The Pennsylvania State University

The Graduate School

College of Engineering

QUANTITATIVE SURFACE ENHANCED RAMAN SPECTROSCOPY COMBINED WITH MICROFLUIDICS FOR LAB-ON-A-CHIP TECHNOLOGIES

A Thesis in

Engineering Science

by

Stephen Michael Koytek

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2010

The thesis of Stephen Michael Koytek has been reviewed and approved* by the following:

Melik C. Demirel
Associate Professor of Engineering Science and Mechanics
Thesis Advisor

W. Brian Reeves
Professor of Medicine

Jian Xu
Assistant Professor of Engineering Science and Mechanics

Judith A. Todd
P. B. Breneman Department Head Chair
Professor, Department of Engineering Science and Mechanics

*Signatures on file in the Graduate School

Abstract

A highly accurate, real time sensor for biomarker detection is required for early detection of disease. Creatinine concentration in urine can provide important information on the status of the kidney for patients suffering from any disease that affects the kidneys. We prepared nanostructured, surface-enhanced Raman spectroscopy (SERS) substrates without template or lithography that provide controllable, well-organized nanostructures on the surface. This allows for the quantitative analysis of creatinine concentration in urine. Here, we present our work on sensitivity and reliability of the SERS substrate to urine samples collected from diabetic patients and a healthy control group. We report the preparation of a new type of SERS substrate which provides fast (<10s), highly sensitive (creatinine concentration < 0.5 μg/mL) and reproducible (<5% variation) detection of urine. Our method is in good agreement with the contemporary enzymatic method.

Lay Abstract

A device for biomarker detection is required for early diagnosis of disease. A common component of urine, creatinine, can provide important information on the status of the kidney for patients suffering from any disease that affects the kidneys. For this project, a surface using very small fibers, not easily distinguishable with a microscope, was designed and used without the use of the usual expensive and timely methods. These surfaces, also known as surface-enhanced Raman substrates, allow us to quantitatively analyze extremely low creatinine levels in urine using an analysis method known as surface-enhanced Raman spectroscopy (SERS). Here, we present our work on sensitivity and reliability of the SERS substrate to urine samples collected from diabetic patients and a healthy control group. We report the preparation of a new type of SERS substrate which provides fast (<10s), highly sensitive (creatinine concentration < 0.5 μg/mL) and reproducible (<5% variation) detection of creatinine in urine. Our method is in good agreement with currently used enzymatic method.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to thank Dr. Melik Demirel, Dr. Hui Wang, Niranjan Malvadkar and the rest of the NanoBioMaterials research group for all of their help throughout this project. I would also like to thank Dr. Reeves for providing the samples tested. Finally, thanks to all of my family and friends for all of their encouragement.

Epigraph

Our imagination is stretched to the utmost, not, as in fiction, to imagine things which are not really there, but just to comprehend those things which are there.

~Richard Feynman

1—Introduction

1.1—Problem Statement

Civilizations across the world are plagued by disease. Some diseases are inherited, others "caught," but all can be prevented through research. Disease prevention starts with early detection; the earlier a given method detects a disease, the more manageable the symptoms are. Contemporary methods take time, and are not immune to some subjectivity; additionally, detection devices can be expensive and bulky. Both methods and devices can only detect after a disease has produced large amount of its signature agent.

Raman spectroscopy has been well used since its conception in the early seventies, but initial results were unreliable at best. (Belyanin, 1979) Now, with advancements in nanotechnology and materials, new methods and materials are being created to increase the reliability of Raman spectroscopy. Modifying the surface of a substrate by some means to increase testing reliability or signal, is known as surface enhanced Raman spectroscopy, or SERS. Using SERS is advantageous in biological and chemical detection because every organic and nonorganic material has unique a Raman spectra. Another advantage is SERS's potential for single molecule detection; precise detection to a single molecule has huge implications in being able to detect agents early.

Surface enhanced Raman spectra also were known to be unreliable due to random hot spot formations which can increase a signal up to fourteen or fifteen magnitudes. If these hot spots can be controlled, these increased signals can be seen more often. The

reason for these hot spots is widely unknown, but they cause the molecule to be captured on the surface of the substrate, causing the molecules bonds to vibrate with more intensity allowing for easier detection.

Detection of these agents sometimes isn't enough to diagnose someone with a disease; the agent must be quantitatively analyzed to determine if the concentration is too high compared to normal levels. This thesis will elaborate on the analysis of human urine for the detection of renal function in diabetic patients, and report the promising results thereof.

Creatinine detection is important do to its usefulness in diagnostics of kidney function. Kidney failure is a very prevalent in humans, and can be caused by many different ailments. However, creatinine is common to all patients, both healthy and sick. Therefore, a method of quantitatively analyzing urine for creatinine concentration is an important tool.

1.2—Literature Review

1.2.1 Raman Spectroscopy

Discovered near the end of the 19th century, light scattering was well observed and experimented with. (Young, 1981) When photons encounter a molecule, more specifically the electric dipole of a molecule, they will scatter. This scattering can be either elastic or inelastic. The reason for the sky being blue is attributed to the elastic scattering of light, known as Rayleigh scattering. The other form, inelastic scattering, is now known as Raman scattering. (Surface Enhanced Raman Spectroscopy, 1995) A visual depiction can be seen in Figure 1.2.1-1.

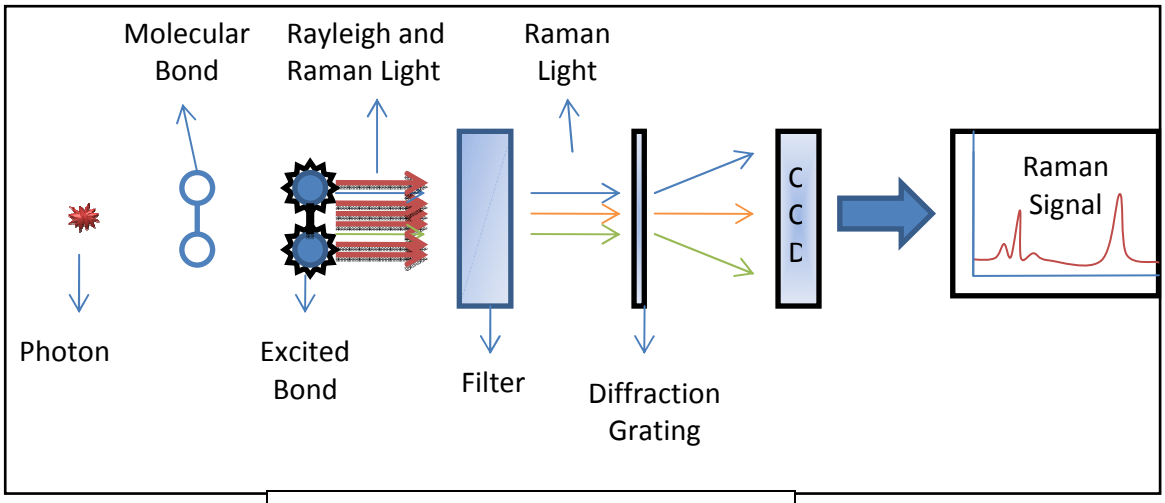

Figure 1.2.1-1: Diagram of Raman Scattering

Raman light scattering (RLS) was discovered in 1928 by K. Krishnan and C.V. Raman in India as well as independently by L.I. Manel'shtam and G.S. Landsberg in the USSR. (Belyanin, 1979) Inelastic scattering of light was deemed unique for its properties and

