Drew University College of Liberal Arts

The Construction and Utilization of a Raman Spectrometer for the purpose of testing Surface-Enhancement

An Honors Thesis in Physics

by

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Raman scattering is a form of inelastic light scattering in which a photon loses some of its energy in a collision with a molecule. The difference between the wavelength of the incident light and the scattered light reveals the vibrational states of the molecule. As these vibrational states correspond to specific bonds between atoms in a molecule, the Raman spectrum can be used to identify molecules. There are several forms of Raman spectroscopy which attempt to enhance the signal, including surface-enhanced Raman spectroscopy (SERS). Raman spectroscopy is a very useful tool in the analysis of many different materials because samples require very little preparation. This means that Raman spectroscopy can be used in applications in which it is important to analyze a sample nondestructively. However, these applications frequently utilize high cost commercially available spectrometers which may not be accessible to many researchers. In order to overcome this obstacle, Raman spectroscopy systems have been constructed less expensively using an excitation source, various optical elements and a spectrometer. For this honors thesis, I constructed such a system and tested it with two spectrometers which offered varying degrees of ease of use and resolution of data. In order to construct the spectrometer, I used a 633 nm laser as an excitation source. The spectrometer was found to be able to obtain spectra from both solid and liquid samples. In order to test this spectrometer, aspirin, pyridine, benzene and naphthalene were used as samples. The set-up was then tested with a SERS substrate. The substrate was analyzed both with and without pyridine. In both cases, a spectrum was obtained which consisted of two intense peaks not in agreement with the spectra of pyridine. These peaks were investigated and do not match known background signals for these substrates as reported by their manufacturer. The exact nature of these peaks is still unknown.

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Raman Spectroscopy/the Raman Effect

The interaction of light with molecules is very important to the study of physics and chemistry. One of the ways in which light and molecules can interact is that the photons may have elastic or inelastic collisions with the molecules. In elastic collisions, the scattered light does not lose energy and therefore retains the same wavelength as the incident light [1]. This is called Rayleigh scattering. Rayleigh scattering is very common, in that the vast majority of light scattered from a sample is Rayleigh scattered and, for this reason, the same wavelength as the incident light. However, a tiny fraction of the scattered light is of a different frequency and wavelength to the incident light. This is what is known as Raman Scattering.

In 1928, Sir Chandrasekhara Venkata Raman made an important discovery [1]. Raman was interested in the blue color of the sky and ocean and as such he began experimenting with the scattering of light [2]. In an experiment with a mercury lamp, he observed discreet lines of the scattered light and was able to photograph the spectra of benzene and other liquids [2]. For this discovery, Raman won the Nobel Prize in physics in 1930. Raman also found that these spectra were “practically a description in spectroscopic form of the chemical composition of the molecule” and he believed that the bands in the spectra corresponded to the vibrational frequencies of the molecule [2]. In fact, these bands correspond to transitions between the vibrational states of the molecule [2].

Molecules are made of atoms which are bound to one another. These bonds can vibrate and these vibrations take place at specific frequencies known as group frequencies [4]. A molecule vibrating at one of these frequencies is said to be in one of its vibrational modes [4]. Each of these frequencies is specific to a certain bond moving in a particular way [4]. This means that if the group frequencies of a molecule can be determined, it is possible to determine the

molecular structure [4]. As each molecule will have a different collection of bonds and therefore will have different sets of vibrational frequencies, it is possible to identify molecules using their vibrations.

In the case of Raman Scattering, the electromagnetic field from the incident light interacts with the molecule, causing it to vibrate in one of its vibrational modes [3]. This scattering is shown in Figure 1.

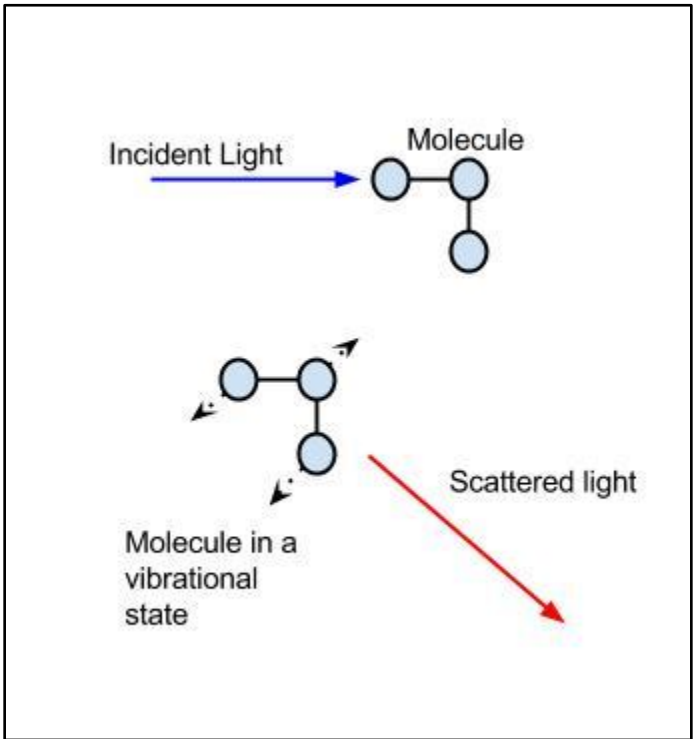

Figure 1: A photon of a certain wavelength interacts with a molecule. The molecule is raised to a vibrational state and the photon is scattered at a different wavelength.

The total energy, E of the incident light is given by the sum of the energy of the vibrational state E_{vib} and the energy of the scattered light $E_{scattered}$.

$$E = E_{vib} + E_{scattered} \quad (1)$$

Using this relationship in equation 1, it can be shown that the energy of vibrational states can be determined if the energy of the incident and scattered photons are known.

The electromagnetic field of a laser can distort the electron cloud within the molecule. This creates virtual energy levels which do not exist for the molecule when not in the presence of an electromagnetic field. A photon may raise a molecule initially in its ground state to this virtual state. It can then drop back down to an excited vibrational state with energy given by $h\omega_1$, where $\hbar$ is Planck's constant and ω_1 is the frequency of the vibration in the excited vibrational state . The laser has energy $\hbar\omega_0$, in which case, the scattered light has energy $E_{scattered}= \hbar(\omega_1- \omega_0)$ [3]. This process is shown in Figure 2 and is known as Stokes Scattering [3]. The molecule may also initially be in an excited state of energy, $\hbar\omega_1$, and scatter back to the ground state after being raised to the virtual state which gives the scattered light an energy of $E_{scattered}= \hbar(\omega_1+ \omega_0)$ as is illustrated in Figure 2 [3]. This is known as anti-Stokes Scattering [3]. As the photon loses energy after the collision with the molecule, Raman scattering is called inelastic scattering while Rayleigh scattering is elastic scattering.

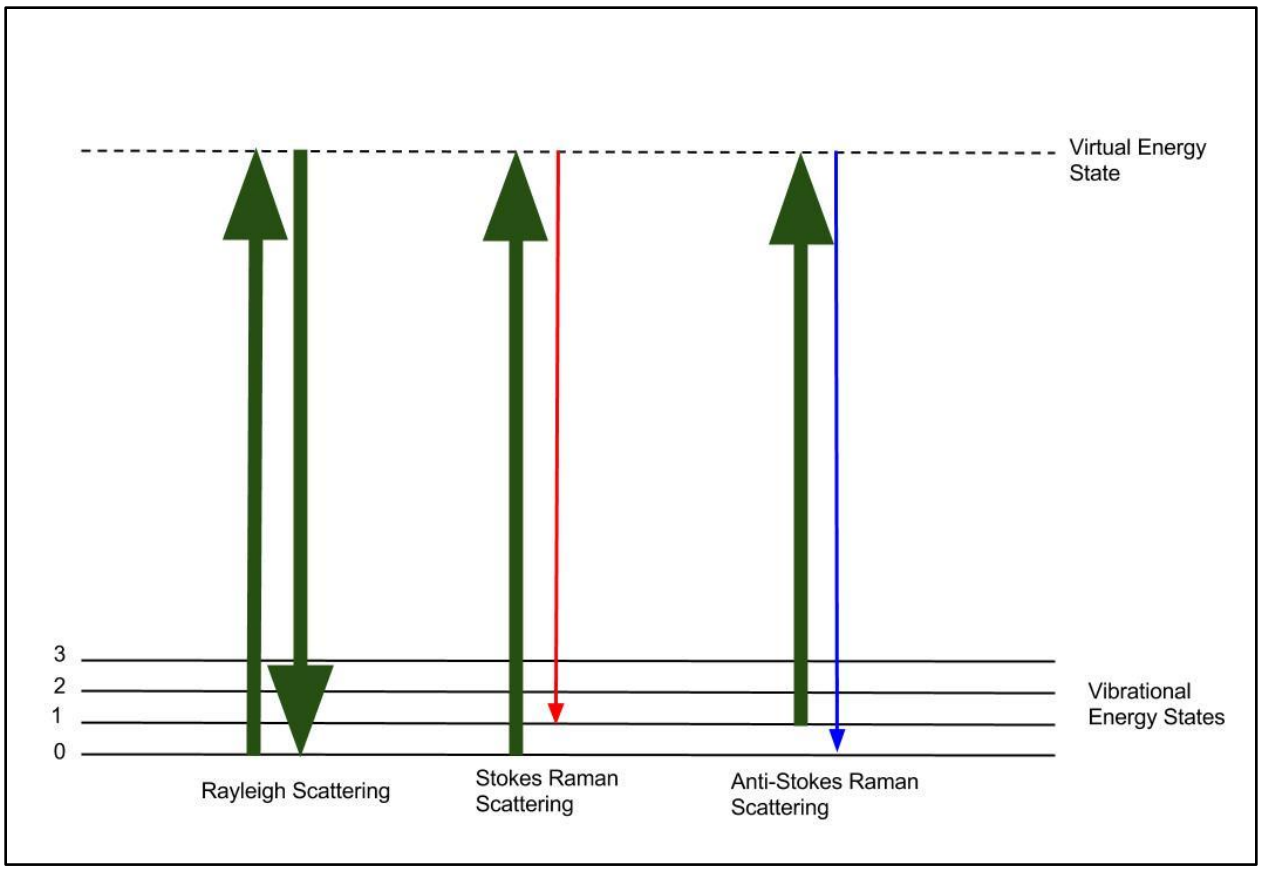

Figure 2: The mechanisms of Raman and Rayleigh scattering. On the left is Rayleigh scattering, an incident photon is scattered without losing energy therefore the scattered light is of the same wavelength as the incident light. In the center is Stokes Raman scattering in which an incident photon raises the molecule to a virtual state from which it drops into an excited vibrational state. As some of the energy of the photon is given to raise the state of the molecule, the scattered photon has a lower energy and longer wavelength. Finally, in anti-stokes Raman scattering, a molecule in an excited vibrational state is raised to a virtual state and drops to the ground state which gives energy to the photon. This scattered photon will have more energy than the incident light.

In order to perform Raman Spectroscopy, the scattered light must be collected and the wavelengths of the scattered light must be recorded. When the wavelength is plotted against the intensity, this should produce peaks at wavelengths which correspond to the frequencies of the vibrational modes of the molecule. From this spectrum, it is possible to identify the structure of the molecule. Obtaining a spectrum however is difficult because at most only 1 in 10^6 of the incident photons will be Raman scattered [4].

Raman and Infrared Spectroscopy:

Infrared (IR) spectroscopy can also be used to determine the composition of molecules; however, it differs from Raman spectroscopy in a number of important ways. To begin with, Raman spectra are created by the scattering of light while IR spectra are created when light is absorbed by molecules [3]. When the energy of the photon corresponds to the energy difference between vibrational modes, the IR light will be absorbed [3]. Raman and IR spectroscopy are complementary forms of spectroscopy as many bonds which are Raman active are IR inactive [8].

