2013

Development of surface-enhanced Raman based sensors

Elizabeth C. (Elizabeth Cecilia) Wellner
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Development of Surface-Enhanced Raman Based Sensors

By

Elizabeth Cecilia Wellner

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Steven R. Emory

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Dr. David Patrick
Master’s Thesis

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Elizabeth Cecilia Wellner

November 15, 2013
Surface Enhanced Raman Based Sensors

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Elizabeth Cecilia Wellner
November 2013
Abstract

While the medical field incorporates various imaging methods for diagnosis and treatment, there is a need for continual development of real-time in vivo imaging techniques in this field. A surface-enhanced Raman scattering (SERS) based fiber optic sensor is reported. An optical fiber was coated in gold nanoparticles, capable of Raman scattering. SERS active molecules were adsorbed onto the gold nanoparticle film, and SERS spectra obtained. Using 4-mercaptopyridine as a SERS reporter molecule, the chemical environment surrounding the probe was determined by the analysis of the pH sensitive SERS spectra. The methods of preparation and characterization of the SERS-based fiber sensors are presented, as well as an analysis of the optimal geometry for the tip of the probe.
Acknowledgments

Special thanks is extended to Dr. Steven Emory, who has guided and encouraged me both in the classroom and laboratory, and who has continued to challenge me to broaden my capacities as a scientist.

I also thank Dr. David Rider and Dr. David Patrick for their assistance in reviewing this work as part of my graduate committee.

Thanks is also extended to Dr. Mark Peyron, whose mentorship was key in my decision to become a chemist.

In addition, I thank the past and present members of Dr. Emory’s research group who have worked on this or related projects, most notably Hannah Sturtevant and Alicia Mangubat, also Nicole Koeppen, Noah Schorr, Anzhela Storozhenko, Ryan Sumner, and Polly Wiltz.

I would be negligent not to especially thank my husband and son, Jeremy and Aleksandr Wellner, respectively. Their patience and assistance have provided the time and focus that I needed to complete this project.
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Chapter 1: Introduction

1.1 Purpose

Numerous strides have been made in cancer research in recent decades, but the disease remains a serious concern around the world. While certain types of cancers are decreasing in rates of occurrence, others continue to increase, such as breast and prostate cancer.\(^1\) Thus, people still have a very valid fear of cancer and the scientific community must persevere in its efforts to find methods to prevent, diagnose, and cure it. As current progress has shown us there appears to be no one single solution that cures all kinds of cancers.

Typically cancers are treated via radiation, chemotherapy, surgery, and cell transplantation, such as a bone marrow transplant. Depending on the type and stage of the cancer, different treatments are indicated for the individual patient. For example, hypoxia renders cancer tumors difficult to remove via radiation or chemotherapy.\(^2\) Radiation works by breaking the double strands of DNA in the tumor. Breaking the bond produces a radical on the DNA strand. The radical then undergoes oxidation, which renders the breakage permanent, destroying the cancer cell. Thus, without the oxygen present, the process is interrupted and cancer cell apoptosis often will not proceed.\(^3\) At present there is no \textit{in vivo} method that can be used in real-time to analyze an individual tumor to determine whether or not hypoxia is present in an individual cancer tumor. Doctors must
often resort to invasive procedures, such as biopsies, to do so. This can cause extra complications and strain on the patient, who now must heal from a surgical procedure that can lead to infection in addition to waiting for the biopsy results.

1.2 Current Methods of Bioimaging

At present fluorescence spectroscopy is often used to examine a microenvironment in vivo. With fluorescence spectroscopy, a fluorophore that is designed to interact with a particular species that is under observation enters the body and is monitored. It can be excited using a given light frequency, and will relax emitting a different frequency of light. This change can be measured and can provide information concerning the environment. Organic fluorescence biomarkers include proteins and small dyes, while fluorescent quantum dots are a common inorganic semiconductor that can be used.\(^4\)

Several problems limit use of fluorophores when performing in vivo imaging. Fluorophores are limited in the chemical information that they can yield. Often they can show a site where activity is occurring, but cannot provide useful information on, for example, pH levels and what elements are involved in a particular reaction in the cell. Several molecules to which the fluorophores bind are themselves fluorescent, and their emission interferes with monitoring the desired fluorophore. Light often photo-bleaches the fluorophore, rendering it useless after a period of time, as it breaks down and no longer emits radiation of the frequency being monitored.\(^5\) In addition, the area under examination must be close to the surface, sometimes even exposed by surgery, because the
excitation radiation often cannot deeply penetrate the system. Deep tissue analysis in the body is difficult with fluorescence spectroscopy.\textsuperscript{6,7}

Quantum dots are less prone to photo-bleaching. However, there are presently limitations with them as well. While quantum dots themselves are small, on the scale of 2-10 nm,\textsuperscript{8} they have a tendency to aggregate, yielding them more difficult for use \textit{in vivo}.\textsuperscript{4} Quantum dots also contain metals toxic to the biological system, such as cadmium, arsenic, lead, and selenium.\textsuperscript{4,8}

This research project focuses on the development of an optical fiber probe that can be used via Raman spectroscopic techniques for \textit{in vivo} monitoring that will be pH-sensitive and thus able to examine chemical changes occurring in microenvironments.

1.3 Background

Sir C.V. Raman discovered Raman scattering in 1928 and in 1930 won the Nobel Prize for this discovery. His experiment was a simple one, but its implications have proven useful. First he focused sunlight using telescope lenses and used two filters, blue-violet and yellow-green, to stop this light from reaching a sample. No light was detected on the other side of the sample, since none could reach it. Next he kept the blue-violet filter in place and moved the yellow-green filter to the other side of the sample.\textsuperscript{9} This allowed blue-violet light to reach the sample. Had blue-violet light been elastically scattered by the sample, it would have been observed on the other side, and the yellow-green filter would have blocked it. However, yellow-green light passed through the yellow-green
filter, meaning that while blue-violet light was absorbed by the sample, yellow-green light was scattered by it. Thus, the frequency and wavelength of the scattered light differed from the incident light, and the scattering must be inelastic.

1.4 Raman Theory

When incident light of frequency $\nu_{ex}$ is shone, and $E_m$ is the amplitude of the light wave, the electric field of the light is\(^\text{10}\)

$$E = E_m \cos(2\pi \nu_{ex} t) \quad (1)$$

The photon strikes the sample, where its electric field interacts with the electron field of the molecule, exciting it. This induces a dipole moment, $\mu_{in}$, in the polarizable substance:\(^\text{10}\)

$$\mu_{in} = \alpha E = \alpha E_m \cos(2\pi \nu_{ex} t) \quad (2)$$

In (2), $\alpha$ is the polarizability of the molecule, that is, how much the electron cloud surrounding the molecule can be disturbed upon excitation. The units of $\alpha$ are C$^2$m$^2$/J, while the units of $\mu_{in}$ are Cm.\(^\text{10}\) As will be discussed later, note that the induced dipole moment is directly proportional to both the polarizability of the molecule and the electric field. It is these variables that can be manipulated in order to enhance the Raman signal provided by a given molecule.

The polarizability changes according to internuclear separation distance:

$$\alpha = \alpha_0 + (r - r_e) \left( \frac{\partial \alpha}{\partial r} \right)_e + \cdots \quad (3)$$

In (3) $r_e$ is the bond distance between the atoms involved in the transition at equilibrium, and $\alpha_0$ is the polarizability at that distance. The variable 'r' is the
internuclear separation distance in question. To find the change in internuclear separation, equation (4) is needed:

$$r - r_e = r_m \cos(2\pi v \nu_v t)$$

Here $r_m$ represents the greatest internuclear separation distance compared to the separation distance of the involved atoms when at equilibrium and $v_v$ is the vibrational frequency.

