

# A RAMAN SPECTROSCOPIC INVESTIGATION OF NEUROTRANSMITTERS AND PHOTON MIGRATION

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## **Abstract**

Neurotransmitters are an integral part of the brain and its ability to properly function. Imbalances in these chemicals can result in neurological diseases such as depression, bipolar disorder, and Parkinson's disease. Earlier detection of neurotransmitter imbalances could result in better prognoses for patients. Surface-enhanced Raman spectroscopy (SERS) shows promise in its ability to nondestructively, and potentially non-invasively, detect physiological levels of neurotransmitters in the brain. The aim of our study was to measure the limits of detection for four individual neurotransmitters on gold nanoparticles in solution with SERS. The ability to detect Raman active molecules in subsurface layers also shows great promise in many applications, including in the medical field to probe layers underneath bone. Spatially offset Raman spectroscopy (SORS) allows subsurface layers in diffuse scattering media to be probed, giving information on the molecules deeper than the surface. The SORS study presented here aimed to determine how deep polytetrafluoroethylene (Teflon) could be detected through a fragment of deer bone and slices of agarose gel. This study increased our understanding of the relationship between spatial offset, probing depth, photon migration in diffuse scattering media such as bone and agarose gel, and its potential application in the non-invasive probing of the brain through the skull.

## **Introduction**

### **A. Raman Spectroscopy**

Raman spectroscopy is a form of vibrational spectroscopy that takes advantage of inelastic scattering of photons. In 1923, Austrian physicist Adolf Smekal first predicted the theoretical inelastic scattering of photons <sup>1</sup>. Five years later, the inelastic scattering of light was first detected by Sir C.V. Raman and K.S. Krishnan <sup>2</sup>. This inelastic scattering, now known as Raman scattering, is the result of electrons relaxing to a different vibrational ground state energy level than the vibrational energy level it was in before excitation by a monochromatic light source. Since these different vibrational energy levels have differing energies, the resulting photon emitted during relaxation has a change in energy equal to the difference in energies of the two vibrational energy levels. Therefore, the emitted photon has a different wavelength than the excitation wavelength <sup>3</sup>. Figure 1, a Jablonski diagram, shows the relaxation of electrons into vibrational energy levels and the two types of scattering that photons can undergo. The first, Rayleigh scattering, by far the most common scattering effect, corresponds to when the molecule gets excited by a photon, rising to a certain virtual energy level proportional to the incident photon's energy, then immediately relaxes to the same vibrational ground state it was in previously. This releases a photon of the same wavelength of the laser (elastic scattering) and yields no molecular information. The other, less common type is inelastic Raman scattering. A key advantage of Raman scattering is that almost every molecule has a unique Raman spectrum, due to the fact that every molecule has different vibrational and rotational modes that can be excited to a higher energy, metastable virtual state. This is what is unique to every molecule. In order to detect these photons from the different vibrational and rotational modes,

it is essential to filter out the simultaneously scattered Rayleigh photons. However, Raman spectroscopy is inherently very weak as only approximately 1 in every 10 million excitations results in an inelastic Raman scattering <sup>4</sup>.

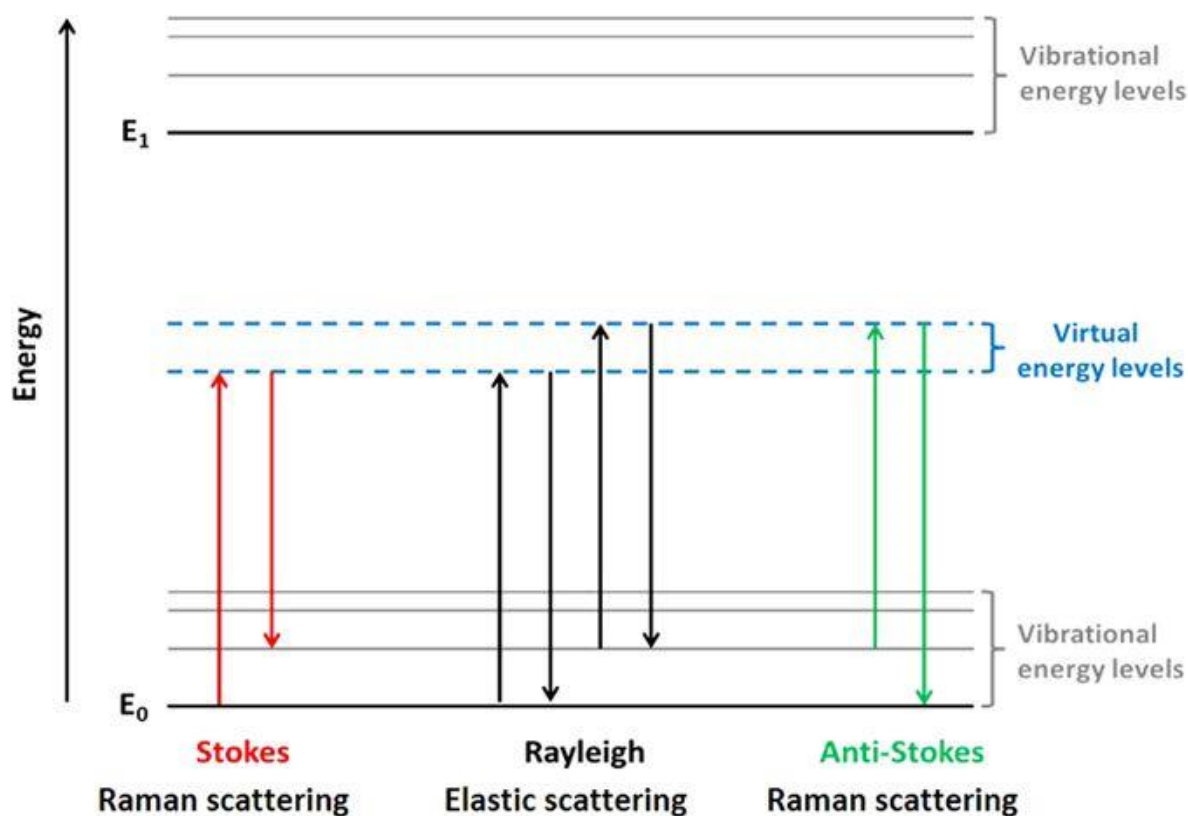


Figure 1: Jablonski Diagram <sup>5</sup>

There are also two types of Raman scattering: Stokes and anti-Stokes. Stokes Raman scattered photons occur from the electron relaxing to a higher vibrational ground state energy level and releasing a photon with a longer wavelength than the incident photon. Anti-Stokes Raman scattered photons occur from the electron relaxing to a lower vibrational ground state energy level and releasing a photon with a shorter wavelength than the incident photon. Anti-Stokes Raman scattering occurs even less frequently than Stokes Raman scattering.

Vibrational and rotational modes are Raman active only if they cause a change in polarizability in the molecule. According to equation 1, a change in polarizability,  $\alpha$ , causes a change in the induced dipole moment,  $\mu_{induced}$ . Equation 1 is a simplified equation with the higher power terms neglected that accurately estimates the induced dipole moment.

$$\mu_{induced} = \alpha * E_{field} \quad (1)$$

$E_{field}$  is the constant electric field produced by the excitation light source. Equation 1 can be expanded using the fact that polarizability is a function of the nuclear coordinate,  $q$ , and that  $E_{field}$  is an oscillating electric field. After manipulation, the induced dipole moment can then be written as:

$$\mu_{induced} = \alpha_0 E_0 \cos 2\pi\nu_0 t + \frac{1}{2} \left( \frac{\partial \alpha}{\partial q} \right)_0 q_0 E_0 [\cos(2\pi(\nu_0 + \nu_m)t) + \cos(2\pi(\nu_0 - \nu_m)t)] \quad (2)$$

| VARIABLE                             | DEFINITION                                                       |
|--------------------------------------|------------------------------------------------------------------|
| $\mu_{induced}$                      | Induced dipole moment                                            |
| $\alpha_0$                           | Initial polarization                                             |
| $E_0$                                | Magnitude of electric field                                      |
| $\nu_0$                              | Frequency of excitation laser                                    |
| $t$                                  | time                                                             |
| $\frac{\partial \alpha}{\partial q}$ | Derivative of polarization with respect to<br>nuclear coordinate |
| $q_0$                                | Initial nuclear coordinate                                       |
| $\nu_m$                              | Frequency of vibrational mode                                    |

Table 1: Definition of Variables

Equation 2 shows mathematically the three types of scattering. The Rayleigh scattering is the first part of the equation that is not dependent on  $\frac{\partial \alpha}{\partial q}$  and does not show frequency change. The Anti-Stokes and Stokes Raman scattering are the second and third parts of the equation, respectively. Both only are present if  $\frac{\partial \alpha}{\partial q} \neq 0$ . This shows that the change in polarizability is responsible for the Raman scattering. Raman intensity is a product of the Raman scattering cross-sectional area and the intensity of the incident photons from the light source, according to equation 3 <sup>6</sup>.

$$I_{RS} = \sigma * I_0 \quad (3)$$

Equation 3 explains the inherent weakness of Raman spectroscopy because the Raman scattering cross-sectional area,  $\sigma$ , is incredibly small.

