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Surface Enhanced Raman Scattering (SERS) for Narcotic detection and applications to Chemical Biology.

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Raman spectroscopy is rapidly finding favour for applications in the life science due to the ease with which it can be used to extract significant data from tissue and cells. However, the Raman effect is an inherently weak effect, which hinders the analysis of low concentration analytes. The Raman sensitivity can be improved via the Surface Enhanced Raman Scattering (SERS) effect. In SERS, Raman spectra are dramatically increased when a molecule is adsorbed onto nanoroughened noble metal surfaces such as silver and gold. The degree of enhancement enables single molecule detection, which offers the potential for the unambiguous identification of analytes at concentrations that are useful in both a forensic and a chemical biology context. Here we discuss some of the practical applications of SERS to both low-level narcotic detection, and how this can be applied to chemical biology.

Abbreviations:

DNA	Deoxyribonucleic acid
FIA	Flow Injection Analysis
FT-IR	Fourier Transform Infrared
GFP	Green Fluorescent Protein
HPLC	High Pressure Liquid Chromatography
NIR	Near Infrared
ppm	Parts per million
SERS	Surface Enhanced Raman Scattering
SERRS	Surface Enhanced Resonance Raman Scattering
TLC	Thin Layer Chromatography

Introduction

Raman spectroscopy is now a well established analytical technique in the physical sciences, as it offers many advantages over other vibrational spectroscopic techniques such as FT-IR and Near Infrared (NIR) absorption spectroscopy [1,2]. The adoption of Raman techniques for life science applications is also attracting attention due to the ease with which it can be used to extract significant data from complex environments such as living tissue and cells. However, the most significant drawback with Raman spectroscopy it is that it is an inherently weak effect, which makes it unsuitable for trace analysis. The discovery of the Surface Enhanced Raman Scattering (SERS) effect in the 1970's offered the exciting possibility of

overcoming this lack of sensitivity. The SERS enhancement is due to a combination of chemical and electronic effects and which are described in more detail in various reviews [3, 4, 5]. Coupling the high sensitivity of SERS with the instrumental benefits of Raman spectroscopy (including the sensitivity to small structural changes, non-invasive sampling capability, minimal sample preparation, and high spatial resolution) yields a methodology that can provide highly specific molecular information on the microscopic scale. It is this unique combination of sensitivity and specificity that makes it such an attractive technique for a wide variety of applications from forensics to healthcare. The degree of sensitivity that can be achieved in carefully controlled environments is very impressive with atto mol (10^{-18} M) concentrations being observed in many cases [6, 7].

SERS is achieved by carefully controlling both electronic and chemical effects, first by careful design of the physical substrates, and second the manner in which the analyte is adsorbed. This is a very dynamic field of research and there is no universal best SERS substrate/method, and so careful consideration of the analyte is required before choosing a SERS method. One recent example of a significant advance in SERS methodology concerned the use of aggregating agents. For metal colloids (the simplest, easiest to prepare, and most sensitive SERS substrate) optimum SERS signals are usually obtained only after an aggregating agent is added to the colloid. The most widely used agent is NaCl, which is employed in most of the studies discussed in this review. However, Koo and co-workers [8] showed that by replacing NaCl with LiCl, SERS signals could be dramatically enhanced (10 fold or more). This underlines the very fluid nature of SERS research, where dramatic improvements in sensitivity occur on a regular basis, and that established methods can be rapidly outperformed by seemingly trivial changes in the experimental method.

This review focuses on the use of SERS for narcotics and chemical biology applications from the point of view of analyte size. It begins by looking at SERS for small molecule detection (drugs & narcotics), then progress to amino acids, and from there to oligonucleotides, proteins, and eventually to whole organisms such as bacteria. It is hoped that this will provide an insight into the potential application of SERS and how it can be applied for the analysis of a wide range of simple and complex analytes in chemical biology.

SERS for small molecule detection & analysis

It is in the domain of small molecule analysis that SERS is most widely utilised. One of the key application areas in small molecule detection is in the area of narcotics and drug detection. In a forensic environment the specificity of Raman coupled with the sensitivity of SERS offers considerable potential. Bell and Sirimuthu [9] for example, have demonstrated the quantitative analysis of nicotine in the 0.1-10 ppm range using a very reproducible polymer encapsulated silver colloid substrate. However, in many cases testing for the presence of narcotics requires the analysis of physiological fluids such as blood, urine, or saliva. In all these cases, analytes are present in very small quantities, and there is the added complication of these fluids containing a wide range of possible interferences. Therefore, sample clean up and isolation may be required before SERS can be used effectively. The use of HPLC with SERS has been explored by Schneider and co-workers [10, 11, 12], to study a wide range of narcotics including cocaine, heroin, amphetamines, 1,4-benzodiazepines, and various metabolites of the drugs. They have shown that HPLC can generate separate out sufficient amounts of material from blood and urine samples for reproducible SERS analysis.