was taken even then to be a major scientific discovery. Raman was later awarded the Nobel Prize in 1930 for his discovery.

Whenever light is incident upon a molecule, the molecule will expel two primary forms of scattered photons, Rayleigh and Raman. Rayleigh scattered photons are re-scattered at the same energy as the incident photons, whereas Raman photons experience a shift in energy. This is due to the vibrational mode of the molecule's dipole. It is widely known that light experiences particle duality thus acting as both a particle and a wave. When the wavelength of light is much larger than that of the size of the molecule it is incident on, the light will cause the dipole to vibrate due to polarity.

While detecting Raman light, the Rayleigh photons must be filtered out. Due to their large number, these photons can easily flood the detector, giving too many photons at that energy. A small fraction of the light scattered by the molecules is scattered at a different energy than the incident photons, this is the Raman effect. It has been discovered that Raman spectroscopy offers great levels of selectivity; (Demirel, 2008) meaning that all molecules and organisms have unique Raman spectra. Each individual spectrum is due to the unique chemical makeup of corresponding molecules and organisms. In Raman spectra, peaks at certain wavelengths are seen and are attributed to different bonds in the mixture. These peaks can be increased if the vibrations of the bonds, and therefore the molecules, are constrained. This knowledge is utilized through surface enhanced Raman spectroscopy (SERS).

1.2.2 Surface Enhanced Raman

Surface enhanced Raman spectroscopy was discovered in 1977 independently by two groups, similar to the discovery of Raman scattering. Van Duyne and Jeanmaire explained the enhancement by an electromagnetic method, whereas Albrecht and Creighton described it as a chemical phenomenon. (Campion & Kambhampati, 1998)

Most of today's SERS substrates consist of some nanostructure with a precious metal layer, such as gold, silver or copper. The nanostructured layer is usually a rough surface to induce capturing of the analyte. The roughness usually accounts for what are called hot spots (see Figure 1.2.2-1).

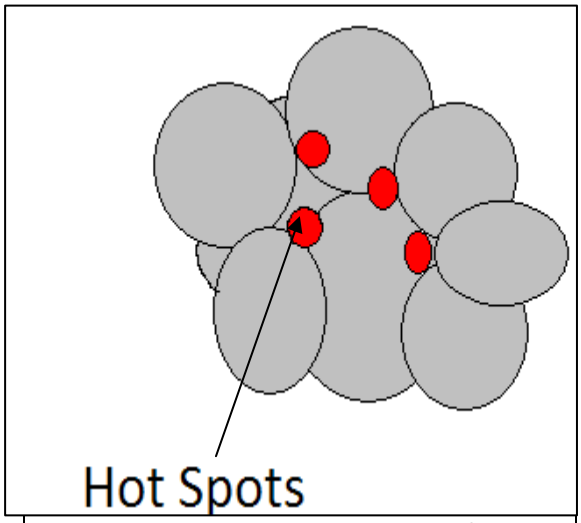

Figure 1.2.2-1: Visual Depiction of Hot Spots where red circles are analyte.

These hot spots are what give SERS its highest signal enhancement factor; some have been observed with a 10^{15} order of magnitude increase in signal. (M. Moskovits, 2002) With detection limits this low, it is theorized that single molecules can be detected.

In a paper by Netti and Stanford, they were able to detect trace amounts of cocaine in an aqueous solution. ((Netti & Stanford, 2006)) In the Figure 1.2.2-2, seen below, as

Netti and Stanford decrease the concentration from 30 ppm to 30 ppb it can be seen that the same peaks are still visible even at extremely low levels of concentration.

This finding applies greatly to the thesis at hand, as well as to detection applications critical to the Department of Defense. Imagine if a chemical or biological warfare agent could be detected at a single molecule level, or if the biomarker for some disease could be detected when it is initially produced. Although the mechanisms of SERS are not completely understood; there are two contributing theories towards this enhancement in signal, chemical and electromagnetic enhancement.

Chemical enhancement was proposed by Albrecht and Creighton in 1977. (Campion & Kambhampati, 1998) This method involves charge transfer from the chemisorbed species and the metal surface. When molecules are adsorbed to the surface, their electronic states can interact with the states in the metal and produce new transition energy levels. To this day, the true nature is of this mechanism is not fully understood, but contributes at least one order of magnitude to the increase in signal.

Electromagnetic enhancement was theorized by Jeanmarie and Van Duyne, also in 1977. (Campion & Kambhampati, 1998) These enhancements arise from the surface plasmons present. The plasmons are created when the incident photons excite the electron gas of the metal. Plasmons are waves that propagate along the surface, parallel to the metal interface. When the plasmons and the substrate are placed in close proximity to each other, an enhanced electromagnetic field is created that produces an enhanced scattered Raman field.

Upon its discovery in 1977, SERS was considered a break through, but the results received were unreliable. With accurate way of understanding the results, and no way to control the roughness of the substrate, SERS was all but forgotten for the better part of the last thirty years. Recently, with advances in nanotechnology, surface roughness can be controlled yielding better, more reliable results.

Two common methods for making these surface enhancements are either modifying the substrate with a metal film, usually gold or silver, or putting the analyte in a gold or silver colloidal solution. The surface modifications seem to provide more stable results, while the colloidal solutions have larger enhancement factors; however, the colloidal solutions yield very unreliable results. The colloidal solution provides the ability for the colloids to aggregate in a standing solution, therefore making particles of varying size inside the solution, all of which yield different results. This can be seen in Figure 1.2.2-3 (Culha, 2007) The unfavorable effects in colloidal solutions have recently been corrected by using microfluidic channels to keep the colloids moving in order to prevent them from aggregating. (Chen, 2007) With this unique application of microfluidics, SERS using colloids are more useful and can be more reliable.