Substitution of (4) into (3), followed by the result of this being substituted into (2) leads to another expression for the induced dipole moment, $\mu_{in}$:

$$\mu_{in} = \alpha_0 E_m \cos(2\pi \nu_v t) + E_m r_m \left( \frac{\partial \alpha}{\partial r} \right)_c \cos(2\pi \nu_v t) \cos(2\pi \nu_v t)$$

(5)

Applying the identity for the product of two cosines, this simplifies to

$$\mu_{in} = \alpha_0 E_m \cos(2\pi \nu_v t) + \frac{E_m}{2} r_m \left( \frac{\partial \alpha}{\partial r} \right)_c \cos(2\pi (\nu_v + \nu_v) t$$

$$+ \frac{E_m}{2} r_m \left( \frac{\partial \alpha}{\partial r} \right)_c \cos(2\pi (\nu_v - \nu_v) t)$$

(6)

The expression contains three terms. The first term is that for Rayleigh scattering, which is inelastic. The molecule excites upon interaction with the incident photon, then relaxes back to the state in which it originated when it scatters light. Therefore, there is no overall change in where it begins and ends the transition. The second term is that of anti-Stokes scattering, where the light scattered is of a higher frequency than the incident light. The third term is Stokes scattering, where the scattered light is of a longer wavelength, lower frequency, than the incident light.
1.5 Raman Scattering

There are two types of scattering that a molecule can undergo: 1) Rayleigh scattering and 2) Raman scattering. Elastic scattering of light is referred to as Rayleigh scattering. This occurs when a particle absorbs a photon of a certain wavelength, is excited to a higher energy state, then emits a photon of the same wavelength as that absorbed as it relaxes back to its original state. Rayleigh is the more common type of scattering since the molecule is absorbing and emitting a photon of the same energy.

The less common type, Raman, is an inelastic type of scattering, and can occur by either of two methods, Stokes or anti-Stokes. The Stokes shift occurs when a molecule in the ground state is excited to a virtual state within the ground electronic state and emits a photon of less energy (longer wavelength) when it relaxes. In this case the particle relaxes to a state higher in energy than its original state. Anti-Stokes emission is the opposite. The particle is originally in an excited vibrational level within the ground electronic state. Upon absorption of a photon the particle is excited, but it relaxes to an energy state lower than its original state. In doing so it emits a photon of higher energy than that absorbed.

Since most molecules are in the ground state, the Stokes shift is more probable than the anti-Stokes. As the temperature is increased, more molecules achieve higher energy, so increased temperature increases the probability of anti-Stokes emission.
Incident radiation (green) strikes the molecule, resulting in one of three effects. In the Stokes effect the particle is excited from the ground electronic state to a virtual higher energy state, and then relaxes back to a higher energy level within the ground electronic state (red). With the anti-Stokes effect the particle is excited from the ground electronic state to a virtual higher energy state, then relaxes back to a lower energy level within the ground electronic state (blue). In Rayleigh scattering the particle relaxes to the same energy level as that in which it began, scattering radiation of the same energy as that which was absorbed.
Raman scattering is about fourteen orders of magnitude less intense than fluorescence.\(^{11}\) As such, its analyzable utility was severely restricted until the invention of the laser. The light emitted from a laser source is intense and monochromatic, significantly increasing the yield of Raman scattered photons.

### 1.6 Polarizability

Molecules that have Raman-active vibration modes must experience a chance in polarizability during a vibration \(\frac{\partial \alpha}{\partial r}\) in equation 2, that is, the electron density in the molecule must distort from its normal shape (inducing a dipole). Molecules with symmetrical bends and stretches, therefore, are generally better Raman scatterers. The more polarizable a given molecule is, the better a Raman scatterer it will generally be.

Infrared (IR) spectroscopy is complementary to Raman. It is sensitive to vibration modes in which the permanent dipole changes \(\frac{\partial \mu}{\partial r} \neq 0\). To be IR-active, an overall vector change must result from the bend or stretch, which requires a change in dipole moment. Symmetric molecules do not have strong dipoles. Therefore highly Raman-active modes will be only weakly IR-active, and vice versa.
1.7 Surface-Enhanced Raman Scattering (SERS) Spectroscopy

Surface-enhanced Raman scattering (SERS) spectroscopy was first reported by Fleischmann in 1974, when he obtained spectra from pyridine on silver coated electrodes.\(^{12}\) This phenomenon allows the Raman signal to be amplified as much as fifteen orders of magnitude. Two sources are thought to contribute to the enhancement, electrochemical enhancement (EME) and chemical enhancement (CE). EME concerns the roughened metal surfaces necessary for SERS. The surface plasmon (valence electrons bound to a metal atom loosely such that they have some free movement) of the roughened metal generates an evanescent field when struck by photons. This field interacts with and enhances the electromagnetic field (E in equation 2) of the adsorbate molecules, resulting in a magnified Raman spectrum of the adsorbate molecules. CE utilizes a charge transfer between the adsorbate molecule and the metal surface to enhance the Raman spectrum. The transfer changes the oxidation state of the adsorbate molecule, which augments the polarizability of the molecule, and therefore the Raman signal obtained from it.\(^{13}\)
Incident light strikes the surface, causing localized surface plasmon to be excited, enhancing the electric field. The enhancement is greatest when the plasmon frequency is in resonance with the radiation frequency.

For EME to occur, the reporter molecules must be close to the surface of the metal, within the evanescent field. CE is only possible if the molecule will actually adsorb to the metal surface. This requires a negative charge or lone pair of electrons on the molecule to provide attraction to the metal. Thus, some molecules that are Raman-active are not SERS-active. For example, benzene is a highly symmetrical molecule and is Raman-active. However it cannot strongly adsorb to a metal surface, so it is not highly SERS-active.

Pyridine provides an excellent example of a molecule that is both Raman active and SERS active. While it is a symmetrical molecule, it also contains a lone pair of electrons, via the nitrogen atom, that allow it to adsorb to a metal
Several factors contribute to the SERS phenomenon. First, the metal particles must be significantly smaller than the wavelength of the incident light. When the metal particles are substantially small, the surface plasmon of the metal oscillates with the same frequency as the incident light ($\nu_{ex}$). Then the induced dipole will be greatest and the Raman scattered light will significantly increase. Under these conditions the electric field component of light is concentrated on a very small space "just outside" the metal nanoparticle (where the surface plasmon exists). Increased local electric field yields a larger SERS signal. Since the surface plasmon field resonance is specific to each metal, metals with surface plasmon that oscillates with a frequency similar to that of the incident light are most useful for SERS.\textsuperscript{14} These include alkali metals, such as lithium, sodium, potassium, and the noble metals copper, silver, and gold.\textsuperscript{14} Gold is used here because it is already approved for biological applications.
Figure 1.3. Classical Raman Pyridine Spectrum.
Spectrum is normalized and was obtained using $\nu_{ex} = 633$ nm.

Figure 1.4. Surface Enhanced Raman Pyridine Spectra.
These spectra are normalized and were obtained using $\nu_{ex} = 633$ nm. The green spectrum is from Figure 1.3; the others are SERS spectra of 50, 100, and 150 mM pyridine in 45 nm gold colloid.
The intensity of the spectra was normalized by dividing the intensity of each spectrum by the integration time and concentration (M) of each sample. It is apparent that the spectra of pyridine adsorbed to gold nanoparticles are enhanced compared to the classical Raman spectrum of pyridine. To determine the enhancement factors of each SERS-active sample, the normalized intensity of the SERS spectra were divided by the normalized intensity of the classical Raman spectrum.

**Table 1.1. Enhancement Factors for Pyridine in Gold.**

<table>
<thead>
<tr>
<th>Pyridine Frequency (cm⁻¹)</th>
<th>Enhancement Factor</th>
<th>Pyridine Frequency (cm⁻¹)</th>
<th>Enhancement Factor</th>
<th>Pyridine Frequency (cm⁻¹)</th>
<th>Enhancement Factor</th>
<th>Pyridine Frequency (cm⁻¹)</th>
<th>Enhancement Factor</th>
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<tr>
<td>995</td>
<td>87</td>
<td>1010</td>
<td>49</td>
<td>1008</td>
<td>30</td>
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<td>47</td>
<td>1032</td>
<td>28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

While the Raman signal is certainly enhanced in all solutions of gold colloid and pyridine, the enhancement factors are greatest in the 50 mM pyridine solution. There are a limited number of active sites available on the metal surface, thus, as the concentration of pyridine increases, the active sites on the metal are saturated and excess pyridine remains free in the solution. Therefore, per the number of moles of pyridine in solution, fewer are resonance enhanced, and the enhancement factors will decrease as concentration increases.