In summary, Raman spectroscopy gives a unique spectrum for every molecule because every molecule has different functional groups with different vibrational and rotational modes. Each Raman active mode in a molecule inelastically scatters photons of different wavelengths. The collection of all the inelastically scattered Raman photons released from a molecule should result in a unique spectrum, often referred to as a molecular fingerprint. In this way, Raman spectroscopy is similar to Infrared (IR) spectroscopy, however one advantage Raman has over IR is that Raman spectroscopy can also be done in aqueous environments. While there are many advantages to Raman scattering, one main disadvantage is that Raman scattering is inherently very weak, so efforts have been made in order to increase the sensitivity of Raman spectroscopy. According to equation 3, one can either increase the Raman scattering cross-sectional area or increase the intensity of the incident photons. Increasing the intensity of the laser does show some improvement of signal, but high intensities can damage or destroy

analytes, so efforts to increase the Raman scattering cross-sectional area have been made. One effective approach is to use Surface-enhanced Raman Spectroscopy.

### **B. Surface-enhanced Raman Spectroscopy**

Surface-enhanced Raman spectroscopy (SERS) can enhance the intensity of Raman photons by magnitudes of up to  $10^8$ . A common approximation is that the SERS effect enhances by the magnitude of the local electric field to the fourth power <sup>7</sup>. The technique relies on adsorption of the analyte to metal nanoparticle surfaces. These nanoparticles can be as simple as spheres or cubes or quite complex <sup>8</sup>. The phenomenon was first observed in 1973 by Martin Fleischmann when he detected pyridine on a roughened silver electrode <sup>9</sup>. However, Fleischmann simply noted the enhanced pyridine signal, but failed to recognize anything novel in the result. In 1977, Jeanmaire and Van Duyne proposed the electromagnetic theory of enhancement <sup>10</sup>. This commonly accepted theory for the mechanism of enhancement is based on the existence of a localized surface plasmon. The localized surface plasmon is made up of the conduction band electrons on a metal nanoparticle surface. When irradiated with near-infrared or visible incident light, the localized surface plasmon oscillates with the incident light's electric field creating a much stronger local electric field <sup>11</sup>. This local electric field is very concentrated and decays rapidly, with the best enhancement within 2 nm of the nanoparticle <sup>12</sup>. Figure 2 shows the localized surface plasmon resonance (LSPR) of a spherical nanoparticle being irradiated by incident light. The electron cloud on the nanoparticle oscillates in resonance with the incident electric field.

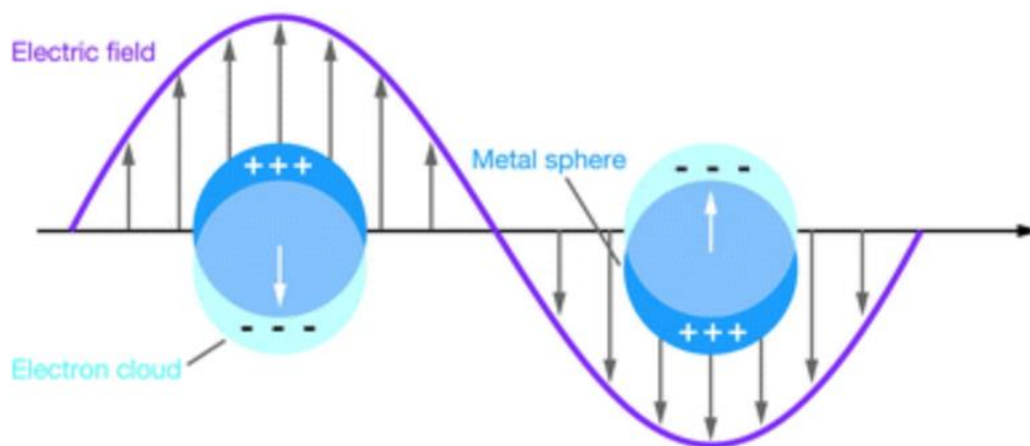


Figure 2: LSPR of a spherical nanoparticle <sup>13</sup>

The reason the local electric field enhances the Raman signal so greatly is because it increases the induced dipole moment, as shown in Equation 2. This increased induced dipole moment effectively increases the Raman scattering cross-sectional area, so, from equation 3, the intensity of the Raman signal is increased. The enhancement factor of SERS is so great that single molecule detection has been shown <sup>14</sup>. The challenge with SERS is choosing a nanoparticle that will allow the analyte to adsorb sterically and electrostatically in a place that will also have significant enhancement. SERS also causes peak shifts, as adsorption to a nanoparticle affects how the molecule vibrates. Although SERS shows very powerful enhancement, Raman spectroscopy is still mainly a surface detection technique. In order to detect Raman scattering deeper in diffuse scattering media, another technique must be used. That technique is Spatially Offset Raman Spectroscopy.

### C. Spatially Offset Raman Spectroscopy

Spatially Offset Raman Spectroscopy (SORS) is a relatively new technique that was first demonstrated in 2005 by Pavel Matousek <sup>15</sup>. In SORS, spectra are collected from a point that is spatially offset from the illumination site on a diffusely scattering medium. The purpose of

offsetting the collection point is to non-invasively detect photons from deeper inside the medium. A diffusely scattering medium is any medium that causes photons to migrate. In other words, the medium causes photons to change direction randomly. This phenomenon is shown in Figure 3. These photons are more likely to be from deeper inside the medium because of the photon migration that is occurring. Photons that are deeper in the media will have more opportunities to migrate, so there is a direct relationship between spatial offset and probing depth.

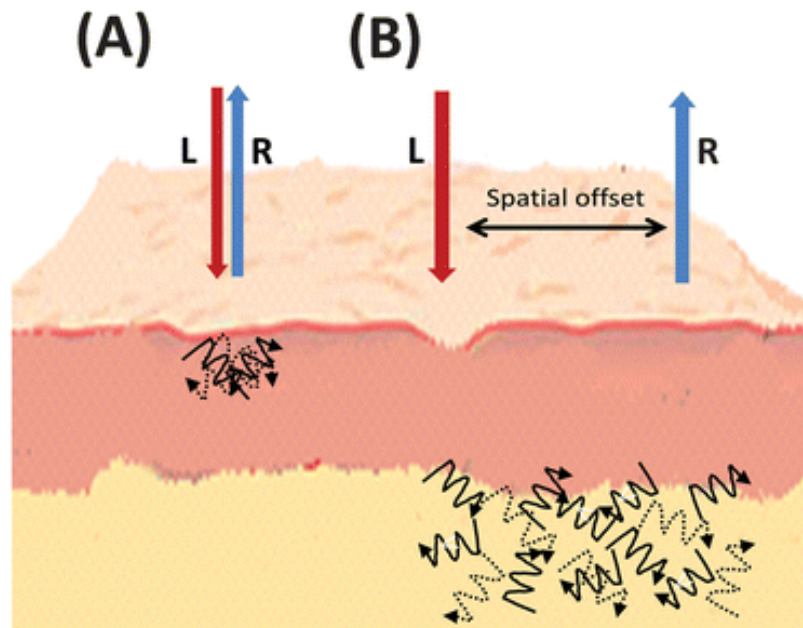


Figure 3: (A) Surface scattering in normal Raman spectroscopy, while (B) shows the mechanism of spatially offset Raman spectroscopy.<sup>16</sup>

The original method of detecting photons at a point offset from the illumination point, as shown in part B of Figure 3, was later modified to enhance the signal-to-power ratio. Regular SORS requires high powered light in order to create enough Raman scattering to detect at the offset collection site. Using inverse SORS, where a ring of light created by an axicon is illuminated on the sample and the collection site is the center of the ring, has shown to

increase the signal-to-power ratio <sup>17</sup>. The axicon works through refraction of light. Normal Raman Spectroscopy only detects scattered photons from up to 200  $\mu\text{m}$  deep in diffuse scattering media <sup>18</sup>. SORS has been shown to detect through several millimeters of media <sup>19</sup>. Currently, the mechanism for photon migration is still debated, but regardless it is a property of diffuse scattering media that can be used to investigate subsurface layers. One drawback of SORS is the relatively weak signal, as some photons stay on the surface, and migration is random, so those are compounded with the already unlikely chance of Raman scattering occurring. Nanoparticles can also be injected into media to perform surface-enhanced spatially offset Raman spectroscopy (SESORS) <sup>20</sup>.

#### **D. Applications**

##### **a. SERS**

SERS is a very effective technique for probing biological samples because of its high sensitivity and selectivity <sup>7</sup>. It is also promising for *in vivo* applications as it can be applied in aqueous environments, since water does not have a strong Raman spectrum, and it also requires virtually no sample preparation besides injection of the nanoparticles in some way <sup>8</sup>. SERS has been studied for use in detection of many diseases including various cancers, Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and viral diseases <sup>7,8</sup>. Many of the neurological diseases result from imbalances in neurotransmitters, so earlier detection of these imbalances could provide better prognoses for patients with these diseases <sup>21</sup>. We applied SERS towards the detection of physiological-relevant concentrations of four neurotransmitters. The four neurotransmitters and their structures are given in Table 2.

