

Surface-enhanced Raman scattering: A new tool for biomedical spectroscopy

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Surface-enhanced Raman scattering (SERS) is a phenomenon resulting in strongly increased Raman signals when molecules are attached to nanometer-sized metallic structures. The effect combines the structural information content of Raman spectroscopy with ultrasensitive detection limits allowing Raman spectroscopy down to the single molecule level. Surface-enhanced Raman scattering opens up exciting opportunities also in the field of biomedical spectroscopy where it allows to study structural-functional properties of biologically relevant molecules which are often available in extremely small amounts only. This review will deal with SERS studies in life sciences performed within the recent half decade. Before discussing examples for applications of SERS in biology, medicine and pharmacy, in a first chapter, the theoretical and experimental background of SERS studies at ultrasensitive detection limits in the biomedical field are briefly summarized. Potential and limitations of SERS as a tool in biomedical spectroscopy will be considered.

AN important field of application of lasers in medicine is related to laser spectroscopic characterization of biomedically relevant molecules and processes in order to study structural-functional properties. In that way, processes such as development of diseases, treatment and therapy control can be investigated based on molecular structural information¹. Laser spectroscopic methods can be applied non-invasively under ambient conditions in a biological environment. Furthermore, they can achieve space and time resolution in biologically relevant dimensions: By using a confocal microscope, spectroscopic signals from about $1\ \mu\text{m}^3$ can be observed, enabling spatially resolved measurements in chromosomes and cells². In the time domain, optical spectra can be measured on the femtosecond time scale, providing information on extremely fast biophysical and biochemical process and short-lived intermediate species, for instance, for understanding protein function³. Vibrational spectroscopic techniques such as Raman spectroscopy can provide high structural information content on the bio-

logical material and allow to monitor small changes within the molecular structure indicating early stages of diseases⁴. An important challenge facing modern biomedical spectroscopy is detecting and characterizing trace amounts of biomedically relevant molecules down to the single molecule level. The most spectacular applications might appear in rapidly spectroscopic characterization of specific DNA fragments down to structurally sensitive detection of single bases in order to elucidate the human genome sequence.

About 50 years after the discovery of the Raman effect^{5,6}, the novel phenomenon of the so-called surface-enhanced Raman scattering^{7,8} (SERS) attracted considerable attention from both basic and practical viewpoints. SERS is a phenomenon resulting in strongly increased Raman signals from molecules which have been attached to 'rough' metal surfaces or to nanometer-sized metallic colloidal particles. The effect showed promise in overcoming the low-sensitivity problems inherent in Raman spectroscopy and combined the structural information content of a vibrational spectroscopy with extremely high sensitivity. Recently, new methods for determining cross sections effective in SERS resulted in unexpectedly large values of at least $10^{-16}\ \text{cm}^2$ per molecule corresponding to enhancement factors of about fourteen orders of magnitude compared with 'normal' non-resonant Raman scattering⁹. Such extremely large cross sections are sufficient for single molecule Raman detection¹⁰⁻¹⁴.

Since its early days, the SERS effect was also particularly appealing in the field of biophysics and biochemistry in order to study biologically relevant molecules which are often available in extremely small amounts only.

There are excellent reviews that summarize SERS studies on biological molecules in the eighties and early nineties¹⁵⁻²⁰. Therefore, this review will mainly deal with SERS studies in life sciences performed within the recent six years. Before discussing examples of applications of SERS in biology, medicine and pharmacy, in a first chapter, the theoretical and experimental background of SERS studies at ultrasensitive detection limits in the biomedical field are briefly summarized. Potential and limitations of SERS as a tool in biomedical spectroscopy will be considered.

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Surface-enhanced Raman scattering

Brief introduction to the SERS effect

In SERS, Raman signals can be increased by many orders of magnitude for molecules which have been attached to nanometer-sized metallic structures (for an overview see refs 21, 22).

Figure 1 *a* and *b* illustrate schematically 'normal' (non-surface-enhanced) Raman scattering and surface-enhanced Raman scattering, respectively. The conversion of laser light I_L into Stokes scattered light I_S is proportional to the Raman cross section σ^{free} , the excitation laser intensity I_L and the number of molecules in the probed volume N . In SERS, molecules are attached to so-called SERS-active substrates. In Figure 1 *b* SERS-active substrate is a colloidal silver particle. N' is the number of molecules which are involved in the SERS process. It is generally agreed that more than one effect contributes to the observed enhancement of the Raman signal^{21,22}. In Figure 1 *b*, $A(\nu_L)$ and $A(\nu_S)$ represent field enhancement factors of the laser- and Stokes-fields. The electromagnetic or field enhancement arises from enhanced local optical fields at the place of the molecule due to excitation of electromagnetic resonances in the metallic nanostructures which appear due to collective excitation of conduction electrons, also called surface plasmon resonance²³⁻²⁷. Because excitation field as well as the Raman scattered field contribute to this enhancement, the SERS signal I_{SERS} is proportional to the fourth power of the field enhancement factor and decreases with the 12th

power of the distance between molecule and metal surface.

In many experiments, SERS-active substrates consist of a collection of nanoparticles exhibiting fractal properties, such as colloidal clusters formed by aggregation of colloidal particles or metal island films^{12,13,28,29}. In those SERS experiments, estimates of electromagnetic enhancement must be based on the exciting properties of electromagnetic fields in fractal small-particle composites³⁰. Optical excitation in such structures tends to be spatially localized in very small areas, resulting in stronger electromagnetic field enhancement effects than for isolated colloidal particles³¹⁻³⁵. Most of SERS applications in the biomedical field seem to benefit from the high field enhancement on fractal colloidal silver and gold structures.

Additional to an electromagnetic enhancement, so-called chemical enhancement can appear due to involvement of metal electrons into the Raman process. The effect can be considered as metal electron-mediated resonance Raman effect via a charge transfer intermediate state³⁶ which often takes place at special adsorption places, so-called active sites. These sites are attributed to atomic scale roughness such as defects or co-adsorbed ions on the surface of the nanostructures providing adsorption places with special high-affinity binding³⁷. In Figure 1 *b*, chemical SERS enhancement is expressed as an increased Raman cross section σ_{ads}^R of the adsorbed molecule compared to the cross section in a 'normal' Raman experiment σ_{free}^R . It should be noted that a full understanding of SERS and quantitative theoretical

