below 50 nm. This enables one to detect, e.g., single carbon nanotubes or even spatially resolve bacterial cell walls.

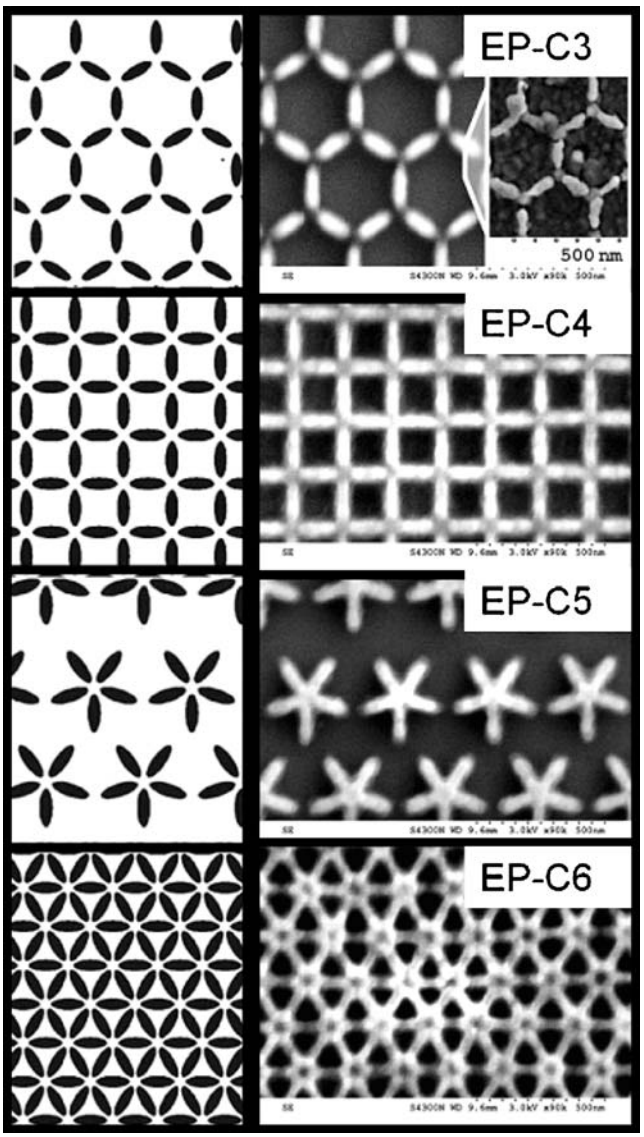

Fig. 3 Scanning electron micrographs of elliptical resist patterns with different symmetries (*right*). The *insets* (*left*) are the Auto-CAD drawings used for the EBL pattern generation. Each pattern has an aspect ratio of 1:3 with a spacing and short axis diameter of 50 nm. All micrographs were collected at ×90k magnification. The *top-right inset* shows a ×40k micrograph after deposition of 25 nm silver (adapted from [35])

Detection of DNA

The specific detection of DNA has gained importance in recent years because more and more DNA sequences of different organisms are being determined. Such sequence information can be used for the identification of the genus or species of microorganisms, diseases, or even a single individual. Fast and reliable DNA detection is not only used in research and development, it also finds increasing applications in areas like forensics, food safety control, and agriculture.

SERS offers several advantages for the detection of biomolecules: it is a rapid non-destructive tool, it yields compound-specific information, and has the potential for multi-component analysis. Vo-Dinh et al. reported on the use of SERS for the detection of specific nucleic acid sequences [36, 37]. However, SERS-active labels were used to detect the hybridization of complementary DNA strands. This approach demonstrated the possibility of using SERS-active labels as specific gene probes [37]. This technique has been further developed to detect DNA fragments of the human immundeficiency virus (HIV) [36]. In another approach Wabuyele and Vo-Dinh [38] used the vanishing of the SERS signal for the detection of a specific HIV DNA fragment (see Fig. 4). For this, they modified gold nanoparticles with single-stranded DNA hairpin structures. These hairpin structures would bind to the surface of the gold nanoparticle with a thiol modification. On the other end of the DNA strand a SERS-active label is attached. As long as the hairpin structure is closed the label remains in close proximity to the metal surface and a SERS signal of the label can be detected. Upon hybridization of a complementary strand of DNA the hairpin structure opens and the label is removed from the metal surface, which leads to the disappearance of the SERS signal.

Cao et al. [39] also reported on a SERS-based detection scheme for DNA that involves gold nanoparticles. They used gold nanoparticles specifically modified with special oligonucleotides with a thiol modification, for binding to