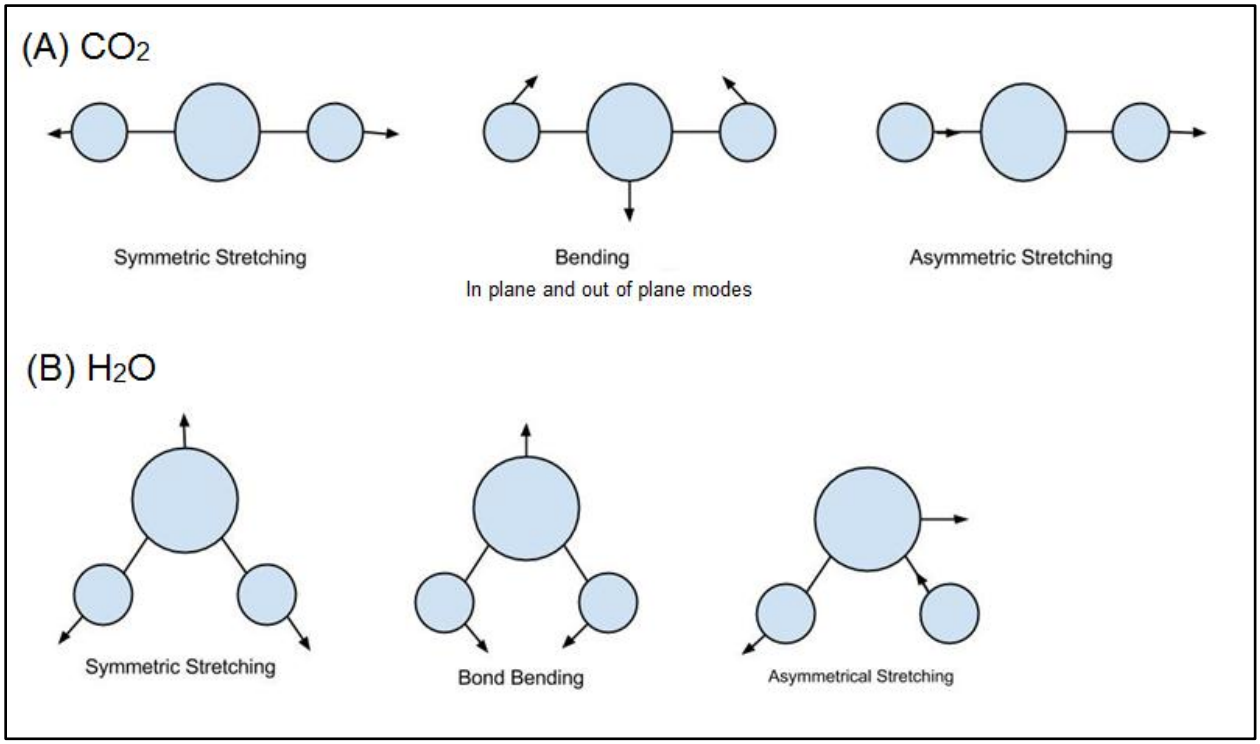

Figure 3: (A) vibrational modes of CO_2. As CO_2 is a molecule with a center of symmetry, the Rule of Mutual Exclusion applies so no modes are both Raman and IR active. (B) vibrational modes of H_2O. H_2O does not have a center of symmetry and does not obey the Rule of Mutual Exclusion..

In Figure 3, the vibrational modes of two different molecules composed of 3 atoms are illustrated. In order to determine the number of vibration modes of a linear molecule, it is necessary to use equation 2 [3].

$$Number\ of\ Vibrational\ Modes = 3n - 5 \qquad (2)$$

In this equation, 3n gives the number of degrees of freedom and the 5 which are subtracted away are rotations and translations [3]. For a 3 atom molecule like CO_2, this gives the 4 vibrational modes show in Figure 3 (A). The frequencies of these modes are unique to the molecule and are dependent on the bonds between the atoms [3]. Similarly, the number of vibrational modes of a non-linear molecule is given by equation 3.

$$Number\ of\ Vibrational\ Modes = 3n - 6 \quad (3)$$

For water, a non-linear molecule composed of 3 atoms, this gives 3 vibrational modes [3]. The set of these vibrational modes identify water uniquely [3]. The identification of these modes gives Raman spectroscopy its ability to identify molecules.

Different vibrations are active in Raman and IR spectroscopy because the two operate under different selection rules. One important rule is the rule of mutual exclusion which states that in molecules with a center of symmetry, no vibration will be both Raman and IR active [3]. A molecule has a center of symmetry if a line drawn from any atom through the center of the molecule will encounter an identical atom [4]. This rule applies to many molecules such as CO_2 as shown in Figure 3, and many other molecules such as benzene [4]. In a symmetrical vibration, such as the symmetrical stretching vibration shown in Figure 3, the polarization of the molecule changes as the electron cloud becomes distorted, however, the electric dipole is unaffected as the symmetry of the molecule remains, therefore, this mode is Raman active and IR inactive [3]. On the other hand, the antisymmetrical stretching creates an oscillating electric dipole however; this does not affect the polarizability so this mode is Raman inactive [3]. This illustrates the importance of Raman spectroscopy as a complement of IR spectroscopy for molecules with a center of symmetry.

An important difference between Raman and IR spectroscopy is that water has a very weak Raman activity. Therefore it is possible to take Raman spectra of samples dissolved in water while IR spectroscopy cannot be fully utilized as the water absorbs nearly all of the incident radiation of the IR at particular wavelengths. In addition, samples in Raman spectroscopy require very little, if any, preparation [3]. All of these advantages point to the importance of Raman spectroscopy in analyzing molecular structure and identifying molecules. However, Raman spectroscopy is limited in that the signal is quite weak and can be drowned out by the fluorescence of the sample [3].

Fluorescence

One of the major challenges in performing Raman spectroscopy is caused by fluorescence. Fluorescence occurs when the incident light is absorbed by a molecule raising it to an excited state. When the molecule returns to the ground state, it emits energy in the form of a photon as is illustrated in Figure 4 [3]. This light forms a broad spectrum without distinct peaks as the emitted light does not correspond to specific energy level transitions. This is because the molecule can be excited to any vibration state in the excited state [3]. The molecule can then drop down to any vibrational state in the ground state [3]. This means that there are no specific transitions and wavelengths form a broad spectrum [3].

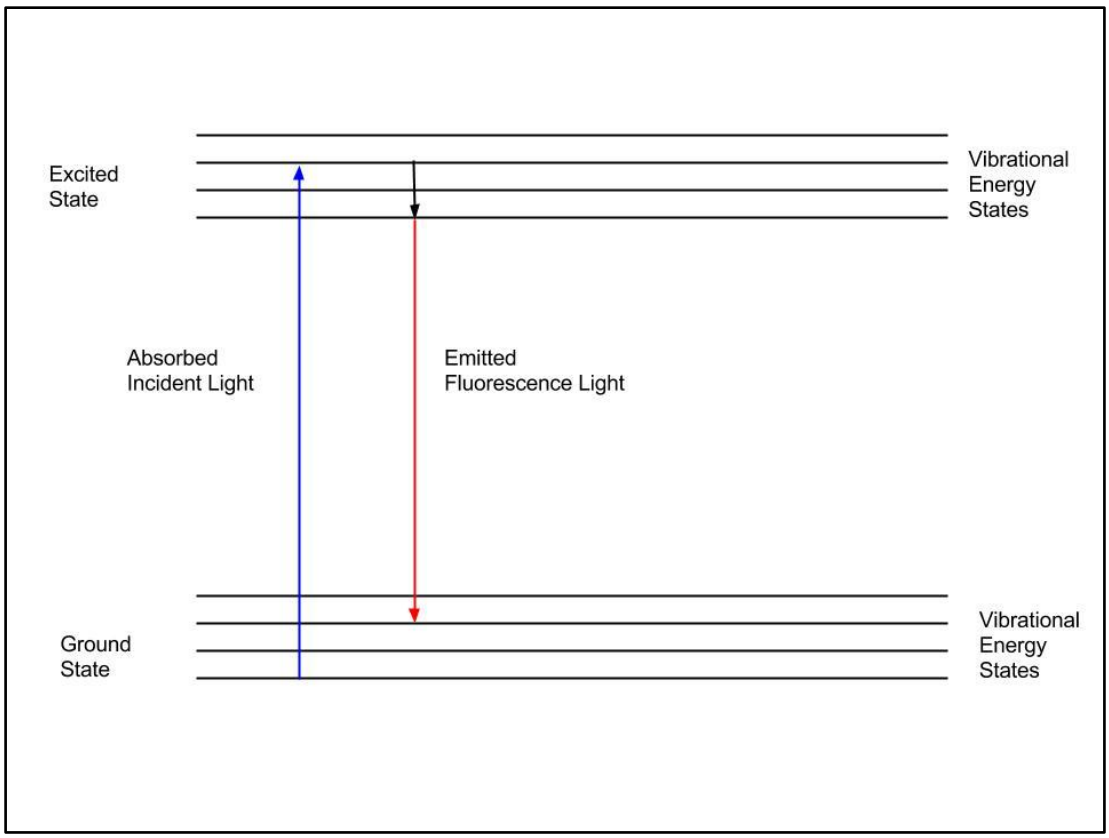

Figure 4: This figure shows the mechanism of fluorescence. An incident photon is absorbed raising the molecule to an excited state. The molecule then returns to the ground state emitting a second photon of a different wavelength.

With fluorescence being at least 10^6 times larger than the Raman signal this can become problematic in Raman spectroscopy [4, 5]. There are several techniques which may be used to reduce the fluorescence of a sample to the point where a Raman signal may be obtained [6]. One such method is exposing the sample to a laser source for a period of time which may range from minutes to days to cause decay of fluorescent molecules [4, 8]. As these fluorescent molecules are often impurities in the sample, their destruction does not affect the sample of interest [7]. This process is known as photo bleaching [7]. This process is illustrated in Figure 5.

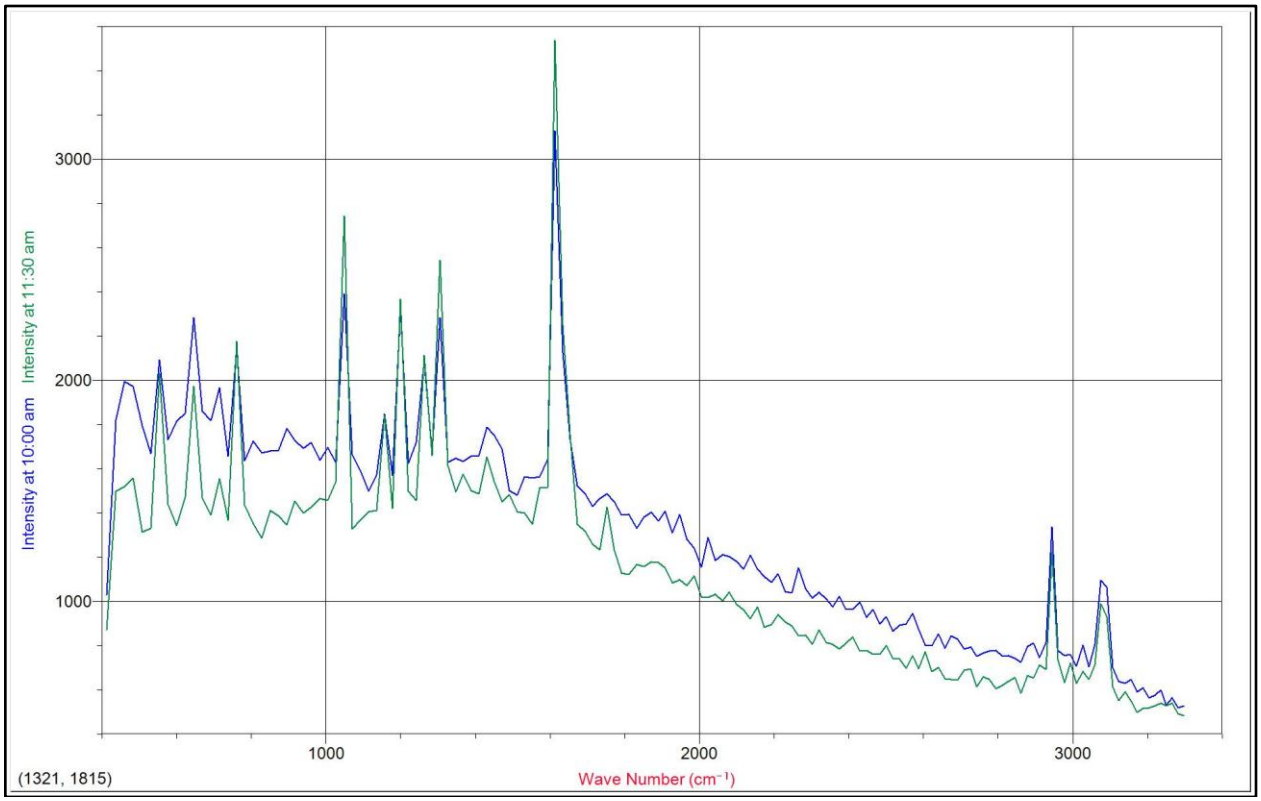

Figure 5: The Raman spectrum of aspirin was taken twice. The first spectrum (in blue) was taken an hour and a half before the second spectrum (in green). The sample was photo bleached in between the two runs. The fluorescent background is reduced in the photo bleached sample however; the peak intensities are unaffected.

Photo bleaching reduces the amount of fluorescence given by a sample while the Raman intensity remains the same which allows the Raman signal to be seen more clearly. As shown in Figure 5, after photo bleaching the background signal is reduced. Nevertheless, the Raman peaks maintain the same intensity which increases the signal to noise ratio. For this reason, Raman peaks are much more clearly defined.