They also make the point that acetonitrile based elutants are best avoided for SERS based detection. This is because acetonitrile can bind to the SERS substrate and can be difficult to displace by the analyte of interest at low concentrations. In addition, there is also the possibility of an interfering SERS spectrum from acetonitrile. Other instrumental techniques that can be coupled with SERS detection methods include capillary electrophoresis [13], and flow injection analysis (FIA) [14]. The latter study is particularly interesting because it involves using FIA with SERS for the quantitative analysis of cyanide levels from plant extracts, which is important for food safety with plants that contain cyanogenic glucosides.

Saliva is another physiological fluid from which clinically useful analytical data can be obtained. SERS has been demonstrated to be capable of measuring 5-fluorouracil at concentration levels (in water) similar to that found in saliva. In saliva however, there is a complication from the presence of physiological thiocyanate [15]. Despite this there is obvious potential for using SERS for saliva based analytical methods, once the interfering species can be accounted for. Another point to consider when conducting SERS experiments on large or small molecules is the influence of pH on SERS spectra. For example, D-penicillamine shows considerable differences in SERS spectra at different pH, which has obvious implications in the design of SERS experiments [16].

Finally, another interesting area of research is the use of SERS for the study of how various drugs interact with biological molecules and environments. The interaction of various antitumoral drugs [17, 18] with proteins was studied using SERS, which helped elucidate the binding mechanisms. Likewise the interaction of doxorubicin with its DNA complex [19] and ethidium derivatives with G-Quadruplex DNA [20] were studied by SERS to reveal specific information about binding processes and the structure of the complexes.

SERS of small biological molecules

A detailed examination of the SERS behaviour of amino acids, homodipeptides, and heterodipeptides was covered by Podstawka, Ozaki, and Proniewicz [21, 22]. In these studies, there is extensive discussion on vibrational assignments of bands in the Raman spectra. One interesting observation is the time dependant changes in some SERS spectra, where the initial adsorption of the analyte onto the silver colloid is via carboxylate groups, but that rearrangement can occur leading to adsorption via amine groups. This observation has obvious implications into the application of SERS for the study of complex biological molecules such as proteins where there are multiple possible adsorption mechanisms, each of which may yield different SERS spectra. The use of SERS for the analysis of amino acids on Thin Layer Chromatography (TLC) substrates revealed that NIR excitation is much preferred over visible excitation, and that the TLC substrates must be chosen with care [23]. SERS has also been demonstrated for the quantitative detection of excitatory amino acids (glutamate and aspartate) at concentration levels in the 0.4 to 5 $\mu\text{mol/L}$ range [24].

At the next level of analyte complexity, oligonucleotides, there is considerable interest in the use of both SERS and SERRS as a transduction method, which can be incorporated into analytical instrumentation. In SERRS, the presence of a chromophore in the analyte of interest results in a surface induced resonance effect, in addition to the SERS effect. The combination of both effects leads to very high enhancement values. However, SERRS only occurs with analytes that contain a chromophore with an absorption that matches the wavelength of the excitation source. It has been demonstrated that SERRS can be more sensitive than fluorescence measurements made on standard commercial instrumentation [25], can be successfully applied to microfluidic devices [26], and with the proper

chromophores deliver a quantitative analytical method down to 10^{-11} M concentrations [27]. Oligosaccharides are another important class of complex biological molecules where SERS has been used for both the identification of closely related species, and the quantitative analysis of oligosaccharides mixtures [28]. Binding mechanisms in model systems which mimic glutathione peroxidase [29] were also studied by SERS.

SERS of large biological molecules

The SERS behaviour of proteins is not trivial, yet it offers the potential for very specific and sensitive interaction studies. The protein GFP was studied using SERS at the single molecule level [30], and in this case it was observed that the spectrum changes with time. This spectral change was also observed with SERS studies on Cytochrome C extracted from yeast [31]. In both cases, the proteins were adsorbed onto silver colloids and analysed under dry conditions, which may account for the spectral changes, although an exact mechanism has not been fully elucidated. The study of S-S containing proteins adsorbed onto colloid in an aqueous environment shows different behaviour from the dry case, and no spectral changes are observed [32]. This would indicate that the protein conformation may be more stable in an aqueous environment, or that the sampling method results in the study of a large number of colloid-protein aggregates simultaneously and that the SERS spectra thus reported is the sum of many different individual conformations. However, clarification on this will require additional research, but it offers exciting possibilities for the study of individual protein species at very low concentrations, similar to the levels in-vivo.