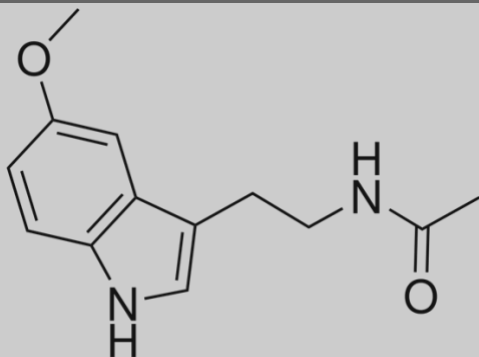
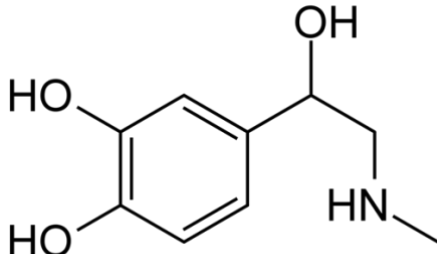
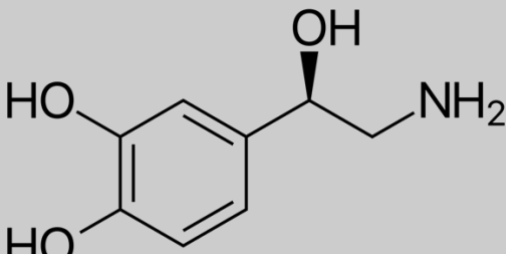
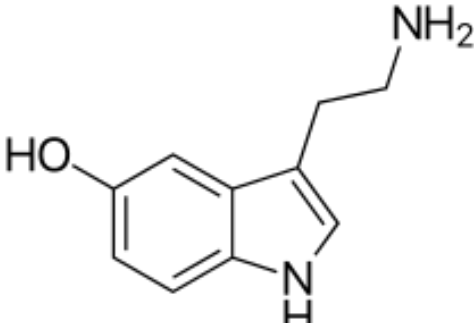
| Name           | Structure                                                                            |
|----------------|--------------------------------------------------------------------------------------|
| Melatonin      |    |
| Epinephrine    |   |
| Norepinephrine |  |
| Serotonin      |  |

Table 2: List of neurotransmitters and their structures used in study

## **b. SORS**

Due to its non-invasive and non-destructive nature, the applications for SORS are very widespread as well. SORS has successfully been used to identify liquid explosives through the container <sup>22</sup>, to confirm quality of pharmaceuticals in their capsule <sup>23</sup>, and determine the quality of salmon through its skin <sup>24</sup>. It also shows great promise in applications for probing the brain non-invasively through the skull. In order to better understand the relationship between probing depth, spatial offset, and photon migration within bone and the brain, the SORS study presented here aimed to characterize how the thickness of agarose gel, a common brain mimic, affected the detection of polytetrafluoroethylene (Teflon), a common, strong Raman scattering material.

## **Methods and Materials**

### **A. Laser and Table Set-up**

The system used for the SERS study was composed of a 785 nm laser, as 785 nm has been determined to give the maximum enhancement with gold nanoparticles <sup>21</sup>, the system of optics to direct the laser onto the sample and direct the Raman scattered photons into the spectrometer, a microscope, and the spectrometer. A diagram of the set-up is shown in Figure 4.

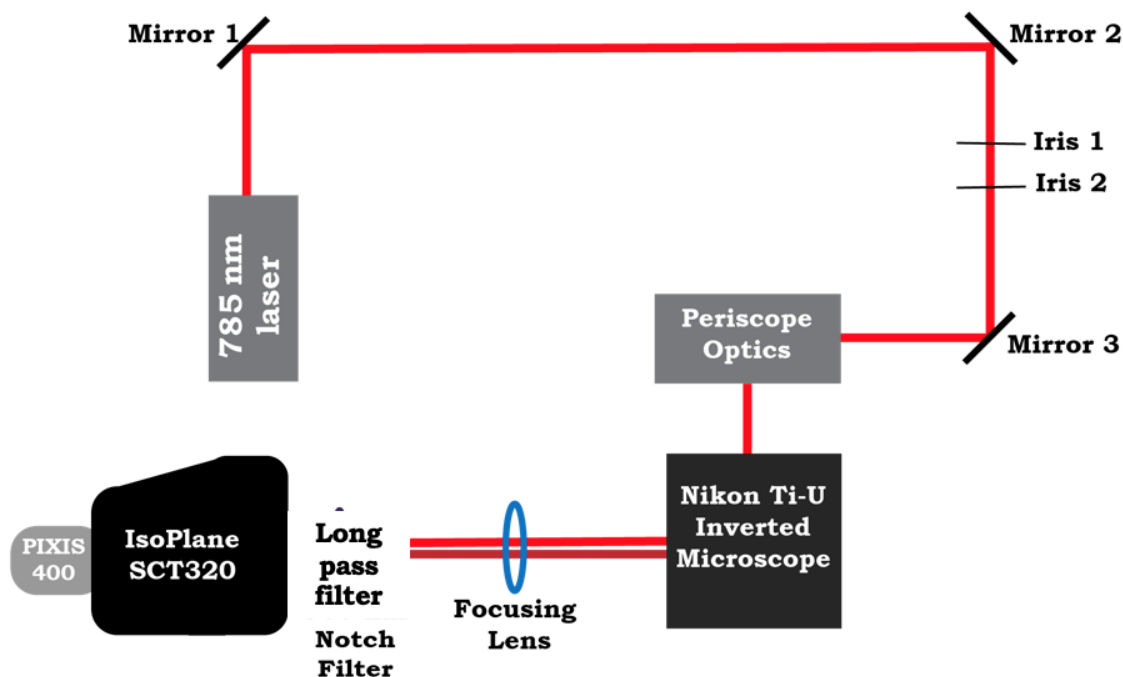


Figure 4: Micro-Raman Set-up

The mirrors and periscope optics direct the laser into the microscope, while the irises are used to confirm the direction of the laser line and adjust the power. The 20X magnifying lens on the microscope was used to concentrate the laser line into a focused point onto the sample, where the Raman photons are backscattered and sent through the focusing lens along with the laser and Rayleigh scattered photons. The focusing lens, which is a converging lens whose focal length is the exact distance to the spectrometer, directs all of the light onto the slit in the spectrometer. The long pass filter and notch filter block the laser photons, Rayleigh scattered photons, and anti-Stokes Raman photons and allow the Stokes Raman photons to pass through. The Stokes Raman photons then enter the spectrometer, where a diffraction grating diffracts the photons based on their wavelength onto a CCD camera.

The system used for the SORS study was very similar. Its diagram is shown in Figure 5.

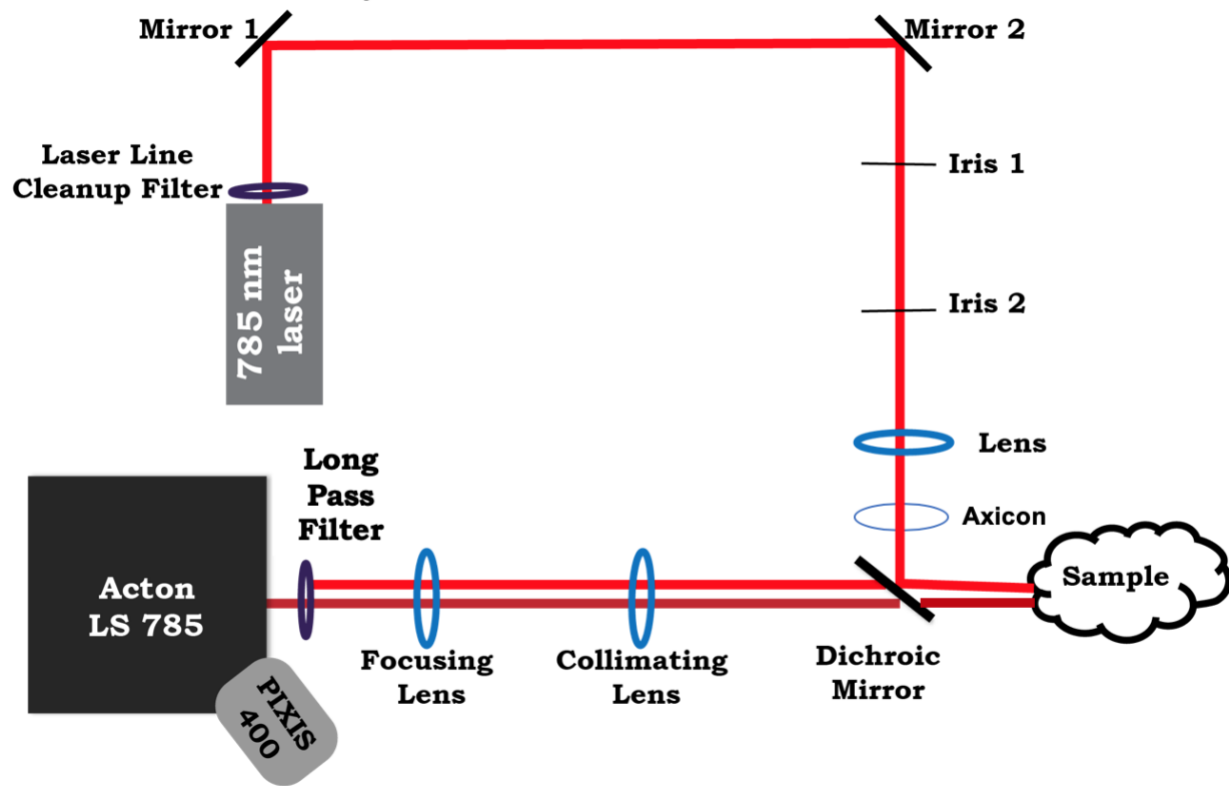


Figure 5: Macro-Raman Set-up

The first lens focuses the laser light onto the sample. The dichroic mirror serves two purposes. It directs the laser onto the sample, and it also serves as a long pass filter as it reflects some of the 785 nm light and allows the longer wavelength Stokes Raman photons through. However, it is not the best filter, so on the diagram the laser light is still shown going through the mirror. The collimating lens collects the Raman scattered photons and makes them travel parallel to the table so that they are incident on the focusing lens at the proper, perpendicular angle. The focusing lens and long pass filter serve the same purpose as on the Micro-Raman set-up, and the same type of CCD camera is used to detect the photons.