Types of Raman

Stimulated Raman

Stimulated Raman is a form of Raman spectroscopy in which a sample is illuminated with a very high intensity laser of frequency ω_p [9]. When this very high intensity laser interacts with the sample, the intensity of the Stokes Raman Scattering becomes so great that the scattered light interacts with the incident beam and causes further scattering [9]. The high intensity

incident laser creates a high intensity Raman scattered beam [9]. This beam of Raman scattered light can then interact with molecules and cause further Raman scattering [9]. This process can increase the rate of Raman scattered photons from one in 10^{12} to as many as half of the incident beam being Raman scattered [9]. However, in stimulated Raman scattering, often only the strongest band will be visible [8]. This is because once the threshold for stimulated emission for one mode is reached all further increases in energy goes into that vibrational mode [8].

Resonant Raman

Resonant Raman takes place when the excitation line is near one of the electronic absorption bands [8].These are frequencies which correspond to the energy difference between energy levels of the molecule [3]. This means that the molecule will be raised to a real excited state rather than a virtual one [8]. In the case of Resonance Raman spectroscopy, the spectrum may shift however; the intensity is greatly increased making it useful for applications in which there may be low concentrations [8]. This is especially useful in biological and biochemical settings because many biological samples are difficult to obtain or are not very soluble [3]. Resonance Raman can be used to detect concentrations as low as 10^{-5} Molar (M) while regular Raman has a limit at around 10^{-3} M [3]. Resonant Raman is also useful in that the enhancement occurs around an electronic absorption band which means that certain areas of a cell, particularly colored areas, can be investigated [3]. This is because a highly colored sample will absorb certain wavelengths of light. Therefore, it is possible to use a laser which will correspond to a particular area [3]. A major drawback of Resonant Raman spectroscopy is that the laser may cause the sample to heat excessively which may decompose it [3].

Inverse Raman

In Inverse Raman Spectroscopy, a sample is irradiated with a laser of a specific frequency (ω_0) along with a second source which ranges from ω_0 to ω_0+4000 cm^{-1} which is the typical range for a Raman spectrum [8]. There will then be absorption at frequencies which correspond to the Raman active vibrational modes [8]. In this method, fluorescence does not cause problems as the spectra of obtained by measuring absorbed light rather than scattered or emitted light[8]. However, it is difficult to find applications for this method as the absorption is weak. In addition it is difficult to get the two lasers to coincide in both space and time [8].

CARS: Coherent anti-Stokes Raman spectroscopy

Coherent anti-Stokes Raman spectroscopy (CARS) is a form of Raman spectroscopy in which two lasers are used. One laser, referred to as the pump laser is of a fixed wavelength [3]. The second is a tunable laser set to the Stokes frequency [3]. The Stokes laser must scan through various wavelengths in order to obtain each vibrational mode of the sample [3]. A photon is emitted from the pump laser which brings the system to a virtual state [3]. The molecule then returns to an excited vibrational mode and a photon is Raman scattered with the Stokes frequency [3]. A second photon from the pump laser brings the system to another virtual state where it can emit back down to the vibrational ground state [3]. This process is shown in Figure 6.

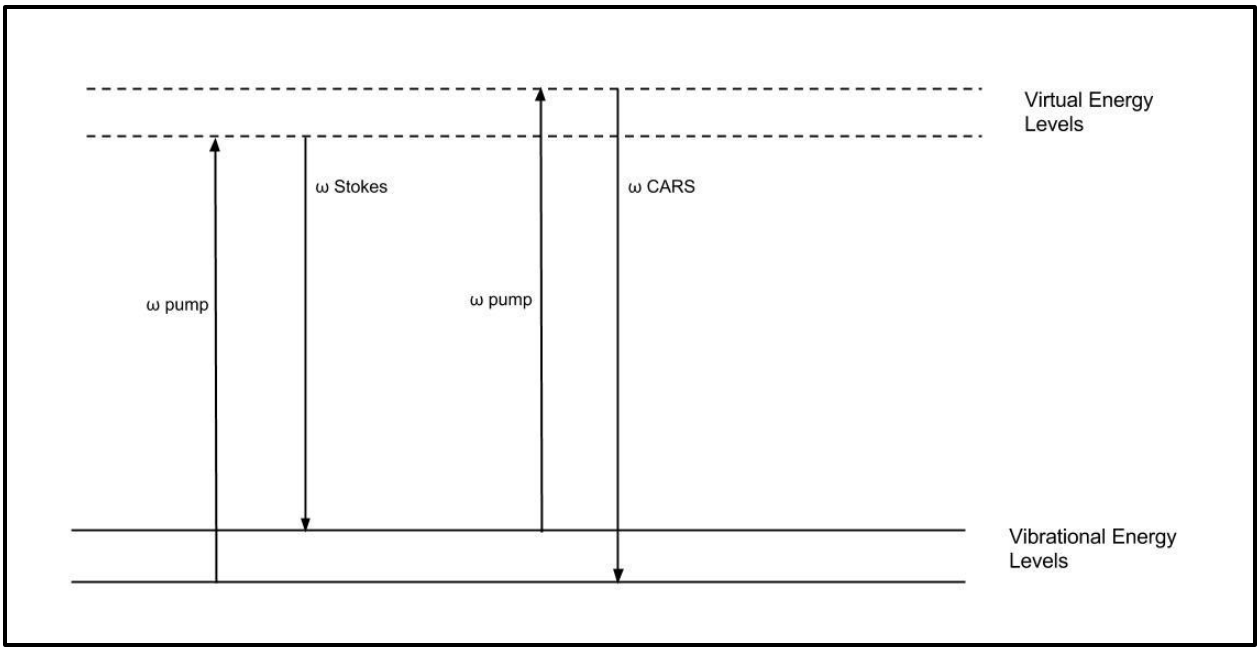

Figure 6: Coherent Anti-Stokes Raman Scattering. In CARS, a pump laser, (ω pump) and a laser at the Stokes frequency (ω stokes) are used to populate an excited vibrational state with molecules. A second photon from the pump laser then raises the molecule to a virtual energy level. The molecules then return to the ground state, emitting light at the anti-Stokes frequency.

Since both the pump and Stokes frequency lasers are of high power, there are many molecules which have been raised to an excited vibrational state [3]. Since this is the transition from an excited vibrational state to the ground state, the Raman lines which appear in the spectrum will be anti-Stokes lines. CARS is useful as it is extremely sensitive and can detect concentrations as low as 10^{-3} M [3]. CARS is commonly used in applications where the molecular structure of gasses is of interest such as in combustion processes [3].

SERS: Surface Enhanced Raman Spectroscopy

In 1974, M. Fleischmann, P.J. Hendra, and A.J. McQuillan published a paper in which they found that a sample of pyridine which had been adsorbed onto a silver electrode produced a much larger Raman signal than found in pyridine alone [10]. They also found that the spectrum shifts from that of a pure liquid sample. For example, they found the peaks corresponding to vibrational modes of pyridine were at 991 and 1030 cm^{-1} in a liquid sample [9]. However these peaks shifted to 1008 and 1037 cm^{-1} for pyridine which had been adsorbed on a silver electrode

[9]. This enhancement effect has come to be known as Surface Enhanced Raman Spectroscopy (SERS). The exact cause of this enhancement effect is not known, but the prevailing theory is that there is a combination of an electromagnetic effect and a chemical effect with the electromagnetic effect being dominant.

It is thought that the electromagnetic effect comes from the electromagnetic field of the laser interacting with surface plasmons of the metal nanoparticles [11]. Surface plasmons are electrons at the surface of a metal which oscillate together. This causes the electromagnetic field around the metal to extend to as much as 20 nm from the surface of the metal. This increases the Raman signal [8] by a factor of 10^4 on average. However there are areas on the surface called "hot spots" where the enhancement may be increased by as much as 10^{11} for single molecules due to nanoparticle junctions [8]. This enhancement is site specific [12]. There is also an enhancement effect when the incident light has the same frequency as the resonant frequency of the surface plasmons [9]. This means that SERS enhancement is dependent on the wavelength of the laser and metal used.

As SERS creates a much larger signal than regular Raman spectroscopy, it can be used in situations where only a small amount of the substance to be detected is present.

Applications:

Raman Spectroscopy has a number of different applications. As Raman spectroscopy determines the molecular structure of a material, it is used in settings where it is important to determine the composition of a material. This can range from tests of food quality and safety [9, 10] to forensic science [15] and security [16] to determining the materials used in paintings [17]. The reason for the popularity of Raman Spectroscopy is the ease of preparation of samples as well as the fact that it does not destroy or contaminate materials [13]. For some applications,

such as the detection of explosives, it is also important that the spectroscopy can be performed at a distance [18].

Food

One of the advantages of Raman Spectroscopy is that it can be used to detect structural differences between different molecules. This is useful in questions of food quality as the spectrum from a pure product will differ from that one that has been adulterated. Ismail Hakkai Boyaci et al. demonstrated the value of this in a study in which it was shown that Raman spectroscopy could be used to differentiate between horsemeat and beef and beef to which horsemeat had been added [13]. In this study, meat was taken from both horses and cows and the fat was extracted [13]. Raman spectra from the samples showed that there were differences between the beef and horsemeat. In particular, a 974 cm^{-1} band attributed to =C-H out-of-plane deformation and 1015 cm^{-1} band attributed to antisymmetric phosphoryl stretching appeared in horsemeat only [13]. From this result, it is possible to determine whether or not a sample has come from beef or a horse. Horsemeat is cheaper to produce, however the horses used for meat are often old and their meat lacks nutrients beneficial to humans [13]. This study also tests whether Raman spectroscopy is capable of detecting whether horsemeat has been added into beef [13]. To this end, four samples were prepared in 0%, 25% 50%, 75% and 100% horsemeat [13]. Raman spectra were taken of all samples and the results were analyzed and it was shown that it was possible to differentiate between these samples [13]. Raman is a powerful tool in this setting as it does not require large samples and it does not cause alterations [13]. This has implications for the use of Raman spectroscopy in food quality and safety. It is capable of detecting differences between similar products and these differences can then be used to determine whether products have been adulterated. This process is fast and does not require excessive sample preparation or large samples making it ideal for product quality testing [8, 9].

In another study, Raman was used to detect whether butter was adulterated through the addition of margarine [12]. This is a common occurrence as margarine is significantly less expensive to produce than butter, so it is cheaper to adulterate butter with margarine [12]. There are currently other methods for analyzing food products such as liquid or gas chromatography, mass spectroscopy, or nuclear magnetic resonance however; these methods are time consuming or difficult to perform [12]. On the contrary, Raman spectroscopy requires no chemicals, is relatively simple to prepare and preform and gives an analysis quickly [12]. This means that if Raman spectroscopy is capable of detecting the presence of margarine in a sample of butter, it would simplify the process of quality control of butter. This study found that there are notable differences in the spectra of butter and margarine [12]. These differences were evident in the peaks which appeared in the spectra. In particular, the peak corresponding to C=C stretching is much more intense in margarine samples than butter due to the lower concentration of unsaturated fatty acid in butter. In addition, the margarine (and the margarine adulterated samples) had higher fluorescence than butter due to the added ingredients [12]. The margarine spectra were also shown to vary in intensity and distinctiveness between brands due to the fact that margarine can have different oil compositions [12]. On the other hand the butter samples did not vary across brands because butter has a very specific composition (82% milk fat, low water and low salt) and there are not additional ingredients [12]. This study found that as margarine concentration increased some of the peaks (at 608 and 730 cm^{-1}) that were found in the butter spectrum began to disappear [12].

Security

Raman spectroscopy can also be used to detect dangerous substances. One of the uses which takes advantage of the ability of Raman spectroscopy to detect the composition of a material at a long distance is explosives detection [17]. In the years since September 11th, 2001,

the United States has made great progress in the use of Raman spectroscopy in the identification of explosives in the field [14]. A Raman spectrometer has been built which can be used to test for explosives from up to 12 meters away [17]. Raman is useful in this kind of application as it is capable of investigating many kinds of samples (solid, liquids and gases) as well as detecting many different kinds of chemicals [14]. In explosives detection, certain groups such as the nitro-group, which has a history of being used as an oxidizer in explosives, it was able to be identified [14]. This functionality can be quantified in a range of explosive compounds [14]. The nitro group has a symmetrical vibrational mode (between 1270 and 1375 cm^{-1}) and an anti-symmetrical mode (between 1450 and 1600 cm^{-1}). Raman requires minimal sample preparation so samples can be tested quickly and nondestructively. Thus samples may be preserved and subjected to further analysis [14]. Sample sizes may also be quite small which allows for the detection of traces of explosives [17]. Also of value is that Raman spectroscopy can be performed through translucent materials meaning that security personnel could perform an initial analysis of substances through packing without opening it, and so they can reduce risk [14]. Portable instruments have been invented which allow results in seconds or minutes [14].

