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Point-of-Care Diagnosis of Urinary Tract Infection (UTI) Using Surface Enhanced Raman Spectroscopy (SERS)

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Abstract— **There are three stages to a complete UTI diagnosis: (1) identification of a urine sample as positive/negative for an infection, (2) identification of the responsible bacterium, (3) antibiogram to determine the antibiotic to which the bacteria are most sensitive to. Using conventional methods, all three stages require bacterial cultures in order to provide results. This long delay in diagnosis causes a rise in ineffective treatments, chronic infections, health care costs and antibiotic resistance. In this work, SERS is used to identify a sample as positive/negative for a UTI as well as to obtain an antibiogram against different antibiotics. SERS spectra of serial dilutions of *E. coli* bacteria mixed with silver nanoparticles, showed a linear correlation between spectral intensity and concentration. For antibiotic sensitivity testing, SERS spectra of three species of gram negative bacteria were collected four hours after exposure to the antibiotics ciprofloxacin and amoxicillin. Spectral analysis revealed clear separation between bacterial samples exposed to antibiotics to which they were sensitive and samples exposed to antibiotics to which they were resistant. With the enhancement provided by SERS, the technique can be applied directly to urine samples leading to the development of a new, rapid method for UTI diagnosis and antibiogram.**

Keywords*— *Surface Enhanced Raman Spectroscopy, bacteria, classification, dilutions, negative, positive, antibiogram

I. INTRODUCTION

Urinary tract infections are one of the most common types of infections causing millions of doctors visits and costs of billions of dollars every year in the US alone [1,2]. Diagnosis of a UTI requires a quantitative urine culture which produces results after 24 hours. A finding of a minimum of 10^5 colony forming units per ml of urine is considered positive for a UTI [3]. Identification of the responsible pathogen involves a series of biochemical tests which require another 24 hours. Determination of the most appropriate antibiotic for treatment requires an antibiogram which involves additional culturing and exposure of the bacteria to a series of antibiotics. Due to the prolonged period of diagnosis, physicians, suspecting an infection, usually prescribe broad spectrum antibiotics before any definitive diagnosis and antibiogram results are obtained. This practice has many undesirable consequences such as: (i) unsuccessful treatments leading to chronic infections, (ii) increased health care costs, and, most importantly, (iii) increased antibiotic resistance by a growing number of bacterial strains [4]. There is, therefore, a pressing need for a fast, accurate, automated and relatively inexpensive method of UTI diagnosis and antibiogram. Development of such a method would provide both short term and long term benefits for public health and would help control the rising problem of antibiotic resistance.

Vibrational spectroscopic methods have recently shown to be a more powerful approach for generating fingerprints of bacteria with minimal sample preparation [5]. Raman scattering, one of the whole organism fingerprinting techniques used, is observed when a very small number of photons incident on a molecule (about 1 in 10^7) are inelastically scattered [6]. A Raman spectrum is a plot of the intensity of scattered light versus the energy difference between incident photons and Raman scattered photons and contains information about the chemical composition and the molecular structure of a substance. A major limitation of regular Raman is the weakness of the Raman effect which results in very low signals, often below the limit of detection for dilute biological samples. Surface enhanced Raman Spectroscopy (SERS) is a variation of the technique which offers great enhancement of the signal (up to 10^{14}) due to plasmon resonance, the unison oscillation of electrons on the surface of a metallic nanostructure as a result of incident light of the right, resonant frequency [7,8]. SERS has had a variety of bioanalytical applications in recent years including the detection and identification of bacteria [9-23]. For this purpose, several classification approaches have been evaluated. A successful classification methodology adequately captures the similarities between spectra which belong to the same species of bacteria, and at the same time highlights the dissimilarities between the spectra of two different species of bacteria. In addition, it may be valuable to devise a classification scheme which does not require extensive pre-processing of the data. Such an approach could eliminate the pitfalls of user bias. No studies have been reported using either regular Raman or SERS for antibiotic sensitivity testing of bacteria, however, except work from this