### 1.8 Surface-Enhanced Resonance Raman Scattering (SERRS)

Surface-enhanced resonance Raman scattering (SERRS) arises when the molecule adsorbed to the metal surface has an electronic resonance (i.e. absorbance) at the excitation frequency. When the particle is excited at the
resonant frequency it does not excite to a virtual state of higher energy, but to a quantized energy state in the first (or higher) electronic state (Figure 1.5). Since this is a more stable state for the molecule, the transition occurs more frequently, leading to more Raman scattering and larger enhancement factors.

Unfortunately, fluorescence contributes to a large background. In fluorescence the molecule also excites to the first excited electronic state. Whereas Raman scattering involves relaxation on the order of picoseconds, with the molecule relaxing directly from the excited to the ground state, fluorescence takes longer (on the order of nanoseconds, where Raman scattering is on the order of picoseconds), relaxing to the lowest energy level within the excited electronic state via non-radiative energy loss, then relaxing back to the ground state, releasing a photon (Figure 1.5).

Because fluorescence is about fourteen orders of magnitude stronger than Raman scattering, this fluorescent background could overpower SERRS. However, metals have many available energy levels. When an excited molecule relaxes to the lowest level of the excited state and would otherwise fluoresce, it can undergo internal conversion and relax non-radiatively through the metal’s energy levels, thereby quenching the fluorescence.
Figure 1.5. Raman and Fluorescence.
A) Non-resonant Raman scattering occurs when the particle is excited to a virtual state and then relaxes back to the ground electronic state. B) Resonant Raman occurs when the excitation frequency allows the particle to excite to the first electronic excited state from which it directly relaxes back to the ground electronic state. C) In fluorescence the particle excites as it does in resonant Raman, then relaxes via non-radiative methods to the lowest level of the excited state. From there it relaxes back to the ground electronic state. In SERRS the metal provides several additional energy levels through which the particle can continue to relax by non-radiative means, thus fluorescence is quenched.

Thus, the use of laser excitation corresponding to a molecular resonant frequency can significantly increase the probability of the energy transitions needed for Raman scattering. The metal surface quenches fluorescence that would otherwise hinder the ability to detect the Raman signal. Crystal violet (CV) is an ideal molecule for these purposes, as explained below, and takes a central role in this research project as a result.
Crystal violet undergoes SERRS when excited using $\nu_{ex} = 633$ nm. Figure 1.6 depicts the classical Raman spectrum of CV, $\nu_{ex} = 785$ nm. This spectrum is normalized using the same methods as those for pyridine in Figures 1.3 and 1.4. The spectrum could not be obtained at 633 nm because at that frequency, CV free in solution will fluoresce and drown out any Raman signals. The signals obtained at 785 nm were converted to where they would appear at 633 nm using

$$\sigma_{633} = \frac{\nu_{633}^4 E_{633}}{\nu_{785}^4 E_{785}} \sigma_{785}$$

(7)

where $\nu$ is the frequency of incident light in Hz at the wavelength indicated by the subscript, $E$ is the power of the laser used in mW, and $\sigma$ is the normalized intensity of a given data point at the wavelength specified by the subscript.\textsuperscript{15}
Figure 1.7. Classical Raman Crystal Violet Spectrum. Spectrum is normalized and was obtained using $\nu_{ex} = 785$ nm.

Figure 1.8. Surface-Enhanced Resonant Raman Crystal Violet Spectra. These spectra are normalized and were obtained using $\nu_{ex} = 633$ nm. The green spectrum is from Figure 1.7; the others are SERS spectra of 50, 175, and 100 nM crystal violet in 45 nm gold colloid.
Table 1.2. Enhancement Factors for Crystal Violet in Gold.

<table>
<thead>
<tr>
<th>100 mM Crystal Violet Frequency (cm⁻¹)</th>
<th>50 nM Crystal Violet Frequency (cm⁻¹)</th>
<th>Enhancement Factor</th>
<th>75 nM Crystal Violet Frequency (cm⁻¹)</th>
<th>Enhancement Factor</th>
<th>100 nM Crystal Violet Frequency (cm⁻¹)</th>
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<tr>
<td>1168</td>
<td>1170</td>
<td>1.91x10⁶</td>
<td>1169</td>
<td>2.10x10⁶</td>
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<td>5.81x10⁶</td>
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<tr>
<td>1233</td>
<td>1222</td>
<td>3.91x10⁶</td>
<td>1221</td>
<td>3.95x10⁶</td>
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<td>3.35x10⁶</td>
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<td>1441</td>
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<td>1443</td>
<td>1.48x10⁷</td>
</tr>
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<td>1534</td>
<td>8.17x10⁶</td>
<td>1534</td>
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</tr>
<tr>
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<td>1584</td>
<td>6.55x10⁶</td>
<td>1584</td>
<td>6.78x10⁶</td>
<td>1584</td>
<td>1.49x10⁷</td>
</tr>
<tr>
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<td>1622</td>
<td>1.06x10⁷</td>
<td>1623</td>
<td>2.55x10⁷</td>
</tr>
</tbody>
</table>

Whereas the enhancement factors for pyridine showed a decrease as the concentration increased, the same trend is not observed with these CV data. With CV, the concentrations in the solutions were much lower, nanomolar rather than the micromolar concentrations used in the pyridine solutions. Thus, the binding sites available for the CV were not saturated, and no CV was left free in the solution. This is necessary because any CV not adsorbed to the metal will fluoresce, as there is no means to quench the fluorescence. Fluorescence would overpower any Raman peaks in the spectra.

1.9 Research Goals

The purpose of this project is to develop a SERS-based optical fiber sensor that can be used as a probe in procedures that examine biological
microenvironments. Raman spectroscopy has potential implications in this area because biological systems are watery environments, and water is not very Raman-active since many of its vibrational modes do not alter the polarizability of the molecule. Specifically, efforts to develop a pH-sensitive SERS probe are described. It is important to monitor pH change because since biological systems tend to have a narrow range of ideal pH values, a diversion from that range can give useful information on several conditions, ranging from tumors, to inflammation, to the effectiveness of medications. This project focuses on two areas specifically: 1) investigation on the effects of tapering a probe to improve the signal to noise ratio of the spectra will be discussed. 2) characterization of probes of fabricated template for gold coating will be discussed, including pH-sensing capabilities.
References


15) Emory, S., Western Washington University, Bellingham, WA. Personal communication, 2011.
Chapter 2: Experimental Materials and Methods

2.1 Reagents

**Gold colloid synthesis:** Potassium tetrachloroaurate (99.995%, Aldrich), nanopure water purified with Barnstead NANOpure deionization system, 17.6 MΩ, and 1% w/w trisodium citrate (99.4%, JT Baker).

**Fiber Colloid Coating Method, Type A and B:** Multimode optical fiber (200 µm, 0.37 NA, ThorLabs), nanopure water purified with Barnstead NANOpure deionization system, 17.6 MΩ, concentrated sulfuric acid (95-97% by assay, JT Baker), hydrogen peroxide (30%, Mallinckrodt), HPLC grade methanol (99.9%, Mallinckrodt), 3-(aminopropyl)trimethoxysilane (95%, Arcos Organics), absolute ethanol (200 proof, AAPER alcohol), crystal violet (100%. JC&B).