Raman spectroscopy can also be used to detect drugs. The various drugs of abuse have different molecular structures and therefore, will produce different Raman spectra [15]. The larger the differences in molecular structure, the larger will be the differences in the frequency or intensity of the peaks in the Raman spectra. However, in the case of drug detection, there are often numerous drugs which belong to the same family and therefore have similar structures [15]. The identification of drug samples can also be complicated by added ingredients such as cutting agents [15]. One drug which has been successfully identified with the use of Raman spectroscopy is ecstasy (MDMA) [19]. In a study by Bell et al., both street quality ecstasy and

synthetic ecstasy, which was produced by the authors, were analyzed [19]. These samples were used in order to obtain spectra [19]. It was found that among the seized drug tablets from the streets, there were differences both between tablets and between different points on the same tablet [19]. It was also found that even with these variations, it was possible to obtain spectra in 60 seconds per tablet with acceptably low sampling errors [19]. This means that law enforcement officials can obtain information about possible patterns about criminal drug manufacturing and the identity of a drug sample in very little time while preserving the sample for further analysis or as evidence [19].

Furthermore, Raman spectroscopy can be used to detect biological weapon agents [20]. A well-known example of one such agent is the anthrax spore. These spores come from bacteria and are capable of surviving for a long time and in many environments [20]. The spores are dangerous in that they cause the disease anthrax which requires treatment within 24 to 48 hours of exposure [20]. Thus quick detection of spores is necessary. The anthrax spores contain calcium dipicolinate (CaDPA) which account for around ten percent of their dry weight. This is a good marker to detect the spores [20]. The two traditional methods for detecting anthrax spores are polymerase chain reaction (PCR) and immunoassays [20]. However, PCR requires expensive reagents and molecular fluorophores as well as extensive sample processing before analysis [20]. Immunoassays can analyze samples more quickly, but requires specific antibodies [20]. Raman spectroscopy provides solutions to both of these problems in that it can analyze samples quickly and gives detailed chemical information which can be used to identify the sample [20]. It is also better suited to this application than IR spectroscopy as Raman works well in aqueous solutions [20]. SERS is primarily used in this application as it requires lower laser power and can then be taken into the field in a portable spectrometer [20]. SERS is especially useful as it has been

shown to be able to detect single spores [20]. This is especially promising as humans do not contract anthrax in concentrations until they are exposed to at least 10^4 spores [20]. SERS can be used to detect the CaDPA peak, which is found in anthrax spores, in a time frame as short as 1 minute to detect anthrax spores which were well below the infection dose [20]. This means that Raman spectroscopy can be used to detect anthrax spores in the field, quickly and at all levels which could be dangerous to humans.

Forensics

Raman spectroscopy could be a useful tool in the forensic sciences due to the fact that Raman does not destroy the samples. One application which is currently being explored is the use of Raman Spectroscopy in identifying cosmetic smears found at crime scenes [21]. Cosmetic smears are commonly found at crime scenes on clothing, bedding or other surfaces [21]. If the brand of make-up used can be identified, it can be used to link a suspect to a victim or to a scene, to help reconstruct the crime scene or to confirm witness testimonies [21]. There are other techniques which may be used to test cosmetic samples; however, these methods each test for various components of the cosmetics' composition, so it is necessary to utilize a combination of techniques [21]. Raman spectroscopy is able to analyze very small samples and as portable Raman spectrometers have been developed, it is possible to perform this analysis in the field [21]. In a study by P. Gardner et al., it was found that Raman spectroscopy is capable of differentiating between lipsticks of the same color but from different brands [21].

In the field of forensics, gunshot residue is another important kind of evidence. Gunshot residues are the particles which are created when a firearm is used [16]. These particles can provide evidence which can link a suspect to a crime scene, identify whether a gun was fired or identify a bullet hole [16]. Traditionally, gunshot residue has been analyzed using scanning electron microscopy coupled with energy dispersive x-ray analysis to look for certain heavy

metal particles however, recently new ammunition including lead-free ammunition, which has been released into the market which complicates the identification of gunshot residue using this technique [16]. Raman spectroscopy was investigated as a possible alternative with the limitation that the gunshot particles must be macroscopic. This usually means they must be found at a short distance from the firearm [16]. In order to be a useful technique in identifying gunshot residue, Raman spectroscopy needs to be able to link the residue to unfired ammunition as well as distinguish between gunshot residue and particles which are not gunshot residue [16]. Lopez-Lopez et al. performed a study in which two different kinds of ammunition (SB-T93+, SB 96+) were shot from the same gun at short distances [16]. The gunshot residue was then analyzed using Raman spectroscopy. The ammunition types used had different stabilizers in the gunpowder which was apparent in their Raman spectra [16]. The SB 96+ had a strong band at 1342 cm^{-1} which corresponded to 2-nitrodiphenylamine, a stabilizer found in the SB 96+; this band was not seen in the SB-T93+ as it did not contain 2-nitrodiphenylamine [16]. This shows that Raman spectroscopy is capable of differentiating between different kinds of ammunition provided that the particles are sufficiently large to perform Raman spectroscopy.

Art

Raman spectroscopy is an important technique in the world of art due to its ability to differentiate between various materials without damaging them. One way in which this is utilized is in identifying whether a material is genuine. A particular case of this is differentiating between real lapis lazuli and fake lapis lazuli [23].This is important as a new fake lapis lazuli which is so good that it cannot be differentiated visually from real lapis lazuli has found its way to markets [23]. Other techniques such as acid tests, applying acetone or using a blowtorch can cause damage to both real and fake samples which should be avoided if possible [23]. Lapis lazuli is a blue stone which is valued for its intense color. However, as it is not a crystalline mineral, it does

not have an exact chemical composition. The pigment which is responsible for the blue color is known to be ultramarine which does have a known chemical composition [23]. Raman spectra of lapis lazuli have shown that there are three Raman-active vibrations which are found at 549, 585 and 259 cm^{-1} which correspond to the symmetric stretching, asymmetric stretching and deformation modes respectively [23]. The spectra of lapis lazuli changes based on the excitation laser used. Thus the spectra found using 1064 and 785 nm excitation produced different spectra [23]. The spectra of lapis lazuli and the spectra of a fake lapis lazuli were different at both wavelengths [23]. This shows that Raman spectroscopy can be used to differentiate between real and fake samples of lapis lazuli which is promising as Raman spectroscopy is non-destructive and fast with results being obtained in 10 seconds [23].

Building Raman Spectrometers:

Raman spectrometers are quite expensive to purchase with commercially available Raman spectroscopy systems ranging costing tens of thousands of dollars [23]. These models include all of the components necessary to perform Raman spectroscopy including a spectrometer, laser excitation source, a probe and the necessary software to obtain spectra from samples [24]. However; the price may be a barrier for some. It is also possible to construct a Raman spectroscopy system from parts [21, 22]. One such spectrometer was constructed using a small nitrogen laser, a monochromator with photomultiplier tube, and various lenses [25]. These elements are all commonly available or easily purchasable and inexpensive [25]. In order to obtain spectra, the laser beam was directed and focused onto a sample using a small mirror and focusing lens [25]. The scattered light was then collected and refocused into the monochromator [25]. This measured the intensity of the light through a range of wavelengths [25]. This data was sent to a computer where the intensity was plotted against the frequency [25]. In order to test the accuracy of this spectrometer, several samples were selected which have well known Raman

spectra and it was found that the spectrometer was capable of obtaining spectra which corresponded to those found in the literature [25]. A second simple Raman spectrometer was constructed using a laser pointer as the excitation source [26]. This spectrometer consisted of a green laser pointer which was directed by a small mirror through a focusing lens onto a sample [26]. The light was then collected and passed through a long pass filter which blocks light of the same wavelength of the incident beam and all shorter wavelengths thereby removing Rayleigh scattered light [26]. Finally, the scattered light was then focused onto a fiber optic cable which brought the signal to a charge coupled device (CDD) array which measured the intensity of light through a range of wavelengths [26]. Again, the data was presented graphically on a computer as a plot of intensity versus wavelength [26]. Samples of benzene, aspirin and liquid water were used to test the spectrometer [26]. All of these samples have well studied Raman spectra [26]. The spectrometer performed well, obtaining spectra which were in good agreement with calculated spectra [26]. The construction of these spectrometers shows that spectrometers built from parts are capable of producing reliable spectra for considerably less cost than a commercially available spectrometer. It was found that these spectrometers which were constructed were capable of obtaining spectra which were comparable to the literature [22, 23]. These spectrometers cost significantly less, (less than $5000 [25] compared to between $24,000 and $35,000 [23]).

In addition to these spectrometers, it has also been shown that surface-enhanced Raman spectroscopy (SERS) may also be performed using a low cost set up constructed in the laboratory [28]. A SERS system was constructed using a laser pointer as the excitation source [28]. The light from the laser was directed using a fiber optic probe onto a sample [28]. The scattered light was then collected by a fiber optic cable which then passed the light through a

long pass filter and into a spectrometer [28]. The output of the spectrometer went directly into a handheld computer, where data could be read [28].

It is the goal of this thesis to show the construction and utilization of a Raman spectrometer using a 633 nm helium neon (HeNe) laser, which is capable of reliably obtaining spectra from known samples. This spectrometer will have the advantage of being much more affordable [22, 24]. It will also allow users to see each of the elements which makes it much easier to explain and understand the mechanisms of Raman spectroscopy. In order to test the spectrometer, both liquid and solid samples were used. The samples chosen were aspirin, naphthalene, benzene and pyridine. These samples were chosen for several reasons. The first is that all of them have well known and available Raman spectra. This is important in that the spectra obtained on this spectrometer can be compared to previous spectra. The samples also have strong enough Raman scattering that it would be possible to detect signals. In addition to these two very important qualities, the aspirin sample was also used because it was readily accessible and could serve in applications. At Drew University, chemistry labs synthesize aspirin. The products produced in the lab could be analyzed via this Raman spectrometer. Pyridine was selected to be used as a sample as this Raman spectrometer was intended to be able to be used to perform SERS and SERS was initially discovered with a pyridine sample. As this was the case, it was desired that this spectrometer have a spectrum for pyridine so that when the SERS tests began, there would be data to compare it to. Thus, the pyridine would be used both as a liquid and on a SERS substrate.

Equipment used:

-Acton Spectrometer (Acton Spectra Pro 275)

The Acton Spectrometer used a diffraction grating (1200 grooves/mm) to measure the intensity of the wavelength of light point by point. Light entered the spectrometer from the fiber optic cable through a slit where the light passed through a diffraction grating. This grating diffracts light of different wavelengths at different angles so only one wavelength at a time reaches the detector. This diffraction grating in Figure 7 is on a motor which can rotate it to detect other wavelengths.

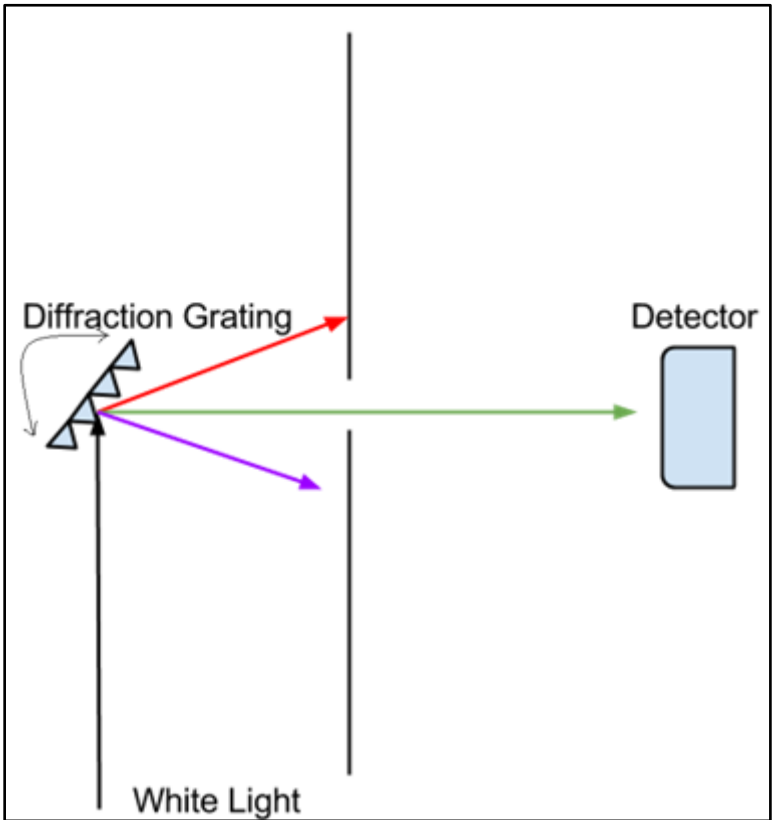

Figure 7: The diffraction grating diffracts light of different wavelengths at different angles. This allows only one wavelength at a time to reach the detector. The diffraction grating is mounted on a step motor which rotates it allowing each wavelength to reach the detector.