1.2.3 Microfluidic Design in Raman Spectroscopy

As mentioned previously, particle size affects the signal received. Since quantitative analysis is dependent on repeatability and robustness, particle size must be controlled. In order to control particle size, fluid flow is used to keep the nanoparticles suspended in solution and to keep them from aggregating.

Lianming Tong and company from Sweden use optical methods to aggregate the nanoparticles and analyte to the surface. (Tong, 2009) With the use of optical tweezers they were able to successfully aggregate silver nanoparticles and Thiophenol (TP) and 2-naphthalenethiol (2-NT) in a microfluidic channel. The silver nanoparticles were used effectively as a Raman active substrate. Tong used two separate lasers, one for tweezing and one for Raman excitation. They see promising results but do mention that several other methods can be used to advance their product, such as silver functionalization or obstacles to break laminar flow and enhance mixing of analyte and silver particles.

There are two ways that microfluidics can be combined with Raman spectroscopy for diagnostics; either as a continuous monitoring technique or singular detection. In the continuous monitoring, the device can be integrated into the flow to a catheter bag for kidney function. The device can also be used to detect for disease as a one time reading.

Other groups, such as Popp and his associates are using more advanced microfluidic systems to detect lower quantities of analyte. (Strehle, 2007) They are using bubbles of oil to mix silver colloid, analyte in water. The bubbles are then analyzed with a Raman laser to receive a signal.

While this group developed very reproducible results, they also showed they receive different signals depending on concentration of analyte in solution as in the figure below.

1.2.4 Raman Quantitative Analysis

One current method for analyzing biofluid, particularly urine, is known as gel electrophoresis. (Patton, 2002) This method is time consuming but effective for doctors in analyzing samples. Other methods have been introduced, such as chemiluminescence-based staining for protein detection, but all current methods take time to prepare and analyze. With the Raman technology that this thesis is proposing, this time can be cut down to minutes, with little-to-no preparation. Due to the uniqueness of every biological entity, Raman spectra are highly specific to each substance. With this specificity, once spectra for a certain biomarker can be identified; the peak, or set of peaks, can easily be pointed out. However, these biomarkers are not always prevalent for every disease and additional quantitative analysis is needed.

The feasibility for the use of Raman spectroscopy for urine analysis has been pursued by several groups. Dou et al. (Dou, Yamaguchi, Yamamoto, Doi, & Ozaki, 1996) and

McMurdy et al. (McMurdy, 2003) studied quantitative Raman analysis in human urine. Premasiri et al. (Premasiri, 2005) used gold active colloids to analyze creatinine levels in urine. With all of these groups doing work in this area, and finding results, it is promising that a less expensive and simplistic method may be developed.

Creatinine levels in urine are important to discuss because the concentration can accurately predict renal function in a human. (McMillan, 2007) When the creatinine concentration is high, kidney failure can be imminent, so it is important for patients with high risk of kidney failure to monitor their creatinine excretion. Such patients include those with diabetes, cystic fibrosis, or people who have been subjected to large amounts of antibiotics. Current tests are extensive in the time and preparation they require.

The research herein focuses upon this need for a simple, easy to use method. Results have been obtained that showing potential for the use of a metalized parylene substrate in urine analysis for renal function. With the simplicity and the lower cost of creation of the nanostructured parylene, it may become a useful method in the future. In the following sections, it will be seen that our substrate is far more reliable with results that are easily quantitatively analyzed. The future goal of this project will be to yield a hand held device that can be used at the patient's bedside for quick diagnosis.

1.3—Design Needs

In order to perform surface enhanced Raman experiments to be used in bedside diagnostics successfully we were required to come up with a suitable substrate to be used that was both reliable and easily manufactured. Today, the major problem with many surface enhanced Raman substrates is that they do not supply very reliable or reproducible results. Those that are accurate are too expensive to produce and use commercially. As will be described later in the Methodology section, the method that was used for substrate preparation was simpler and less expensive than other methods currently used.

2—Methodology

2.1—Film Preparation

What follows is the description of the method used to prepare the substrate required for SERS. The basic setup is a helical nanostructured poly(p-xylylene) chloral (PPX-Cl) substrate of approximately 13 microns on which a 30 nanometer layer of silver is deposited on top of a PPX-Cl film, all grown on a silicon substrate.

2.1.1 Nanostructure PPX-Cl

Silicon wafers act as the substrate for the nanostructured PPX film. The silicon wafers were first washed in deionized water and dried under nitrogen flow then added to a one to one solution of HCl and methanol. After a waiting time of thirty minutes, the wafers were removed and sonicated in deionized water for ten minutes and dried under nitrogen flow. Before being sonicated again for ten minutes, the wafer was kept in concentrated sulfuric acid for thirty minutes. Thoroughly dried under nitrogen flow, the silicon wafers were immersed into toluene solution containing 1% allyltrimethoxysilane and 0.1% acetic acid at room temperature for self assembled monolayer (SAM) formation. These wafers were then removed after an hour, sonicated in anhydrous toluene for ten minutes and dried under nitrogen flow once again. After heated on a hot plate at 150 ^{o}C for four minutes to more tightly bind the SAMs, nanostructured PPX-Cl films were deposited on the wafers using 0.3 g of dichloro[2.2]paracyclophane. The vaporizer and pyrolysis chamber temperatures were held at 175 and 690 ^{o}C, respectively. The flux angle was maintained at ten degrees.

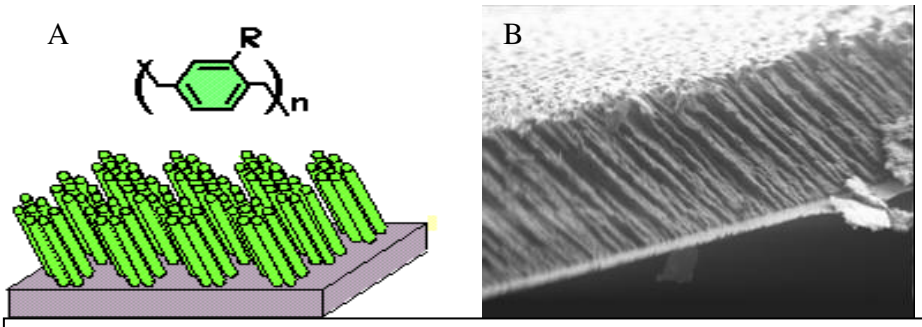

Figure 2.1.1-1: [A] Drawing of the nanostructure before silver deposition [B] SEM image of nanostructured PPX

2.1.2 Metal Deposition

After the PPX-Cl film templates were assembled, the silver was thermally deposited from resistively heated tungsten and tantalum boats onto the surface at about 1 x 10^{-8} Torr base pressure in a cryogenically pumped deposition chamber. A 300 Å layer of Ag was deposited.