**Polymer Based Fiber Coating Method, Type A and B:** Multimode optical fiber (200 µm, 0.48 NA, ThorLabs), tetrahydrofuran (Fisher Scientific), hexanes (99.9%, Fisher Scientific), chloroform (Mallinckrodt), polystyrene(57500)-block-poly-4-vinylpyridene(18500) (1.10 Polydispersity Index, Polymer Source Incorporated), toluene (99.5%, Mallinckrodt), polytetrafluoroethylene filter (0.2 µm with glass microfiber filter, Whatman), hydrogen tetrachloroaurate(III) trihydrate (99.99%, Alfa Aesar), concentrated sulfuric acid (Mallinckrodt), sodium borohydride (Fisher Scientific), 1-3dithiolpropane (98%, Arcos Organics), 4-mercaptopypyridine (96%, Arcos Organics).
2.2 Gold Colloid Synthesis

Methods in the literature were adapted to synthesize the colloids. The reaction is not balanced.¹

Synthesis, 60-nm nanoparticles.

\[ \text{KAuCl}_4(\text{aq}) + \text{Na}_3\text{C}_6\text{H}_5\text{O}_7(\text{aq}) \rightarrow \text{Au colloid}(\text{aq}) \]

Clean glassware is essential to the process. Scratches or other impurities can cause the metal to aggregate or nucleate rather than form monodisperse nanoparticles. Into 50 mL nanopure water, 5.55 mg potassium tetrachloroaurate (KAuCl₄) was dissolved, and the solution brought to a boil. While stirring the boiling solution, 0.25 mL of 1% w/w trisodium citrate (Na₃C₆H₅O₇) was added slowly dropwise, and the flask allowed to continue boiling for 5 minutes while the reaction proceeded. The solution changed colors as the reaction proceeded, from clear and colorless to clear faint grey to cloudy reddish-burgundy. This synthesis method was also used to form a colloid of 71.5 nm gold particles, with changes to the amounts of KAuCl₄ and Na₃C₆H₅O₇ solution added.

Synthesis, 22.4 nm nanoparticles.

The 22.4 nm gold colloid was made using the same process outlined above, except that here 5.20 mg of KAuCl₄ was added to the nanopure water, and 1.58 mL 1% w/w Na₃C₆H₅O₇ was slowly added to the boiling mixture.
The colloids are synthesized via the boiling of an aqueous solution of KAuCl₄ and Na₃C₆H₅O₇. The color of the colloid depends on the size of the gold nanoparticles.

### 2.3 Fiber Colloid Coating Method, Type A

A section of optical fiber was cut to about 30 cm. The polymer outer coating was stripped off for approximately the first 1 cm of the fiber with a fiber stripper, exposing the inner glass. This stripped tip was cleaned by soaking it in “piranha”, (4:1 concentrated sulfuric acid (H₂SO₄) to 30% hydrogen peroxide (H₂O₂)), then thoroughly rinsed with nanopure water followed by methanol. The fiber was air dried.

Following cleaning, the exposed fiber tip was allowed to react for two days in a solution of 4:1 3-(aminopropyl)trimethoxysilane (APTMS) to methanol, which functionalized the tip by depositing onto the surface positively charged amino groups, making it receptive for the gold particles to be coated onto it. After the soaking in the APTMS solution, the fiber was rinsed with ethanol followed by another rinse in nanopure water.

Next the fiber tip was placed in a 60-nm diameter gold colloid solution (Section 2.2) for at least one hour. The tip showed evidence of a gold coating as
it was no longer clear and colorless but now had a dark greyish tinge. The fiber was rinsed with nanopure water and set to dry. The fiber was ready to have the reporter molecule (crystal violet or 4-mercaptopyridine) adsorbed onto the metal surface. The fiber was set in a solution of 10 mM CV in nanopure water for one hour, and then rinsed with nanopure water.

2.4 Tapered Fiber, Colloid Coating Method, Type B

The process for fabricating the tapered fiber was the same as that described above (section 2.2) for the other fiber. However, to taper it the middle of an optical fiber (approximately 60 cm) was held over a flame on a Bunsen burner. The fiber became hot and was pulled apart, yielding two tapered fibers of about 30 cm each. The tapered fiber was coated with the 22.4 nm gold particles.
**Figure 2.2. Fiber Coated with Gold Nanoparticle Colloid.**
The exposed fiber tip was cleaned with a solution of $\text{H}_2\text{SO}_4$ and $\text{H}_2\text{O}_2$, and then coated in APTMS. Next the fiber tip was immersed in gold nanoparticle colloid, and the negative particles bonded to the positive amino groups. From here the fiber was set in a solution of CV, and these reporter molecules adsorbed to the gold surface.
2.5 Polymer Based Fiber Coating Method, Type A

This preparation method was adapted from those outlined by Aizawa and Buriak. A fiber was cut to about 13 cm long and the polymer cladding stripped off for the first 1 cm. To clean the fiber, the exposed tip of the cladding was set in three separate solutions of pure tetrahydrofuran (THF) for 30 minutes each. In between each solution it was rinsed with pure THF. Next the fiber was rinsed with nanopure water and allowed to dry. The fiber was set in a 3:1 solution of H$_2$SO$_4$ to 30% H$_2$O$_2$ for 30 minutes, and then rinsed with nanopure water and left to dry again. Next a polymer solution was coated onto the fiber to lay a foundation of micelles over which gold could be coated. A solution of 4 mg/mL polystyrene(57500)-block-poly-4-vinylpyridene(18500) (PS-B-P4VP), was made by dissolving 8.0 mg of PS-B-P4VP in 2 mL of toluene, and was filtered with a polytetrafluoroethylene (PTFE) filter. The exposed and cleaned fiber tip was dipped in this solution for 3 seconds, then left standing, with the exposed tip pointed up, to dry overnight.

The block copolymer is dissolved in toluene because the polystyrene (PS) is hydrophobic. Since poly-4-vinylpyridene (P4VP) is hydrophilic it turns inward to escape the toluene, thus forming the center of a micelle. The micelles are then dried on a surface film, with the P4VP ends of the polymer are at the center of the micelles, while the PS ends stick out.

Next the fiber was set in methanol for 20 minutes, causing the hydrophilic core of the micelles to open, yielding a toroid shape (ring-shape) and exposing
the hydrophilic centers of the micelles. The fiber was left to dry for ten minutes, then soaked in 10 mM hydrogen tetrachloroaurate(III) tridydrate (HAuCl₄·3H₂O) in 0.1 M H₂SO₄ for ten minutes to plate gold onto the toroids. PS-b-P4VP is a base, and it extracts a proton from HAuCl₄·3H₂O. In doing so, Au³⁺ ions attract and attach to the surface.

The fiber was rinsed with nanopure water, then dried for ten minutes, then set in methanol again for 20 minutes to reinforce the toroid structure and allowed to dry. The fiber was set in a solution of 0.5 M NaBH₄ in nanopure water for 20 minutes to reduce the Au³⁺ to Au⁰. Then the fiber was set in an argon plasma etcher (Harrick Plasma Cleaner/Sterilizer, PDC-32G), where the plasma reduced any remaining Au³⁺ to Au⁰ and to etch away the polymer base.

Next, the fiber tip was allowed to coat in a solution of 1,3-dithiolpropane for four hours, then rinsed with nanopure water and dried. The fiber was set in a solution of 71.5 nm gold colloid (made in section 2.2) for 6.5 hours to allow thorough coating, and then rinsed with nanopure water and allowed to dry. The fiber was set in a solution of 25 mM 4-mercaptopyridine (4-MPy) with a stirbar and left overnight. The fiber was rinsed with nanopure water and air dried.