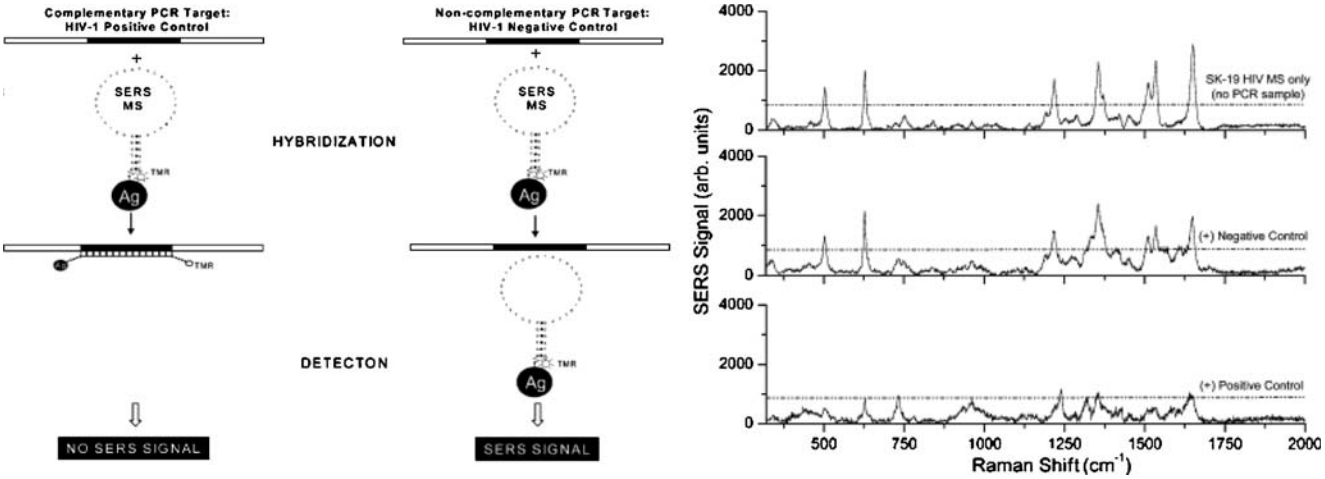

Fig. 4 *Left* schematic representation of the hybridization and optical detection scheme. Binding of nanoprobes to a capture region of the PCR product quenches the optical signal, whereas unbound nanoprobes generate a detectable SERS signal. *Right* SERS spectra of HIV-1 SERS MS nanoprobe with no target DNA sequence (blank) and in the presence of a noncomplementary DNA target sequence (negative diagnostic) and a complementary HIV-1 DNA target (positive diagnostic). The threshold level of the SERS signal from the SERS MS nanoprobe is indicated by the *dotted line* (adapted from [38])

the gold surface, and a SERS-active label (a fluorescent dye) integrated in the DNA strand. Those gold nanoparticle labels were then used to label a DNA chip. However, the bound nanoparticles could not be detected via SERS after binding. The authors assumed that the nanoparticles were not packed closely enough on the surface to give an electromagnetic field enhancement. After an additional silver deposition step, very specific SERS signals were detected [39]. This detection scheme was also transferred from chip-based detection to a glass-bead-based parallel detection [40]. The authors were able to show that they could specifically detect defined mixtures of SERS-active labels thereby enabling a vast number of possible labeling combinations and the possibility of a highly multiplexed detection scheme. It was pointed out that this detection technology could be easily adjusted to the detection of proteins and other biomolecules [40, 41]. Recently, the read out of a DNA chip using SERS without any additional labels has been reported. The authors used unique silver torus nanostructures as substrates for the chip and a shift in the SERS signal for the detection of the binding of complementary DNA to a complementary surface immobilized capture DNA [42].

An approach using surface-enhanced resonance Raman scattering (SERRS) has been reported by Graham and co-workers [43–46] in which they used specific Raman-active labels for detection. Even so the possibility of the detection method is demonstrated in their work; to our knowledge no sequence-specific approach detecting the hybridization of a complementary capture and target probe has been reported by them yet. However they were able to demonstrate the detection of labeled oligonucleotides in a lab-on-a-chip approach using microfluidics [43].

The ever growing demand for fast and accurate techniques for the detection of nucleic acids and other biomolecules is a great opportunity to develop SERS into a valuable tool for the detection of biomolecular interactions. Further improvements have to be made especially in producing reproducible substrates and developing easy-to-use detection devices as well as automating the detection process. Such obstacles might be overcome in the future to make SERS an interesting alternative in the investigation of DNA.

Surface-enhanced resonance Raman scattering (SERRS)

In comparison to SERS, where the excitation wavelength used is far away from the electronic absorption state of the analyte, SERRS uses the additional enhancement when the excitation wavelength is in or near an electronic vibration of the analyzed molecule. Therefore, the detection limit can be lowered.

SERRS studies have been carried out in numerous research fields. Many investigations used dyes such as triphenylmethane, rhodamine, fluorescein, phthalocyanine, and azo dyes adsorbed on colloidal silver, silver island films, or silver electrodes to investigate orientation effects or influences of chromophores and substituents on the SERRS spectra. Even coverage dependence, the usability for qualitative and quantitative analysis, and the detection of dye-labeled DNA have been the focus of investigation [47–49]. SERRS was also used to detect indicator molecules for the presence of an analyte [50] or for pH determination [51]. Cotton and co-workers studied various biomolecules, e.g., cyanine dyes, chlorophyll a, bacteriochlorophyll a, and bacteriopheophytin a, in Langmuir–Blodgett monolayers on silver films [52].

Many investigations were performed on proteins such as heme proteins [53, 54], phytochrome [55], and non-heme metalloenzymes [56]. In addition, investigation of the potent drugs hypericin and emodin on aqueous silver colloid [57] and the interactions of hypericin with DNA [58] where performed by Bertoluzza and co-workers.

SERRS is also used for detection in flow injection analysis in a spectroelectrochemical cell [59], as an extra HPLC detector for nitrophenol compounds [60], or for characterizing compounds separated by column liquid chromatography [61].

SERRS investigations of inorganic complexes adsorbed on silver electrodes or colloids represents a wide field of investigation [62, 63]. Studies on the interactions between DNA and ruthenium complexes were performed by Cordier and co-workers [64].

Single-molecule detection

One application of surface-enhanced resonance Raman scattering (SERRS) is the detection of single molecules (SM SERS). Most tests of SM SERS use excitation wavelengths located close to the surface plasmon resonance and also to the optical transition of the analyzed molecule. Nie and Emory acquired an enhancement factor in the order of 10^{14}–10^{15} by the exploration of single rhodamine 6G molecules adsorbed on single silver nanoparticles [65]. During their work they observed that a few particles, so-called hot particles, offer exceptionally high enhancement efficiencies. It was shown that only larger aggregates, which are created by salt-induced aggregation, exhibit large SERS intensities [66–68]. Käll and co-workers investigated proteins such as hemoglobin or horseradish peroxidase by incubating the analyte with silver colloidal solution [69, 70]. In these cases the proteins are used to induce aggregation of the nanoparticles to utilize the enhanced electromagnetic field that exists between particles, which is predicted to be up to 10^3 higher than the incident optical field. Unlike most works, in which silver colloids are used, Aroca and co-workers applied Langmuir–Blodgett monolayers on silver island films to detect single perylene dyes [71, 72].

Another possibility to detect single molecules by means of SERS was executed by Kneipp and co-workers. They exploited the huge SERS cross section obtained on colloidal clusters (100- to 150-nm-sized silver clusters prepared from a silver colloidal solution with NaCl as aggregating agent) to detect non-resonant single molecules [73]. SERS enhancement factors of 10^{14} were observed for dye molecules attached to the silver clusters. Further studies by the same group investigated anti-Stokes signals of single molecules for a better understanding of the localized surface plasmon resonance of the silver clusters at 830 nm excitation [74] and hyper-Raman scattering [75].