SERS has also demonstrated an ability to distinguish closely related proteins such as human insulin and its analogue insulin lispro. These two insulins differ only in the interchange of two neighboring amino acids; specifically, the propyl-lysyl sequence at the C-terminus of the B-chain in insulin lispro is inverted as compared to human insulin [33]. In the study it was observed that the protein solution (in 0.1mM HCl) deposited on the silver film substrate caused aggregation. The fact that very small differences in protein structure can be discriminated by SERS, offers potential for highly specific proteomic sensors.

One way in which the complexity of protein SERS can be avoided is to use SERS labels which are conjugated to biomolecules or biotin. In this way, specific cellular proteins or environments can be targeted using the SERS label (such as Cresyl violet), then incubated with silver colloid and then the SERS signal from the label attached to the protein detected [34]. This method has the obvious advantage that it can avoid non-specific interactions that can hinder SERS, and that the SERS signal will be very well defined, thus enhancing reproducibility. A similar approach has been used with a Raman-active dye labelled gene for cancer diagnostics [35]. One area of biotechnology where the label effect finds extensive application is in the area of immunoassays, where many groups are demonstrated SERS based approaches [36, 37, 38],

SERS in and of cells & bacteria

SERS can also be applied to the study of more complex environments and organisms such as mammalian cells and bacteria. In this case, SERS has an advantage over fluorescence microscopy in that less harmful NIR excitation can be used. SERS can be applied to the study of physiological conditions such as pH [39], where silver nanoparticles were functionalised with 4-mercaptobenzoic acid, and the SERS spectrum is sensitive to pH

changes in the range of 6-8. The principle is inherently simple and involves the use of SERS labels that change structure in response to environmental factors. It should be possible to extend the scheme to a wide variety of ions and small molecules in cells.

Another cell based SERS study involved observing mitoxanthrone adsorption on the plasma membrane [40]. From their studies the authors were able to discriminate cells that were resistant or sensitive to mitoxanthrone uptake by the SERS spectra. An alternative method for probing specific localities with cells involves the use of tapered optical fibres coated with a SERS substrate [41]. Combining these novel fibre probes with a micromanipulation system enables the acquisition of SERS spectra from very precise cellular compartments. In this study the authors deal with plant cells and demonstrate a portable system for field use, however, it is clearly applicable to human and or mammalian cells.

SERS can be applied to the analysis of more complex structures than proteins such as whole bacteria. SERS coupled with chemometrics has been successfully applied to the discrimination of different strains of bacteria [42]. In this study models were generated by cluster analysis for genus-level classification (six different types) and strain level discrimination of seven *Escherichia coli*. The work showed that care needs to be taken in the collection of SERS spectra from bacteria because there are differences in spectra when sampled from the same batch of bacteria. This is obviously a result of the large size of the bacteria relative to the colloidal Ag particles, and because of the heterogeneity of the cell envelope. A more detailed study utilising gold-based colloids showed enhancement factors of 10^4 , demonstrated that the SERS spectra were much simpler than the Raman spectra of the bulk bacteria, and that single bacterium analysis is possible [43]. A further study investigated in detail the specific biochemical components in the bacterial cell wall that produced the SERS signals [44]. A potential application of this work in the bioterrorism field involves the use of SERS to quantitatively measure dipicolinic acid (DPA) concentration in the ppm concentration range. DPA is a marker for bacterial spores and therefore SERS could be used as a transduction method for sensitive anthrax spore detection [45]. A second anthrax SERS study [46] used a portable battery operated Raman spectrometer and demonstrated a sensitivity of $\sim 1 \times 10^4$ spores, by extracting the calcium dipicolinate marker from spores. The folding behaviour of mycolic acids (aliphatic biological molecules) isolated from *Mycobacterium tuberculosis* and *M. kansasii* has also been studied using SERS [47].

Conclusions

SERS offers considerable potential in the area of molecular identification and analysis down to the single molecule level, which has significant applications in both biomedical diagnostics and forensics applications. Further development in the areas of substrate design, protocol development, and instrumentation will lead to more robust SERS methods that will be employed across the life and physical sciences. The ability to analyse small pharmaceutical and large biological molecules using the same methods may lead to a totally new method of observing chemical changes at a cellular level, which may rival the established fluorescence techniques.