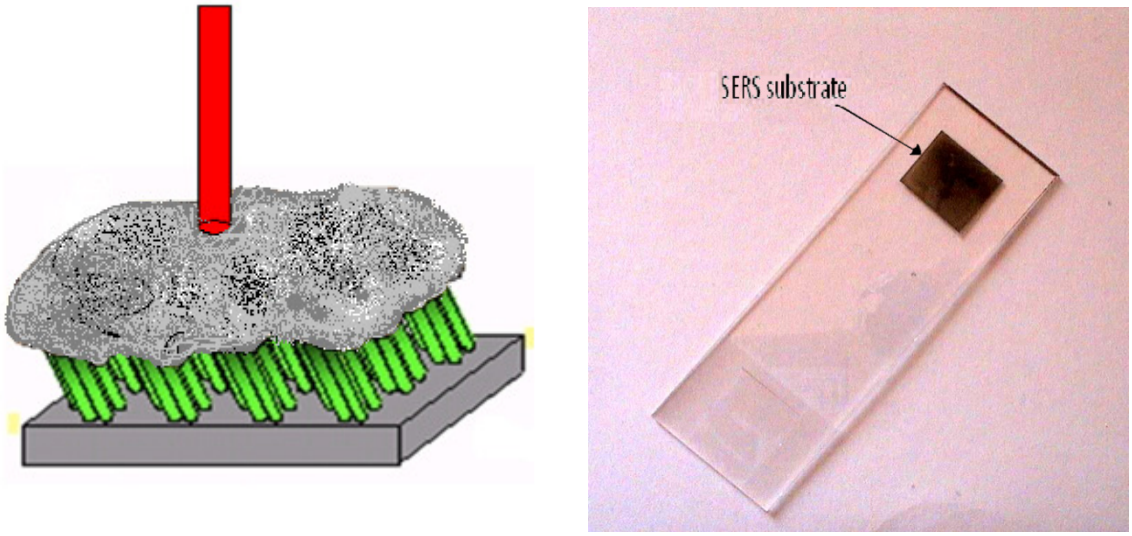

Figure 2.1.2-1: (Left) Visual depiction of parylene surface with silver layer deposited on its surface. (Right) Example of substrate used for analysis.

2.1.3 Channel Preperation

Cut glass micro slides purchased from VWR into three equal parts. Clean individual pieces with ethanol. Dynamically spin coat the Shipley 1805 photoresist before baking on hot plate at 140 ^{o}C for 1 minute. Place in position about two inches underneath UV lamp (UVP B-100A) and place shadowmask directly on top of photoresist layer. Expose for 1 minute and 25 seconds. Dip in 0.1 M solution of NaOH and move back and forth, up and down for about 10-15 seconds, clean in water. Post bake for around 1 minute or more. Etch in BHF for required depth (1 um/minute). Clean with acetone, then with distilled water, dry under N_2 flow before sonicating in ethanol. If substrate still appears dirty, a post process clean with piranha solution must be done.

2.2—Measurement

Prior to any Raman measurement, the substrates were treated by a UV-Ozone cleaner for around two minutes and a reading was taken to assure no contamination. Once a clean substrate was assured, five microliters of urine were added to the substrate and dried naturally. The urine was not previously treated or diluted. A Renishaw inVia microRaman instrument was used for studying the substrate. The Renishaw consisted of a 35mW HeNe laser (632.8 nm) as the light source, a motorized microscope stage sample holder, and a CCD detector. Spectrum was collected between five and ten percent laser power. The parameters of the instrument were a 50x objective and ten second acquisition time. To obtain high signal to noise ratios, up to sixteen scans were performed.

2.3—Sample Collection

Patient urine samples were collected from the Nephrology Department of The Pennsylvania State University Medical School. The artificial urine was prepared based on 36.4 g of urea, 15.0 g or sodium chloride, 9.0 g of potassium chloride, and 9.6 g of sodium phosphate that were dissolved into 1.5 L of distilled water.

3—Results

3.1—Data

To check for contaminated samples, the substrates were analyzed without urine first to check for unusual peaks. An example of a contaminated spectrum can be seen in Figure 3.1-1.

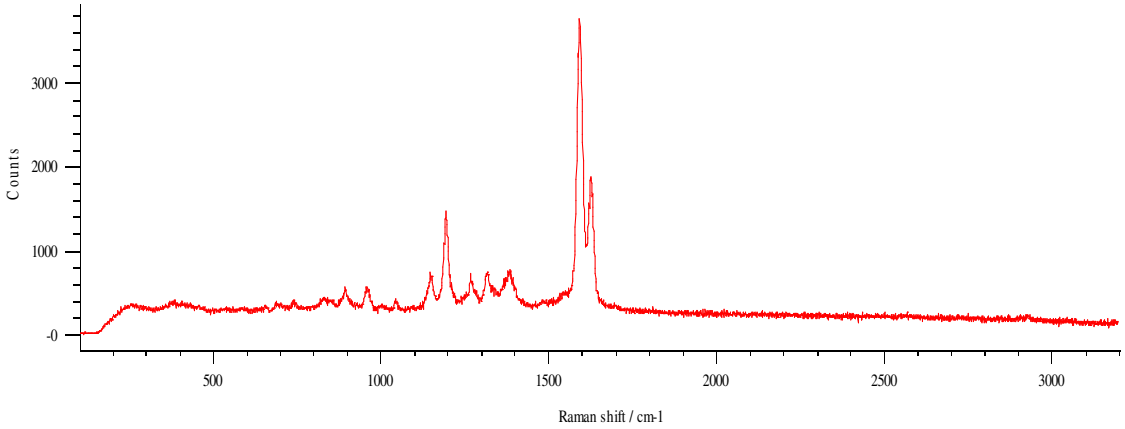

Figure 3.1-1: Image of a contaminated substrate

The substrate is tested once again, after treatment with ozone, to see if the contamination remains. In Figure 3.1-2, the maximum number of counts observed is 110, which is 3% of what was seen in the contaminated sample. So, the sample is considered noncontaminated and viable for testing.