The most important limitation is that only a few clusters of the aggregated nanoparticles, so-called hot particles, show inordinate SERS intensities to allow single-molecule detection. To make SM SERS a qualitative and quantitative analytical tool these clusters must be produced reproducibly and accurately defined.

UV-SERS

Extending the excitation wavelengths from the visible and near infrared spectral region to the ultraviolet would allow SERRS applications in particular on important biological molecules such as DNA or proteins showing absorption in the UV.

Since the SERS enhancement mainly depends on the electromagnetic (EM) enhancement mechanism, which is due to a plasmon excitation in the substrate metal, it is mainly not possible to obtain SERS spectra with coinage metals in the UV as in the visible spectral region. Hence other substrate metals must be investigated for their UV-SERS activity. So far UV-SERS experiments have been performed with 325-nm excitation wavelength. The first UV-SERS enhancement was reported by Ren et al. in 2003 [76] who used roughened rhodium and ruthenium electrodes as SERS substrate and showed an enhancement for pyridine as a model substance. The authors also calculated enhancement factors for silver and rhodium in the UV spectral region and could show that Ag does not show an enhancement factor for excitation wavelengths under 350 nm but Rh does. In addition, Wang and Wu [77] presented SERS spectra from an electrochemically roughened gold electrode with the thiocyanate ion as model substance. In agreement with the results of Ren et al. [76] they showed by the use of extended Hückel molecule orbital calculations that for gold in the UV spectral region, only the charge transfer (CT) mechanism contributes to the SERS enhancement. Wen and Fang [78] also presented studies of an Au electrode with UV excitation on *p*-hydroxybenzoic acid (PHBA). The authors claim, like Wang and Wu, that the increased intensities of the Raman bands in the surface Raman spectrum in comparison to the UV-Raman spectrum is due to the non-radiative CT mechanism. Another reason for this is that the relative band intensities vary strongly with the given potential at the electrode and the bands shift with the electrode potential about 3–10 cm^{-1}. It should be pointed out that the potential at which resonance occurs varies with the vibrational mode. Therefore, the PHBA molecules are chemisorbed on the Au surface and the metal PHBA–bond is influenced by the potential. In addition, Lin et al. [79] published UV-SERS experiments of pyridine on Rh electrodes as well as thiocyanate on Rh, Ru, and Co electrodes. Recently, a different approach with excitation in the deep UV at 244 nm

and sputtered aluminum surfaces as SERS-active metal was reported [80].

SERS as an analytical tool

Both Raman and SERS spectroscopic investigations can be performed in the presence of water, and thus these techniques can be applied for the investigation of organic and biochemical substances in their natural environment [81, 82]. The pH-dependent absorption behavior of various biomolecules has been studied by means of SERS [83, 84].

To perform a quantitative analysis more subtle methods have to be applied, since the SERS signal depends largely on the age and the state of aggregation of the used substrate [85, 86]. Up to now a lot of work has been done on the production and synthesis of reproducible SERS-active substrates (see SERS-active substrates). Unfortunately highly significant surface enhancement needs aggregated hot spots (see Single-molecule SERS), which could not be performed reproducibly.

One possibility for a quantitative analysis of, e.g., organic trace pollutants in aqueous solutions is the implementation of a fluidic cell. In fluidic systems one can generate a mean spectrum over a defined period of time, where the inhomogeneous particle and particle aggregate distribution of the colloidal solutions due to hot spots can be circumvented. In addition to a constant mixing behavior the time period between activation and measurement can also be kept constant. In order to minimize the needed sample volumes the fluidic systems have been recently adapted into chip-based systems. Even colloid synthesis has been implemented [87, 88]. Figure 5a schematically depicts the setup of such a microfluidic device. Via an external syringe pump system the different solutions are pumped into the channels of the chip, where they are combined and mixed thoroughly before the SERS spectrum is detected with a laser focused into the channel. Choo and co-workers successfully detected nicotine, cyanide ions, and methyl parathion in an alligator-teeth-shaped PDMS channel [89–91], whereas Docherty et al. succeeded in detecting labeled oligonucleotides in a microfluidic lab-on-a-chip system [43]. The analysis of mitoxantrone, an anti-cancer agent, in blood serum has been performed by McLaughlin et al. [92]. To circumvent the problem of a memory effect caused by sedimentation of nanoparticle aggregates [92] we implemented a two-phase liquid/liquid segmented flow system for a quantitative detection of crystal violet [93]. A concentration dependency plot is shown in Fig. 5b.

In order to prevent poor batch-to-batch reproducibility a different approach is to add an internal standard to the analyte solution which shows a similar binding behavior to the SERS substrate. Bell et al. detected dipicolinic acid in the presence of CNS^- anions as an internal standard to compensate for changes in experimental parameters such as fluctuations in the laser power [94]. To minimize the effect of fluctuations in the SERS substrate isotopically labeled internal standards were applied which have nearly the same binding affinity to the metal colloid [95, 96]. In Fig. 6 SERS spectra of the individual isotopomers of creatinine are shown as well as a mixture of both spectra. Different force constants of the isotopes lead to a small wavenumber shift of the respective bands.

Another interesting topic concerning SERS and its possible application to quantify unknown concentrations of molecules is presented by van Duyne and co-workers. They developed a silver nanostructured substrate modified by a self-assembled monolayer (SAM) of alkanethiolates for the detection of glucose [97]. In comparison to the bare silver surfaces, which show only a very weak or nonexistent binding affinity to the glucose molecules, the SAM-modified structures can reversibly bind glucose. Another advantage is that the SAM stabilizes the silver surface and protects it against oxidation [98]. By combining these SAM-modified structures with a flow cell, the group succeeded in producing a reversible glucose sensor even in the presence of blood serum protein mimic conditions [99].