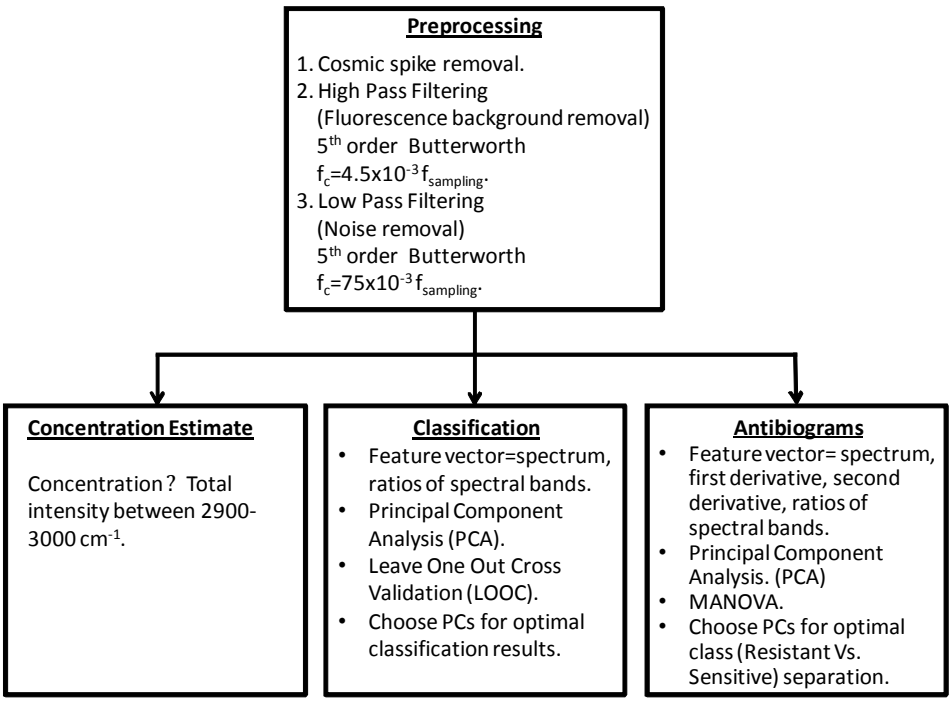

Figure 1. Block diagram of the pre-processing and data analysis.

group which reported the use of bulk Raman for classifying UTI bacteria and determining their sensitivity to an antibiotic [24-27].

The aim of this research was the use of SERS for the identification of samples of bacteria as negative or positive for a UTI and determination of their sensitivity to antibiotics using colloidal silver and gold nanoparticles. Using SERS is expected to provide the necessary signal enhancement not possible with regular Raman which would allow detection and identification of bacteria and development of their antibiogram from dilute samples like urine eliminating the need for cultures. A series of concentrations of E. coli bacteria (10^3-10^8 cells/ml) were correlated with the total intensity in the high-wave region (2900 to 3000 cm^{-1}) of their SERS spectra indicating an almost linear relationship. Sensitivity to the antibiotic ciprofloxacin and resistance to amoxicillin exhibited by *E. coli*, *K. pneumoniae* and *Proteus spp.* strains was confirmed using their SERS spectra after just 4 hours of exposure to the antibiotics. These results may lead to the development of a fast and accurate diagnosis and antibiogram method for UTI based on SERS.

II. Methodology

Clinical bacterial isolates from patients with UTI were identified by biochemical tests and obtained on LabM blood agar from affiliated clinical laboratories. Bacterial strains were subcultured on Mueller Hinton agar Petri dishes, cultured in Mueller Hinton broth and frozen stocks were created in 15% glycerol and stored at -80 ^{0}C. For classification with SERS, 1 strain of E. coli was first streaked on Mueller Hinton agar Petri dishes. 5 ml liquid cultures in Mueller Hinton broth were grown for 15-18 h at 37 ^{0}C and was subsequently washed 3X with sterile deionized water. Bacterial sample concentration was determined after obtaining their optical density at 600 nm in a spectrophotometer (an OD of 1 corresponds to 10^8 bacteria/ml). Serial dilutions were prepared to obtain the following concentrations: 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 bacteria/ml. For collection of SERS spectra of bacterial samples colloidal silver nanoparticles were prepared as follows: 90 mg of $AgNO_3$ was dissolved in 500 ml dH_2O and brought to boiling. 10 ml of a 1% sodium citrate solution was added and allowed to boil for one hour [28]. Bacterial dilutions were resuspended in 10 µl sterile deionized water and mixed with an equal volume of colloidal silver nanoparticles spotted on glass slides and allowed to dry. SERS spectra were collected directly from the spots.

Nine bacterial strains were chosen, 3 E. coli, 3 K. pneumonia, and 3 Proteus spp., and were treated with Ciprofloxacin or Amoxicillin and used for SERS. All samples were incubated for 0, 2 and 4 hours at 37^{0}C in the presence or absence of the two antibiotics. 10 µl of bacteria were mixed with an equal volume of gold nanoparticles ($5.7x10^9$ GNP/ml, Nanopartz), spotted on glass slides and allowed to dry. SERS spectra were collected directly from the spots.