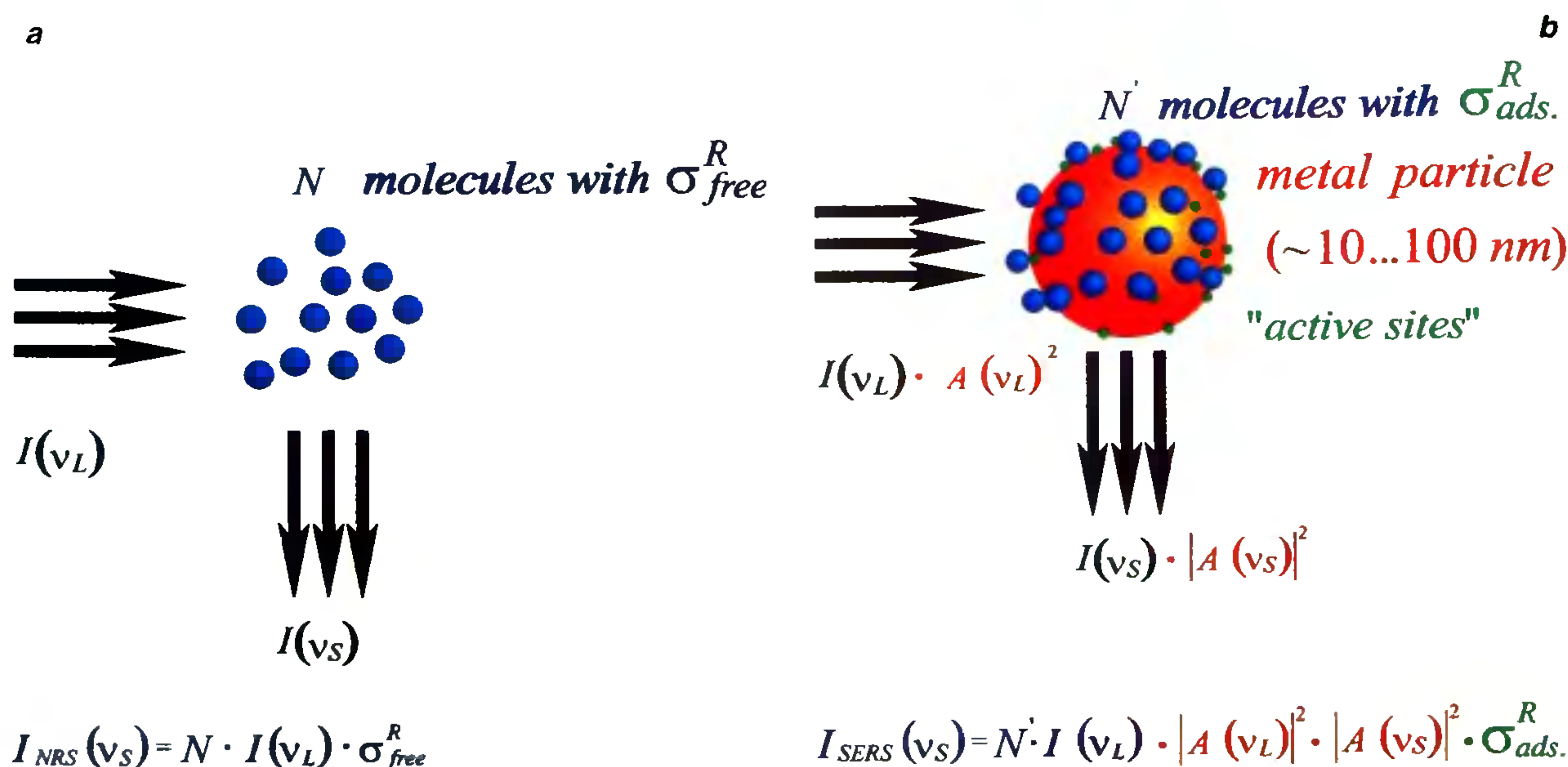


Figure 1. Schematic of 'normal' (*a*) and surface-enhanced (*b*) Raman scattering (for explanation see text). [Reprinted with permission from ref. 39, Copyright 1998 IOP Publishing Ltd.]

estimate of total enhancement factors are still under investigation.

Frequency shifts, line widths and relative intensities of the Raman lines characterize the Raman spectrum of a molecule. Due to non-uniform enhancement level for different vibrations and also due to the interaction between molecule and metal surface, an SERS spectrum can show some deviations from the normal Raman spectrum of the molecule. But in most cases, (in spite of that changes) an SERS spectrum provides still a clear 'fingerprint' of the molecule.

SERS enhancement factors and effective SERS cross sections

SERS enhancement factors for isolated small (10–100 nm diameter) spherical silver and gold colloidal particles have been estimated to be of the order of 10^6 and 10^3 , respectively²⁶. The values were experimentally confirmed in solutions of colloidal silver or gold using crystal violet and adenine as target molecules^{13,38}. In many SERS experiments, so-called chemical SERS enhancement additionally contributes one to three orders of magnitude enhancement^{21,22}. If the target molecule has electronic transitions in the range of the excitation laser, the total enhancement factor can be additionally increased for two to four orders of magnitude by exploitation of the resonance Raman effect (surface-enhanced resonance Raman scattering (SERRS)).

Recently, we observed extremely large effective Raman cross sections of the order of 10^{-16} cm^2 per molecule corresponding to enhancement factors of about 10^{14} in experiments using near-infrared (NIR) excitation and colloidal silver cluster as SERS active substrate^{9,10,12,13,39}. Since the NIR excitation wavelength was far away from electronic transition of the target molecule, no molecular resonance Raman effect should contribute to the observed total enhancement. As mentioned above, giant local fields

on the fractal structures might provide a rationale for the large enhancement factors⁴⁰, but the experimental results do not rule out a 'chemical contribution' of one to two orders of magnitude to the total enhancement. Similar enhancement factors have also been reported for rhodamine 6G at resonant visible excitation¹¹. Since the enhancement was observed for R 6G molecules on spatially isolated so-called 'hot' silver nanoparticles, other mechanism(s) should play an essential role in those experiments. In general, for full understanding of extremely large SERS enhancement factors, further investigations are crucial.

SERS-active substrates for biomedical application

A key problem in application of SERS in biomedical spectroscopy is developing SERS active substrates that provide reproducible and large enhancement factors and that are compatible to biological environment such as, for example, cells or body fluids. Silver and, sometimes gold, have been prepared in a variety of ways to generate SERS active substrates for applications in life sciences. The most common SERS substrates used for biological systems include roughened silver electrodes^{41,42} and colloidal silver particles in solution, 'dry' on a surface, or self-assembled into polymer-coated substrates^{43,44}. SERS microprobes have been designed in μm dimensions as metal films on silicon substrates for insertion into small openings or as silver wire microelectrodes as small as 0.75 μm (refs 29, 45, 46).