The detector in this spectrometer was a photomultiplier tube (Acton PD-439, $1080) which uses the photoelectric effect to measure the intensity of light. The scattered photon passes through the slit in the spectrometer and hits a photosensitive plate which releases an electron through the photoelectric effect [33]. A high voltage source (PHV400) creates a high voltage difference between the ends of the tube (the anode and the cathode) which accelerate the electron

[33]. Between the two ends are metal plates called metal channel dynodes [33]. When the electron strikes these dynodes, more electrons can be released meaning that each additional dynode causes more electrons to be released [33]. All of these electrons are accelerated towards the anode where the current is measured [33]. This current is usually proportional to the number of photons which are reaching the detector [33].

This spectrometer allowed for high integration time, resolution and averaging. The integration time is the amount of time that the spectrometer needs to measure the intensity of each wavelength. A greater integration time means higher intensities as more photons will be counted at each wavelength. The resolution of this spectrometer was 0.1 nm. This means that the spectrometer was able to sample wavelengths which were as few as 0.1 nm apart. The averaging allows the intensity of the wavelength to be taken several times and these intensities are then averaged. This technique can help to remove noise from the spectra. The integration times ranged from 500 ms to 10000 ms. These settings gave a much larger signal and Raman was detected in more circumstances.

-Ocean Optics (727 733 2447 Red Tide USB 650, $1154 [23])

This spectrometer uses a CCD array to measure the amount of light. The light was dispersed by a diffraction grating (600 gratings/mm) and detected by a CCD array. The CCD detector is displayed in Figure 8.

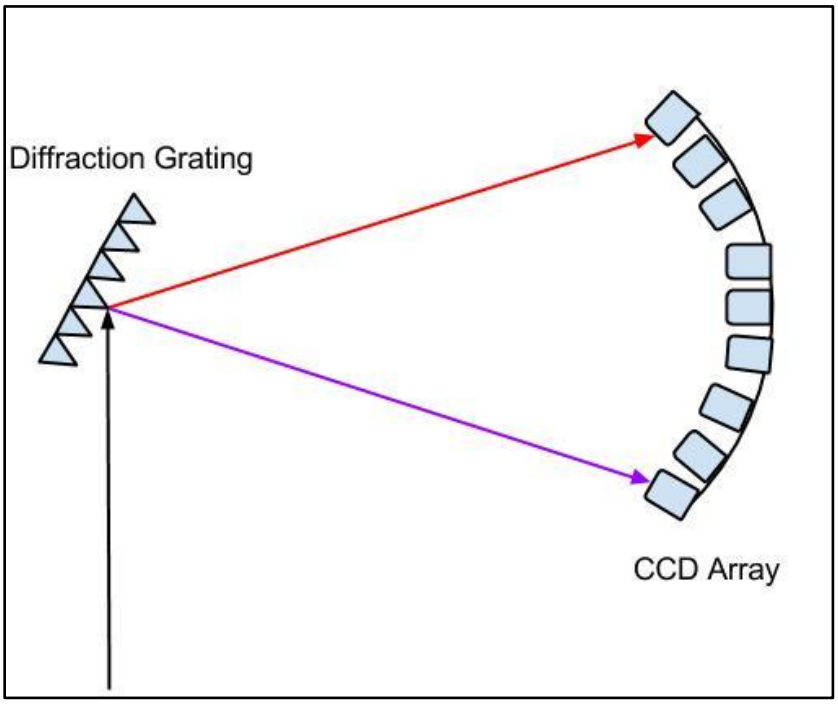

Figure 8: The incident beam is diffracted by a static diffraction grating. This grating diffracts different wavelengths at different angles. A CCD array is set of detectors arranged together which capture light diffracted through a range of angles.

Instead of recording the intensity of each wavelength individually, the whole spectrum was recorded simultaneously when using the Ocean Optics spectrometer. It also had a maximum resolution of 1nm. This means that this spectrometer was very fast (a spectrum could be obtained in 10 seconds or less), but also lacked resolution. As this spectrometer was able to display the entire spectrum very quickly it was useful for aligning the laser because any changes made to the alignment of the system could be seen very quickly. The differences in the spectra obtained from these two spectrometers can be seen in Figure 9.

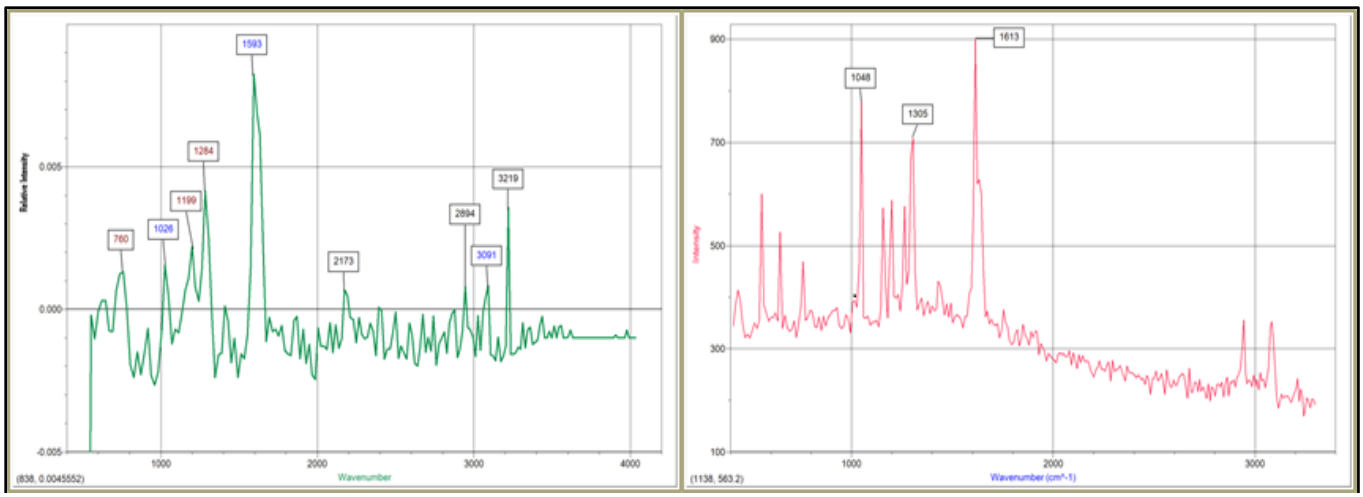

Figure 9: A comparison of the aspirin spectrum from the Ocean Optics Spectrometer (left) and Acton Spectrometer (right)

-Melles Griot HeNe Laser (25-LHP-828-249, $8304 [34]): 633nm

For this set-up a 650 nm long pass filter (Thor Labs FEL0650, $73 [35]) and a 633 laser line filter (Thor Labs FL632.8-3, $135 [35]) were used. The HeNe was better suited to the Q-SERS substrates (which were designed for a 785nm laser). The power measured for the output of this laser was 16 mW.

-Argon Ion Laser (Spectra-Physics 161B-05): 488nm

In this set-up, a 490nm long pass filter (Semrock BLP01-488R-20-D, $420 [36]) was used before the detector to block Rayleigh scattered light. A 488nm laser line filter (Thor Labs FL488-3, $176 [35]) was used to ensure the light to the molecule was monochromatic. The Argon Ion had adjustable power and was higher powered than the HeNe laser. This laser was used with its power set to the highest setting. The output power at this setting was 160 mW. The higher power made it easier to detect the Raman signal as the higher the power of the incident light is, the stronger the Raman scattering will be.

-Q-SERS Substrate (G1101, G1100, $50/2 slides [31])

These substrates were a gold nanoparticle chip mounted on a glass slide. The gold nanoparticles were a combination of 60 nm and 15 nm particles which were deposited on a

silicon chip [31]. Two varieties were used, hydrophobic and hydrophilic. This property helps to control the dispersion of the sample which leads to more predictable residue concentration and reproducibility of results [31]. The hydrophobic slides have a droplet with a much smaller diameter leading to a more concentrated sample which creates higher reproducibility of results [31]. The hydrophilic substrates create a wider droplet and are useful when analyzing hydrophobic samples [31].

-Klarite

Klarite substrates, similarly to Q-SERS, were composed of a gold nanoparticle chip mounted on a glass slide. The manufacturer stated that these substrates were ideal for a 633 nm laser. On these substrates, the gold nanostructure took the form of 1.5 μm diameter pyramids [32]. This gives the advantage of being a very uniform surface which increases the reproducibility of results [32]. These substrates were significantly more expensive than the Q-SERS substrates, costing $493 for 5 substrates [32].

-Wave plate (ThorLabs WPH10M, $513 [35])

The half wave plate changed the polarization of the laser incident on the sample. Raman is polarization dependent. Thus changing polarization should change the amplitude of the Raman peaks.

Costs

The equipment utilized in this work cost in total on the order of $30,000. However, this figure includes two spectrometers: both the Ocean Optics spectrometer and the Acton spectrometer. This means that there was flexibility in the speed and resolution of the data acquired. It also allowed for a choice of wavelength and power for the excitation source. As a result, this Raman spectroscopy set-up could be used to create four different configurations which would individually cost between around $10,000 (for a set-up using the HeNe laser and Ocean Optics

Spectrometer) and around $21,000 (for a set-up using the Argon Ion laser and Acton Spectrometer). These prices could be further reduced if the laboratory were already equipped with any of these elements.

Building the Raman Spectroscopy Set-Up

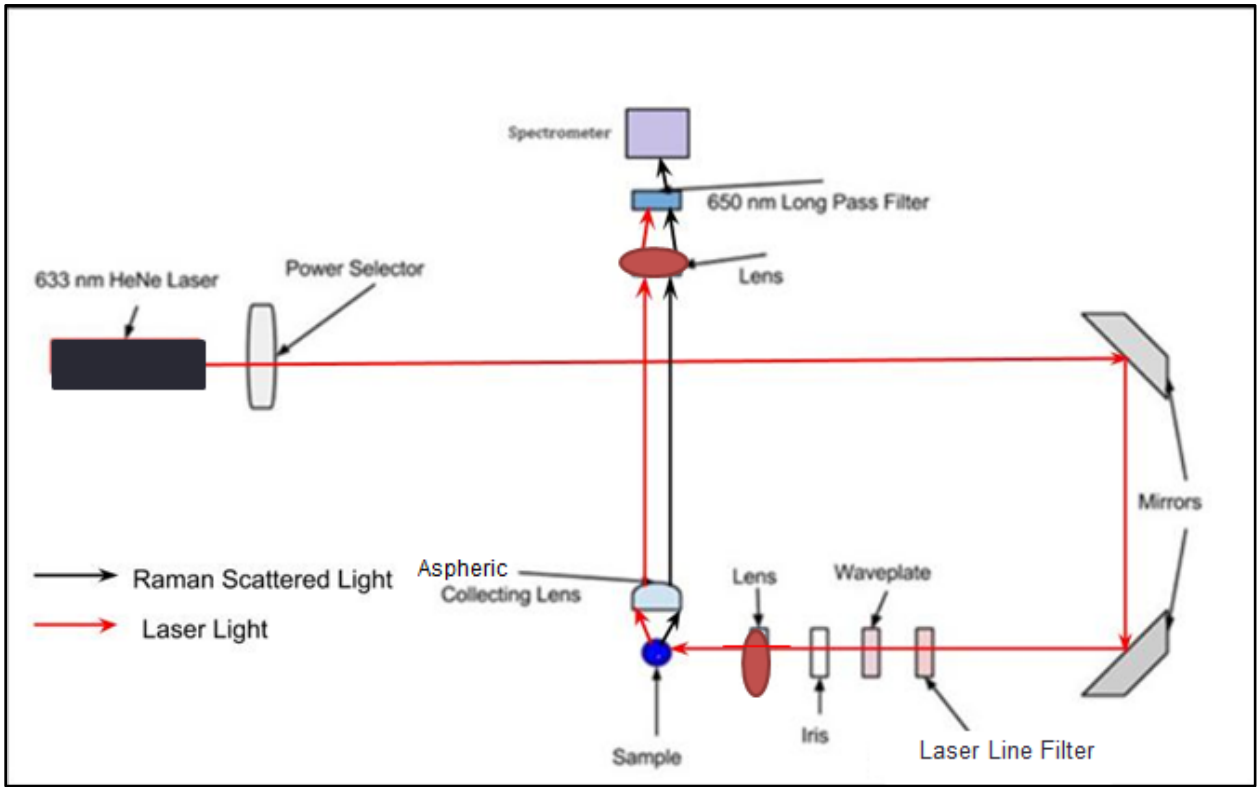

Figure 10: A schematic of the Raman spectrometer. Light leaving the laser is steered by two mirrors through a series of optical elements which filter and focus the light onto the sample. The scattered light is then collected and focused onto the detector.

The first step in building a homemade Raman spectroscopy set-up is to acquire the necessary parts. The entire set-up pictured in Figure 10 is mounted on an optics table. The optics table is stable to prevent the elements from being moved out of alignment. The table top also has an arrangement of holes ¼ x 20 thread screws which are spaced 1 inch apart. These holes allow for optical elements to be attached by screwing bolts through holes in their bases into the table.