SERS spectra from the serial dilutions of bacteria were collected using the iRaman system (BWTek, Inc) with a laser source at 532 nm and 3.0 cm^{-1} resolution. For classification of E. coli bacteria as positive or negative for a UTI, SERS spectra were collected from 18 samples by exposure to laser for 60 s X 4 averages. For classification of bacteria as sensitive to

Ciprofloxacin and resistant to Amoxicillin, SERS spectra were collected using the Enwave Raman Analyzer (Enwave Optronics, Inc) with a laser source at 785 nm and 4.5 cm^{-1} resolution from 9 samples belonging to 3 different species of bacteria (E. coli, K. pneumoniae, Proteus spp.). SERS spectra were collected from all samples treated or not treated with the two antibiotics after spots of bacteria and gold nanoparticles were exposed to laser for 10 s and a total acquisition time of 5 min.

III. Data Analysis

The 18 raw SERS spectra obtained from serial dilutions of two strains of E. coli bacteria consisted of data ranging from 300 cm^{-1} to 3000 cm^{-1} inclusive. Spectra were pre-processed by removing cosmic spikes and filtering to remove the background and the high frequency noise. Cosmic spikes, i.e. sharp peaks caused by the electronics of the system, were identified in advance and removed by interpolating the values between the beginning and end of each spike from all spectra. The fluorescent background was eliminated using a high-pass filter (5^{th} order Butterworth, $f_c = 4.5x10^{-3}f_{sampling}$) and the high frequency noise was removed using a low-pass filter (5^{th} order Butterworth, $f_c = 75x10^{-3}f_{sampling}$). The various steps of the algorithm are also illustrated in the block diagram of Figure 1.

The concentration of bacterial samples was estimated by summation of the total intensity of the spectra in the high-wave region, from 2900 to 3000 cm^{-1}. In addition, samples were classified as "positive" or "negative" for bacteriuria using Linear Discriminant Analysis. The spectra were preprocessed as described above. A feature vector was created which included the Raman spectrum and the ratios between 30 cm^{-1} sections of that spectrum. Principal component analysis of the data preceded the classification. This was important in order to improve the accuracy of the results, but was also necessary for creating a positive definite feature matrix required for the Discriminant Analysis algorithm. The principal components describing the highest variance of the original data were retained and the rest were discarded leading to optimal classification results. A leave-one-out cross-validation procedure was performed and the results for each of the 18 classification exercises were recorded.

Preprocessing of the SERS spectra of all samples of bacteria (E. coli, K. pneumonia, Proteus spp.) used in the antibiotic sensitivity study was done as described above. The feature vector in this case included the Raman spectrum as well as its first and second derivatives A principal components transformation was used and only principal components (PCs) describing the highest variance were retained. The MANOVA algorithm was subsequently applied to calculate a score for each sample in order to maximize the separation between groups of bacteria which were sensitive to an antibiotic with groups of bacteria which were resistant or were not treated with antibiotic.

IV. Results

The data confirms that bacterial concentration correlates well with spectral intensity. 18 SERS spectra were collected from serial dilutions of E. coli bacteria. Dilutions of 10^3 and 10^4 bacteria/ml are considered "negative" as they are below the level of "significant bacteriuria" which is 10^5 cfu/ml according to current, clinical standards. Dilutions 10^5-10^8 bacteria/ml are considered "positive" as they are at the level of significant bacteriuria. Spectra were pre-processed and classified as explained before. Figure 2 shows the SERS spectra from all bacterial dilutions. Using Linear Discriminant Analysis, the samples could be classified as positive or negative with perfect accuracy. Figure 3 the correlation between the total intensity of SERS spectra in the 2900-3000 cm^{-1} range with the log of concentrations of bacteria. A linear relationship exists in the 10^3-10^7 bacteria/ml range which suggests that SERS spectra could be used not only to determine whether a sample contains bacteria or not but also the concentration of bacteria in the sample.

SERS spectra of samples of bacteria (E. coli, K. pneumonia, Proteus spp.) were collected after treatment in the presence or absence of Ciprofloxacin or Amoxicillin. A MANOVA score was calculated for all samples and the results are shown in Figure 3. The results show that the MANOVA score of bacteria incubated with Ciprofloxacin is significantly higher than that of untreated bacteria or bacteria treated with Amoxicillin especially after 4 hours of incubation with antibiotics. These results suggest that SERS could be used in the determination of antibiotic susceptibility of bacteria after a short treatment with antibiotic.