Figure 2 shows typical SERS-active silver colloidal structures in different aggregation stages. A comparison between Figure 2b and c, which were recorded in the nanometer and micrometer scale by electron and light microscopy, respectively, demonstrates the self-similarity of the structures. As discussed above, colloidal metal particles, particularly fractal clusters formed from colloids by aggregation can provide very high SERS enhancement

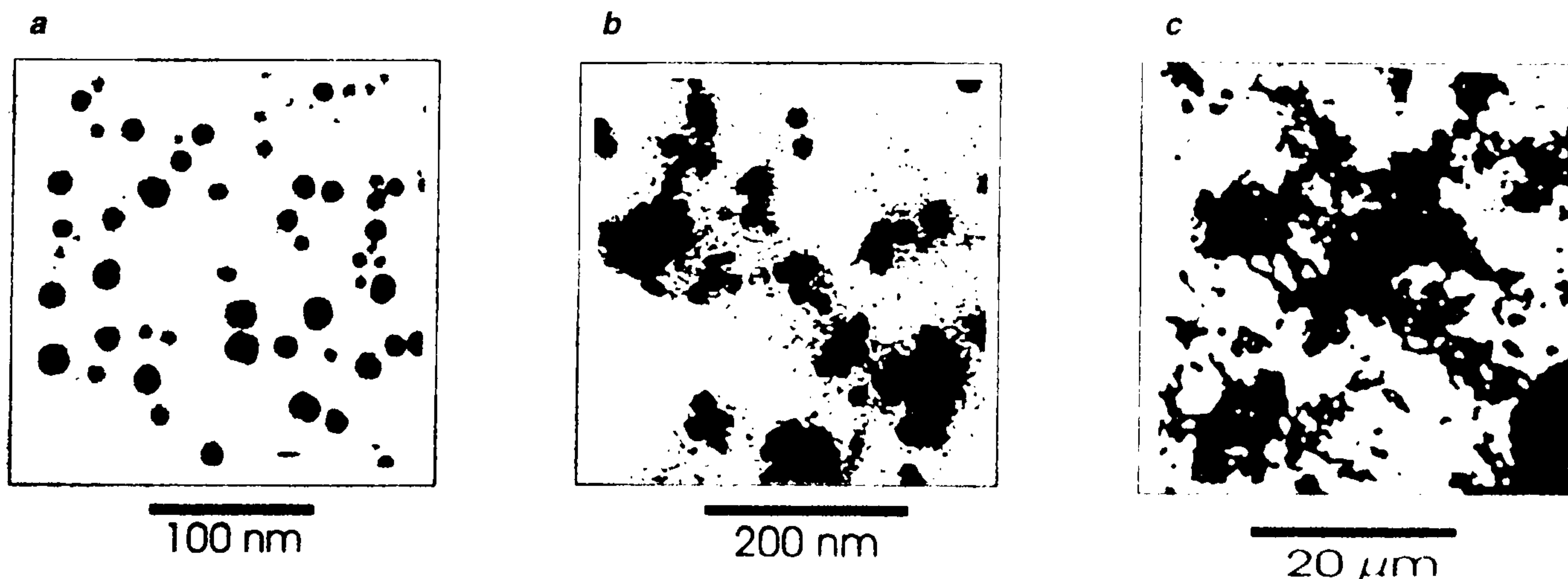


Figure 2. SERS active colloidal particles in different aggregation stages

levels. Recently, we found that at near-infrared excitation, colloidal gold clusters can provide an enhancement level comparable to that of colloidal silver clusters³⁸. This finding suggests colloidal gold clusters as a substrate for high-sensitivity SERS which can provide an enhancement level sufficient for Raman single molecule detection. Due to its chemical inactivity, gold might have some advantages compared to silver, particularly in biomedical spectroscopy. Recently, SERS behaviour of glycine on colloidal silver and gold and particularly the nature of the interaction between the amino acid and the colloidal particles of both metals have been studied⁴⁷.

Examples for application of SERS in the biomedical field

The potential of SERS for studying biomedically interesting molecules was exploited since the early eighties. SERS spectra have been measured from amino acids and peptides, from purine and pyrimidine bases, but also from 'large' molecules such as proteins, DNA and RNA. For DNA and RNA, it can take hours until stable and strong SERS spectra appear. This might be explained by very long times that are needed by these large molecules to achieve stationary adsorption states⁴⁸. SERS was also studied from many 'intrinsically colored' biomolecules such as porphyrins, chlorophylls and various pigments, as well as from dye-containing larger molecules such as heme-containing protein. In these SERS experiments, molecular resonance Raman effect provides an additional enhancement. Other medical applications include such as SERS detection of stimulating drugs⁴⁹ and selective analysis of antitumor drug interaction with living cancer cells⁵⁰⁻⁵². These are only a few examples. For a complete overview on SERS in the biomedical field up to 1993, we refer to a number of instructive and comprehensive review papers¹⁷⁻²⁰.

In the following, we discuss some selected examples for biomedical applications of SERS performed within the recent five years.

Probe of lipid chemistry at high spatial selectivity using silver microprobes

Lipids are chemical compounds secreted by sebaceous glands in most mammals. Typically these lipids are fatty acids, triglycerides and wax esters. The chemistry of these compounds, particularly monitoring of *in vivo* chemical changes, is of great interest. In ref. 46, the authors demonstrate that SERS is a potentially useful probe of lipid chemistry. Classes of lipids in synthetic mixtures imitating sebaceous glandular secretion and their bacterial transformation products have been spectroscopically resolved. Special silicon substrate silver microelectrodes

and fine silver wire probes with tip diameters as small as 0.75 μm are small enough and provide useful geometry and mechanical strength and flexibility for direct insertion in lipid-secreting structures. SERS spectra at very good signal-to-noise ratios have been measured from lipid mixtures as monolayers on silver silicon-substrate microprobes in 180 s acquisition time. The laser intensity at the sample was 1 $\mu\text{W}/\mu\text{m}^2$.

Probe of neurotransmitters in physiologically relevant concentrations and close to the time scale of neuronal processes

The detection and quantification of neurotransmitters in brain extracts or in a brain dialysates is an important question in neurochemistry. Of particular interest are measurements of concentration changes in correlation to neuronal events, i.e. measurements in the time scale of neuronal processes⁵³. It has been shown that artificial neural networks are capable of accurately identifying the Raman spectra of aqueous solutions of neurotransmitters⁵⁴. However, the detection limits in spontaneous Raman scattering are of the order of 10^{-1} M concentrations, i.e. orders of magnitude too high compared to physiologically relevant values. The detection limits could be decreased for many orders of magnitude by exploiting the SERS effect. SERS spectra of different neurotransmitters have been measured on silver electrodes and on colloidal silver particles in water⁵⁵⁻⁵⁸. Figure 3 shows SERS spectra of dopamine and norepinephrine on colloidal silver clusters. SERS spectra of these two neurotransmitters were measured at concentrations between 5×10^{-6} and 5×10^{-9} M with accumulation times between 0.025 and 5 s (ref. 58). A fiber optic probe was used to provide about 100 mW cw near infrared excitation laser light (830 nm) and to collect the Raman photons. Scattering signals were detected by a charge-coupled device (CCD). Probed volumes were in a size of about 200 picoliters which results in fewer than 10^6 molecules (atomols) contributing to the Raman signal. Recently, similar detection limits for dopamine and epinephrine have been reported from two photon-excited fluorescence experiments⁵⁹. In the SERS experiments, albumin was added to the aqueous sample solutions in order to make them more similar to a real biological environment. The low concentrations and fast data acquisition are in the order of physiologically relevant concentrations and close to the time scale of neuronal processes, respectively. Fluorescence background from other compounds in real body fluids should be drastically reduced with NIR excitation of the SERS spectra.