The first element in this arrangement was the excitation source which is a 633 nm Helium Neon (HeNe) laser. The power of this laser was measured to be 16 mW. This laser had 5 neutral density filters ranging from 0.5 to 4.0 optical density mounted in a filter wheel (ThorLabs FW1AND) directly in front of the aperture. These filters block some light at every wavelength and can therefore be used to reduce the intensity of the laser. Depending on which hole is in front of the laser, more or less light passes through which changes the power of the laser This acts as a power selector for the laser which does not have adjustable power. In order to perform Raman spectroscopy, the laser needs to be directed onto a sample. In order to do this, two steering mirrors (ThorLabs BB1-E02) were placed in the path of the laser to steer it. The mirror configuration is shown in Figure 11.

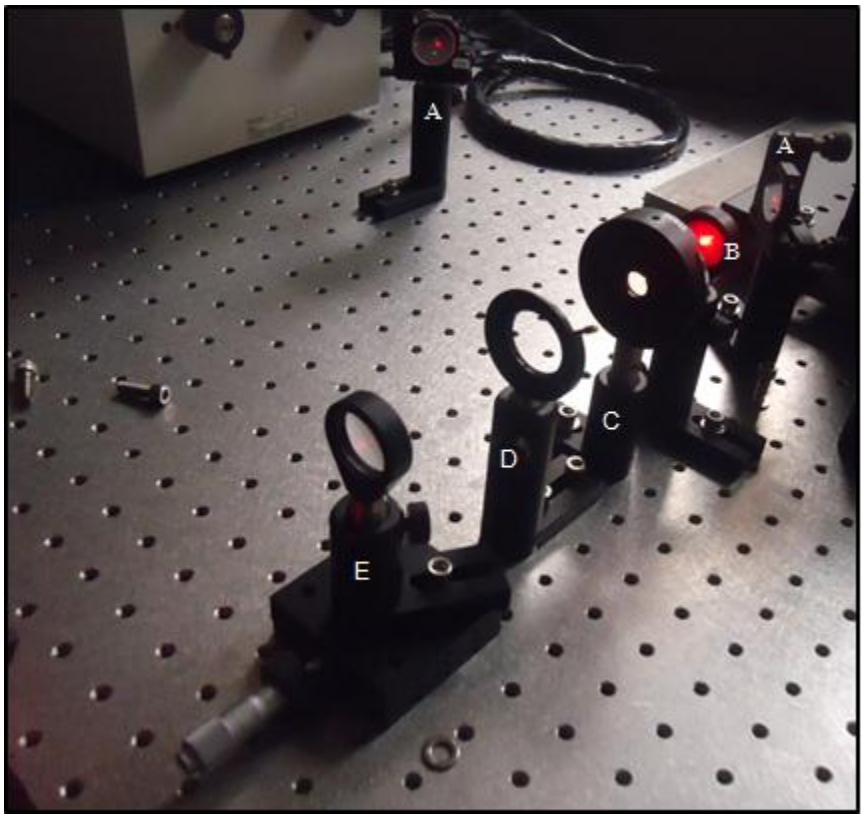

Figure 11: The incident light leaves the laser and is steered by two mirrors (A) to travel through the laser line filter (B) which only allows light of the same frequency of the incident beam to pass through. Then it passes through a

wave plate (C) which changes the polarization of the light, the iris which was used to align the laser (D) and a lens (E) which focuses the beam onto the sample.

In order to ensure the proper alignment of the set-up, it was necessary to make sure that the height of the laser beam at the mirrors was the same throughout the set-up. In order to insure this, the height was measured at the mirrors, then the height of the iris (C in Figure 11) was set such that the center was the same. The iris (ThorLabs ID25) was used to make sure that the beam was traveling straight after leaving the second mirror. To this end, a second iris was mounted at the other end of the table (about a meter away) and on the same row of holes as the first so that if the beam were straight, it would pass through the centers of both. In order to make the beam travel the path which was required to perform Raman spectroscopy and at a constant height, it was necessary to adjust the mirrors as pictured in Figure 12.

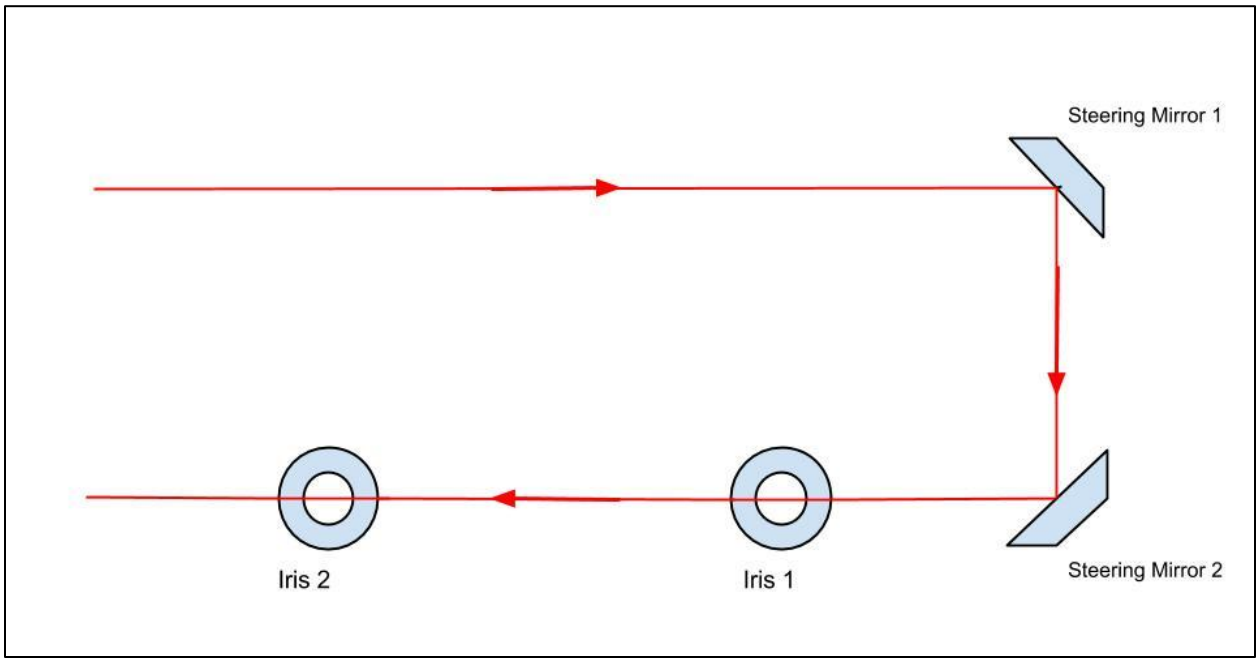

Figure 12: The laser is steering by two steering mirrors to travel through a pair of irises. The two irises determine the line that the laser travels. The two irises were placed on either side of the sample holder to ensure that the sample would lie in the lasers path.

Each of the steering mirrors pictured in Figure 12 had two screws which allowed adjustment either left and right or up and down. The tilt of the mirror changed the path of the laser accordingly. Once the mirrors had been adjusted such that the laser passed through the

center of both irises and was therefore straight and parallel to the table, more optical elements were added. The first piece after the second mirror is a 633 nm laser line filter (ThorLabs FL632.8-3) with a spectral width of 0.6 nm. The point of this filter is to prevent laser noise from reaching the sample. This filter only allows light with a wavelength of 633 nm to pass through. After the laser line filter a half wave plate (ThorLabs WPH10M) was placed, which can be used to change the polarization of light. As Raman spectroscopy is polarization dependent, it is important to be able to adjust the polarization. After the wave plate and the iris, there is a lens. This lens is used to focus the beam on the sample.

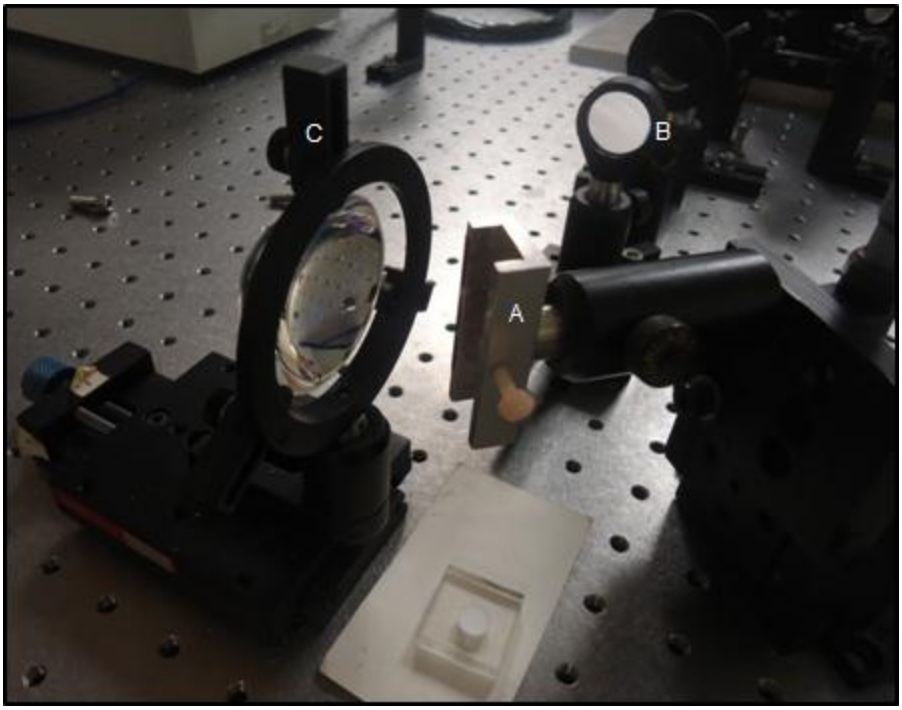

Figure 13: The sample potion of the Raman spectrometer is pictured. The incident laser beam will travel through the focusing lens (B) which will focus it onto a sample which is held in the holder (A). This holder is mounted on a stage capable of motion in two dimensions. Any scattered light will be collected by the collecting lens (C) and steered towards the detector.

In figure 13 above, it is possible to see the focusing lens (ThorLabs LA1433) (B) with a focal length of 15 cm directly behind the sample holder. This lens was mounted on a base which was bolted to the table; however, the base was capable of motion in one dimension (towards and

away from the sample) which is achieved by turning a small screw. As the screw turns it compresses a spring (or allows the spring to expand) which moves the base. This capability was used to focus the beam on the sample. The collecting lens (ThorLabs AL5040-A) (C in Figure 13) with focal length of 3 cm shown in figure 13 on the left is bolted to the table, so it was only necessary to place the sample holder and stage (A) in Figure 13 so that the sample would be in the center of the collecting lens. The sample holder itself, in the center of Figure 13, was mounted on a stage. This stage had could be adjusted up and down or in and out. In order to do this, a screw on either the top or back of the stage is turned and this in turn compresses a spring or allows its expansion which moves the stage. The distance that the stage moved can then be read off the micrometer. The next element in the set-up is the collecting lens. This lens is very important as it collects the light scattered from the sample. In order to adjust this lens such that the Raman scattered light is focused onto the fiber optic cable, a sample (initially a small piece of wood) was placed into the holder and the laser light was reflected off the surface and through the collecting lens as show in Figure 14. The collecting lens can be moved towards or away from the sample (through a mechanism similar to that of the lens stage described above) to the lens, which focuses the beam on the sample; the collecting lens is mounted on a stage, which can be moved forwards or backwards by turning a screw. The beam leaving the collecting lens should be as wide as the lens and not decrease in diameter by the time it reaches the second lens. This means that the light leaving the collecting lens is a parallel beam. In order to make sure that this happens, it is necessary to place the sample one focal length away from the collecting lens. This second lens (ThorLabs LA1050) has a focal length of 10 cm and it is placed directly in line with the collecting lens. The light which enters it is then refocused on the fiber which sends the light to the detector.

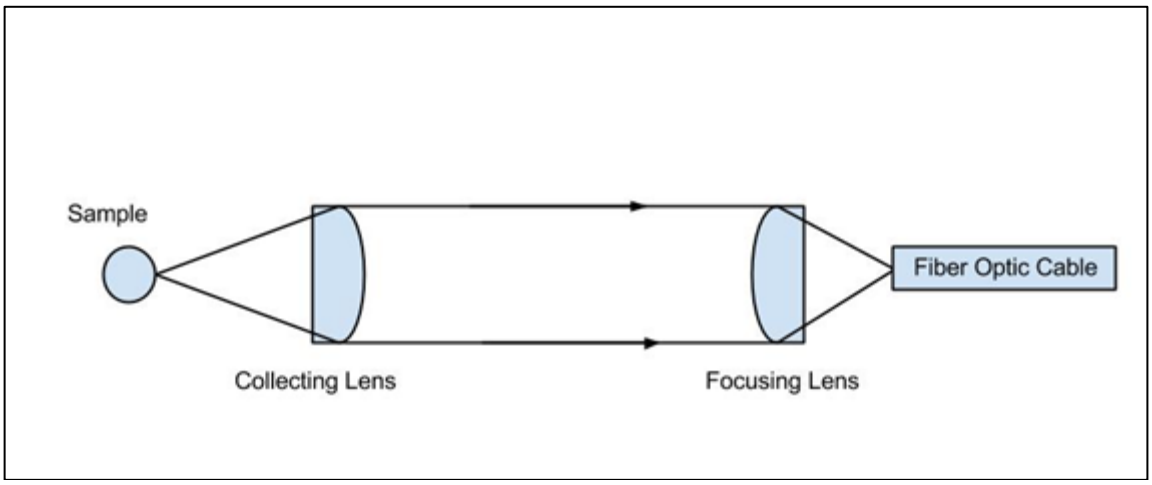

Figure 14: The light scattered from the sample goes in many directions. This light is then collected by the collecting lens. As the sample is at the focal point of this lens, the beam leaves parallel. This parallel beam can then be focused onto the fiber optic cable which is found at the focal point of the focusing lens.