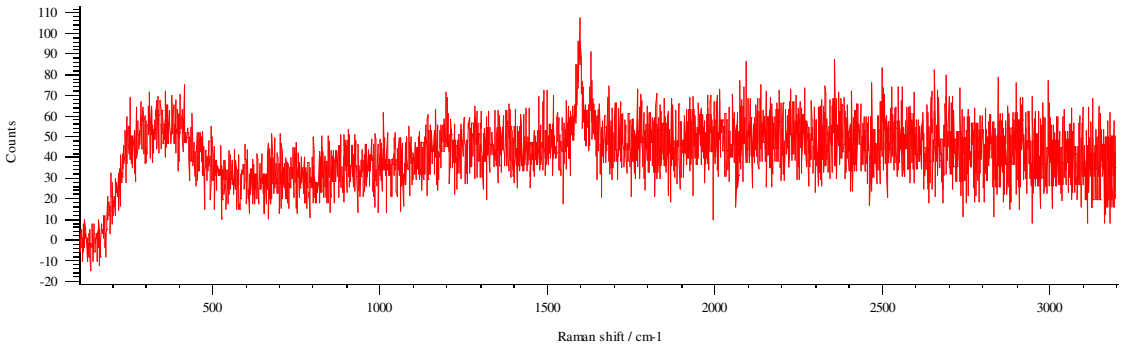

Figure 3.1-2: Image of a noncontaminated substrate after treatment with ozone

Prior to analysis of the actual urine samples, artificial urine was created and analyzed to determine what peaks were distinctive to each substance. The spectra can be seen in Figure 3.1-3. The graphs are, from top to bottom, creatinine, artificial urine, and urine from a diabetic patient.

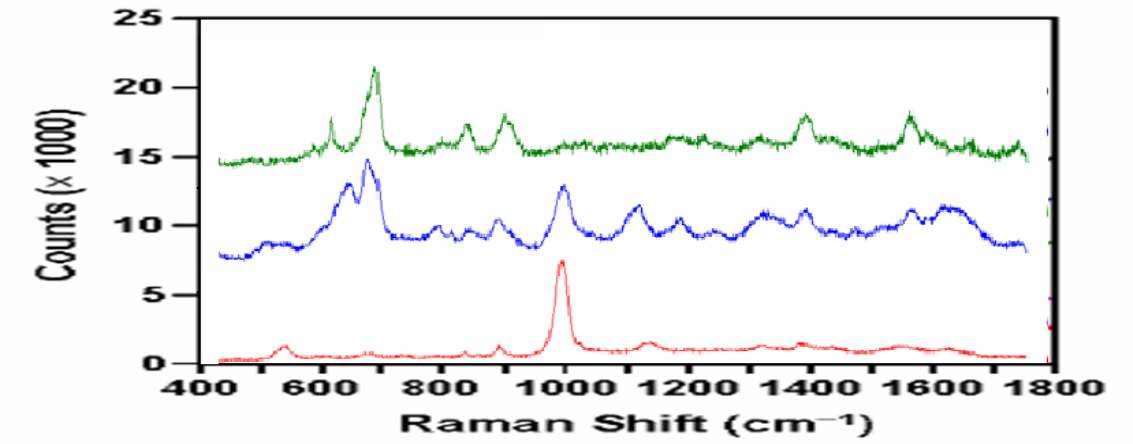

Figure 3.1-3: Spectra, from top to bottom, creatinine, artificial urine and urine from a diabetic patient. (Spectra shifted for visibility) (Wang, 2010)

Once the substrate is cleaned, urine is deposited and dried on the film. The sample is then placed in the Renishaw Raman microscope to obtain a sample for analysis. A spot

on the substrate is selected and a scan is taken for approximately ten seconds. A result

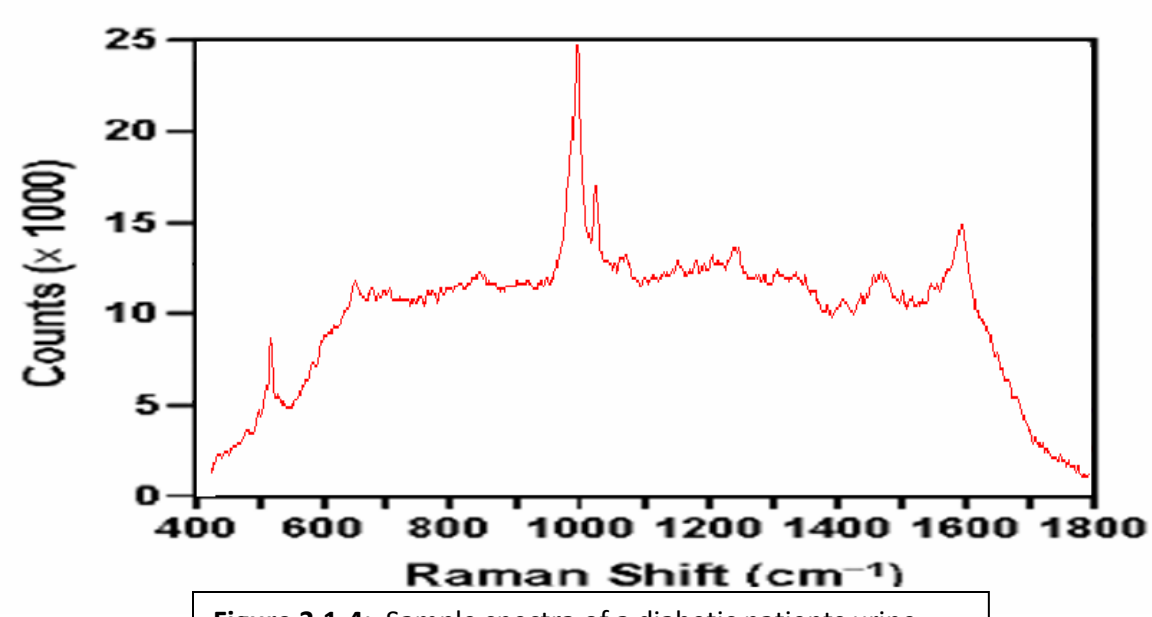

Figure 3.1-4: Sample spectra of a diabetic patients urine

similar to Figure 3.1-4 is collected.

This spectrum is then taken and analyzed through the WiRE program selecting a best fit analysis to obtain peak heights.

3.2—Analysis

Upon collection of several patients' data, the samples were analyzed in the WiRE program, thereby obtaining peak heights and ratios. After a baseline is selected, this is used as a reference for the peak heights. Figure 3.2-1 shows the example of a screen hot after the curve fit was performed.