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References and recommended reading:

Papers of particular interest, published within the annual period of the review, have been highlighted as:

- of special interest
- of outstanding interest

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- 1 Petry R, Schmitt M, Popp J: **Raman Spectroscopy - A prospective tool in the life sciences**. *Chemphyschem* 2003, **4**:14-30.
 - 2 Baena JR, Lendl B: **Raman spectroscopy in chemical bioanalysis**. *Curr Opin Chem Bio* 2004, **8**:534-539.
 - 3 Schatz GC, Van Duyne RP: **Electromagnetic mechanism of Surface-enhanced Spectroscopy**. In *Handbook of Vibrational Spectroscopy*. Edited by Chalmers JM, Griffiths PR. Chichester: John Wiley & Sons Ltd, 2002.
 - 4 Vo-Dinh T: **Surface-enhanced Raman spectroscopy using metallic nanostructures**. *Trac-Trend Anal Chem* 1998, **17**:557-582.
 - 5 Campion A, Kambhampati P: **Surface-enhanced Raman scattering**. *Chem Soc Rev* 1998, **27**:241-250.
 - 6 Etchegoin P, Maher RC, Cohen LF, Hartigan H, Brown RJC, Milton MJT, Gallop JC:
•• **New limits in ultrasensitive trace detection by surface enhanced Raman scattering (SERS)**. *Chem Phys Lett* 2003, **375**:84-90.
The paper demonstrates measurement of single molecule SERS (SM-SERS) at concentrations of 10^{-18} M. What is interesting about the paper is the fact that experiments were carried out over 8 hours with the SM-SERS events clearly occurring only twice during this period. The paper also advances a method for single molecule counting using SM-SERS but also concedes that more work is required to understand the 'hot spot' mode of SERS enhancement and the other physiochemical factors that affect analyte adsorption on metal colloids.
 - 7 Futamata M, Maruyama Y, Ishikawa M: **Metal nanostructures with single molecule sensitivity in surface enhanced Raman scattering**. *Vib Spectrosc* 2004, **35**:121-129.
 - 8 Koo TW, Chan S, Sun L, Su X, Zhang JW: **Specific chemical effects on surface-**
•• **enhanced Raman spectroscopy for ultra-sensitive detection of biological molecules**. *Appl Spectrosc* 2004, **58**:1401-1407.
This papers major contribution is the fact that changing the aggregation agent to LiCl results in dramatic increases in SERS signals. It will be interesting to see if other research groups find the same levels of enhancement on more complex biological molecules.
 - 9 Bell SEJ, Sirimuthu NMS: **Rapid, quantitative analysis of ppm/ppb nicotine using surface-enhanced Raman scattering from polymer-encapsulated Ag nanoparticles (gel-colls)**. *Analyst* 2004, **129**:1032-1036.
 - 10 Sagmuller B, Schwarze B, Brehm G, Trachta G, Schneider S: **Identification of illicit drugs by a combination of liquid chromatography and surface-enhanced Raman scattering spectroscopy**. *J Mol Struct* 2003, **661**:279-290.
 - 11 Trachta G, Schwarze B, Sagmuller B, Brehm G, Schneider S: **Combination of high-performance liquid chromatography and SERS detection applied to the analysis of drugs in human blood and urine**. *J Mol Struct* 2004, **693**:175-185.
 - 12 Trachta G, Schwarze B, Brehm G, Schneider S, Hennemann M, Clark T: **Near-infrared**