2.6 Polymer Based Fiber Coating Method, Type B

This coating method is similar to that outline in section 2.5, with a few differences. Once the cladding was removed from the 1 cm tip of the 13 cm long fiber, the very edge of the tip, about 1 mm, was cut off to make it less jagged. The tip was wiped 15 times with a Kimwipe soaked in pure THF until no friction
was sensed. Then the fiber tip was wiped 15 times with a Kimwipe soaked in hexanes, followed by being wiped 15 times with a Kimwipe soaked in chloroform. The fiber was set in pure THF for 20 minutes with a stir bar. The fiber tip was rinsed with hexanes, then set in hexanes for 20 minutes with a stir bar. Next the fiber was rinsed with chloroform and set in chloroform with a stirbar for 20 minutes. Finally, the fiber was rinsed with a 3:1 solution of H$_2$SO$_4$ to 30% H$_2$O$_2$.

From this point the fiber was cleaned and coated using the same method as that in Polymer Based Fiber Coating Method, Type A (Section 2.5), starting with a 20 minute soak in a 3:1 solution of H$_2$SO$_4$ to 30% H$_2$O$_2$ and culminating with time in the plasma etcher to etch away the polymer molding and reduce any remaining Au$^{3+}$. To finish, the fiber underwent one of two treatments. In the first type, the fiber was set in a solution of 10 mM CV in nanopure water for one hour, then rinsed once more with nanopure water. In the second possible treatment, the sensor tip was allowed to coat in a solution of 1,3-dithiolpropane for four hours, then rinsed with nanopure water and dried. Next it was set in a solution of 71.5 nm gold colloid (made in section 2.2) for 6.5 hours to allow thorough coating, and then rinsed with nanopure water and allowed to dry. The fiber was set in a solution of 25 mM 4-mercaptopyridine (4-MPy) with a stirbar and left overnight. The next morning it was rinsed with nanopure water and left to dry.
Figure 2.3. Polymer Base Fiber Coating Process.
The block copolymer forms micelles in solution, which adsorb to the surface of the cleaned optical fiber. Gold binds to the micelles, forming an array, from which the polymer is removed via plasma etching.
2.7 Instrumentation

*Scanning Electron Microscopy and Energy Dispersive X-ray Analysis:* The fibers coated in sections 2.3 and 2.4 were examined using scanning electron microscopy and energy dispersive X-ray analysis (SEM-EDX). The SEM was a Tescan VEGA model 5136 with variable pressure and an accelerating voltage of 15.0 kV. Attached to the SEM was the EDX; the model was a Genesis XMS 200.

*Atomic Force Microscopy:* The fiber coated according to section 2.6 was analyzed on a NanoScope III multimode atomic force microscope (AFM), by Digital Instruments. The instrument was operated in tapping mode using an Ultrasharp noncontact silicon cantilever, NSC12/50.

*Benchtop Raman Spectroscopy:* Functionalized fibers were analyzed using a Delta Nu Advantage (633-nm, 5 mW) benchtop Raman spectrometer. The spectrometer had a NuScope microscope attachment, and the fiber was placed on a separate translational stage to allow for movement in all directions (x, y, z) under the laser beam.
The sensor was stabilized on a glass microscope slide, and placed on the stage. 633 nm light was focused onto the functionalized tip, and scattered light was collected back into the detector.
**Direct Excitation Spectroscopy Configuration:** The fiber was held in position with a fiber chuck within the coupler (F-1015, Newport). Laser excitation at 633-nm radiation from a HeNe laser passed through a laser line filter and into the objective (633-nm, ND 3.0, Melles Griot). The objective focused the laser beam onto the surface of the fiber optic sensor (coated in gold and reporter molecules), exciting the molecules. The Raman scattered photons were coupled back into the fiber and directed through a dichroic mirror (640 DRLP, Omega optical). Then the photons passed through two filters (3RD 650-750, 3RD 650 LP, Omega Optical) to reduce stray light and a focusing lens (c280, TM-B, thorlabs). The photons entered a single-stage spectrograph (Triax 320, Jobin Yvon, 1200 g/mm, 500 blaze), connected to the CCD camera (100 x 1024 pixels, DV401-BV, Andor Technology). The setup is shown in **Figure 2.5.**
Figure 2.5. Direct Excitation Spectroscopy Laser Configuration. 633 nm radiation from the HeNe laser excited the particles on the functionalized tip of the fiber. The resulting scattered photons traveled through the rest of the fiber, the dichroic mirror, and then the bandpass filter and lens before reaching the spectrograph and CCD detector. Inset: The laser radiation excited the particles on the fiber tip directly, rather than traveling through the non-functionalized tip before reaching the particles.
Optode Spectroscopy Configuration: Excitation radiation of 633-nm from a HeNe laser passed through a laser line filter and a dichroic mirror, then on to the fiber coupler objective (160/0.17, 10/0.25, Melles Griot). The light was coupled into the end of the fiber that was not functionalized. Once inside the fiber, the light underwent internal reflection, until it reached the tip of the fiber that was functionalized with the gold and reporter molecule. There it excited the particles, and inelastically scattered light returned through the fiber. These scattered photons passed once more through the dichroic mirror to same two filters and CCD detector described in the Direct Excitation Spectroscopy Configuration section.
Figure 2.6. Optode Configuration Showing Fiber.
The radiation was coupled into the non-functionalized end of the fiber via an objective lens. The light traveled through the fiber until it excited the reporter molecules on the functionalized tip (just to the bottom left of the picture). Scattered photons coupled back through the fiber to the detector, which was behind the black cardboard that helps prevent outside light from interfering. (photo courtesy of Hannah Sturtevant)
Figure 2.7. Optode Configuration With Fiber.
The angle of the light penetrating the fiber was adjusted via the black knobs on the top and sides of the coupler. (photo courtesy of Hannah Sturtevant)
Figure 2.8. Optode Spectroscopy Laser Configuration.
Light from the HeNe laser (633 nm) passed through a filter and was then redirected to the untreated end of the fiber via a dichroic mirror. The light traveled down the fiber to the functionalized tip and interacted with the molecules there. Inelastically scattered light traveled back through the dichroic mirror, then through a filter and into the detector. Photo: Laser light traveled through the functionalized tip of the fiber. (photo courtesy of Hannah Sturtevant)
2.8 References


Chapter 3: Results and Discussion

3.1 Gold Colloid Coated Fibers

Both the non-tapered and tapered fibers coated via the gold colloid method (Sections 2.3 and 2.4) were analyzed using scanning electron microscopy (SEM) and energy-dispersive x-ray (EDX) instruments. On both fibers the clusters of gold coating are apparent, and EDX confirms that gold has in fact coated onto the fiber surface for the non-tapered fiber.

Figure 3.1. SEM Imaging of Non-tapered Fiber.
Fiber was set in a solution of gold nanoparticles. Evidence of gold clusters is apparent in the images.

Figure 3.2. SEM Imaging of Tapered Fiber.
Tapered fiber was set in a solution of gold nanoparticles. Evidence of gold clusters is apparent in the images.
3.2 SERS Analysis of Tapered and Non-tapered Fibers

Crystal violet spectra were obtained from both fibers by aiming 633-nm light into the non-functionalized tip of the fiber, allowing it to react with the gold nanoparticles and the reporter molecules, then coupling the scattered light back through the fiber to the detector, as discussed in Section 2.7. The peaks are consistent with those reported in the literature, particularly the peaks at 937, 1196, 1606, and 1636 cm\(^{-1}\). The spectra for both the tapered and non-tapered fibers are shown in Figure 3.4, the tapered fiber spectrum in red. The tapered fiber yields a spectrum with a far higher signal-to-noise ratio than the non-tapered fiber. This was consistent amongst several spectra obtained with these fibers.
**Figure 3.4. SERRS Spectra for CV on Both Tapered and Non-tapered Fibers.**
The results indicate that using the tapered fiber the spectra obtained show far more detail, far more of the CV peaks, and that the intensity is improved.