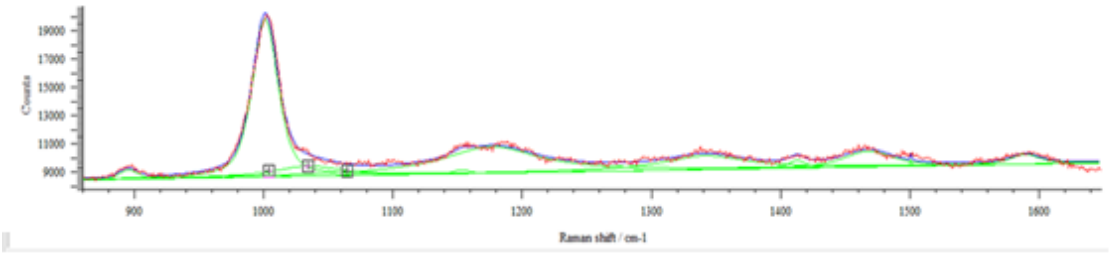

Figure 3.2-1: Spectrum after curve fit.

Heights and areas relative to the baseline are given in a table for each peak selected by the user. These heights and areas are copied into excel and ratios are taken between the albumin and creatinine data. Using these ratios, and using the silicon reading as a reference peak for each substrate, it is possible to get reliable data.

Additionally we experimented to determine if different creatinine concentrations could be detected by the Raman analysis. To do this, artificial creatinine was added, in different amounts, to an artificial urine sample to test for a change in peaks. It was observed that the peaks of creatinine at 820 cm^{-1} and 900 cm^{-1} became bigger in both peak height and area. This can be seen in Figure 3.2-2. The samples had known creatinine concentrations of 0.5 µg/ml, 5.1 µg/ml and 10.2 µg/ml.

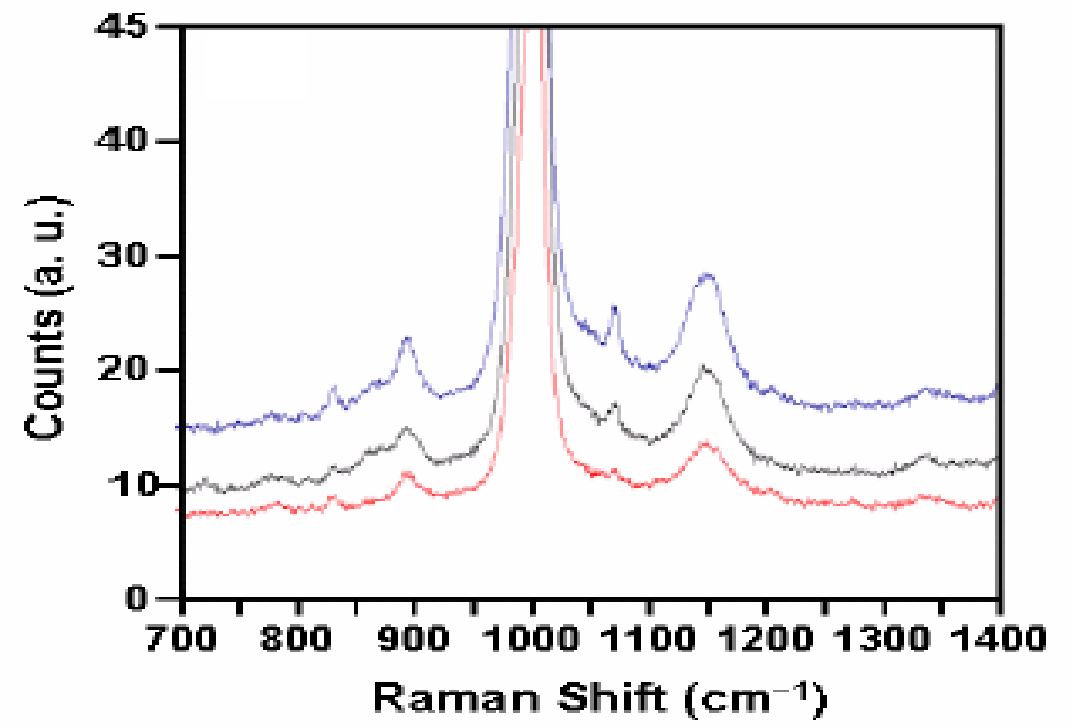

Figure 3.2-2: Blue line shows creatinine concentration of 10.2 µg/ml, black is 5.1 µg/ml and the red is 0.5 µg/ml. (Wang, 2010)

A common problem in analyzing urine is that salt content can affect the results thereby yielding false data. In order to check the substrate against this influence, additional KCl was added to the solution to observe the affect the salt had. The graph can be seen in Figure 3.2-2 below. Salt content added, from top to bottom is: 0, 0.5, 5.0, 50.0, 500.0 mM. It is seen that the salt has no effect on the results meaning that this method is feasible for all urine salt concentrations.

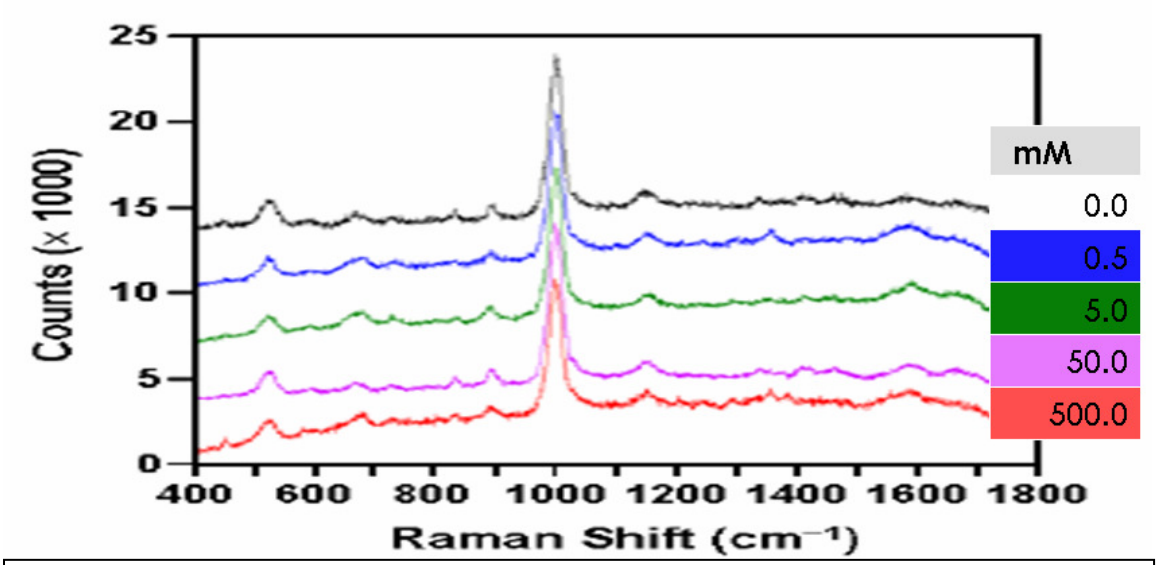

Figure 3.2-3: This graph shows salt content fluctuating from 0-500 mM from top to bottom. (Spectra is shifted for visibility) (Wang, 2010)

Due to the robustness of our substrate, we are able to get reproducible results which are not typically seen using surface enhance Raman of metalized structures. Figure 3.2-2 shows an example of one patient's urine taken at twelve separate locations on the substrate. Most substrates exhibit hot spots at random locations which would cause much higher enhancements in certain spots, but with the substrate used in this experiment, we see results are within 5% of each other.