- **Fourier transform surface-enhanced Raman scattering spectroscopy of 1,4-benzodiazepine drugs employing gold films over nanospheres.** *J Raman Spectrosc* 2004, **35**:368-383.
This volume of the Journal of Raman Spectroscopy is a special issue for Raman applications in pharmaceutical analysis.
- 13 Dijkstra RJ, Gerssen A, Efremov EV, Ariese F, Brinkman UAT, Gooijer C: **Substrates for the at-line coupling of capillary electrophoresis and surface-enhanced Raman spectroscopy.** *Anal Chim Acta* 2004, **508**:127-134.
- 14 Thygesen LG, Jorgensen K, Moller BL, Engelsen SB: **Raman spectroscopic analysis of cyanogenic glucosides in plants: Development of a flow injection surface-enhanced Raman scatter (FI-SERS) method for determination of cyanide.** *Appl Spectrosc* 2004, **58**:212-217.
- 15 Farquharson S, Shende C, Inscore FE, Maksymiuk P, Gift A: **Analysis of 5-fluorouracil in saliva using surface-enhanced Raman spectroscopy.** *J Raman Spectrosc* 2005, **36**:208-212.
- 16 Lopez-Ramirez MR, Arenas JF, Otero JC, Castro JL: **Surface-enhanced Raman scattering of D-penicillamine on silver colloids.** *J Raman Spectrosc* 2004, **35**:390-394.
- 17 Fabriciova G, Sanchez-Cortes S, Garcia-Ramos JV, Miskovsky P: **Joint application of**
 - **micro-Raman and surface-enhanced Raman spectroscopy to the interaction study of the antitumoral anthraquinone drugs danthron and quinizarin with albumins.** *J Raman Spectrosc* 2004, **35**: 384-389.
The paper describes an interesting experimental procedure for collecting good quality SERS spectra from sensitive biological molecules, and might be of interest to anyone using SERS for protein analysis.
- 18 Fabriciova G, Sanchez-Cortes S, Garcia-Ramos JV, Miskovsky P: **Surface-enhanced Raman spectroscopy study of the interaction of the antitumoral drug emodin with human serum albumin.** *Biopolymers* 2004, **74**:125-130.
- 19 Lee CJ, Kang JS, Kim MS, Lee KP, Lee MS: **The study of doxorubicin and its complex with DNA by SERS and UV-resonance Raman spectroscopy.** *B Kor Chem Soc* 2004, **25**:1211-1216.
- 20 Breuzard G, Millot JM, Riou JF, Manfait M: **Selective interactions of ethidiums with G-quadruplex DNA revealed by surface-enhanced Raman scattering.** *Anal Chem* 2003, **75**:4305-4311.
- 21 Podstawka E, Ozaki Y, Proniewicz LM: **Part I: Surface-enhanced Raman spectroscopy investigation of amino acids and their homodipeptides adsorbed on colloidal silver.** *Appl Spectrosc* 2004, **58**:570-580.
- 22 Podstawka E, Ozaki Y, Proniewicz LM: **Part II: Surface-enhanced Raman spectroscopy investigation of methionine containing heterodipeptides adsorbed on colloidal silver.** *Appl Spectrosc* 2004, **58**:581-590.
- 23 Istvan K, Keresztury G, Szep A: **Normal Raman and surface enhanced Raman spectroscopic experiments with thin layer chromatography spots of essential amino acids using different laser excitation sources.** *Spectrochim Acta A* 2003, **59**:1709-1723.
- 24 O'Neal PD, Cote GL, Motamedi M, Chen J, Lin WC: **Feasibility study using surface-enhanced Raman spectroscopy for the quantitative detection of excitatory amino acids.** *J Biomed Opt* 2003, **8**:33-39.
- 25 Faulds K, Barbagallo RP, Keer JT, Smith WE, Graham D: **SERRS as a more sensitive technique for the detection of labelled oligonucleotides compared to fluorescence.** *Analyst* 2004, **129**:567-568.