In spite of their very similar chemical structure and very similar 'normal' Raman spectra, clear differences appear between dopamine and norepinephrine SERS spectra. Such differences are probably due to small

differences in the adsorption behavior of these molecules. SERS, therefore, can improve the structural sensitivity and selectivity for similarly structured molecules. SERS spectra from mixtures containing the two neurotransmitters allowed quantitative information on the mixtures of the two compounds.

SERS study of membrane transport processes

Understanding of transport of molecules through both biological and synthetic membranes is of basic interest for questions such as drug delivery across stratum corneum and the release of drugs from synthetic delivery systems such as patches and polymer implants. It is actually important to enhance drug penetration through human skin in order to achieve therapeutic level for local, regional or transdermal topical therapy. Structure-penetration relationships and mechanisms for increase of drug penetration have been developed. For instance, lipid solubility, molecular size and hydrogen bonding seem to be important parameters for diffusion across skin⁶⁰⁻⁶².

Membrane permeability studies are usually performed *in vitro* by determination of diffusion profiles using Franz-type diffusion cells⁶³. In order to improve understanding of the penetration process, new techniques to elucidate the process are required. It has been demonstrated that SERS can be used to observe interfacial arrival times of molecules⁶⁴. In the experiments, the transport of a mixture of pyridine and diphenyldisulphide (DPDS) through a Silastic (polydimethylsiloxane) membrane was studied. Aggregated colloidal silver solution at the receiving interface of the membrane was used to enhance the Raman signal of the molecules that had arrived at this side. A plot of SERS intensity against time showed that two mechanisms of diffusion are observed, a rapid diffusion perhaps through channels or pores within the membrane structure and a diffusion through the bulk of the membrane giving rise to a slower onset of growth of the penetrant but responsible for the greater part of the mass transport. Also, the individual interfacial arrival times of both pyridine and DPDS could be distinguished. The results show that SERS can discriminate between the movement of different molecules across a membrane and to observe different interfacial arrival times and concentration growth rates in the receiving (colloidal silver) solution.

Enzyme immunoassay utilizing SERS

Because of its high content of 'chemical information', Raman spectroscopy and particularly SERS is an attractive method for enzyme immunoassays. Dou *et al.* describe a new enzyme immunoassay employing SERS spectra of the enzyme reaction product⁶⁵. Figure 4a illustrates the proposed system. Antibodies immobilized on a solid substrate react with antigen (mouse-IgG) which binds with other antibodies labeled with peroxidase. If these immunocomplexes are subjected to reaction with *o*-phenylenediamine, azoaniline is generated. The enzyme reaction is shown in Figure 4a (B). The reaction product is adsorbed on colloidal silver particles providing a strong SERS spectrum of the azo-compound as shown in Figure 4b. In that way, the concentration of antigen is determined indirectly via the SERS signal of azoaniline. Figure 4c plots the intensity of the 1442 cm⁻¹-SERS line versus the concentration of the antigen resulting in a 'good' straight line. The correlation coefficient was calculated to be 0.999. Detection limit was estimated to be 1.8×10^{-12} M.

DNA and gene probes based on SERS-labels

Rapid progress in DNA and genome research results in strong interest in the development of methods for probing DNA and DNA fragments, particularly also in techniques that avoid radioactive labeling. In addition to the potential

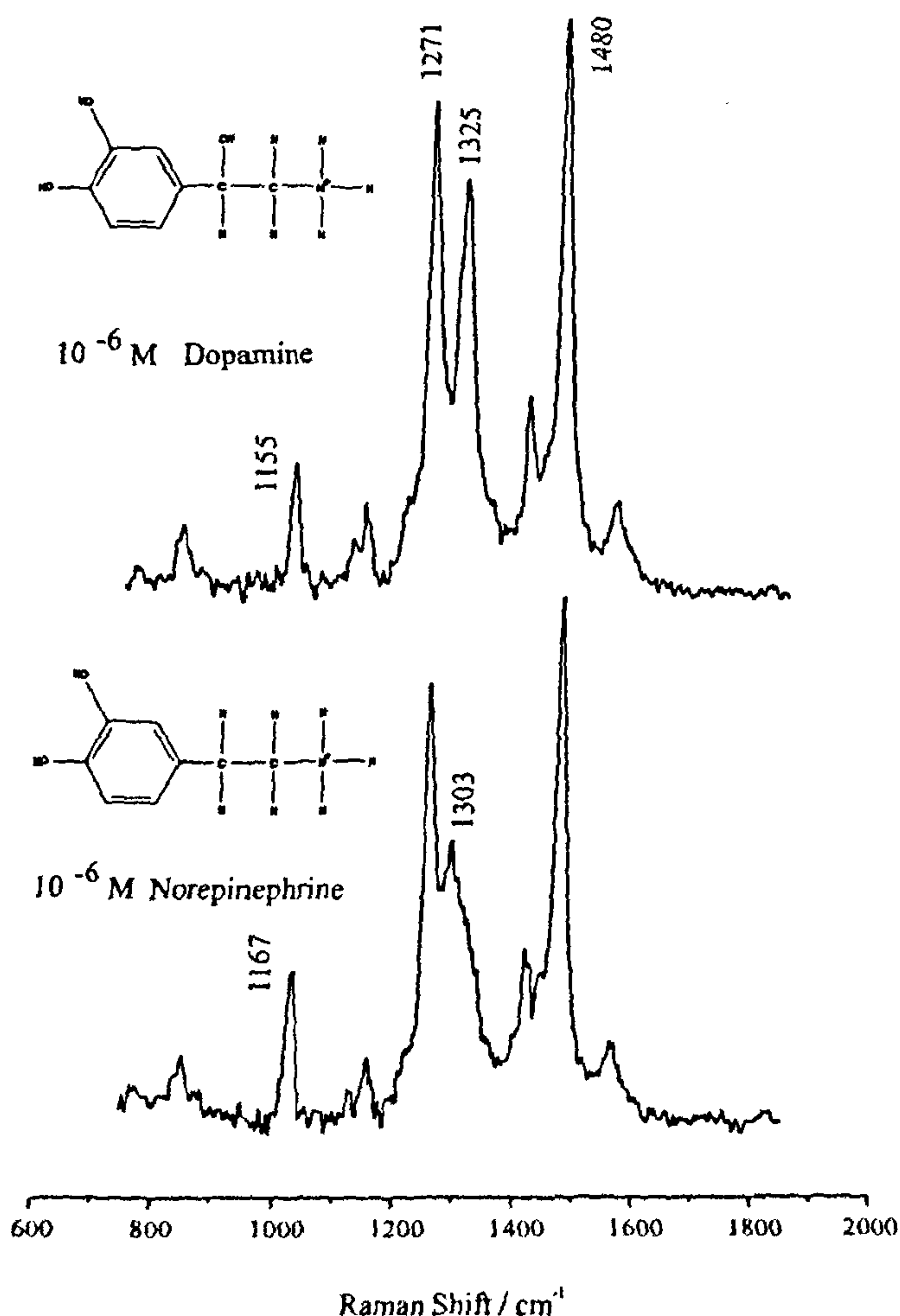


Figure 3. SERS spectra of neurotransmitters in silver colloidal solution. Spectra were collected within 50 ms using 100 mW 830 nm excitation.