### 3.3 Geometric Analysis of Tapered Versus Non-tapered Fibers

Sources in the literature indicate that the optimal geometry to enhance the spectra is a tapered fiber. When light traveling through one medium reaches another, transparent medium, the light bends as it enters said new medium. Both the angle at which the light enters the new medium and the indices of refraction of the two materials affect how much the light will bend. Snell’s law explains the relationship between the incident light and the refracted light:

\[
n_1 \sin(\theta_1) = n_2 \sin(\theta_2) \tag{7}\]

where \(n_1\) and \(n_2\) are the indices of refraction of the two materials and \(\theta_1\) and \(\theta_2\) are the angles of the light before and after passing through the boundary.
between the media, respectively, measured from normal to the interface of the two media.³

When light is shone into a transparent medium, some light will be refracted while other light will be reflected. As the angle at which light is entering the medium, in this case a fiber (Figure 3.5), is increased more light reflects while less refracts. For each material there is a critical angle at and above which all light will reflect. Once all light is reflected in the medium, the light is said to undergo total internal reflection. At the critical angle the light will reflect at 90° from the angle of incidence. Thus, using 90° as an angle in Snell’s law, one can determine the critical angle between two materials if the indices of refraction are known.⁴

Snell’s law is central to the end goal of this research project. Since the fiber is ultimately intended for analysis of in vivo environments, a flexible fiber through which light can be coupled is imperative. Laser light aimed into the fiber interacts with the gold nanoparticles and reporter molecules on the tip of the fiber in the body. The scattered light then couples back through the fiber to a CCD detector, yielding spectra that can be used to determine chemical information about the microenvironment.
As discussed in Section 1.7, light incident on the fiber tip interacts with the surface plasmon of the roughened metal, generating an evanescent wave and an electronic field. The electronic field interacts with the reporter molecules (in this case crystal violet) adsorbed to the metal, exciting them, and as they relax they undergo Raman scattering. However, the radiation can only penetrate so far into the metal and adsorbed molecules. The depth of penetration \( d_0 \) depends on several variables: wavelength of incident radiation \( \lambda \), indexes of refraction in both the starting medium \( n_1 \), and the medium into which the radiation will refract \( n_2 \), and the angle of incident light on the fiber \( \theta \), which is also the angle at which light will penetrate the sample. These give the radiation penetration depth as summarized in equation (8):
Thus far this project has only obtained data on crystal violet using a tapered fiber, but eventually the principle will be applied with a gold-coated fiber with 4-MPy, set in a buffer solution to detect pH change. In order to explain how a taper is the optimal geometry for a fiber, and therefore yields a stronger spectrum than a non-tapered fiber, calculations for light penetration depth were performed for a fiber with 4-MPy and a buffer solution.

Determining the penetration depth \( d_e \) for an un-tapered fiber is straightforward. Any light reflected in the fiber is reflected at 90°, due to the law of reflection, thus the radiation penetrating from the glass to the space outside the glass does so at the same angle as it was originally incident into the fiber.\(^5\)

With the taper, this is not the case; the angle at which light penetrates the space outside the glass is different from that at which it enters the fiber, precisely because the taper is not parallel to the rest of the un-tapered length of the fiber. Geometrical analysis indicated that the angle at which light penetrates the taper \( (\theta_2) \), is equal to \( \theta_1-\Phi \), where \( \theta_1 \) is the angle of light incident on the fiber and \( \Phi \) is the taper angle of the fiber (Figure 3.6).\(^6\)
Light strikes the top of the taper and reflects back at an angle of $\theta_2$ from normal to the taper of the fiber. This angle can be used to determine the penetration depth ($d_e$) of the radiation into the gold and buffer solution, the microclimate to which the fiber is exposed.

For an un-tapered fiber, calculations can be performed using (8) while varying $\theta_2$ from $0^\circ$-$90^\circ$. The penetration depth of the radiation into the sample can be determined for each angle of entry. The results are shown in Figure 3.7.

For calculations in which $n_2$ was the index of refraction gold coated on the surface and the buffer in which it was submerged, the value for $n_2$ was taken to be half-way between the indexes of refraction for a buffer solution and gold, 1.33 and 0.33 respectively, giving an index of refraction of 0.83. The index of refraction for gold is an estimate, as the value varies depending on several
factors, including particle size and shape. The value of 0.33 is used because it is the index of refraction for gold at 633 nm. Despite the other factors that can alter this index of refraction, using 0.33 for these calculations does not alter the validity of the geometry established, as the angles contributing to the penetration depth of the evanescent field are independent of the indexes of refraction.

Figure 3.7. Radiation Penetration Depth, Un-tapered Fiber.
For an un-tapered fiber, the angle of incidence of the radiation is the same as the angle at which light penetrates the sample, for light undergoing total internal reflection. Thus, the radiation penetration depth for the fiber can be determined by varying the incident angle from 0°-90°.

To show that the geometric determination for \( \theta_2 \) is consistent (\( \theta_2 = \theta_1 - \Phi \)), the opposite calculation was performed, that is \( \theta_1 \), the angle of light incident on
the fiber, was set at 90° (light shone straight down the fiber, parallel to the un-tapered portion), and the taper angle varied from 0°-90°. The results should be the opposite of those in Figure 3.7, and they are, as shown in figure Figure 3.8.

Figure 3.8. Radiation Penetration Depth, Tapered Fiber.
For a tapered fiber, setting θ₁ at 90°, and varying the taper angle (Φ) from 0°-90° should have the complimentary result as that shown in Figure 3.7.
Table 3.1 summarizes the results.

Table 3.1. Radiation Penetration \( (d_e) \) Depth Comparison for Tapered and Un-Tapered Fiber, Light Penetrating from Glass to Gold and Buffer Solution.

<table>
<thead>
<tr>
<th>Incident Angle ( (\Theta_1) ) (degrees)</th>
<th>Penetration Depth ( (d_e) ) (nm)</th>
<th>Taper Angle ( (\Phi) ) (degrees)</th>
<th>Penetration Depth ( (d_e) ) (nm)</th>
</tr>
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<tbody>
<tr>
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</tr>
<tr>
<td>34</td>
<td>549.809</td>
<td>56</td>
<td>549.809</td>
</tr>
</tbody>
</table>

Having established that the geometrical analysis is consistent, that the data sets were complimentary, the penetration depth \( (d_e) \) was determined in three dimensions, that is, the taper angle was plotted along with the angle of radiation incident on the fiber to determine the penetration depth of the radiation (Figures 3.12, 3.13, and 3.14). Since the incident light only undergoes total internal reflection above the critical angle, the critical angle for the fiber (0.48 NA) was calculated by first using (9):

\[
(9)
\]

to find the maximum half acceptance angle of the fiber \( (\theta) \). NA is the numerical aperture of the fiber, and \( n_1 \) is the refractive index of the air outside the fiber (approximately 1.0). The maximum half acceptance angle is also the critical
angle, since the entire acceptance cone is in all directions surrounding the axis.\textsuperscript{10} The result is a critical angle of 28.7°. Thus, to determine the penetration depth for light that will undergo total internal reflection in the fiber, calculations were performed for incident light angles between 30° - 90°.

However, the challenge of the index of refraction for gold (n\textsubscript{2}), that it depends on the shape and size of the particles, remained. In order to account for a broad range of possible indexes of refraction, three dimensional calculations using equation 8 were performed to find the depth of the evanescent field for taper angles from 0° - 90° with n\textsubscript{2} varying from 0.2-1.6. These calculations are shown in \textit{Figures 3.9 – 3.11}. 
Figure 3.9. Light Penetration Depth, Incident Light Angle 45°.
With incident light on the fiber at 45°, light will penetrate the fiber and generate an evanescent field until the taper angle exceeds 35°, at which point light completely escapes the fiber.

Figure 3.9 indicates that if the incident light enters the fiber at 45°, then as the taper angle (Φ) and index of refraction (n₂) increase, the evanescent field depth will also increase until the taper angle exceeds 37°. At a taper angle greater than 37°, the light will escape the fiber. Once the index of refraction for gold is greater than 1.0, the light will also escape the fiber.
Figure 3.10. Light Penetration Depth, Incident Light Angle 60°.
With incident light on the fiber at 60°, light will penetrate the fiber and generate an evanescent field until the taper angle exceeds 45°, at which point light instead escapes the fiber.