## B. Synthesis and Characterization of Gold Nanoparticles

Citrate-reduced colloidal, spherical gold nanoparticles (AuNPs) were synthesized according to the method described by Turkevich et al <sup>25</sup>. The LSPR of the nanoparticles was characterized using UV-Vis spectrophotometry. The LSPR of the nanoparticles is shown in Figure 6.

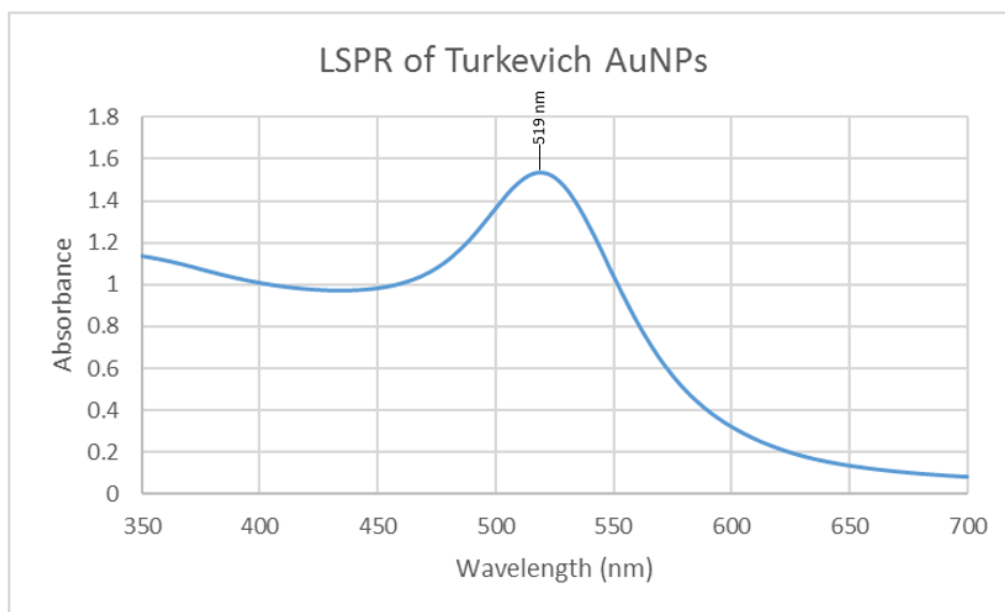


Figure 6: LSPR of Turkevich AuNPs at 519 nm

The nanoparticles were also characterized using scanning electron microscopy (SEM). An example SEM of Turkevich AuNPs are shown in Figure 7.

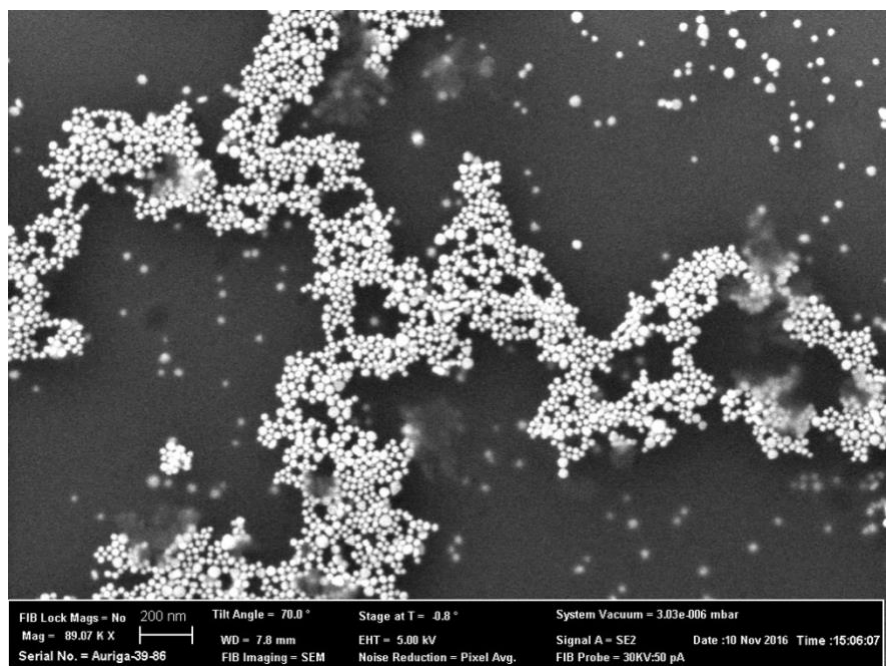


Figure 7: SEM of AuNPs

The AuNPs were tested for SERS activity with 100  $\mu$ M benzenethiol, whose normal Raman limit of detection is much higher than 100  $\mu$ M. The spectrum is shown in Figure 8.

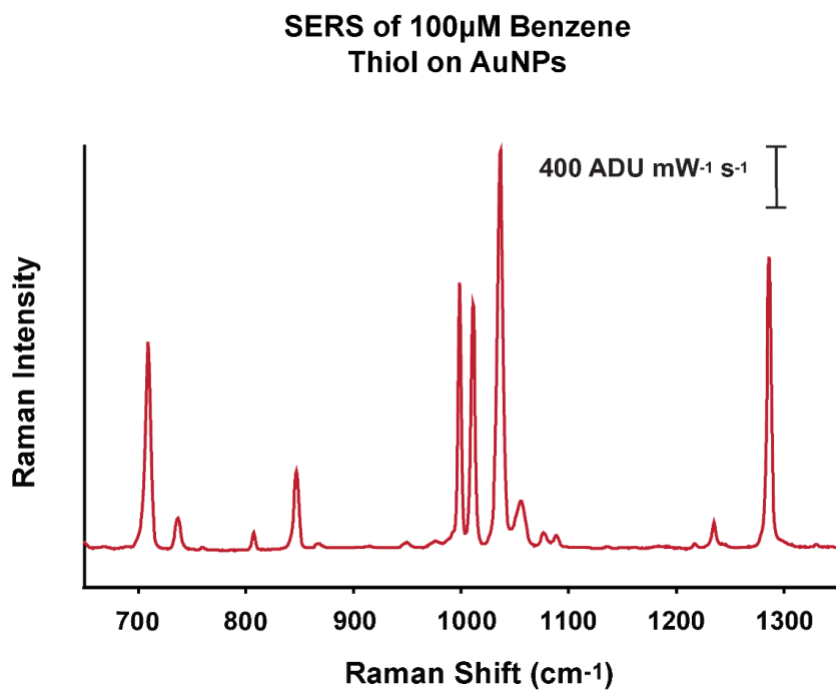


Figure 8: SERS of Benzenethiol at 785 nm with 5mW for 10s on AuNPs

### **C. Preparing Neurotransmitter SERS Solutions**

1 mL solutions of 500  $\mu$ M, 100  $\mu$ M, 50  $\mu$ M, 10  $\mu$ M, 1  $\mu$ M, 600 nM, 100 nM, 50 nM, and 1 nM were prepared for each neurotransmitter. Next, two 2 mL aliquots of AuNPs for each concentration of neurotransmitter were centrifuged at 4200 rpm for 15 minutes. The supernatant was removed, and 100  $\mu$ L of a neurotransmitter solution was placed onto the AuNPs and vortexed to return the AuNPs into solution. This was done twice for each concentration of neurotransmitter and combined, so each neurotransmitter SERS solution had 200  $\mu$ L of solution with 4 mL worth of AuNPs. The solutions were then taken to the Micro-Raman set-up. Immediately before taking the spectrum of each solution, the solution's pH was lowered with 0.75  $\mu$ L of 3 M hydrochloric acid. This helps protonate the neurotransmitters and electrostatically adsorb to the negatively charged AuNPs. A spectrum of only AuNPs in water was also taken. All spectra were taken at 785 nm at 50 mW for 60 seconds.