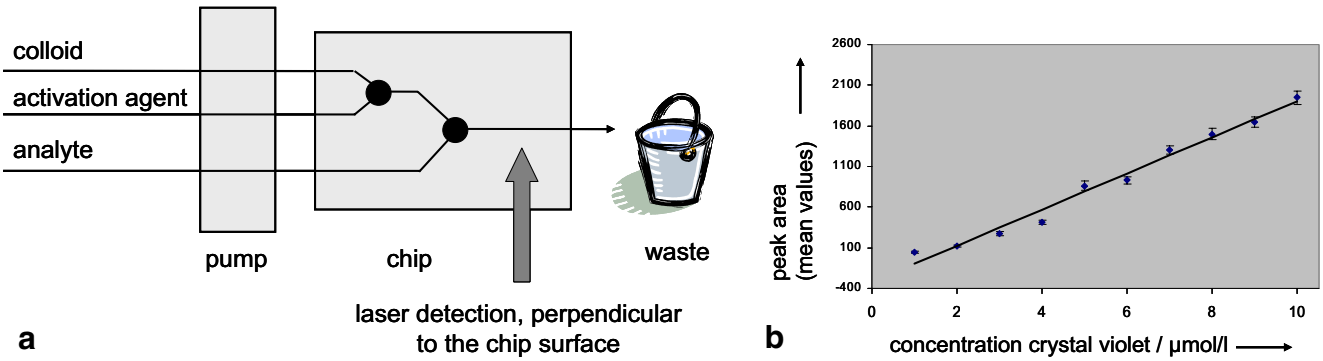

Fig. 5 Schematic depiction of a lab-on-a-chip integrated microfluidic device (**a**) and a quantitative analysis of crystal violet measured in such a chip (**b**)

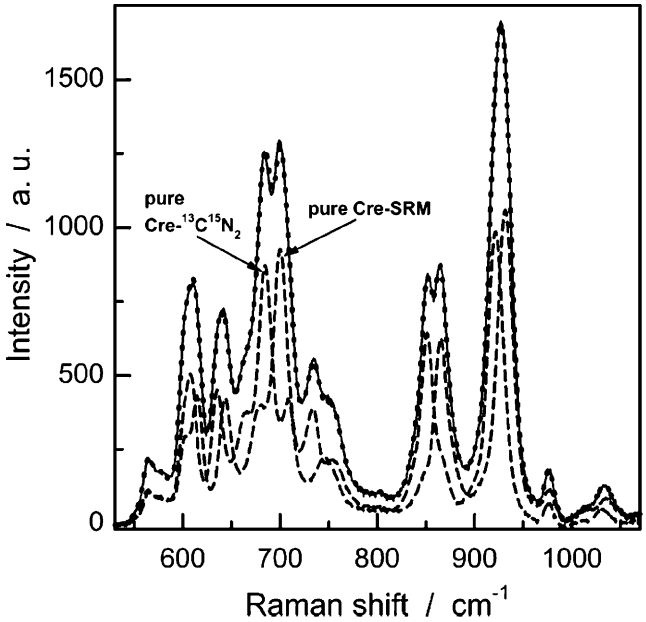

Fig. 6 SERS spectra of the two isotopomers (---) and a SERS spectrum of the sum of both (•••) are compared to the experimental SERS spectrum of an equimolar mixture of both isotopomers (—) (adapted from [95])

Investigation of bacteria by means of SERS spectroscopy

The first SERS spectra of whole bacterial cells were published in 1998 by Efrima and Bronk by coating Gram-negative *E. coli* cells with silver nanoparticles [100]. In the following years several research groups continued the investigations of *E. coli*, and other bacteria. Mainly silver or gold nanoparticles are used as SERS-active substrates. They are prepared directly in the outer cell wall of the bacteria by external coating [101–103] or adsorption of these nanoparticles to the cell wall [104–106]. In addition, solid substrates like immobilized nanoparticles on a microscope glass slide [107], metal deposited island films [108], and metal FON (film over nanospheres) [109] layers are mentioned as SERS-active substrates for investigations of bacterial cells.

Most of the SERS measurements on bacteria were performed in a glass cuvette or on bulk material resulting in an averaging over a lot of bacterial cells which increases the SERS intensity [103–105]. However, SERS spectra of single bacterial cells or spores using optical tweezers [110] or confocal microscopy [108, 111] have been reported in several studies.

A TEM image of an *E. coli* cell coated with silver nanoparticles by adhesion on the outer cell wall is depicted in Fig. 7a [106]. The nanoparticles are in close contact with the outer cell wall, so that only components of the outer membrane contribute to the SERS spectra. In Fig. 7b several SERS *E. coli* spectra obtained from different research groups are shown. Zeiri et al. [103] used the external silver coating to prepare SERS-active nanoparticles directly in the outer cell membrane. In comparison Sengupta et al. [106] and Jarvis et al. [104] applied silver colloids as SERS-active substrate for coating the bacteria. As shown in Fig. 7b [106] the band positions are nearly identically but the band intensities vary due to different SERS-active substrates.

Investigation of living cells and tissues

Beside the characterization and identification of bacteria, eukaryotic cells and tissues have also been analyzed by surface-enhanced (resonance) Raman spectroscopy. One approach uses silver colloids to minimize fluorescence in living tissues and enhance signals of components of interest, respectively. This technique has, for example,

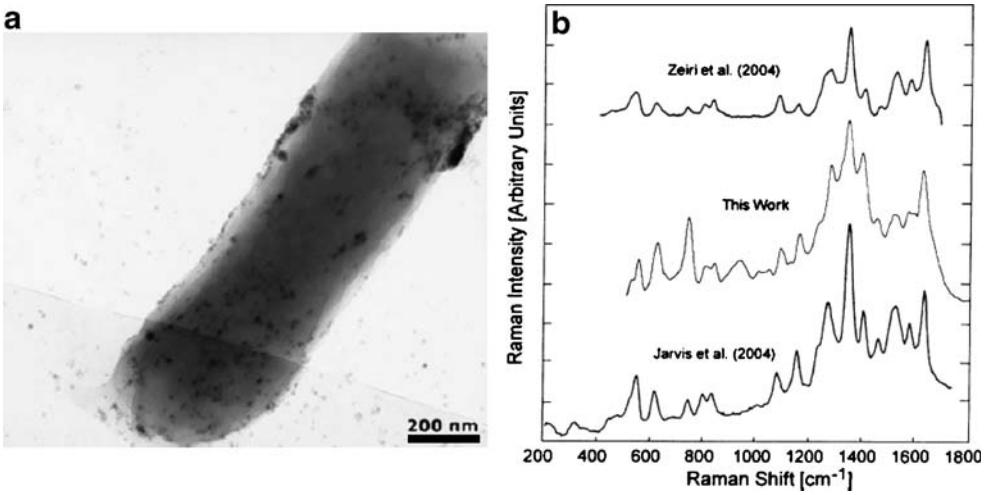

Fig. 7 SERS on *E.coli* cells: **a** TEM images of MC4100 *E. coli* cell coated with Ag nanoparticle, **b** comparison of the SERS *E. coli* spectra obtained from several research groups (adapted from [106])

allowed mint taxas to be characterized by identifying the composition of the essential oil inside their glandular trichomes [112, 113].