However, before the fiber, there is a 650 nm long pass filter (ThorLabs FEL0650). This filter only allows light with a wavelength of 650 nm or longer to pass through. This means that the 633 nm light from the laser is prevented from reaching the detector as is any Rayleigh scattered light. This is important as Rayleigh scattering is significantly stronger than Raman scattering.

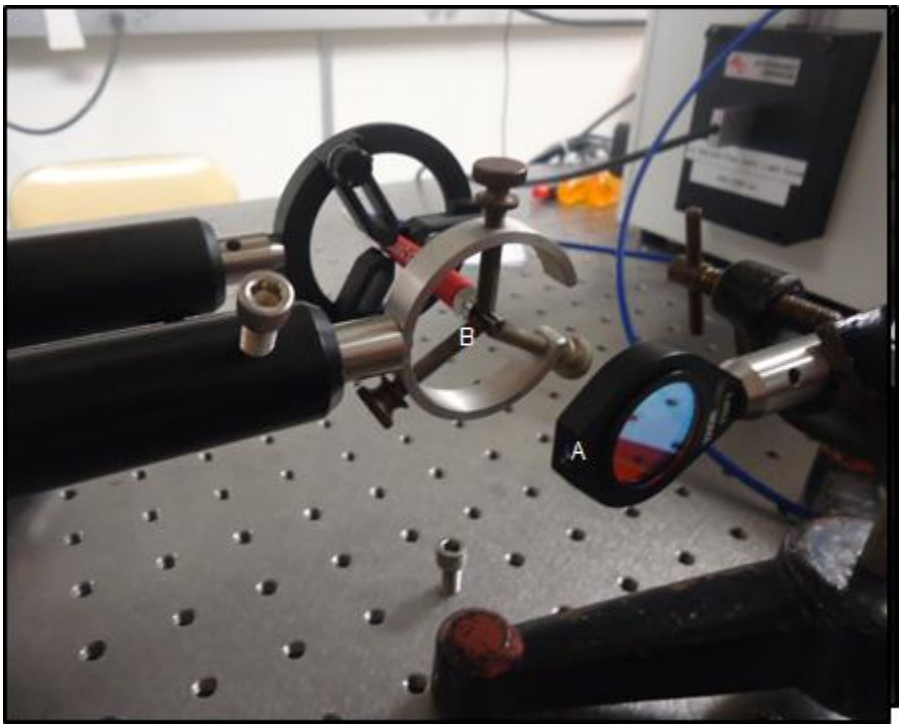

Figure 15: The detector portion of the spectrometer. The light collected from the sample passes through the long pass filter (A) into the fiber optic cable which then passes the light to the detector (B). The detector sends data to the computer. The detector is held in place by two holders to prevent accidental motion.

The detector pictured in Figure 15 is the fiber optic cable of an Ocean Optics Spectrometer. This device is an array of pixels which record the light hitting the detector. It is held in place by two holders through the use of three screws. Initially, there was only one of these holders, however, that arrangement was insecure and caused misalignment. In order to remedy this, a second holder was added to hold the fiber optic cable more securely. Both of the holders were bolted to a stage which can move in three dimensions. This allowed the fiber optic cable to move into the exact focus of the second focusing lens. In order to find this point, a sample was placed into the holder and the appropriate spectrometer was turned on. The computer then displayed a spectrum and adjustments were able to be made in each direction. The effects of these changes could be seen in the spectrum on the computer screen. In order to align the detector, it is necessary to maximize the intensity of the Raman scattered light collected by the

spectrometer. When the spectrum is at a maximum then the detector is receiving the most Raman signal.

Data Collection Methods:

In order to obtain a spectrum, the light from the laser must travel throughout the set-up to the sample. The laser passes through the laser line filter which does not allow light to pass unless it is the same wavelength as the laser. The light then passes through the wave plate which has the ability to change the polarization of the light. It is then focused by a small lens. The laser then reaches the sample. Liquid samples are placed into a small cuvette with four transparent glass walls. It is aligned so that the laser hits one of the walls perpendicularly. Solid samples are placed into the holder and the screw is tightened until the sample is held fast. The solid samples are prepared in order to have a flat side for the laser to be incident on. The light then strikes the sample and is scattered. The scattered light is collected by the collecting lens and travels to a second lens which refocuses the light onto a fiber optic cable which sends the light to a detector.. This data is detected by a detector and sent to a computer.

In order to collect data, a commercial computer program was utilized. Each of the spectrometers has a separate program which is used to record and analyze the data. The first program which was used was LoggerPro. This program recorded the data from the Ocean Optics spectrometer and displayed it graphically. LoggerPro allows users to save data from past runs. It also allows ones to manipulate the output in various ways such as converting the x-axis from wavelength (nm) to wave number (cm^{-1}). The equation which is used to make this conversion is as follows in equation 4.

$$Wave\ Number = \left(\frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scattered}}\right) * 10^7 \qquad (4)$$

In order to use LoggerPro, a user must input a starting and ending wavelength, an integration time (up to 1000 ms) and a number of averages (up to 10). Therefore the runs took a maximum of 10 seconds. The program continues to take data until the user stops it. Once the program is stopped, the data is saved both graphically and numerically. This data can also be exported as a spreadsheet.

The second computer program used was connected with the Acton spectrometer. This program is called SpectraSuite. Similarly to LoggerPro, SpectraSuite requires a starting and ending wavelength, integration time and number of averages. However, SpectraSuite also allows the user to select a step size. This program also does not limit the integration time to 1000 ms. SpectraSuite, once started, takes a run and then stops. The user then has the option of saving the data in Microsoft Excel. This data is displayed graphically. This graph can only be manipulated, however, by zooming in or out. The x-axis is recorded only in wavelength, so, in order to obtain a spectrum with wave numbers, the user must save the data, calculate wave number and graph it in a separate program such as LoggerPro or Excel. SpectraSuite also has the option to plot the intensity at a single point over time. This was used for alignment as the effect of the adjustments made could be seen in the intensity of the peak.

One of the major challenges with the solid samples was the significant amount of fluorescent background. Fluorescence occurs when a material absorbs electromagnetic radiation at one wavelength and emits it at another. In most cases, fluorescence is much stronger than the Raman signal, so it is necessary to find a way to reduce the background fluorescence. Fluorescence was reduced was by photo bleaching the sample between half an hour and an hour. In order to see the spectrum without the fluorescent background on the Ocean Optics spectrometer, two spectra were obtained as shown in the top of Figure 16; one in which data was

taken normally (a reading was taken for every wavelength and displayed) and a second one in which a setting called “wavelength smoothing” was set to 20. This setting averaged neighboring amplitudes which gave a background spectrum. This comes about because fluorescence forms are broad background curve while the Raman peaks ought to be sharp. The wavelength smoothing essentially averages away the Raman peaks leaving only the fluorescent background. These two could then be either subtracted or divided in order to obtain just the Raman spectrum.

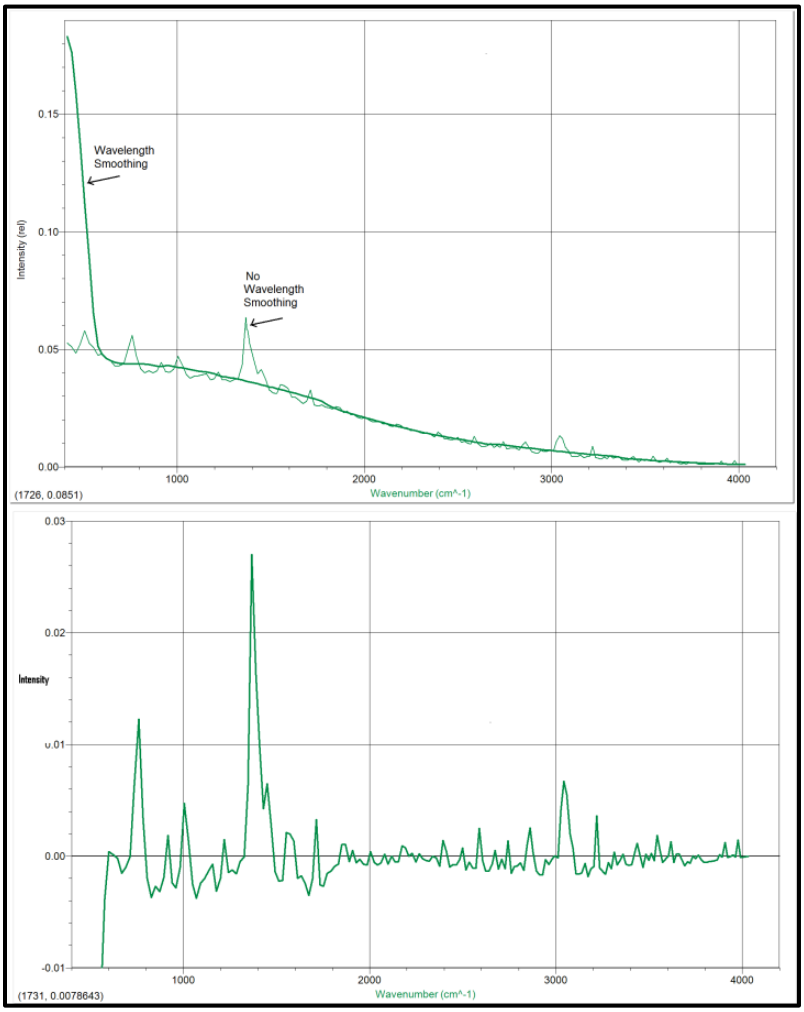

Figure 16: Naphthalene Spectrum taken on Ocean Optics Spectrometer. (above) The Spectra with and without wavelength smoothing. (below). The result of subtracting the background obtained by using wavelength smoothing from the spectrum. The spectra were obtained with the HeNe laser.

The primary difficulty in constructing a Raman spectrometer is that it must be able to detect a very weak signal. The set-up as described here was designed to separate the Raman signal from the much stronger Rayleigh scattering by using a low pass filter. This filter blocks all wavelengths shorter than a certain threshold. For the 633 nm laser, a 650 nm long pass filter was used; therefore only wavelengths longer than 650 nm would be allowed to pass which effectively filters out the Rayleigh scattered light. In addition, the spectra were also obtained in a dark room so that stray light from the overhead lights would not be detected. There was a problem encountered in that the computer must be running in order to obtain spectra, and the computer also gives off light. This light can add to the background in the spectra which makes the Raman peaks less clear. This problem was overcome by using a black piece of felt to cover the computer screen while the spectra were recorded.

In addition to preventing as much light as possible from reaching the detector, it was also necessary to collect and record as much of the Raman signal as possible. In order to make sure that the maximum intensity of light was reaching the detector, the system was adjusted. As the detector and laser are both small, any slight changes could greatly alter the intensity of the light reaching the spectrometer. Due to this sensitivity, the Raman spectrometer was realigned daily. After the first use and alignment of each sample, the intensity of a peak was recorded. For realignments, the previous maximum amplitudes were used as a gauge. The system was adjusted until the peaks were as high as or higher than the previously recorded intensities. As all of the previous intensities were recorded, it was possible to tell when spectra were not working as they should.

In inspecting the spectra, there was also a pattern of certain peaks appearing consistently on the Ocean Optics spectrometer. These peaks are recorded in Table 1.

Table 1: The background peaks which were found in spectra obtained on the Ocean Optics spectrometer

Wave Number (cm^{-1})
916
1019
1220
1409
1713
2587
2861
3219

These peaks were found on several spectra where they were not expected. This finding led to spectra being taking with nothing in the sample holder. The peaks still appeared, so it is thought that they are noise created by the Ocean Optics spectrometer itself. It is important to note in the spectra taken on the Ocean Optics spectrometer that these are not Raman peaks and they can be confused with weak Raman peaks. The Acton spectrometer did not have this noise.