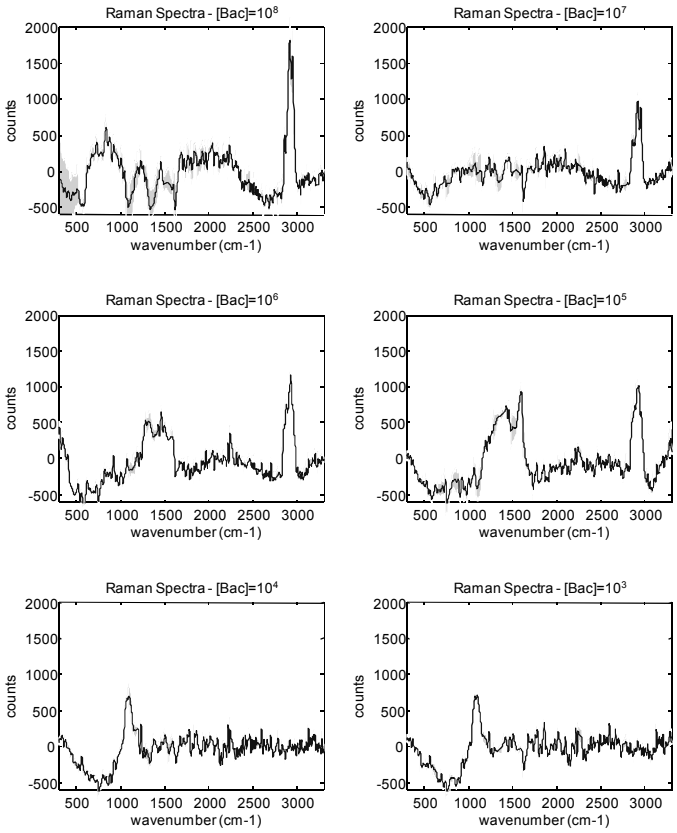

Figure 2. SERS spectra of the 18 bacterial dilutions collected for this study. The solid line is the average and the light blue background is the range of values of all samples

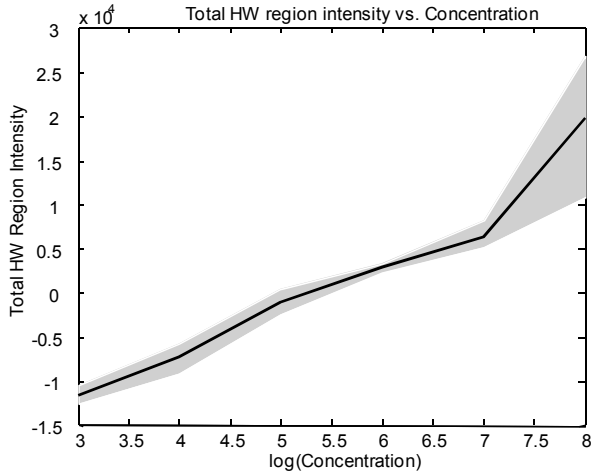

Figure 3. Correlation between the total intensity of the 2900-3000 cm^{-1} range of the SERS spectra with various concentrations of E. coli samples. The shaded region illustrates the range between minimum and maximum numbers whereas the black line is the mean value.

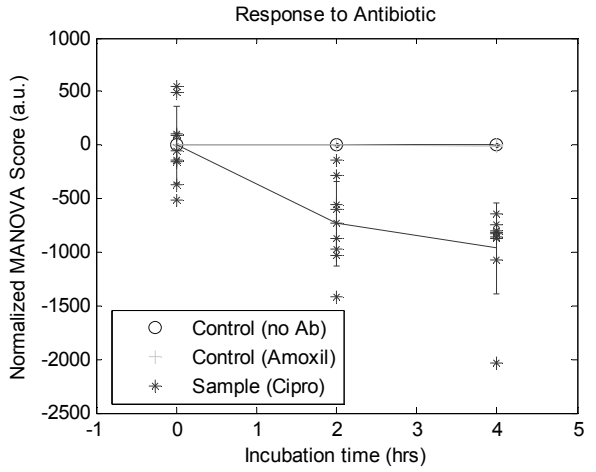

Figure 4. Normalized MANOVA score of SERS spectra of E. coli, K. pneumoniae. and Proteus spp. strains after their incubation in the presence of 0.128 μg/ml Ciprofloxacin (*), in the presence of 64 μg/ml Amoxicillin (+), or in the absence of any antibiotic (○).