According to Figure 3.10 if the incident light on the fiber is 60°, then, again, as the taper angle (Φ) and index of refraction (n₂) increase, the evanescent field depth will also increase until the taper angle exceeds 52°. At a taper angle greater than 52°, the light will escape the fiber. Once the index of refraction for gold exceeds 1.4, the light will also escape the fiber.
Figure 3.11. Light Penetration Depth, Incident Light Angle 75°.
With incident light on the fiber at 75°, light will penetrate the fiber and generate an evanescent field until the taper angle exceeds 65°, at which point light instead escapes the fiber.

The same trend is observed in Figure 3.11. If the incident light on the fiber is 75°, then as the taper angle (Φ) and index of refraction (n₂) increase, the evanescent field depth will also increase until the taper angle exceeds 67°. At a taper angle greater than 67°, all light escapes the fiber and no evanescent field is generated. Once the index of refraction is greater than 1.4, the light will escape the fiber.
Having established that the incident light will generate an evanescent field for a range of indexes of refraction for gold \((n_2)\), calculations in three dimensions for the evanescent field depth for light transitioning to gold, to buffer, and to gold and buffer proceeded. These calculations were completed using an index of refraction of 0.33 for gold, as this is the index of refraction for gold using 633-nm excitation.\(^8\)

For calculations where the light passes from one medium to the next (such as for light going from glass to gold), both the excitation wavelength (633 nm) and the indexes of refraction for the media are constants. Only \(\theta\) (called \(\theta_2\) according to the determined geometry) changes. Since \(\theta_2 = \theta_1 - \Phi\), setting \(\theta_1\) constant (30°, 35°, 40°, etc.) and varying \(\Phi\) from 0° - 90° will result in the 3-dimensional graph of radiation penetration depth for fibers with several different geometries. No single range of ideal taper angle and incident light angle was found. Instead, for any given angle of incident light \(\theta_1\), as the taper angle \(\Phi\) increases, \(\theta_2\) decreases, thus \(\sin(\theta_2)\) decreases. Since \(\sin(\theta_2)\) is in the denominator, this leads to an increase in \(d_e\), the penetration depth of the light. Thus, an increase in the taper angle leads to an increase in the penetration depth of the incident laser light, as reported in the literature.\(^2\) This same trend is observed for each of the three examined scenarios (light traveling from glass to gold, glass to a buffer solution, and glass to gold and a buffer solution). \textbf{Figures 3.12, 3.13, and 3.14} summarize these results.
Figure 3.12. Light Penetration Depth, Light Traveling From Glass to Gold.
For a given angle of incident light, as the taper angle increases, the light penetrates deeper into the medium surrounding the fiber. The lowest penetration depth here is 67.9 nm, at a taper angle of 0° and an incident light angle of 90°.
Figure 3.13. Light Penetration Depth, Light Traveling From Glass to Buffer Solution.

For a given angle of incident light, as the taper angle increases, the light penetrate deeper into the medium surrounding the fiber. The most shallow penetration depth here is 136.9 nm, at a taper angle of 0° and an incident light angle of 90°.

In Figure 3.13, for incident light angles of 60° and lower, the light does not generate an evanescent field in the surrounding medium when traveling from glass to buffer solution, regardless of taper angle. Instead, at these angles all light will escape the fiber.
**Figure 3.14. Light Penetration Depth, Light Traveling From Glass to Gold Surrounded by Buffer Solution.**

For a given angle of incident light, as the taper angle increases, the light penetrates deeper into the medium surrounding the fiber. The lowest penetration depth here is 79.1 nm, at a taper angle of 0° and an incident light angle of 90°.

According to **Figure 3.14**, incident light at an angle of 30° the light does not penetrate the surrounding medium, regardless of taper angle. Like with the light penetrating from the glass to the buffer solution, light penetrating at this angle into the gold and buffer solution will escape the fiber and not provide an evanescent field, because the angle at which light penetrates from the glass into the surrounding medium drops below the critical angle of the fiber. To more easily visualize the trend, the data from **Figure 3.14** is plotted in two dimensions in **Figure 3.15**.
Figure 3.15. Two-Dimensional View of Light Transitioning From Glass into Gold and Buffer Solution.
The data from Figure 3.14 is shown, for incident light angles ($\Theta_1$) of 45°, 60°, 75°, and 90°.

Figure 3.15 allows more simple visualization of Figure 3.14. Again, as the angle of incident light on a fiber sensor is increased and the taper angle of the fiber increases, the penetration depth of the evanescent field also increases, each time to the same depth. This is key to the design of the fibers. While a fiber with a taper angle of nearly 40° with an incident light angle of 75° (633 nm) will generate a deep evanescent field, this fiber will have less surface area than a fiber with a taper angle around 25°. But with such a fiber, the same evanescent...
field can be generated by using 60° incident light of 633 nm. Since the fiber with the more shallow taper will have an increase in surface area, more gold and reporter molecules can be coated onto it, yielding a stronger Raman signal, thus a better spectrum.

Investigation of how the index of refraction for $n_2$ influences the penetration of the light (Figures 3.9, 3.10, and 3.11) spurred the question of at what taper angle the light would escape from the fiber, for a given incident light angle and index of refraction, $n_1$. This will occur where equation 8 is undefined, which happens when:

$$\sin^2(\theta_1 - \Phi) - \left(\frac{n_2}{n_1}\right)^2 = 0$$  \hspace{1cm} (9)

To determine at what taper angle ($\Phi$) this occurs, the incident light angle ($\Theta_1$) was varied from 30° - 90°, based on the fiber critical angle, $n_1$, the index of refraction for glass, was set at 1.52, and $n_2$, the index of refraction for gold, varied from 0.2 – 1.5.

*Figure 3.16* shows the result.
Figure 3.16. Taper Angle (Φ) at Which Light Escapes the Sensor.
For a given angle of incident light on a fiber coated in gold and a given index of refraction for gold, the taper angle at which all light escapes the fiber varies.

Figure 3.16 shows the taper angle at which light escapes the fiber and does not generate an evanescent field, depending on the index of refraction and angle of light incident on the fiber. It can be compared to Figure 3.12 to cross check the results. Figure 3.12 is calculated using 0.33 as the index of refraction for gold. According to Figure 3.16, at an index of refraction of about 0.33 and an incident light angle of 60°, light will escape the fiber at a taper angle at 47.5°.

Figure 3.12 confirms that the light will escape the fiber at this taper angle. These results are consistent for the other angles of incident light shown in Figure 3.16.
Figure 3.17. Penetration Depth for Non-tapered and Tapered Fibers.
For a given angle of light incident on the fiber, as the taper angle of a fiber increases, the light will penetrate deeper into the media surrounding the fiber, such as the gold and buffer solution used in this project.

3.4 pH Change Detection Analysis

Fibers used for pH measurements were developed using the polymer-coating method described in sections 2.5 and 2.6. The fiber was characterized using atomic force microscopy. A complete, uniform gold coating was observed on the surface of the fiber (Figure 3.18). Figure 3.18 (A) has an average interparticle space of 5x10^2 ± 1x10^2 nm.
Figure 3.18. AFM Image of Polymer Templated Fiber.