safety hazard associated with radioactive labels, there is only one principal label for gene probe. That means that DNA can only be probed for one sequence at a time. An alternative approach is based on optical labels such as dye

molecules that allow fluorescence detection of DNA fragments⁶⁶. But fluorescence also suffers from poor spectral selectivity of fluorescence labels. Recently, SERS has been suggested as a new optical probe. DNA and DNA fragments are now tagged with an 'SERS-label' and the fluorescence signal is replaced by the surface-enhanced Raman signal. SERS probe provides the high spectral selectivity of the Raman spectra of various different molecules that potentially can serve as 'SERS-labels'.

Vo-Dinh *et al.*⁶⁷ applied SERS to probe DNA fragments by hybridization which involves joining of strands with the corresponding mirror images that are simultaneously tagged with an SERS label. The technique has been demonstrated for hybridization of $p(dA)_{18}$ oligonucleotides attached on a nitrocellulose surface. Nucleic acid fragments consisting of 18 deoxyribonucleotide oligomers of thymine, $p(dT)_{18}$, which is the appropriate mirror strand, have been tagged with cresyl fast violet as SERS label. The labeled $p(dT)_{18}$ that hybridized to the $p(dA)_{18}$ oligomers were recovered by washing the nitrocellulose and deposited on an SERS active silver substrate. A strong SERS peak appears from the labeled $p(dT)_{18}$ that have hybridized to $p(dA)_{18}$ oligonucleotides. For example, labeled $p(dC)_9$ oligonucleotides were also analysed and no SERS signal was detected since $p(dC)_9$ oligonucleotides do not hybridize to $p(dA)_{18}$.

Dou *et al.*⁶⁸ proposed an SERS label-based method for quantitative monitoring the concentration of double-stranded (ds) DNA amplified by a polymerase chain reaction (PCR). The amplification of DNA by PCR is usually determined by agarose gel electrophoresis. Regarding this relatively inconvenient and time-consuming method, there is a strong interest in alternative techniques for confirmation of DNA amplification. In the proposed method, a DNA intercalator, 4',6-diamidino 2-phenylindole dihydrochloride (DAPI) was employed in order to form a complex with the ds-DNA. DAPI gives rise to a strong SERS signal in silver colloid solution but it is also a so-called deeply intercalating dye. Such strong intercalation prevents a strong contact between DAPI and silver colloids and the SERS signal of the dye vanishes with intercalation. Therefore in the solution containing a mixture of PCR, DAPI and colloidal silver, the SERS signal of the intercalator is inversely proportional to the concentration of ds-DNA. A good straight line fit was obtained between DAPI SERS signals versus concentration of ds-DNA amplified by PCR.

Detection of a single adenine molecule based on its intrinsic Raman spectrum

Detecting and identifying a single DNA base is of great scientific and practical interest. For example, one approach in rapid DNA sequencing is based upon

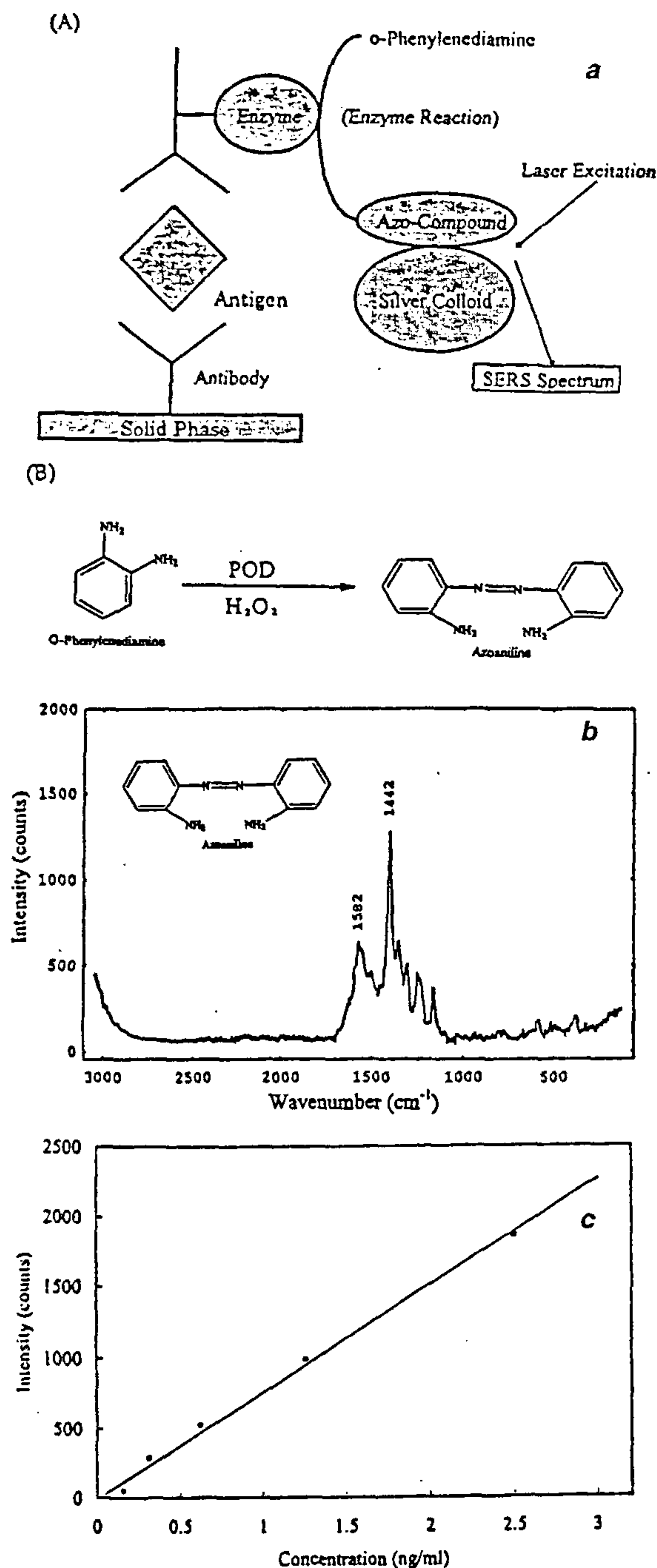


Figure 4. *a*, An SERS enzyme immunoassay system (A) and example of an enzyme reaction (B). *b*, SERS spectrum of the enzyme reaction product adsorbed on the silver substrate. *c*, Plots of the intensity of the 442 cm^{-1} line versus the concentration of antigen. [Reprinted with permission from ref. 65, Copyright 1997 American Chemical Society.]

spectroscopic single molecule detection. To detect and to identify single bases by fluorescence, they must be labeled by fluorescent dye molecules to achieve large enough fluorescence quantum yields and distinguishable spectral properties⁶⁹.