A number of different SERS experiments have been performed localizing the colloids inside or outside the investigated cells. Incubating rat PC 12 cells with silver colloids allows for the monitoring of the neurotransmitters dopamine, adrenaline, and noradrenaline at the outer cell wall [114]. Kneipp et al. [115, 116] used gold colloids inside epithelial cells to investigate native chemicals and their distribution inside a cell.

A different approach for the SERS investigation of single cells is the use of SERS labels. Specific SERS labels which are selective for, e.g., ketones inside cell surface proteins were prepared and linked to silver colloids. This allows the selective localization of these proteins inside HeLa cells [117]. For a more complex approach, dyes like rhodamine 6G or cresyl violet were used with metal colloids. The localization not only of the native chemicals like DNA bases or amino acids but also of different concentrations of rhodamine 6G as a probe molecule can be investigated by using gold colloids inside lymphocytes [118]. Different receptors inside endothelial cells were localized by linking these Raman labels to target molecules like biotin or other substances [119]. Also antibodies linked to a labeled gold colloid can be used [120]. With this method specific cancer markers can be localized in, e.g., kidney cells in order to investigate tumor development [121, 122].

The use of metal colloids always leads to a contamination of the whole cell or tissue. In order to minimize the partly degenerative effect of metal colloids inside cells a highly localized SERS substrate would be preferable. Therefore, Geßner et al. [123, 124] localized silver colloids on the surface of an etched glass fiber tip for the investigation of yeasts, red blood cells, and plant tissue. With this SERS tip it was possible to combine a high spatial resolution with a high enhancement factor.

Tip-enhanced Raman spectroscopy (TERS)

In order to obtain even higher spatial resolutions single metallic nanoparticles on a cantilever were used to combine SERS spectroscopy with atomic force microscopy (AFM) in the so-called tip-enhanced Raman spectroscopy (TERS). The first results applying the TERS technique were published in 2000 by the research group of Zenobi and Deckert [5]. For TERS different approaches and tips like AFM cantilevers with deposited silver nanoparticles or etched thin metallic wires were investigated [5]. TERS spectra of C_{60} (bucky balls) were measured with a tuning fork set up to handle the TERS tip [5].

In the following years a lot of research groups have improved the TERS technique using different kinds of analytes both inorganic and organic substances. Some of the most investigated analytes are carbon-based clusters like carbon nanotubes [125–128], bucky balls (C_{60}) [129, 130], or carbon onions [131]. Using the TERS technique it is possible to investigate single carbon clusters in order to get information about the deformation of these structures with increasing tip pressure [132] and investigate encapsulated biomolecules in single-wall carbon nanotubes [133]. In addition, TERS spectra of organic dyes were recorded and the signal-to-noise ratio can be increased by choosing the excitation wavelength nearby the absorption maximum [134–137]. Additionally, DNA components like the DNA bases were investigated using the TERS technique [138–140].

Preliminary studies of biomolecules pointed out that more complex biological systems can also probably be probed by means of TERS. First results investigating single bacterial cells using the TERS technique are shown in Fig. 8 [141]. TERS spectra with a high signal-to-noise ratio can be achieved and are dominated by contributions of the outer cell wall. However, over time the TERS signal changes due to fluctuations of cell components on the outer cell wall [141, 142].

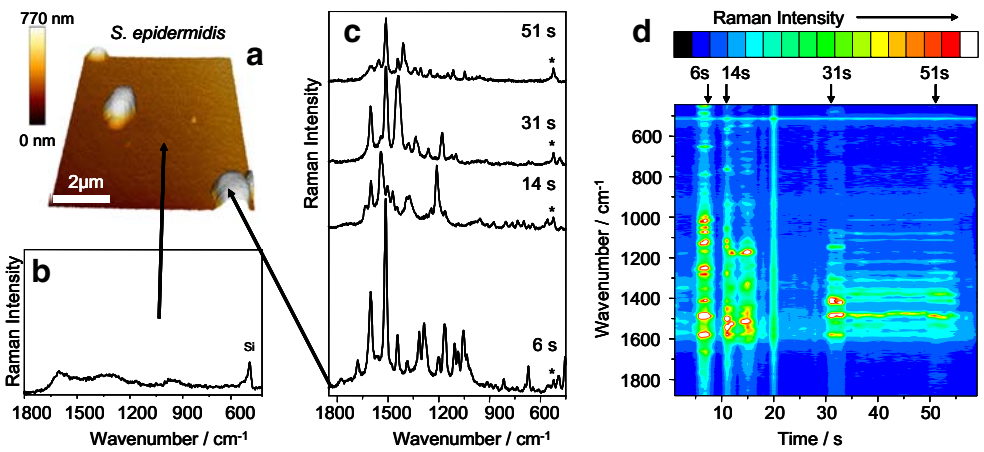

Fig. 8 TERS on single *S. epidermidis* cells: **a** pseudo-three-dimensional AFM image of single *S. epidermidis* cells on a glass surface, **b** reference TERS spectrum of the glass surface, **c** single TERS spectra measured on the top of a single bacterial cell using a silver-coated AFM cantilever, **d** false-color plot of the evolution of the TERS signal during the measurement period. The ever-present Raman band around 520 cm^{-1} is attributed to the silicon tip

Conclusion

In recent years the developments in the field of SERS spectroscopy have opened a great variety of different applications. New and reproducible substrates with high enhancement factors were designed, leading to applications not only in surface analysis but also in a wide field of biospectroscopy. The extension of laser excitation into the ultraviolet range is a promising approach for further investigations of biomolecules. In addition to substance analysis, SERS is also used in the field of bacteria research and identification. The possibility to analyze living cells and tissues by means of conventional or labeled colloids enables further insight into cell metabolism. TERS as the combination of surface-enhanced Raman spectroscopy with atomic force microscopy enables new research possibilities with a spatial resolution in the nanometer range. With this method even the surface of living cells (e.g., bacteria) can be investigated on the cellular level.