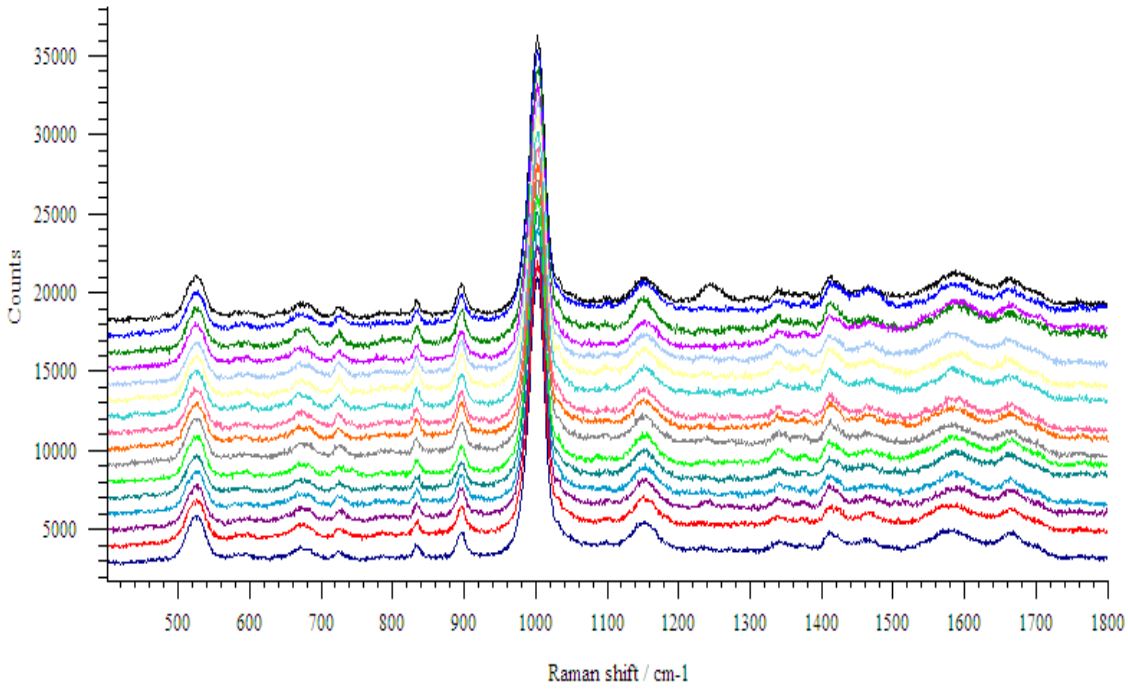

Figure 3.2-4: This graph shows 16 spectra from different spots on the surface. This proves that the substrate has uniform hot spot formation. Urea peak at 1000 cm^{-1} experiences variance of less than 5%.

Even though these results are repeatable and analysis can be performed on the peaks, the peaks themselves are not helpful. Some sort of quantitative analysis must be performed to analyze kidney function. In order to do this, the creatinine levels of the patients were analyzed by both SERS and a chemical assay method.

After the data from both procedures was collected, linear predictive models were produced using the SERS data to predict the concentration of creatinine. The linear relationship between concentrations and peak areas were found to have a percentage of variance, R^2, of 0.943 and 0.780 for the 840 cm^{-1} and 900 cm^{-1} peak areas respectively. These results are seen in Figure 3.2-3a. Figure 3.2-3b shows the value predicted by SERS

and the actual concentration, with a correlation value of 0.971 and 0.883 for the 900 cm^{-1} and 830 cm^{-1} peaks, respectively.

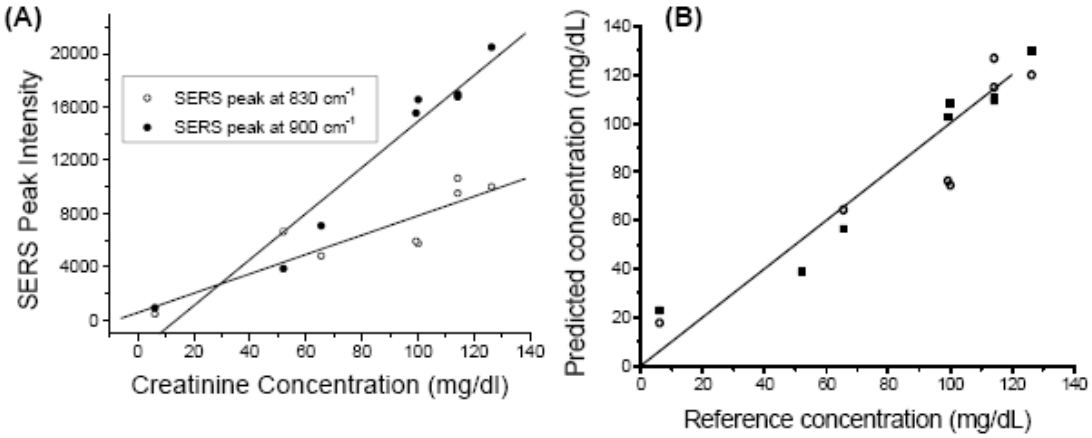

Figure 3.2-5: (A) Linear fit of SERS Peak Intensity versus Creatinine Concentration (mg/dl). (B) Predicted concentration versus Reference concentration to check validity of results. (Wang, 2010)

If Table 3.1 seen below, the patients and healthy controls used are outlined along with the creatinine concentration in mg/dL, scan times (10 s/scan) and laser power (%).