### **D. SORS of Teflon**

The SORS study utilized a fragmented deer skull bone, agarose gel, and Teflon. The 1.72-1.96 mm-thick deer skull bone was cleaned with 3% hydrogen peroxide for 24 hours to reduce fluorescence from residual organic tissue both on and in the bone. The agarose gel was prepared by adding 120 mg of agarose and 180 mg of sodium chloride to 20 mL of water and heated to boiling. Once boiling, 1 mL of the solution was pipetted into a segment of an ice tray and allowed to solidify. These gave uniform agarose gel slices of 2.8 mm thickness. On the Macro-Raman set-up, four offsets were used: 0 mm, 1.5 mm, 3 mm, and 7.5 mm. The three axicons used to create these offsets were 1°, 2°, and 5°. The spectra began with the Teflon behind the bone with no gel in between. Spectra at all four offsets were collected. Agarose

slices were added one at a time, and spectra at all offsets were taken. This process was repeated until no further gel slices would fit on the stage, which was at 25.2 mm of gel. Figure 9 shows the set-up with a 7.5 mm offset and 19.6 mm of agarose gel. All of the spectra were taken at 785 nm with 90 mW of power for 60 seconds.

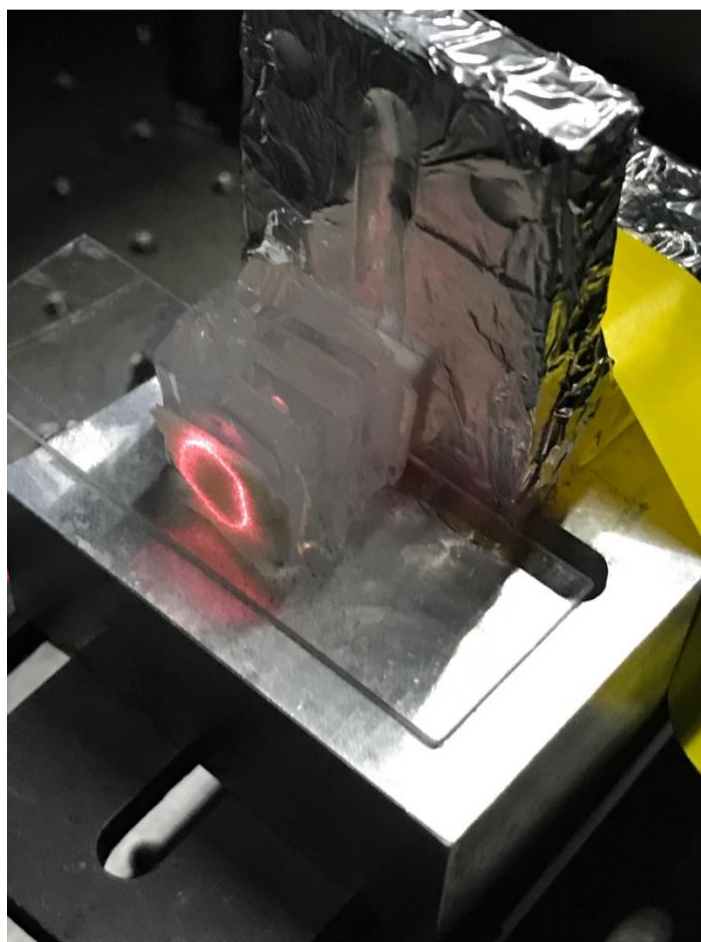


Figure 9: 7.5 mm offset on the bone with 19.6 mm of agarose gel

## **Results and Discussion**

### **A. Limit of Detection of Neurotransmitters**

The spectrum of AuNPs in water was subtracted from all spectra to isolate the peaks solely from the neurotransmitters. The limit of detection was determined qualitatively by identifying peaks from the neurotransmitters according to the peak assignments made in a study recently

published in the Sharma group, where quantitative determination of the limits of detection was also done<sup>21</sup>. Once no peaks were identifiable, the limit of detection was determined as the lowest concentration that still maintained peaks. One or two of the more prominent and consistent peaks were used. For melatonin the chosen peaks were at 905  $\text{cm}^{-1}$  and 1009  $\text{cm}^{-1}$ . For serotonin the chosen peaks were at 812  $\text{cm}^{-1}$  and 1221  $\text{cm}^{-1}$ . For epinephrine and norepinephrine, the peaks used were at 674  $\text{cm}^{-1}$  and 1370  $\text{cm}^{-1}$ , respectively. The results are shown in Figures 10-13. The chosen peaks are marked with a dashed line.