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References

1. Fleischmann M, Hendra PJ, Mcquilla Aj (1974) Chem Phys Lett 26:163–166
2. Moskovits M (1985) Rev Modern Phys 57:783–826
3. Tian Z-Q, Yang Z-L, Ren B, Wu D-Y (2006) Top Appl Phys 103:125–146
4. Otto A (1982) Springer Ser Chem Phys 21:186–195
5. Stöckle RM, Suh YD, Deckert V, Zenobi R (2000) Chem Phys Lett 318:131–136
6. Jeanmaire DL, Van Duyne RP (1977) J Electroanal Chem 84:1–20
7. Wang Z, Rothberg LJ (2006) Appl Phys B 84:289–293
8. Yan W, Bao L, Mahurin SM, Dai S (2004) Appl Spectrosc 58:18–25
9. Wang CH, Sun DC, Xia XH (2006) Nanotechnol 17:651–657
10. Kucheyev SO, Hayes JR, Biener J, Huser T, Talley CE, Hamza AV (2006) Appl Phys Lett 89:053102–053104
11. Bahns JT, Yan F, Qiu D, Wang R, Chen L (2006) Appl Spectrosc 60:989–993
12. Lee PC, Meisel DJ (1982) J Phys Chem 86:3391–3395
13. Steinbrück A, Csaki A, Festag G, Fritzsche W (2006) Plasmonics 1:79–85
14. Jackson JB, Halas NJ (2004) PNAS 101:17930–17935
15. Pergolese B, Bigotto A, Muniz-Miranda M, Sbrana G (2005) Appl Spectrosc 59:194–199
16. Sun Y, Xia Y (2002) Science 298:2176–2179
17. Wiley B, Sun Y, Mayers B, Xia Y (2005) Chem Eur J 11:454–463
18. Jin R, Cao YC, Hao E, Metraux GS, Schatz GC, Mirkin CA (2003) Nature 425:487–490
19. Leverette CL, Jacobs SA, Shanmukh S, Chaney SB, Dluhy RA, Zhao YP (2006) Appl Spectrosc 60:906–913
20. Zhang J, Gao A, Alvarez-Puebla RA, Buriak JM, Fenniri H (2006) Adv Mater 18:3233–3237
21. Fischer UC, Zingsheim HP (1981) J Vac Sci Technol 19:881–885
22. Haynes CL, Van Duyne RP (2001) J Phys Chem B 105:5599–5611
23. Hulteen JC, Van Duyne RP (1995) J Vac Sci Technol A 13:1553–1558
24. Haynes CL, Duyne RPv (2002) Mater Res Soc Proc 728: S10.17.11–S10.17.16
25. Baia L, Baia M, Popp J, Astilean S (2006) J Phys Chem B 110:23982–23986
26. Baia M, Toderas F, Baia L, Popp J, Astilean S (2006) Chem Phys Lett 422:127–132
27. Stuart DA, Yuen JM, Shah N, Lyandres O, Yonzon CR, Glucksberg MR, Walsh JT, VanDuyne RP (2006) Anal Chem 78:7211–7215
28. Li H, Baum CE, Sun J, Cullum BM (2006) Appl Spectrosc 60:1377–1385
29. Kahl M, Voges E, Hill W (1998) Spectrosc Eur 10:8–13
30. Félidj N, Aubard J, Lévi G, Krenn JR, Salerno M, Schider G, Lamprecht B, Leitner A, Aussenegg FR (2002) Phys Rev B 65:0754191–0754199
31. Felidj N, Aubard J, Levi G, Krenn JR, Hohenau A, Schider G, Leitner A, Aussenegg FR (2003) Appl Phys Lett 82:3095–3097
32. Sackmann M, Bom S, Balster T, Materny A (2007) J Raman Spectrosc 38:277–282
33. Haynes CL, McFarland AD, Zhao L, Van Duyne RP, Schatz GC, Gunnarsson L, Prikulis J, Kasemo B, Kall M (2003) J Phys Chem B 107:7337–7342
34. Billot L, Lamy de la Chapelle M, Grimault AS, Vial A, Barchiesi D, Bijeon JL, Adam PM, Royer P (2006) Chem Phys Lett 422:303–307
35. De Jesus MA, Giesfeldt KS, Oran JM, Abu-Hatab NA, Lavrik NV, Stepaniak MJ (2005) Appl Spectrosc 59:1501–1508
36. Isola NR, Stokes DL, Vo-Dinh T (1998) Anal Chem 70:1352–1356
37. Vo-Dinh T, Houck K, Stokes DL (1994) Anal Chem 66:3379–3383
38. Wabuyele MB, Vo-Dinh T (2005) Anal Chem 77:7810–7815
39. Cao YC, Jin R, Mirkin CA (2002) Science 297:1536–1540
40. Jin RC, Cao YC, Thaxton CS, Mirkin CA (2006) Small 2:375–380
41. Cao YC, Jin R, Nam JM, Thaxton CS, Mirkin CA (2003) J Am Chem Soc 125:14676–14677
42. Green M, Liu FM, Cohen L, Kollensperger P, Cass T (2006) Faraday Discuss 132:269–280
43. Docherty FT, Monaghan PB, Keir R, Graham D, Smith WE, Cooper JM (2004) Chem Commun118–119
44. Graham D, Brown R, Smith WE (2001) Chem Commun 1002–1003
45. Graham D, Mallinder BJ, Smith WE (2000) Angew Chem Int Ed 39:1061–1064
46. Faulds K, Smith WE, Graham D (2004) Anal Chem 76:412–417
47. Munro CH, Smith WE, White PC (1995) Analyst 120:993–1003
48. Graham D, Faulds K, Smith WE (2006) Chem Commun 4363–4371
49. Sarkar J, Chowdhury J, Pal P, Talapatra GB (2006) Vib Spectrosc 41:90–96
50. Carron K, Mullen K, Lanouette M, Angersbach H (1991) Appl Spectrosc 45:420–423
51. Mullen KI, Wang D, Crane LG, Carron KT (1992) Anal Chem 64:930–936
52. Uphaus RA, Cotton TM, Moebius D (1985) Thin Solid Films 132:173–184
53. Dick LA, Haes AJ, Van Duyne RP (2000) J Phys Chem B 104:11752–11762
54. Lecomte S, Wackerbarth H, Soulimane T, Buse G, Hildebrandt P (1998) J Am Chem Soc 120:7381–7382
55. Farrens DL, Holt RE, Rospendowski BN, Song PS, Cotton TM (1989) J Am Chem Soc 111:9162–9169
56. Broderick JB, Natan MJ, O'Halloran TV, Van Duyne RP (1993) Biochemistry 32:13771–13776