V. CONCLUSION

The use of SERS for UTI diagnosis and antibiogram is presented in this study. Using this method, the concentration of samples of E. coli bacteria can be determined by calculation of the total intensity of the high-wave region of the spectra. Dilutions which represent "negative" samples correspond to what is commonly considered as a negative urine culture by current clinical standards, that is a culture with less than 105 cfu/ml. Dilutions corresponding to "positive" samples also correspond to what is considered as "significant bacteriuria" and therefore a "positive" sample for UTI by clinical laboratories. These results are therefore significant as they provide a strong indication that SERS can be used to determine the concentration of bacteria in samples. These experiments need to be expanded using a greater variety of UTI causing bacteria and a greater number of samples.

In addition, this method is able to distinguish the SERS spectra of bacteria that are treated and sensitive to an antibiotic from bacteria that are not treated or are resistant to an antibiotic, after a 4 hour incubation time. Apart from work from this group, no other reports have been published documenting the use of bulk Raman or SERS for antibiotic testing of bacteria. Experiments are currently under way to repeat this study using a larger number of samples belonging to a greater number of species which will include both gram negative and gram positive bacteria. In addition, a greater variety of antibiotics will be used to which some bacteria will be resistant and others sensitive. It is expected that SERS spectra of bacteria that are sensitive to antibiotics will be distinguishable from SERS spectra of bacteria that are resistant to antibiotics.

This study strongly suggests that SERS can be used to determine the concentration of bacterial samples which is the first necessary step in the process of identifying a sample as negative or positive for a UTI. SERS can also be used to determine the sensitivity of samples to antibiotics. In order for SERS to become the basis for developing a new method of diagnosis and antibiogram for UTI, experiments have to be done directly on urine samples. Urine samples will be minimally processed by filtering to remove other cells than bacteria and to concentrate bacteria in order to increase the sensitivity of the technique and avoid culturing. Development of a novel, accurate and rapid method of UTI diagnosis and antibiogram would therefore be possible and would have many short term and long term benefits for public health.

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REFERENCES

[1] M. S. Litwin and C. S. Saigal, "Introduction", [Urologic Diseases in America], DHHS, PHS, NIH, NIDDK, NIH publication, Washington, DC, 07-5512, 3-7, 2007.

[2] National Center for Health Statistics, [National Hospital Discharge Survey: 2004 Annual Summary With Detailed Diagnosis and Procedure Data], DHHS, Centers for Disease Control and Prevention, DHHS publication, Hyattsville, MD, 1733, 2006.

[3] M. G. Morgan and H. McKenzie., "Controversies in the laboratory diagnosis of community-acquired urinary tract infection", Eur. J. Clin. Microbiol. Infect. Dis. vol 12, pp. 491-504, 1993.

[4] A. J. Alanis, "Resistance to Antibiotics: Are we in the Post-Antibiotic Era?", Arch. Med. Res. vol 36, pp. 697-705, 2005.

[5] J. T. Magee, "Whole-organism fingerprinting", in Handbook of New Bacterial Systematics, M. Goddfellow and M.G. O'Donell, Eds, London: Academic Press, 1993, pp. 383- 427.

[6] A. Mahadevan-Jansen, "Raman Spectroscopy form Benchtop to Bedside", in Biomedical Photonics Handbook, T. Vo-Dinh, Ed., London: CRC Press, 2002.

[7] M. Moskovits, "Surface-enhanced Spectroscopy", Rev. Mod. Phys. vol. 57, pp. 783-826, 1985.

[8] T. Vo-Dinh, F. Yan, and D. L. Stokes,, "Plasmonics-based nanostructures for surface-enhanced Raman scattering bioanalysis", Methods Mol. Biol. vol. 300, pp. 255-283, 2005.

[9] C. R. Yonzon, C. L. Haynes, X. Zhang, J. T. Walsh Jr, and R. P. Van Duyne, "A glucose biosensor based on surface-enhanced Raman scattering: improved partition layer, temporal stability, reversibility, and resistance to serum protein interference", Anal. Chem. vol. 76, pp. 78-85, 2004.

[10] S. Shanmukh, L. Jones, Y. P. Zhao, J. D. Driskell, R. A. Tripp, and R. A. Dluhy, "Identification and classification of respiratory syncytial virus (RSV) strains by surface-enhanced Raman spectroscopy and multivariate statistical techniques", Anal. Bioanal. Chem. vol. 390, pp. 1551-1555, 2008.