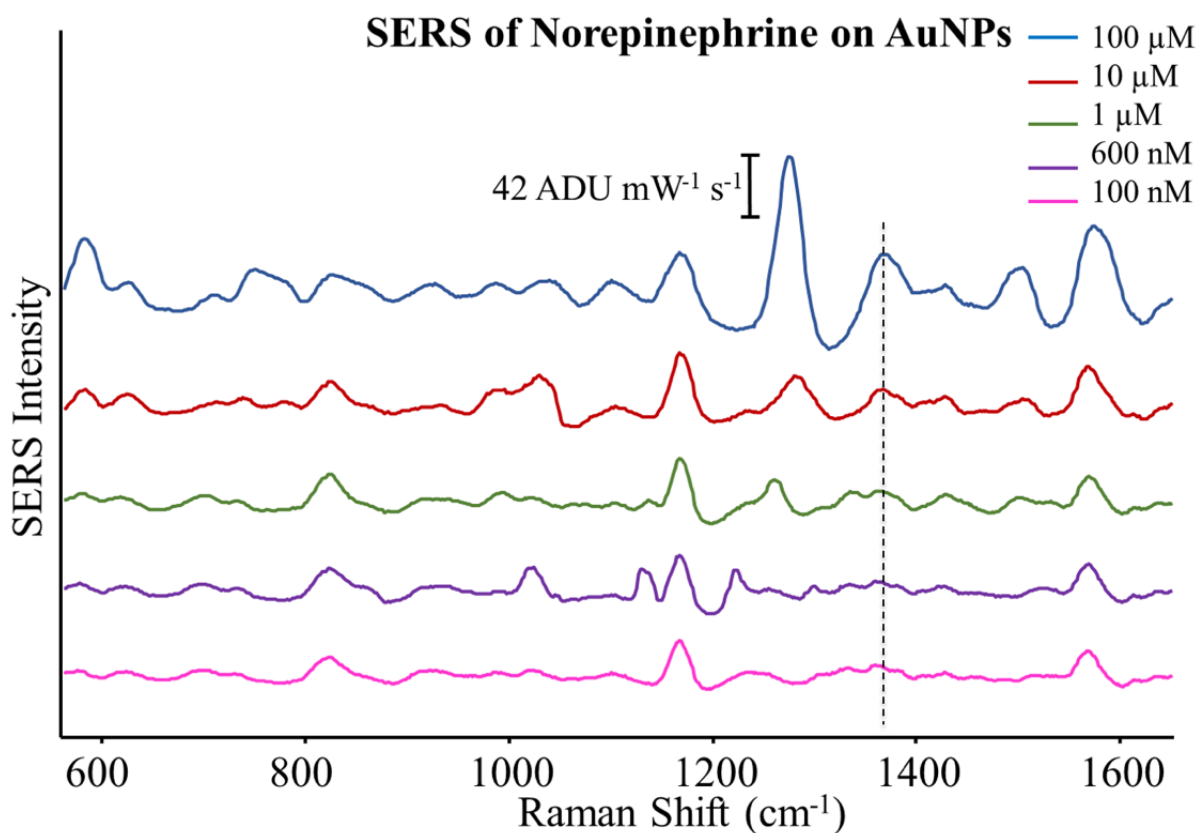


Figure 10: SERS of various concentrations of Norepinephrine on AuNPs

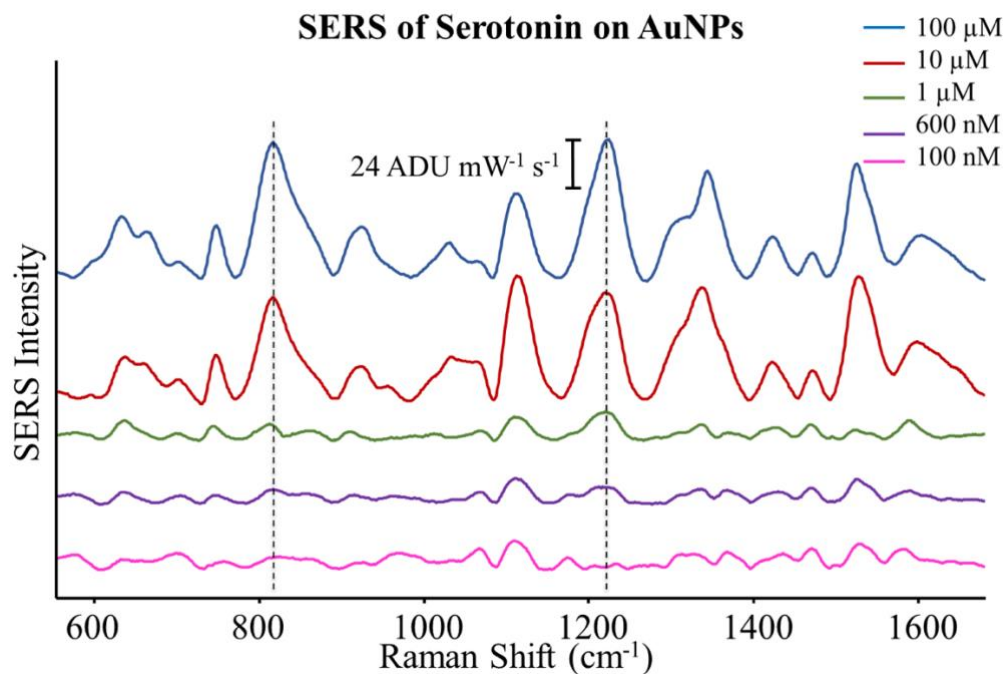


Figure 11: SERS of various concentrations of Serotonin on AuNPs

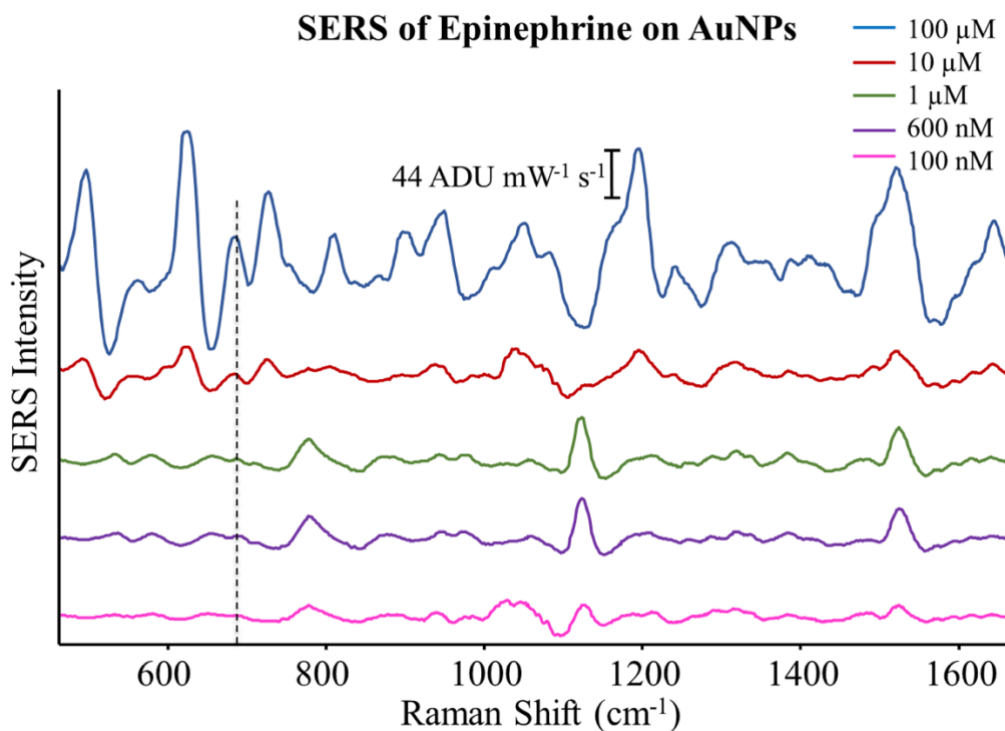


Figure 12: SERS of various concentrations of Epinephrine on AuNPs

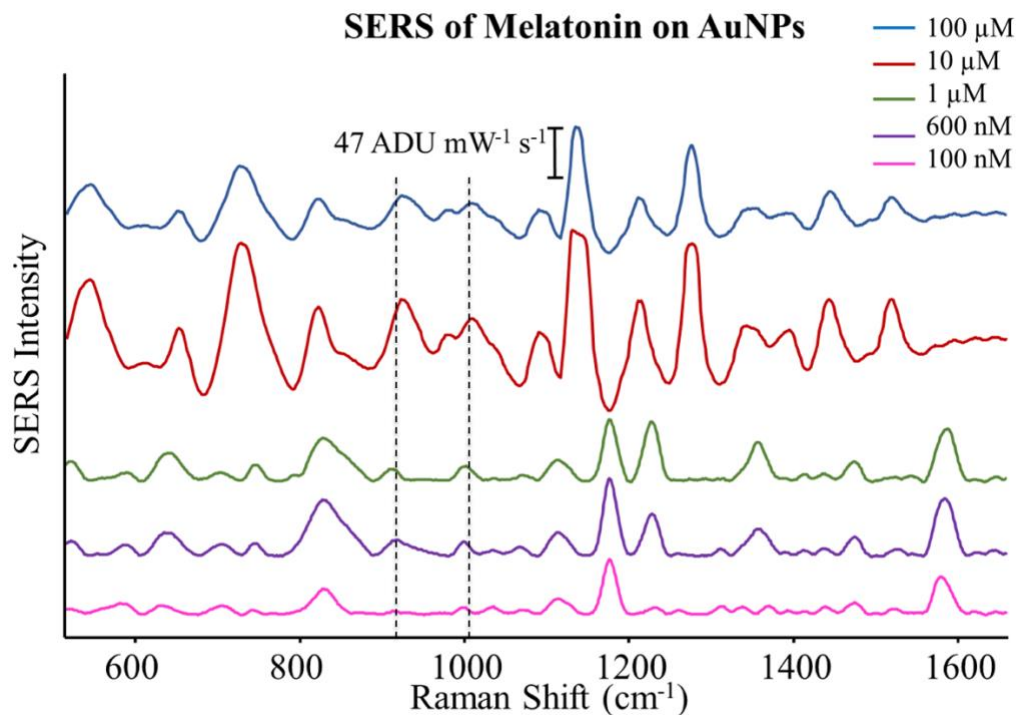


Figure 13: SERS of various concentrations of Melatonin on AuNPs

For norepinephrine in Figure 10, the peak at  $1370\text{ cm}^{-1}$  is very noticeable at  $100\text{ }\mu\text{M}$  and  $10\text{ }\mu\text{M}$ , and it is still present at  $1\text{ }\mu\text{M}$ . At  $600\text{ nM}$ , the peak cannot be distinguished, so the limit of detection was determined to be  $1\text{ }\mu\text{M}$ , which agrees with the literature <sup>21</sup>. For serotonin in Figure 11, the peaks at  $812\text{ cm}^{-1}$  and  $1221\text{ cm}^{-1}$  are clearly present at  $100\text{ }\mu\text{M}$ ,  $10\text{ }\mu\text{M}$ , and  $1\text{ }\mu\text{M}$ . At  $600\text{ nM}$ , both peaks have broadened but can still be distinguished. At  $100\text{ nM}$ , the peaks are not present at all, so the limit of detection was determined to be  $600\text{ nM}$ . The literature states the value of the limit of detection to be  $100\text{ nM}$  <sup>21</sup>. Figure 12 shows the most consistent epinephrine peak at  $674\text{ cm}^{-1}$ . It diminishes quickly and cannot be recognized in the  $1\text{ }\mu\text{M}$  spectrum, so the limit of detection was concluded to be  $10\text{ }\mu\text{M}$ , which agrees with the literature<sup>21</sup>. Figure 13 shows the slightly shifted peaks for melatonin. The shifted peaks at approximately  $905\text{ cm}^{-1}$  and  $1009\text{ cm}^{-1}$  are present through the  $600\text{ nM}$  spectrum but are not

distinguishable from the noise in the 100 nM spectrum, so a limit of detection of 600 nM was determined, which is higher than the 100 nM limit of detection in the literature<sup>21</sup>.

The biggest hurdle with this SERS detection of the neurotransmitters is the adsorption to the spherical nanoparticles. First, there are many different orientations that the molecules can take to adsorb, which leads to relative enhancement of different peaks and peak shifts, and second, the molecules must be in a low pH to adsorb effectively, and low pH can cause the nanoparticles to fall out of solution, due to neutralization of the surface charge that keeps the colloidal nanoparticles suspended in solution. In other words, the neurotransmitters do not adsorb well at physiological pH, which would make *in vivo* SERS very difficult. These challenges could be mitigated in several ways: (1) more complex shapes of nanoparticles could help with the adsorption of the neurotransmitters, or (2) we could functionalize the nanoparticles with antibodies or other capture molecules, which would specifically bind the neurotransmitter of interest. This is work currently on-going in the Sharma lab.

## **B. SORS Depth Study**

We were interested in probing thicker layers of bone to move SESORS sensing into a range that is closer to the physiological range of the human skull, which is 3-14 mm thick, as well as through thicker layers of tissue. We applied the 1.5 mm and 3 mm offsets to our system of bone, agarose gels, and Teflon and found that these offsets were not large enough to completely penetrate through the bone, so only the 7.5 mm offset was used for the depth study. Teflon's largest peak,  $734\text{ cm}^{-1}$ , was used for identification of Teflon. The 0 mm offset was subtracted from the 7.5 mm offset for each gel thickness in order to minimize the influence from the bone. Although the large offset helps to relatively detect more photons from the

Teflon, the absolute number of photons detected is greatly diminished, as photon migration is random and some photons escape, so a scaled subtraction of the 0 mm offset was performed. Figure 14 shows some of the 7.5 mm offset spectra without being subtracted in order to show the dominance of the bone peak at  $960\text{ cm}^{-1}$ .