But, nucleotides and bases show well-distinguished surface-enhanced Raman spectra^{70,71}. Figure 5 shows SERS spectra of the four DNA base measured in silver colloidal solution using NIR excitation. For example, the ring breathing modes of three DNA bases adenine, guanine and cytosine give rise to strong and sharp well-separated Raman lines at 735, 660 and 800 cm^{-1} , respectively. The spectrum of the fourth DNA base, thymine coincides with the cytosine line in the ring breathing region, but lines within the 1200–1400 cm^{-1} region are also quite different and unique for this base and can be used as marker.

Recently, extremely large effective surface enhanced Raman cross sections on the order of 10^{-16} cm^2 per molecule have been derived for adenine and for its nucleotide from the obtained vibrational pumping¹³. Such cross sections are sufficient for single molecule detection. Figure 6 shows a schematic diagram of the experimental set up used for single molecule SERS spectroscopy. Spectra are excited by an argon-ion laser pumped cw Ti:sapphire laser operating at 830 nm with a power of about 100–200 mW at the sample. A microscope attachment is used for laser excitation and collection of the Raman scattered light. Scattering volumes are of the order of femtoliters to picoliters within a small droplet of sample solution which is placed on a microscopic cover slide. Low analyte concentrations result in average num-

bers of one or fewer molecules which are available in the probed volume. The dots in the sample droplet in Figure 3 represent single analyte molecule-loaded silver cluster. Brownian motion of these clusters into and out of the probed volume results in strong statistical changes in SERS signals measured from such a sample in time sequence. Figure 7a represents selected typical spectra collected in 1 s from samples which contain an average of 1.8 adenine molecules in a probed 100-fL volume. The drastic changes disappear for 10 times higher adenine concentration when the number of molecules in the probed volume remains statistically constant. Figure 7b gives the statistical analysis^{10,13} of adenine SERS-signals (100 measurements) from an average of 1.8 molecules in the probed volume and from 18 molecules. The change in the statistical distribution of the Raman signal from Gaussian (below) to Poisson (top) reflects the probability to find 0, 1, 2 (or 3) molecules in the scattering volume during the actual measurement and is an evidence that single molecule detection of adenine by SERS is achieved. Comparing the 1.3 molecule fit with the 1.8 molecule concentration/volume estimate we conclude that 70–75% of the adenine molecules were detected by SERS.

The strong field enhancement provided by colloidal cluster³¹ might be the key for understanding the enormous non-resonant surface-enhanced Raman cross sections exploited in single molecule adenine experiments. Therefore, it should be possible to achieve SERS cross sections on the same order of magnitude for other bases when they are attached to colloidal silver or gold clusters. Thus, NIR-SERS provides a method for detecting and identifying a single DNA base, which does not require any

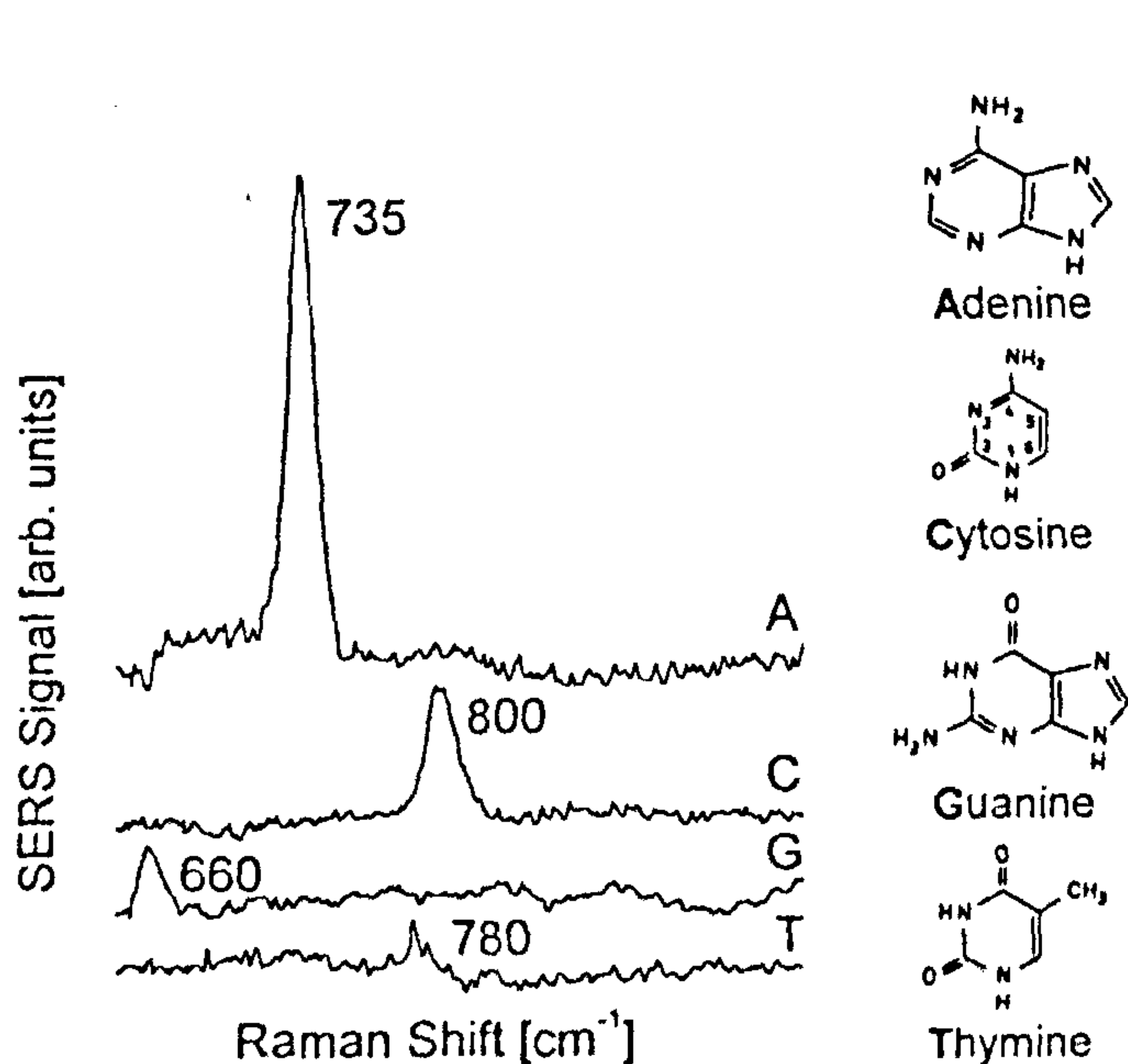


Figure 5. SERS spectra of DNA bases attached to colloidal silver clusters in aqueous solution. Spectra were collected from about 10^{-8} M base concentrations within one second using 100 mW 830 nm excitation.