Patient ID	Standard creatinine concentration (mg/dL)	Scan times (10 s/scan)	laser power (%)
CR24 (M, Diabetes type I)	126.3	8	5
HD46 (F, Diabetes type II)	100.1	8	5
HK17 (F, Diabetes type II)	65.5	1	10
MC42 (F, Diabetes type II)	52.0	16	5
SR45 (M, Diabetes type II)	114.2	4	10
SV29 (F, Diabetes type I)	114.2	1	10
TJ008 (M, Diabetes type II)	6.1	1	5
RN37 (F, Diabetes type I)	54.7	1	10
GC006 (F, Diabetes type II)	73.0	1	10
ML36 (M, Diabetes type II)	158.1	1	10
MB38 (F, Diabetes type II)	41.8	16	5
MR53 (healthy control)	173.0	1	10
AS19 (healthy control)	99.3	1	5

Table 1.1 – Table of patients and healthy controls used as the sample.

3.3—Interpretation and Impact

With the values of R^2 being so close to one, it is seen that a correlation can be made between the actual values and the SERS values. Being able to quantitatively analyze urine strengthens the impact of the results. Instead of just looking for a biomarker in the urine, concentrations of the molecules in the urine can be inspected; therefore providing doctors with the ability to test for a broader scope of diseases. Advances like these allow for doctors to make quicker decisions at the bedside, forgoing extensive lab work.

Impacting a broader scope of diseases allows for doctors to use this method, not only in the advanced medical school and hospitals in the United States, but also out into the field to more quickly and less expensively diagnose a patient who may not have access to typical medical facilities. Third world countries and underserved areas would benefit greatly from such medical advancements.

4—Conclusions

4.1—Summary

Through this research we were able to design a robust and simple substrate that can help quantitatively analyze urine to determine the function of the kidney, with relative accuracy and reliability. Due to the repeatability seen on our substrate, no matter where the substance is analyzed on the surface, it can be assured that all spots will yield similar results. This repeatability deems the substrate reliable enough for diagnostic testing. Through these findings, it follows that the substrate can be used to quantitatively analyze other substances for the diagnosis of other diseases, in addition to determining the health of renal function. Also, because of the nature of surface enhanced Raman spectroscopy, distinct biomarkers can be recognized in the spectra at lower concentrations allowing for quicker diagnostics.

A highly accurate, real time sensor for biomarker detection is required for early detection of disease. We report the preparation of a new type of SERS substrate which provides fast (<10s), highly sensitive (creatinine concentration < 0.5 μg/mL) and reproducible (<5% variation) detection of urine. The linear relationship between concentrations and peak areas were found to have a percentage of variance, R^2, of 0.943 and 0.780 for the 840 cm^{-1} and 900 cm^{-1} peak areas respectively.

4.2—Important Findings

With the findings that our results can be quantitatively analyzed, there is the potential for diseases to be detected with bedside diagnostics. Although this thesis primarily characterizes kidney function through urine analysis, this can be directly correlated to future wide spread disease detection.

4.3—Future Work

The future of this project entails a portable, handheld Raman machine to be used in bedside diagnosis. According to the FDA, a conventional laser pointer is around 5mW with a range of wavelengths from 400-710 nm; both are within the needs of this project. (Important Information for Laser Pointer Manufacturers, January) This does not mean that all that is needed is a simple laser pointer for this device, but provides insight into the approximate size of the laser than can be used. The ability to have a small handheld device, like that shown in Figure 4.3-1, which utilizes the substrates created through this research, provides for quicker detection in both hospital and underserved settings.

Figure 4.3-1: Handheld Raman Diagnostic Device courtesy Nanodevices, Inc.

Bibliography

Belyanin, V. B. (1979). History of the Discovery of the Phenomenon of Raman Light Scattering. *Plenum Publishing Corporation* , 510-515.

Campion, A., & Kambhampati, P. (1998). Surface-enhanced Raman scattering. *Chemical Society Reviews* , 241-250.

Chen, L. (2007). Recent Advances in surface-enhanced Raman scattering detection technology for microfluidic chips. *Electrophoresis* , 1815-1828.

Demirel, M. C. (2008). Bio-organism Sensing via Surface Enhanced Raman Spectroscopy on Controlled Metal/Polymer Nanostructured Substrates. *Journal of Biointerphases* .

Dou, X., Yamaguchi, Y., Yamamoto, H., Doi, S., & Ozaki, Y. (1996). Quantitative analysis of metabolites in urine using a highly precise, compact near-inrared Raman spectrometer. *Vibrational Spectroscopy* , 83-89.

McMillan, J. I. (2007, December). *Acute Renal Failure*. Retrieved April 2009, from MERCK: http://www.merck.com/mmpe/sec17/ch233/ch233b.html

McMurdy, J. (2003). Raman spectroscopy-based creatinine measurement in urine samples from a multipatient population. *Applied Spectroscopy* , 522-525.

Moskovits, M., Tay, L. L., Yang, J., & Haslett, T. (2002). SERS and Single Molecule. In *Optical Properties of Nanostructured Random Media* (p. 215). Springer Berlin / Heidelberg.

Mustafa, C. (2007). Experimental parameters influencing surface-enhanced. *Journal of Biomedical Optics* , 054015-1 - 054015-6.

Netti, C., & Stanford, H. (2006, June 1). *Applications of Reproducible SERS Substrates for Trace Level Detection*. Retrieved November 2008, from Spectroscopy Online: http://spectroscopyonline.findanalytichem.com/spectroscopy/article/articleDetail.jsp?id=368979

Patton, W. F. (2002). Detection technologies in proteome analysis. *Journal of Chromotography B.* , 3-31.

Premasiri, W. (2005). Characterization of the Surface Enhanced Raman Scattering (SERS) of bacteria. *Journal of Physical Chemistry B* , 312-320.

Strehle, Katrin R. (2007). A Reproducible Surface-Enhanced Raman Spectroscopy Approach. Online SERS Measurements in a Segmented Microfluidic System. *Analytical Chemistry* .

Surface Enhanced Raman Spectroscopy. (1995). Retrieved November 2008, from University of Exeter: School of Physics: http://newton.ex.ac.uk/research/biomedical/optics/sers.html

Tong, Lianming. (2009). Optical aggregation of metal nanoparticles in a microfluidic channel. *The Royal Society of Chemistry - Lab on a Chip* .

Wang, H., Malvadkar, N., Koytek, S., Bylander, J., Reeves, W.B., Demirel, Melik C. (2010). Quantitative analysis of creatinine in urine by metalized nanostructured parylene. *Journal of Biomedical Optics*. 15, 027004

Young, A. T. (1981). Rayleigh Scattering. *Applied Optics* , 533-535.