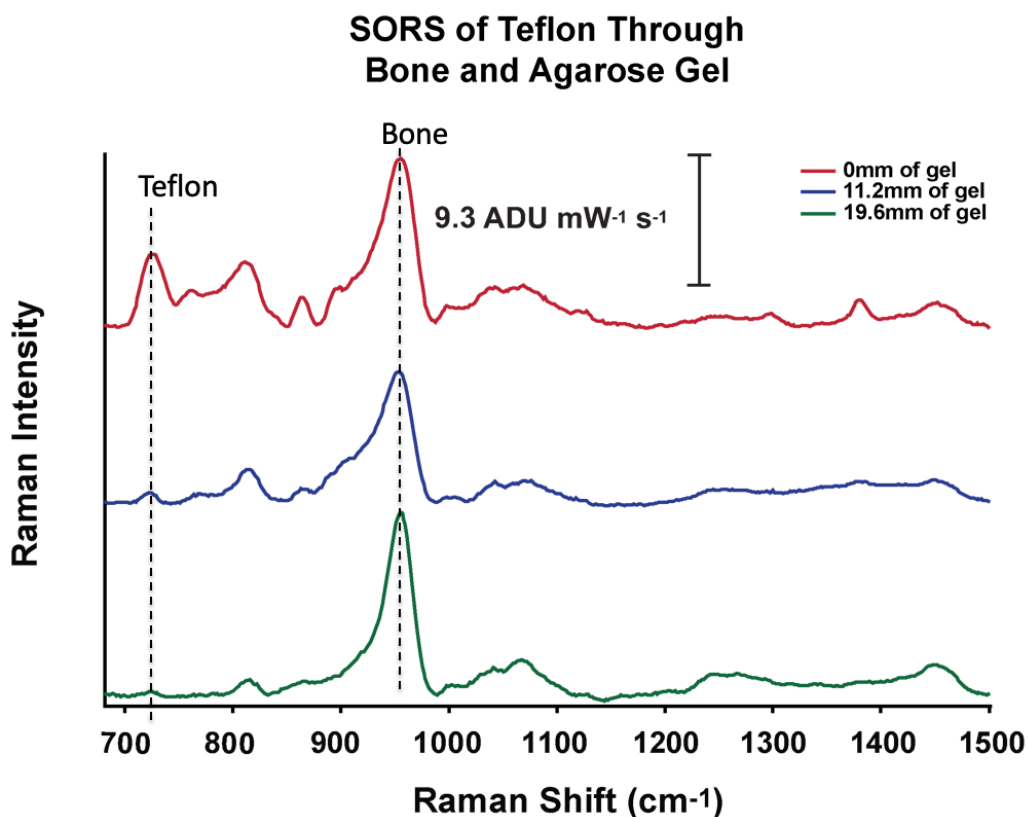


Figure 14: 7.5 mm offset through various thicknesses of gel

Although, the Teflon peak at approximately  $734\text{ cm}^{-1}$  is visible in all 3 spectra, it is much smaller than the bone peak. Figure 15 shows the scaled subtracted spectra.

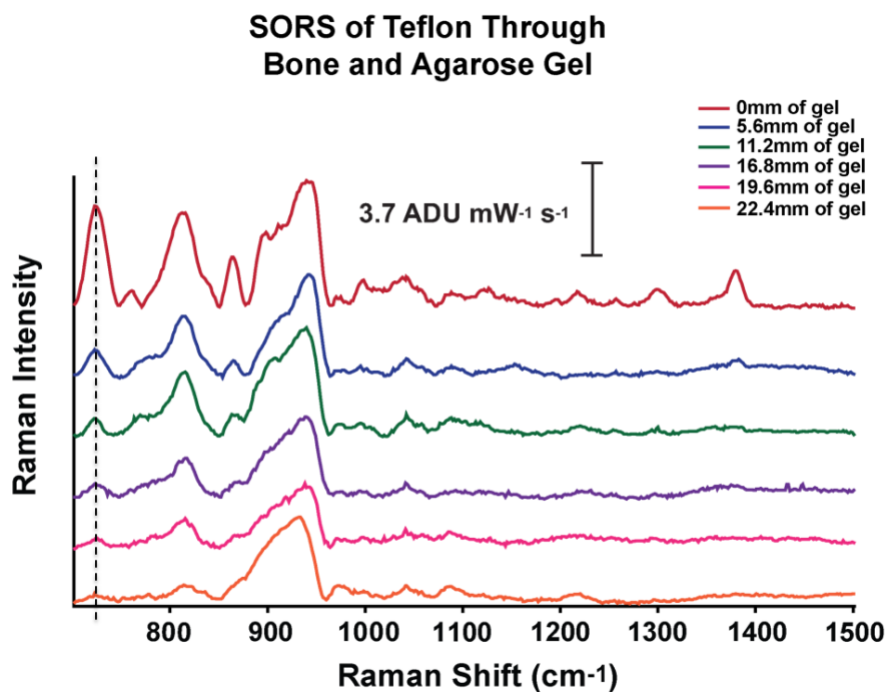


Figure 15: 7.5 mm offset subtracted spectra at various gel thicknesses

The main Teflon peak is much more clearly visible. Figure 15 shows a limit of detection of the Teflon to be 19.6 mm of gel. The peak at 810 cm<sup>-1</sup> is from the gel, and the large broad peak around 930 is from the shoulder of the original bone peak, which reveals an interesting phenomenon. At a larger offset, an overall blue-shift of the Raman scattered photons occurred. Both the Teflon peak and bone peak were shifted by multiple wavenumbers and broadened. The phenomenon is shown in Figure 16.

## SORS of Teflon Through Bone at Various Offsets

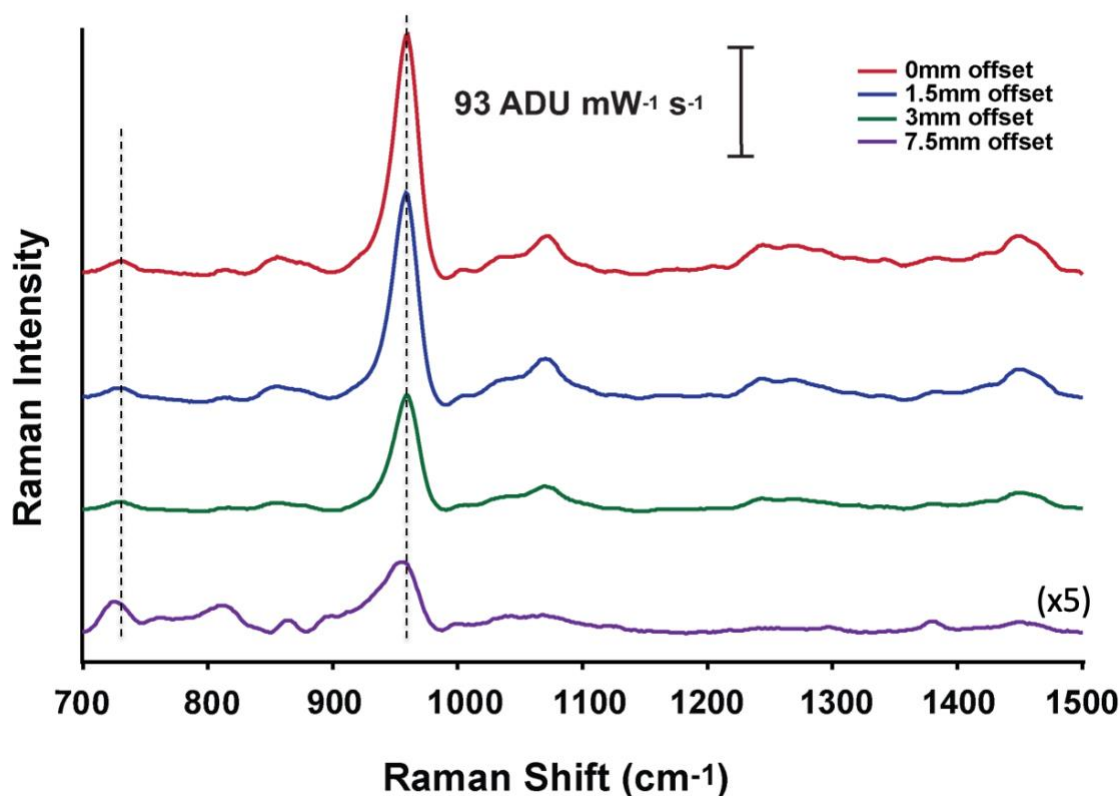


Figure 16: Peak shift of Teflon and Bone at larger offsets

The two peaks are noticeably broadened only to the left and slightly shifted left. This explains why Figure 15 has the broad peak at 930 cm<sup>-1</sup> because the scaled subtraction of the 0 mm offset spectrum did not have anything in the 930 cm<sup>-1</sup> region. This is an interesting phenomenon, which has not been previously demonstrated, as SORS should not show any peak shifts. The Sharma group is continuing to research the origin of this phenomenon.

The limit of detection at 19.6 mm of gel is most likely due to the loss of photons due to photon migration, not due to the absorption by the gel. Figure 17 shows the scattering properties of the gel.