- 26 Docherty FT, Monaghan PB, Keir R, Graham D, Smith WE, Cooper JM: **The first SERRS multiplexing from labelled oligonucleotides in a microfluidics lab-on-a-chip.** *Chem Commun* 2004, **1**:118-119.
- 27 Faulds K, Smith WE, Graham D: **Evaluation of surface-enhanced resonance Raman scattering for quantitative DNA analysis.** *Anal Chem* 2004, **76**:412-417.
- 28 Mrozek MF, Zhang D, Ben-Amotz D: **Oligosaccharide identification and mixture quantification using Raman spectroscopy and chemometric analysis.** *Carbohydr Res* 2004, **339**:141-145.
- 29 Hao YQ, Lia LN, Wu YQ, Liu JQ, Luo GM: **Two-dimensional correlation spectroscopic studies on the recognition mechanism of a glutathione peroxidase mimic bis-cyclodextrin diselenide.** *Vib Spectrosc* 2004, **36**:185-189.
- 30 Habuchi S, Cotlet M, Gronheid R, Dirix G, Michiels J, Vanderleyden J, De Schryver FC, Hofkens J: **Single-molecule surface enhanced resonance Raman spectroscopy of the enhanced green fluorescent protein.** *J Am Chem Soc* 2003, **125**:8446-8447.
- 31 Delfino I, Bizzarri AR, Cannistraro S: **Single-molecule detection of yeast cytochrome c by surface-enhanced Raman spectroscopy.** *Biophys Chem* 2005, **113**:41-51.
- 32 Podstawka E, Ozaki Y, Proniewicz LM: **Adsorption of S-S containing proteins on a colloidal silver surface studied by surface-enhanced Raman spectroscopy.** *Appl Spectrosc* 2004, **58**:1147-1156.
- 33 Drachev VP, Thoreson MD, Khaliullin EN, Davisson VJ, Shalaev VM. **Surface-enhanced Raman difference between human insulin and insulin lispro detected with adaptive nanostructures.** *J Phys Chem B* 2004, **108**:18046-18052.
- 34 Nithipatikom K, McCoy MJ, Hawi SR, Nakamoto K, Adar F, Campbell WB: **Characterization and application of Raman labels for confocal Raman microspectroscopic detection of cellular proteins in single cells.** *Anal Biochem* 2003, **322**:198-207.
- 35 Culha M, Stokes D, Vo-Dinh T: **Surface-enhanced Raman scattering for cancer diagnostics: detection of the BCIL2 gene.** *Expert Rev Mol Diagn* 2003, **3**:669-675.
- 36 Ji XH, Xu SP, Wang LY, Liu M, Pan K, Yuan H, Ma L, Xu WQ, Li JH, Bai YB, Li TJ: **Immunoassay using the probe-labeled Au/Ag core-shell nanoparticles based on surface-enhanced Raman scattering.** *Colloids Surface A* 2005, **257-258**:171-175.
- 37 Xu SP, Ji XH, Xu WQ, Li XL, Wang LY, Bai YB, Zhao B, Ozaki Y: **Immunoassay using probe-labelling immunogold nanoparticles with silver staining enhancement via surface-enhanced Raman scattering.** *Analyst* 2004, **129**:63-68.
- 38 Grubisha DS, Lipert RJ, Park HY, Driskell J, Porter MD: **Femtomolar detection of prostate-specific antigen: An immunoassay based on surface-enhanced Raman scattering and immunogold labels.** *Anal Chem* 2003, **75**:5936-5943.
- 39 Talley CE, Jusinski L, Hollars CW, Lane SM, Huser T: **Intracellular pH sensors based on surface-enhanced Raman scattering.** *Anal Chem* 2004, **76**:7064-7068.
- 40 Breuzard G, Angiboust JF, Jeannesson P, Manfait M, Millot JM: **Surface-enhanced Raman scattering reveals adsorption of mitoxantrone on plasma membrane of living cells.** *Biochem Bioph Res Co* 2004, **320**:615-621.
- 41 Gessner R, Rösch P, Petry R, Schmitt M, Strehle MA, Kiefer W, Popp J: **The application of a SERS fiber probe for the investigation of sensitive biological samples.** *Analyst* 2004, **129**:1193-1199.

This paper shows a very interesting method for obtaining SERS spectra from inside cells and tissue. The tapered tip design enables SERS spectra to be collected from very precise locations inside of living cells. In this work it proved possible to identify the presence of

- pesticide in various localities in living leaves. The method has considerable potential throughout the life sciences.
- 42 Jarvis RM, Goodacre R: **Discrimination of bacteria using surface-enhanced Raman spectroscopy**. *Anal Chem* 2004, **76**:40-47.
 - 43 Premasiri WR, Moir DT, Klempner MS, Krieger N, Jones G, Ziegler LD: **Characterization of the Surface Enhanced Raman Scattering (SERS) of bacteria**. *J Phys Chem B* 2005, **109**:312-320.
 - 44 Zeiri L, Bronk BV, Shabtai Y, Eichler J, Efrima S: **Surface-enhanced Raman spectroscopy as a tool for probing specific biochemical components in bacteria**. *Appl Spectrosc* 2004, **58**:33-40.
 - 45 Bell SEJ, Mackle JN, Sirimuthu NMS: **Quantitative surface-enhanced Raman spectroscopy of dipicolinic acid - towards rapid anthrax endospore detection**. *Analyst* 2005, **130**:545-549.
 - 46 Zhang, XY, Young, MA, Lyandres, O, Van Duyne RP: **Rapid detection of an anthrax biomarker by surface-enhanced Raman spectroscopy**. *J Am Chem Soc* 2005, **127**:4484-4489.
 - 47 Hasegawa T: **Structural analysis of biological aliphatic compounds using surface-enhanced Fourier transform Raman spectroscopy**. *Biopolymers* 2004, **73**:457-462.