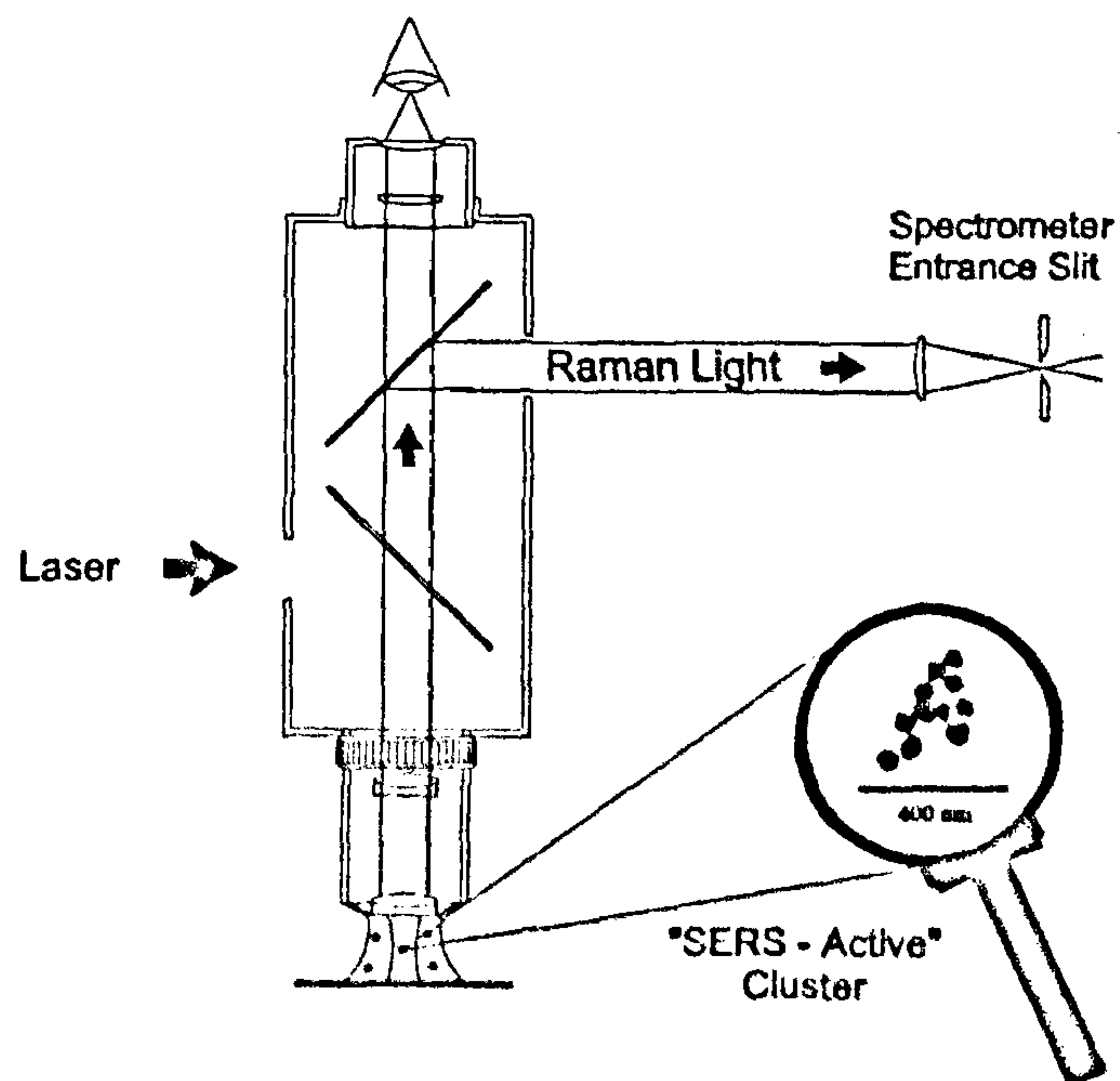


Figure 6. The single molecule SERS experimental schematic diagram. The inset shows an electron micrograph of a part of typical SERS active colloidal clusters.

labeling because it is based on the intrinsic surface-enhanced Raman scattering of the base.

Potential and limitations of SERS as a tool in biomedical spectroscopy

SERS combines fingerprint capabilities of vibrational spectroscopy and ultrasensitive detection limits. Particularly, radioactive labeling of biomolecules can be avoided. Molecules of biomedical interest can be detected and characterized using SERS spectra of an 'optical label' or by the use of their intrinsic surface-enhanced Raman spectra. The last technique is of particular interest because optical labeling is avoided as well. Due to adsorption on the metal particles which results in formation of new and fast relaxation channels for the electronic excitation of the target molecule, in many cases, a strong quenching of the fluorescence has been observed.

Limitations of SERS spectroscopy are related to the fact that target molecules have to be attached to so-called SERS-active substrates such as nanometer-sized silver or gold structures. Due to the mainly electromagnetic origin of the enhancement, it should be possible to achieve a strong SERS effect for each molecule. However, SERS enhancement cannot be observed for all molecules. There is still a molecular selectivity of the effect that cannot be explained yet. Based on empirical findings, SERS seems to work very well for molecules with delocalized pi systems²². Fortunately, many molecules of biomedical and pharmaceutical interest contain unsaturated cyclic electron systems. Therefore, SERS should be applicable for relatively many problems in the biomedical field.