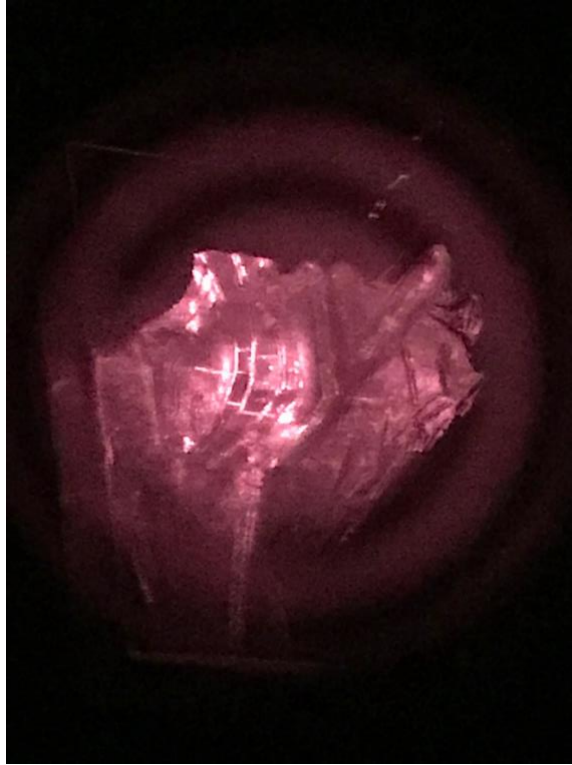


Figure 17: Laser light interacting with agarose gel

Figure 17 shows the agarose gel being illuminated by the laser at 0 mm offset. Although the scattering can only be seen by the naked eye because there is no bone in front, the same random migration is occurring with the bone present. As more gel is added, there is more diffuse scattering media to allow the photons to migrate. Photons are lost out of the gel in all directions, so less photons are getting to the Teflon to create Raman scattering, and the Raman scattered photons have more of a chance of migrating themselves and being lost out of the sides of the gel. Larger offsets lead to a decrease in detection of photons because the photons are already closer to the edges of the material, and the photons have a smaller chance of migrating to the center of the illumination ring for collection because it is farther away.

## **Conclusion**

The detection of neurotransmitters *in vivo* would greatly help for the early detection of many neurodegenerative diseases. Raman spectroscopy shows great potential in this endeavor. However, many challenges must first be overcome. First, the limit of detection for neurotransmitters using regular Raman spectroscopy is much higher than physiological concentrations. This study has shown that surface-enhanced Raman spectroscopy can lower the limit of detection of certain neurotransmitters into the physiological range. Certain other challenges such as adsorbing neurotransmitters onto nanoparticles at physiological pH are areas of future research. Also, neurotransmitters exist in the brain in mixtures of many molecules, so identifying individual neurotransmitters in mixtures and developing nanoparticles that are non-toxic to the brain would be another area of future work. Furthermore, all of this would also require removing part of the skull to probe the brain, which would be incredibly painful and dangerous. The ability to non-invasively see through the skull to detect Raman active molecules would solve that problem. Spatially offset Raman spectroscopy shows promise in this area. Utilizing photon migration, spatially offsetting the collection zone allows deeper media to be probed. This study showed that Teflon could be detected through a 1.72-1.96 mm deer skull fragment and 19.6 mm of agarose gel, a common brain mimic. Areas of future work with SORS include using more offsets and a thicker bone, detecting through skin and bone, and detecting through a less-flat, more rounded bone that would be found in a human skull. SERS and SORS show great potential to be used in tandem to help detect neurological imbalances of neurotransmitters to give patients an earlier and better diagnosis of neurological diseases.

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## References

1. Smekal, A., Zur Quantentheorie der Dispersion. *Die Naturwissenschaften* **1923**, *11* (43), 873-875.
2. Raman, C. V.; Krishnan, K. S., Polarisation of Scattered Light-quanta. *Nature* **1928**, *122* (3066), 169-169.
3. Keresztury, G. b.; Chalmers, J. M., Raman Spectroscopy: Theory. In *Handbook of Vibrational Spectroscopy*, 2006.
4. Harris, D. C.; Bertolucci, M. D., *Symmetry and spectroscopy : an introduction to vibrational and electronic spectroscopy*. Dover Publications: New York, 1989; p xii, 550 p.
5. Lam, Z.; Kong, K. V.; Olivo, M.; Leong, W. K., Vibrational spectroscopy of metal carbonyls for bio-imaging and -sensing. *The Analyst* **2016**, *141* (5), 1569-1586.
6. Engel, T.; Reid, P., *Quantum chemistry and spectroscopy*. 3rd ed.; Pearson: Boston, 2013; p xvi, 507 p.
7. Sharma, B.; Frontiera, R. R.; Henry, A.-I.; Ringe, E.; Van Duyne, R. P., SERS: Materials, applications, and the future. *Materials Today* **2012**, *15* (1-2), 16-25.
8. Moore, T.; Moody, A.; Payne, T.; Sarabia, G.; Daniel, A.; Sharma, B., In Vitro and In Vivo SERS Biosensing for Disease Diagnosis. *Biosensors* **2018**, *8* (2).
9. Fleischmann, M.; Hendra, P. J.; McQuillan, A. J., Raman spectra of pyridine adsorbed at a silver electrode. *Chemical Physics Letters* **1974**, *26* (2), 163-166.
10. Jeanmaire, D. L.; Van Duyne, R. P., Surface raman spectroelectrochemistry. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1977**, *84* (1), 1-20.
11. Haynes, C. L.; McFarland, A. D.; Van Duyne, R. P., Surface-Enhanced Raman Spectroscopy. *Analytical Chemistry* **2005**, *77* (17), 338 A-346 A.
12. Dieringer, J. A.; McFarland, A. D.; Shah, N. C.; Stuart, D. A.; Whitney, A. V.; Yonzon, C. R.; Young, M. A.; Zhang, X.; Van Duyne, R. P., Introductory Lecture : Surface enhanced Raman spectroscopy: new materials, concepts, characterization tools, and applications. *Faraday Discuss.* **2006**, *132*, 9-26.
13. Willets, K. A.; Van Duyne, R. P., Localized Surface Plasmon Resonance Spectroscopy and Sensing. *Annual Review of Physical Chemistry* **2007**, *58* (1), 267-297.

14. Etchegoin, P. G.; Le Ru, E. C., A perspective on single molecule SERS: current status and future challenges. *Physical Chemistry Chemical Physics* **2008**, *10* (40).
15. Matousek, P.; Clark, I. P.; Draper, E. R. C.; Morris, M. D.; Goodship, A. E.; Overall, N.; Towrie, M.; Finney, W. F.; Parker, A. W., Subsurface Probing in Diffusely Scattering Media Using Spatially Offset Raman Spectroscopy. *Applied Spectroscopy* **2016**, *59* (4), 393-400.
16. Ellis, D. I.; Cowcher, D. P.; Ashton, L.; O'Hagan, S.; Goodacre, R., Illuminating disease and enlightening biomedicine: Raman spectroscopy as a diagnostic tool. *The Analyst* **2013**, *138* (14).
17. Matousek, P., Inverse Spatially Offset Raman Spectroscopy for Deep Noninvasive Probing of Turbid Media. *Applied Spectroscopy* **2016**, *60* (11), 1341-1347.
18. Froud, C. A.; Hayward, I. P.; Laven, J., Advances in the Raman Depth Profiling of Polymer Laminates. *Applied Spectroscopy* **2016**, *57* (12), 1468-1474.
19. Sowoidnich, K.; Churchwell, J. H.; Buckley, K.; Goodship, A. E.; Parker, A. W.; Matousek, P., Photon migration of Raman signal in bone as measured with spatially offset Raman spectroscopy. *Journal of Raman Spectroscopy* **2016**, *47* (2), 240-247.
20. Stone, N.; Kerssens, M.; Lloyd, G. R.; Faulds, K.; Graham, D.; Matousek, P., Surface enhanced spatially offset Raman spectroscopic (SESORS) imaging – the next dimension. *Chemical Science* **2011**, *2* (4).
21. Moody, A. S.; Sharma, B., Multi-metal, Multi-wavelength Surface-Enhanced Raman Spectroscopy Detection of Neurotransmitters. *ACS Chemical Neuroscience* **2018**, *9* (6), 1380-1387.
22. Eliasson, C.; Macleod, N. A.; Matousek, P., Noninvasive Detection of Concealed Liquid Explosives Using Raman Spectroscopy. *Analytical Chemistry* **2007**, *79* (21), 8185-8189.
23. Eliasson, C.; Matousek, P., Noninvasive Authentication of Pharmaceutical Products through Packaging Using Spatially Offset Raman Spectroscopy. *Analytical Chemistry* **2007**, *79* (4), 1696-1701.
24. Afseth, N. K.; Bloomfield, M.; Wold, J. P.; Matousek, P., A Novel Approach for Subsurface Through-Skin Analysis of Salmon Using Spatially Offset Raman Spectroscopy (SORS). *Applied Spectroscopy* **2014**, *68* (2), 255-262.
25. Turkevich, J.; Stevenson, P. C.; Hillier, J., The Formation of Colloidal Gold. *The Journal of Physical Chemistry* **1953**, *57* (7), 670-673.