[11] A. Pal, N. R. Isola, J. P. Alarie, D. L. Stokes, and T. Vo-Dinh, "Synthesis and characterization of SERS gene probe for BRCA-1 (breast cancer)", Faraday Discuss. vol. 132, pp. 293-301, 2006.

[12] L. Zeiri, B. V. Bronk, Y. Shabtai, J. Czege, and S. Efrima, "Silver metal induced surface enhanced Raman of bacteria", Colloids Surf. A. vol. 208, pp. 357-362, 2002.

[13] L. Zeiri, B. V. Bronk, Y. Shabtai, J. Eichler, S. Efrima, "Surface-enhanced Raman spectroscopy as a tool for probing specific biochemical components in bacteria", Appl. Spectrosc. vol. 58, pp. 33-40, 2004.

[14] A. A. Guzelian, J. M. Sylvia, J. A. Janni, S. L. Clauson, and K. M. Spencer, "SERS of whole-cell bacteria and trace levels of biological molecules", Proc. SPIE, vol. 4577, pp. 182-188, 2002.

[15] R. M. Jarvis, and R. Goodacre, "Discrimination of bacteria using surface-enhanced Raman spectroscopy", Anal. Chem. vol. 76, pp. 40-47, 2004.

[16] R. M. Jarvis, A. Brooker, and R. Goodacre, "Surface-enhanced Raman spectroscopy for bacterial discrimination utilizing a scanning electron microscope with a Raman spectroscopy interface", Anal. Chem. vol. 76, pp. 5198-5202, 2004.

[17] A. Sengupta, M. L. Laucks, N. Dildine, E. Drapala, and E. J. Davis, "Bioaerosol characterization by surface-enhanced Raman spectroscopy (SERS)", J. Aerosol Sci. vol. 36, pp. 651-664, 2005.

[18] W. R. Premasiri, D. T. Moir, M. S. Klempner, N. Krieger, G. Jones II, and L. D. Ziegler, "Characterization of the surface enhanced raman scattering (SERS) of bacteria", J. Phys. Chem. B, vol. 109, pp. 312-320, 2005.

[19] A. Sengupta, M. Mujacic, and E. J. Davis, "Detection of bacteria by surface-enhanced Raman spectroscopy", Anal. Bioanal. Chem. vol. 386, pp. 1379-1386, 2006.

[20] R. M. Jarvis, A. Brooker, and R. Goodacre, "Surface-enhanced Raman scattering for the rapid discrimination of bacteria", Faraday Discuss. vol. 132, pp. 281-292, 2006.

[21] R. M. Jarvis, N. Law, I. T. Shadi, P. O'Brien, J. R. Lloyd, and R. Goodacre, "Surface-Enhanced Raman Scattering from Intracellular and Extracellular Bacterial Locations", Anal. Chem. vol. 80, pp. 6741-6746 (2008).

[22] R. M. Jarvis, and R. Goodacre, "Characterisation and identification of bacteria using SERS", Chem. Soc. Rev. vol. 37, pp. 931-936, 2008.

[23] A. Walter, A. Marz, W. Schumacher, P. Rosch, and J. Popp, "Towards a fast, high specific and reliable discrimination of bacteria on strain level by means of SERS in a microfluidic device", Lab Chip vol. 11, pp. 1013-1021, 2011.

[24] E. Kastanos, A. Kyriakides, K. Hadjigeorgiou, and C. Pitris, "Urinary tract infection diagnosis and response to antibiotics using Raman spectroscopy", Proc. SPIE vol. 7169, 71690I, 2009.

[25] E. Kastanos, A. Kyriakides, K. Hadjigeorgiou, and C. Pitris, "UTI diagnosis and antibiogram using Raman spectroscopy", Proc. SPIE vol. 7368, 73680U, 2009.

[26] E. Kastanos, A. Kyriakides, K. Hadjigeorgiou, and C. Pitris, "A Novel Method for Urinary Tract Infection Diagnosis and Antibiogram Using Raman Spectroscopy", J Raman Spec. vol. 41, pp. 958-963, 2010.

[27] A. Kyriakides, E. Kastanos, K. Hadjigeorgiou, and C. Pitris, "Classification of Raman Spectra using the Correlation Kernel", J Raman Spec. vol. 42, pp. 904-909, 2011.

[28] P.C. Lee, and D. Meisel, "Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols", J Phys Chem. vol. 86, pp. 3391-3395, 1982.