In spite of that, the main point for the use of SERS in biomedical spectroscopy is to overcome this molecular selectivity and to achieve large enough effective Raman cross sections for each molecule you wish to detect. A better theoretical understanding of SERS in general including adsorption processes might be an important key

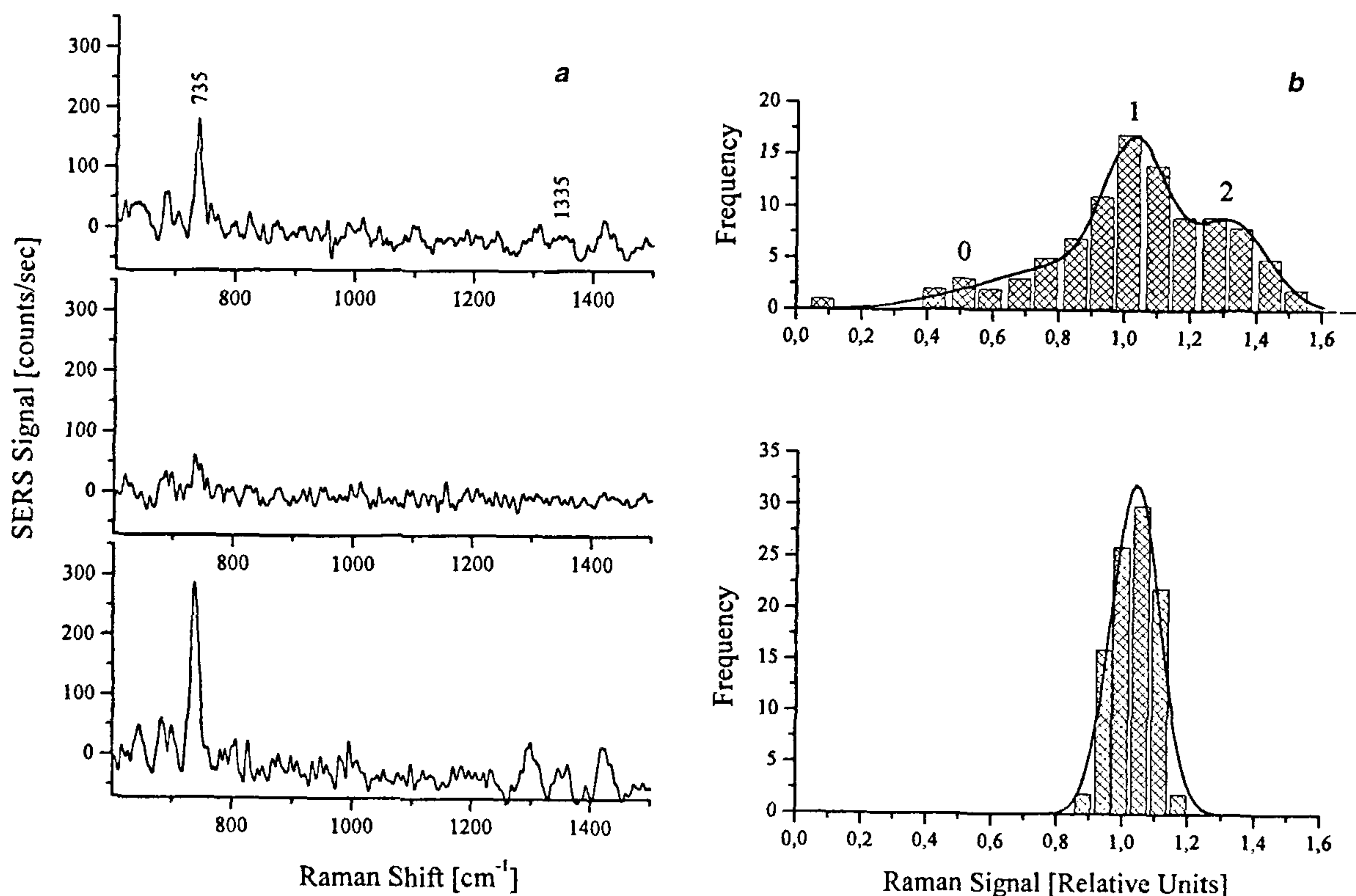


Figure 7. Single molecule SERS spectra of adenine: *a*, Typical SERS Stokes spectra representing approximately '1' (top), '0' (middle), or '2' (below) adenine molecules in the probed volume (collection time 1 s, 80 mW NIR excitation). *b*, Statistical analysis of 100 SERS measurements at an average of 1.8 adenine molecules (top) and for 18 adenine molecules (below) in the probed volume. The experimental data of the 1.8 molecule sample were fit by the sum of three Gaussian curves (solid line) whose areas are roughly consistent with a Poisson distribution for an average number of 1.3 molecules. As expected, the data of the 18 molecules sample could be fit by one Gaussian curve. [Reprinted with permission from ref. 13, Copyright 1998 American Institute of Physics.]

for further development of SERS as a tool in biomedical spectroscopy.

By exploiting extremely large enhancement factors of at least fourteen orders of magnitude, corresponding to effective Raman cross sections of the order of 10^{-16} cm^2 per molecule, SERS can reach single molecule sensitivity. These single molecule capabilities open up exciting perspectives for SERS as tool in laboratory medicine and for basic research in biology.

Summary

A new spectroscopic tool, SERS, has been introduced and briefly discussed. The effect combines the structural information content of Raman spectroscopy with ultra-sensitive detection limits, allowing Raman spectroscopy down to the single molecule level. For generating a strongly increased Raman signal, the target molecule has to be attached to SERS-active substrates, such as, for instance, tiny silver or gold particles. In most biomedical applications, these colloidal particles have been provided in aqueous solutions. An additional favorable aspect for Raman spectroscopy is related to fluorescence quenching for the target molecules due to new relaxation channels between molecule and metal.

The potential of SERS in biomedical spectroscopy has been illustrated by some recent applications such as structurally sensitive detection of lipids and neurotransmitters, studying membrane transport processes, probing DNA fragment using an SERS label, proposing new enzyme immunoassays employing SERS and detecting a single DNA base molecule without any labeling based on its intrinsic Raman spectrum.

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MEETINGS/SYMPOSIA/SEMINARS

2nd International Seminar on Analytical Techniques in Monitoring the Environment

Date: 18–20 December 2000
Place: Tirupati, India

The seminar will be of three days duration comprising keynote addresses, invited talks, oral and poster presentations in the following technical sessions: Spectral methods; Electroanalytical methods; Chromatographic methods; Radioanalytical and other miscellaneous methods; Biosensors; Air quality monitoring; Metal speciation; Environmental specimen banking and preparation of SRMs; Any other related area.

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Fifth IUPAC International Symposium on Bioorganic Chemistry (ISBOC-5)

Date: 30 January–4 February 2000
Place: Pune, India

Topics include: Biomimetic & macromolecular chemistry; Molecular recognition; Structural biology; Bioactive molecules; Organized assemblies; Biomolecular technologies; Drug design and combinatorial chemistry.

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Symposium on Modern Trends in Inorganic Chemistry (MTIC-VIII)

Date: 18–20 January 2000
Place: Bangalore

Topics include: Solid state and materials chemistry; Metal ions in biology: enzymes, proteins and models; Organometallic compounds and catalysis; Main group chemistry; Supramolecular chemistry; Metal clusters; Experimental and theoretical studies of ground and excited states; Mechanism of inorganic reactions.

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