57. Jancura D, Sanchez-Cortes S, Kocisova E, Tinti A, Miskovsky P, Bertoluzza A (1995) Biospectroscopy 1:265–273
58. Sanchez-Cortes S, Miskovsky P, Jancura D, Bertoluzza A (1996) J Phys Chem 100:1938–1944
59. Gouveia VJP, Gutz IG, Rubim JC (1994) J Electroanal Chem 371:37–42
60. Ni F, Thomas L, Cotton TM (1989) Anal Chem 61:888–894
61. Somsen GW, Coulter SK, Gooijer C, Velthorst NH, Brinkman UAT (1997) Anal Chim Acta 349:189–197
62. Aroca R, Clavijo RE, Jennings CA, Kovacs GJ, Duff JM, Loutfy RO (1989) Spectrochim Acta A Mol Biomol Spectrosc 45A:957–962
63. Hidalgo M, Montes R, Laserna JJ, Ruperez A (1996) Anal Chim Acta 318:229–237
64. Gaudry E, Aubard J, Amouri H, Levi G, Cordier C (2006) Biopolymers 82:399–404
65. Nie S, Emory SR (1997) Science 275:1102–1106
66. Michaels AM, Nirmal M, Brus LE (1999) J Am Chem Soc 121:9932–9939
67. Jiang J, Bosnick K, Maillard M, Brus L (2003) J Phys Chem B 107:9964–9972
68. Vosgroene T, Meixner AJ (2005) ChemPhysChem 6:154–163
69. Xu H, Bjerneld EJ, Kall M, Borjesson L (1999) Phys Rev Lett 83:4357–4360
70. Bjerneld EJ, Foeldes-Papp Z, Kaell M, Rigler R (2002) J Phys Chem B 106:1213–1218
71. Constantino CJ, Lemma T, Antunes PA, Aroca R (2001) Anal Chem 73:3674–3678
72. Goulet PJG, Pieczonka NPW, Aroca RF (2005) J Raman Spectrosc 36:574–580
73. Kneipp K, Kneipp H, Deinum G, Itzkan I, Dasari RR, Feld MS (1998) Appl Spectrosc 52:175–178
74. Kneipp K, Kneipp H, Kartha VB, Manoharan R, Deinum G, Itzkan I, Dasari RR, Feld MS (1998) Phys Rev E 57:R6281–R6284
75. Kneipp K, Kneipp H, Itzkan I, Dasari RR, Feld MS (1999) Chem Phys 247:155–162
76. Ren B, Lin X-F, Yang Z-L, Liu G-K, Aroca RF, Mao B-W, Tian Z-Q (2003) J Am Chem Soc 125:9598–9599
77. Wang P, Wu G (2004) Chem Phys Lett 385:96–100
78. Wen R, Fang Y (2005) J Electroanal Chem 576:237–242
79. Lin X-F, Ren B, Yang Z-L, Liu G-K, Tian Z-Q (2005) J Raman Spectrosc 36:606–612
80. Dörfer T, Schmitt M, Popp J (2007) J Raman Spectrosc 38:1379–1382
81. Petry R, Schmitt M, Popp J (2003) ChemPhysChem 4:14–30
82. Schmitt M, Popp J (2006) J Raman Spectrosc 37:20–28
83. Cinta-Pinzaru S, Peica N, Kustner B, Schlucker S, Schmitt M, Frosch T, Faber JH, Bringmann G, Popp J (2006) J Raman Spectrosc 37:326–334
84. Peica N, Pavel I, Pinzaru SC, Rastogi VK, Kiefer W (2005) J Raman Spectrosc 36:657–666
85. Faulds K, Littleford RE, Graham D, Dent G, Smith WE (2004) Anal Chem 76:592–598
86. Schneider S, Grau H, Halbig P, Nickel U (1993) Analyst 118:689–694
87. Ayora Canada MJ, Ruiz Medina A, Frank J, Lendl B (2002) Analyst 127:1365–1369
88. Keir R, Igata E, Arundell M, Smith WE, Graham D, McHugh C, Cooper JM (2002) Anal Chem 74:1503–1508
89. Lee D, Lee S, Seong GH, Choo J, Lee EK, Gweon D-G, Lee S (2006) Appl Spectrosc 60:373–377
90. Jung J, Choo J, Kim DJ, Lee S (2006) Bull Korean Chem Soc 27:277–280
91. Yea K-h, Lee S, Kyong JB, Choo J, Lee EK, Joo S-W, Lee S (2005) Analyst 130:1009 1011
92. McLaughlin C, MacMillan D, McCardle C, Smith WE (2002) Anal Chem 74:3160–3167
93. Strehle KR, Cialla D, Rösch P, Henkel T, Köhler M, Popp J (2007) Anal Chem 79:1542–1547
94. Bell SEJ, Mackle JN, Sirimuthu NMS (2005) Analyst 130:545–549
95. Stosch R, Henrion A, Schiel D, Guettler B (2005) Anal Chem 77:7386–7392
96. Zhang D, Xie Y, Deb SK, Davison VJ, Ben-Amotz D (2005) Anal Chem 77:3563–3569
97. Malinsky MD, Kelly KL, Schatz GC, Van Duyne RP (2001) J Am Chem Soc 123:1471–1482
98. Yonzon CR, Lyandres O, Shah NC, Dieringer JA, Van Duyne RD (2006) Top Appl Phys 103:367–379
99. Yonzon CR, Haynes CL, Zhang X, Walsh JT Jr, Van Duyne RP (2004) Anal Chem 76:78–85
100. Efrima S, Bronk BV (1998) J Phys Chem B 102:5947–5950
101. Jarvis RM, Brooker A, Goodacre R (2004) Anal Chem 76:5198–5202
102. Zeiri L, Bronk BV, Shabtai Y, Eichler J, Efrima S (2004) Appl Spectrosc 58:33–40
103. Zeiri L, Efrima S (2005) J Raman Spectrosc 36:667–675
104. Jarvis RM, Brooker A, Goodacre R (2006) Faraday Discuss 132:281–292
105. Laucks ML, Sengupta A, Junge K, Davis EJ, Swanson BD (2005) Appl Spectrosc 59:1222–1228
106. Sengupta A, Mujacic M, Davis EJ (2006) Anal Bioanal Chem 386:1379–1386
107. Leyton P, Lizama-Vergara PA, Campos-Vallette MM, Becker MI, Clavijo E, Cordova Reyes I, Vera M, Jerez CA (2005) J Chilean Chem Soc 50:725–730
108. Biju V, Pan D, Gorby YA, Fredrickson J, McLean J, Saffarini D, Lu HP (2007) Langmuir 23:1333–1338
109. Haynes CL, Yonzon CR, Zhang X, Van Duyne RP (2005) J Raman Spectrosc 36:471–484
110. Alexander TA, Pellegrino PM, Gillespie JB (2003) Appl Spectrosc 57:1340–1345
111. Premasiri WR, Moir DT, Klempner MS, Krieger N, Jones G II, Ziegler LD (2005) J Phys Chem B 109:312–320
112. Rösch P, Kiefer W, Popp J (2002) Biopolymers 67:358–361
113. Rösch P, Popp J, Kiefer W (1999) J Mol Struct 480–481:121–124
114. Dijkstra Reyer J, Scheenen Wim JJM, Dam N, Roubos Eric W, ter Meulen JJ (2007) J Neurosci Methods 159:43–50
115. Kneipp J, Kneipp H, McLaughlin M, Brown D, Kneipp K (2006) Nano Lett 6:2225–2231
116. Kneipp K, Haka AS, Kneipp H, Badizadegan K, Yoshizawa N, Boone C, Shafer-Peltier KE, Motz JT, Dasari RR, Feld MS (2002) Appl Spectrosc 56:150–154
117. Hu Q, Tay L-L, Noestheden M, Pezacki JP (2007) J Am Chem Soc 129:14–15
118. Eliasson C, Loren A, Engelbrektsson J, Josefson M, Abrahamsson J, Abrahamsson K (2005) Spectrochim Acta A Mol Biomol Spectrosc 61A:755–760
119. Nithipatikom K, McCoy MJ, Hawi SR, Nakamoto K, Adar F, Campbell WB (2003) Anal Biochem 322:198–207
120. Schlücker S, Küstner B, Punge A, Bonfig R, Marx A, Ströbel P (2006) J Raman Spectrosc 37:719–721
121. Kim J-H, Kim J-S, Choi H, Lee S-M, Jun B-H, Yu K-N, Kuk E, Kim Y-K, Jeong DH, Cho M-H, Lee Y-S (2006) Anal Chem 78:6967–6973
122. Lee S, Kim S, Choo J, Shin SY, Lee YH, Choi HY, Ha S, Kang K, Oh CH (2007) Anal Chem 79:916–922
123. Geßner R, Rösch P, Kiefer W, Popp J (2002) Biopolymers 61:327–330
124. Geßner R, Winter C, Rösch P, Schmitt M, Petry R, Kiefer W, Lankers M, Popp J (2004) ChemPhysChem 5:1159–1170
125. Yano T-a, Verma P, Kawata S, Inouye Y (2006) Appl Phys Lett 88:093125/093121–093125/093123