(A) A section of the fiber 1 μm x 1 μm shows very few gold particles can be seen, but they are packed in quite close together. (B) A section 5 μm across shows that the particles are packed close together, but there is some variation in height. (C) A section 10 μm also shows a thorough coating of gold particles.
4-MPy was used as the reporter molecule for pH change. The signal obtained from that compound is not very strong compared to a Raman scatterer like CV, because the radiation used to excite the 4-MPy (633 nm) is not the resonant frequency for 4-MPy, thus the molecules are exciting to a virtual energy state, not to the first excited electronic state, and the transition occurs less frequently, thereby yielding less scattering. Thus, to ensure that the polymer coating method would yield SERS data, a fiber coated in CV (see section 2.6) was tested first, since CV is a very strong Raman reporter, due to its polarizability potential and 633 nm excitation light being its resonant frequency. This fiber did provide strong CV spectra. All spectra from this point onward were obtained using 633-nm excitation on the Delta Nu Advantage 633-nm (5 mW) benchtop Raman spectrometer, described in section 2.7. The CV spectrum is shown in Figure 3.19.
Figure 3.19. SERRS Spectrum of CV on Fiber Made Using Polymer Base Coating Method.
The peaks for CV were obtained using 633-nm excitation and an integration time of 5 seconds, and are consistent with those found previously in the research project and in the literature.¹
Having demonstrated that the polymer coating method provided SERS data for crystal violet, tests proceeded using 4-MPy. 4-MPy can exist in both a protonated and deprotonated form. The two forms have Raman modes at two different frequencies. Thus, as the pH changes the ratios of the two peaks relative to each also change as the 4-MPy establishes a new equilibrium between the protonated and deprotonated species.\textsuperscript{11}

A peak around 1594 cm\(^{-1}\) is the result of a non-aromatic C=C vibration, which only exists in the deprotonated form, thus the intensity of this peak should increase as pH increases. A second peak, around 1618 cm\(^{-1}\), is reported to be caused by C=C and C=N vibrational modes. The C=N vibration is only possible in the protonated species, thus this peak should be more pronounced at lower, more acidic pH levels.\textsuperscript{12}

\textbf{Figure 3.20. 4-Mercaptopyridine.} Depending on the pH of its surroundings, 4-MPy will establish an equilibrium between its protonated and deprotonated forms.
Coating a sensor with a single layer of 4-MPy did not yield a detectable signal. The signal-to-noise ratio was too poor. However, 4-MPy can be layered, where the lone pair of electrons on the nitrogen will adsorb to more gold coated onto it. This allows more 4-MPy to adsorb to that gold via the sulfur in the molecule when another layer of 4-MPy is added to said second layer of gold, and so on. However, when this method was attempted, while 4-MPy was detected, no pH sensitivity was found. In order to detect pH change, the ratios of the protonated to deprotonated peaks must change when the sensor is exposed to buffers at various pH levels. The problem with attempting to coat multilayers onto the fiber this way is that each layer of 4-MPy between the gold layers is entirely protonated, because the gold is bonded to the nitrogen. As a result, the protonated 4-MPy peak is very large relative to the deprotonated peak, regardless of how the pH changes. Only the outermost layer of 4-MPy can actually equilibrate with the solution.

To resolve this issue, a layer of 1,3-dithiolpropane (DTP) was used as a bridge to add a second layer of gold nanoparticles on top of the polymer-based gold nanoparticle layer. DTP is not a strong Raman scatterer in the region where the pH sensitive peaks for 4-MPy are, so it will not interfere with the signal. This increased the surface area of gold nanoparticle binding sites available, and increased the interparticle sites between the gold nanoparticles. Thus the electronic coupling of the gold nanoparticles with the 4-MPy molecules adsorbed to the surface also increased. This ultimately provides a stronger Raman scattering signal because the evanescent field and the number of reporter
molecules that can undergo Raman scattering are both increased. Because only the top layer of molecules consist of 4-MPy, all detected 4-MPy, whether protonated and deprotonated, was from the top layer, thus eliminating the problems associated with multilayers of 4-MPy between gold nanoparticles that yield an intense protonated peak that does not change as the pH level changes. From this fiber, a much more clear spectrum was detected for 4-MPy, typical of that shown in Figure 3.22.

**Figure 3.21. Gold Toroid Coated Fiber.**
Gold particles are coated onto the clean silica surface. 1,3-dithiolpropane is added onto the gold as a bridge, then gold nanoparticles are coated onto the 1,3-dithiolpropane. 4-mercaptopropyridine is coated onto the nanoparticles.
Figure 3.22. SERS Spectrum of 4-MPy Via Polymer Base.
The peaks for 4-mercaptopyridine, obtained with a 633-nm laser, are consistent with those found in the literature.\textsuperscript{11}

Having established that 4-MPy was present on the fiber, testing proceeded on the fiber using buffer solutions of Na\textsubscript{2}HPO\textsubscript{4}, KH\textsubscript{2}PO\textsubscript{4}, and NaClO\textsubscript{4}.\textsuperscript{13} A consistent change in pH for 4-MPy (pK\textsubscript{a} = 1.43)\textsuperscript{14} was found for buffers between pH 3-6.
Figure 3.23. Average Normalized SERS Spectra.
At the four pH levels tested using 633-nm excitation, the peaks clearly changed. As the pH increased, the deprotonated peak (1594 cm\(^{-1}\)) increased as a function of pH, the protonated peak (1618 cm\(^{-1}\)) decreased as a function of pH.
Figure 3.24. Close-up of Average Normalized SERS Spectra.
A closer look at the peaks in question shows that the ratio of protonated to deprotonated 4-MPy is changing consistently as the pH of the buffer solution changes, with the deprotonated peak (1594 cm⁻¹) becoming more dominant as the pH increases. All data are normalized to the peak at 1594 cm⁻¹.
The peak around 1594 cm\(^{-1}\) is lowest around a pH of three. As the fiber is exposed to more basic solutions, this peak becomes more dominant. Since the 1594 cm\(^{-1}\) peak corresponds to the deprotonated form of the species. Correspondingly, around pH three the peak at 1618 cm\(^{-1}\) is greatest, and as the pH increases, this peak becomes less distinct than it was at the more acidic pH levels. This is the expected trend, since this Raman mode is only possible in the protonated species of the 4-MPy.\(^\text{12}^\) Figure 3.25 shows that the log of the ratio of the basic to acidic peaks change consistently with relation to pH change (correlation coefficient 0.978). This is consistent with the linear relationship observed in buffers via the Henderson-Hasselbalch equation:

\[
pH = pK_a + \log \left( \frac{[\text{base}]}{[\text{acid}]} \right) \tag{10}
\]
Figure 3.25. Linear Relationship Between pH Level and Ratio of Peaks at 1618 cm$^{-1}$ to Peaks at 1594 cm$^{-1}$. With a correlation coefficient of 0.978, the data indicates that the ratios of the peaks are changing in a linear manner according to the pH change.
3.5 Conclusions and Future Work

The geometrical relationship between light incident on a fiber and light striking a tapered end of a fiber have been determined. Calculations performed using this relationship have indicated that increasing the taper on a fiber for any given angle of light incident on the fiber, will increase the depth to which the light can penetrate. Understanding how deep the light will penetrate is key to fabricating the sensors because to detect a strong spectrum from certain species, multiple layers must be stacked in order to pack the molecules closer together, resulting in a need for deeper light penetration into the layers when obtaining spectra in optode mode. It is now necessary to develop a method by which consistent tapered fiber sensors can be fabricated, and the angles of the tapers tailored. Hydrofluoric acid could possibly be used to make the taper, but it is unfavorable as it is quite dangerous. A pipette puller may also be an option. A method to fully control the angle of light penetration into the fiber is also necessary.

Non-tapered fibers were coated in a PS-b-P4VP block copolymer. Gold was layered onto the polymer. These sensors have been found to be pH sensitive between pH 3-6 using 4-mercaptopyridine as the reporter molecule. Hopefully this method can also be used to detect a broader change in pH, and can also be tested using other pH sensitive molecules. Work with this fiber has thus far been through direct excitation only, where the laser is shone directly onto the reporter molecules. Work must be done to couple the light into the other end of the fiber,
the end that has not been functionalized, to determine if spectra can be obtained by having the light travel into the fiber, interact with the molecules, and then couple back through the fiber to the detector (optode method). This is necessary if the fiber is to be used in vivo to detect microclimates. In addition, a design is needed to protect the functionalized tip; the coating on it is at present quite brittle. As work has shown that a tapered fiber will allow deeper light penetration into the layer of reporter molecules, it will also be necessary to test tapered fibers coated via the polymer template.
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