126. Anderson N, Anger P, Hartschuh A, Novotny L (2006) Nano Lett 6:744–749
127. Hayazawa N, Yano T, Watanabe H, Inouye Y, Kawata S (2003) Chem Phys Lett 376:174–180
128. Roy D, Wang J, Welland ME (2006) Faraday Discuss 132:215–225
129. Verma P, Yamada K, Watanabe H, Inouye Y, Kawata S (2006) Phys Rev B 73:045416/045411–045416/045416
130. Wang JJ, Smith DA, Batchelder DN, Saito Y, Kirkham J, Robinson C, Baldwin K, Li G, Bennett B (2003) J Microsc 210:330–333
131. Kodama T, Ohtani H (2006) Appl Phys Lett 89:223107/223101–223107/223103
132. Yano T-A, Inouye Y, Kawata S (2006) Nano Lett 6:1269–1273
133. Saito Y, Yanagi K, Hayazawa N, Ishitobi H, Ono A, Kataura H, Kawata S (2006) Jpn J Appl Phys Part 1 45:9286–9289
134. Yeo B-S, Schmid T, Zhang W, Zenobi R (2007) Anal Bioanal Chem 387:2655–2662
135. Pan D, Klymyshyn N, Hu D, Lu HP (2006) Appl Phys Lett 88:093121/093121–093121/093123
136. Hayazawa N, Tarun A, Inouye Y, Kawata S (2002) J Appl Phys 92:6983–6986
137. Pettinger B, Ren B, Picardi G, Schuster R, Ertl G (2005) J Raman Spectrosc 36:541–550
138. Hayazawa N, Watanabe H, Saito Y, Kawata S (2006) J Chem Phys 125:244706/244701–244706/244707
139. Rasmussen A, Deckert V (2006) J Raman Spectrosc 37:311–317
140. Watanabe H, Ishida Y, Hayazawa N, Inouye Y, Kawata S (2004) Phys Rev B 69:155418/155411–155418/155411
141. Neugebauer U, Rösch P, Schmitt M, Popp J, Julien C, Rasmussen A, Budich C, Deckert V (2006) ChemPhysChem 7:1428–1430
142. Neugebauer U, Schmid U, Baumann K, Ziebuhr W, Kozitskaya S, Deckert V, Schmitt M, Popp J (2007) ChemPhysChem 8:124–137