Results:

Naphthalene

The first sample which was used was naphthalene. It was selected because it has strong and fairly distinct Raman peaks. The initial naphthalene tablet used was a mothball. Therefore, it was necessary to smooth one side in order to have a flat surface for the laser to strike. The naphthalene was then placed into the sample holder which was set to be at a 45^0 angle to the incident light.

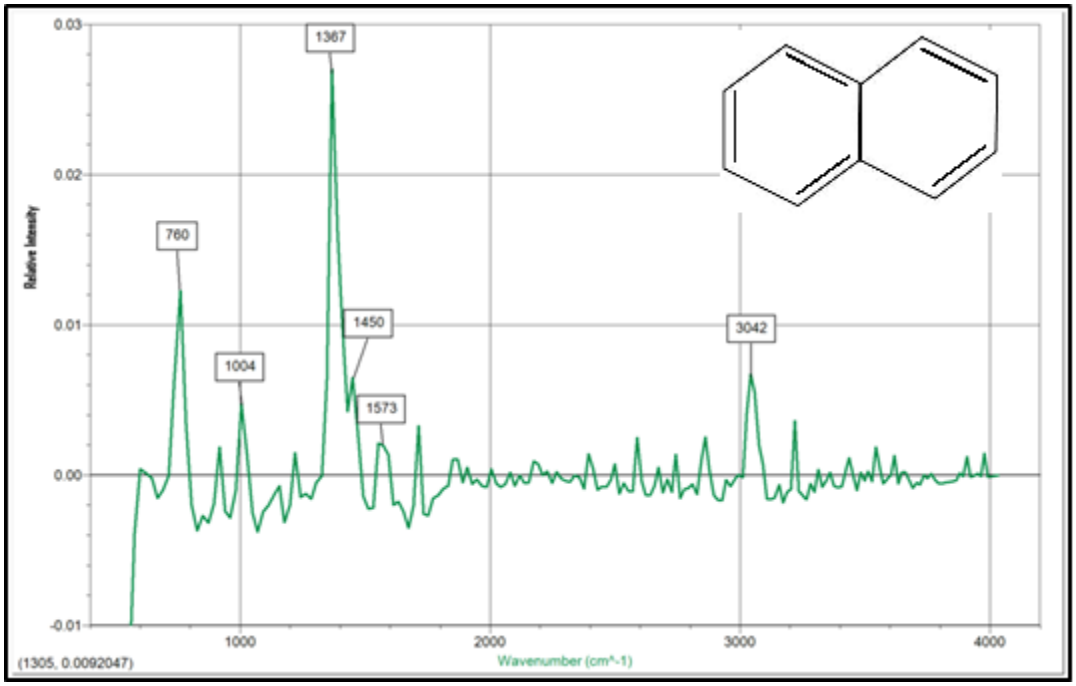

Figure 17: The Ocean Optics Spectrum of Naphthalene after background subtraction obtained using the HeNe laser.

Figure 17 above shows the peaks found in the naphthalene spectrum. These were compared to standard values. Peaks have been previously recorded in the literature are in fairly good agreement with the peaks which are found in the above spectrum [28].

Table 2: Raman Peaks of Naphthalene compared to accepted values

Wave Number of Peaks obtained (cm^{-1})	Wave Number of Peaks in Literature (cm^{-1}) [28]	Assignment [37]	Difference in Wave Number (cm^{-1})	Difference in Wavelength (nm)
760	763	Breathing	3	0.13
1004	1021	CC Stretch	17	0.77
1367	1382	CC Stretch, CH Bend	15	0.72
N/a	1464	CC Stretch, CH Bend	-	-
1573	1576	C=C Stretch	3	0.15
3042	3056	CH Stretch	14	0.86

This data shows that the Raman spectroscopy set up constructed was capable of obtaining Raman spectra which are consistent with established spectra. The wave number of these peaks is what is

recorded in literature; however, it is possible to convert the resolution in wavenumbers using the equation 5.

$$\Delta\nu = \frac{\Delta\lambda}{\lambda^2} \quad (5)$$

Using this equation, the 1nm resolution of the Ocean Optics spectrometer is 23 cm^{-1} at 650 nm. The resolution of the Acton spectrometer, 0.5 nm, is 11 cm^{-1}. The difference between the known wavelengths and the wavelengths of the vibrations obtained with this spectrometer differed however, this difference was below the 1 nm resolution of the Ocean Optics spectrometer on which this spectrum was obtained. This indicates the spectra obtained on the spectrometer fall within the resolution of the spectrometer.

Aspirin

Another sample which was used was an aspirin tablet. In order to prepare the sample, a razor blade was used to scrape off the coating and to smooth the surface. The sample was then placed into the sample holder which was positioned such that the face of the aspirin was at a 45^0 angle to the laser. The aspirin, like the naphthalene, had a significant fluorescent background on the Ocean Optics spectrometer. This was reduced by photo bleaching the aspirin for half an hour before taking the spectrum.

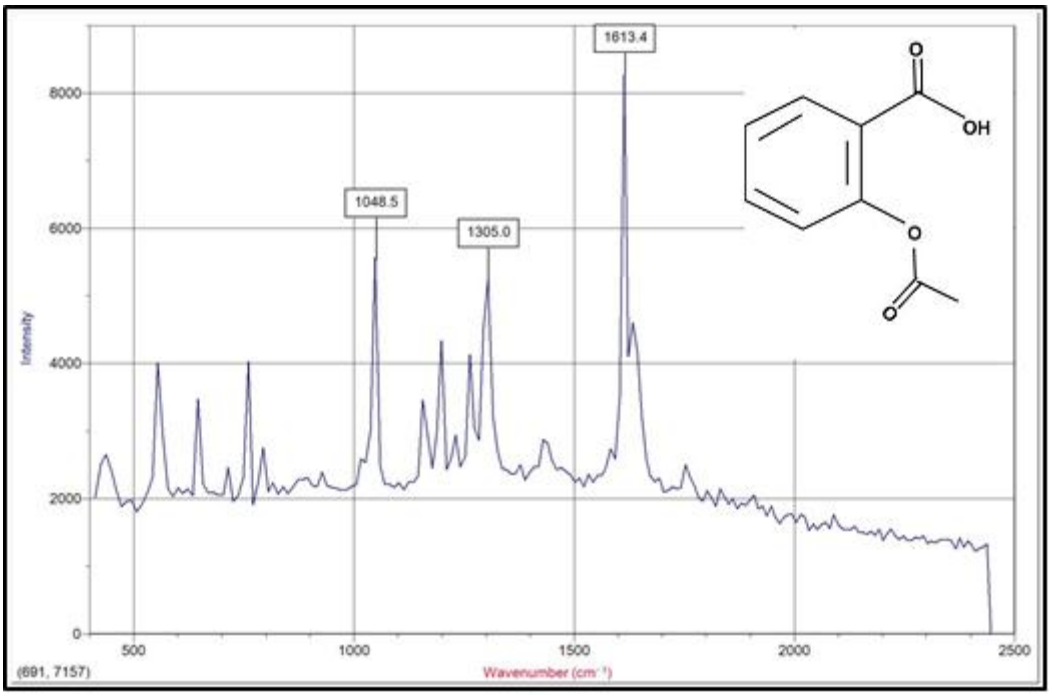

Figure 18: The Raman Spectrum of aspirin taken on the Acton spectrometer using the HeNe laser.

The Spectrum in figure 18 shows a clear peak at 1613.4 cm^{-1} as well as peaks at 1305 cm^{-1} and 1048.5 cm^{-1}. According to the McCrone Atlas of Microscopic Particles, aspirin has a CC-stretching mode at 1606 cm^{-1} [26] and a ring mode at 1035 cm^{-1}, which correspond to the peaks found at 1613.4 and 1035 cm^{-1} [29]. This aspirin spectrum served to confirm that this set-up was capable of obtaining spectra for solid samples. This was considered important as the SERS samples were to be treated as solids. These were used to align the laser and detector and also in order to make sure that Raman signals were being detected.

Liquid Pyridine

Benzene and Pyridine were used as liquid samples. Signals from these liquids were obtained with the Argon Ion laser. It is believed that the HeNe laser was not powerful enough to create a sufficiently strong Raman signal that could be detected. The pyridine was selected as the initial molecule on which SERS was performed.

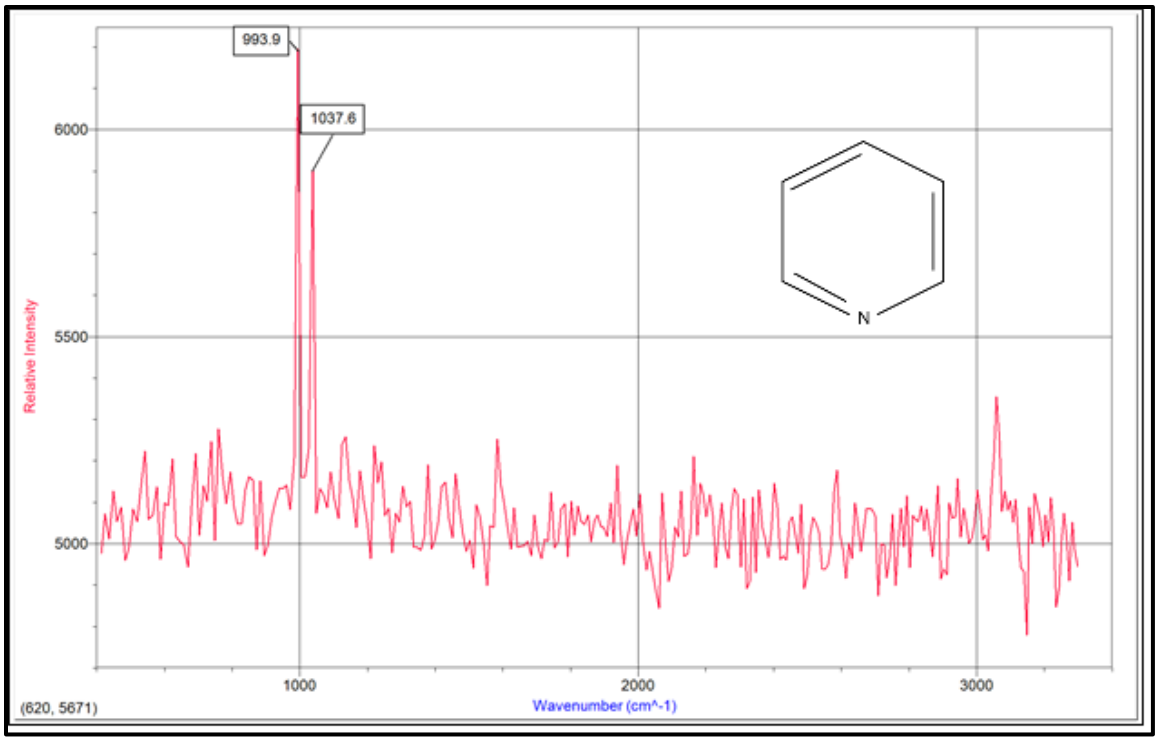

Figure 19: Raman Spectrum of liquid Pyridine taken on the Acton Spectrometer using the Argon Ion laser.

Figure 19 shows the Raman spectrum of liquid pyridine. Two clear peaks were obtained at 993.9 and 1037.6 cm^{-1}. Pyridine peaks have been reported at 991 and 1029 cm^{-1} which correspond to the ring breathing modes of pyridine [30]. Table 3 displays the values of expected peaks and the values that were obtained for these peaks. In this case, the differences in the wavelengths corresponding to these peaks were calculated to be below the resolution used (0.5 nm).

Table 3: Values of Raman Peaks for Pyridine

Wave Number (cm^{-1})	Accepted Value of Wave Number (cm^{-1})	Difference (cm^{-1})	Difference (nm)
993.9	991	2.9	0.13
1037.6	1029	8.6	0.39

The peaks are quite close together in the Raman spectrum of pyridine which means that it is essential to have good resolution as these peaks are only found 2 nanometers apart. Therefore, this sample shows that this spectrometer is capable of differentiating between peaks in close

proximity to one another. This spectrum was used to compare with the SERS data using pyridine on a SERS substrate.

Pond Water:

Pond water was collected from each of the ponds in the Zuck Arboretum located on the campus of Drew University. The samples were obtained during the summer by bringing bottles to the pond and filling them with water. As the samples were collected by hand, the water came from near the shore of the pond. In obtaining the samples, it was attempted to include as little pond sediment as possible. The ponds are called Long Pond and Round Pond. The samples from Long Pond had noticeably more visible particles than those from Round Pond. The pond water samples were then concentrated in order to maximize the amount of particles which would be found in the sample. In order to concentrate samples, the pond water was placed into beakers which were then placed into a low-pressure oven. This oven was equipped with a vacuum hose which was used to decrease the pressure in the oven as well as heating elements which heated the oven. The combination of low pressure and high temperature allowed the water to boil faster. After the samples had been reduced from 20 mL to 5 mL, they were placed into a small glass cuvette. This cuvette was placed into the sample holder such that one side was perpendicular to the laser beam.

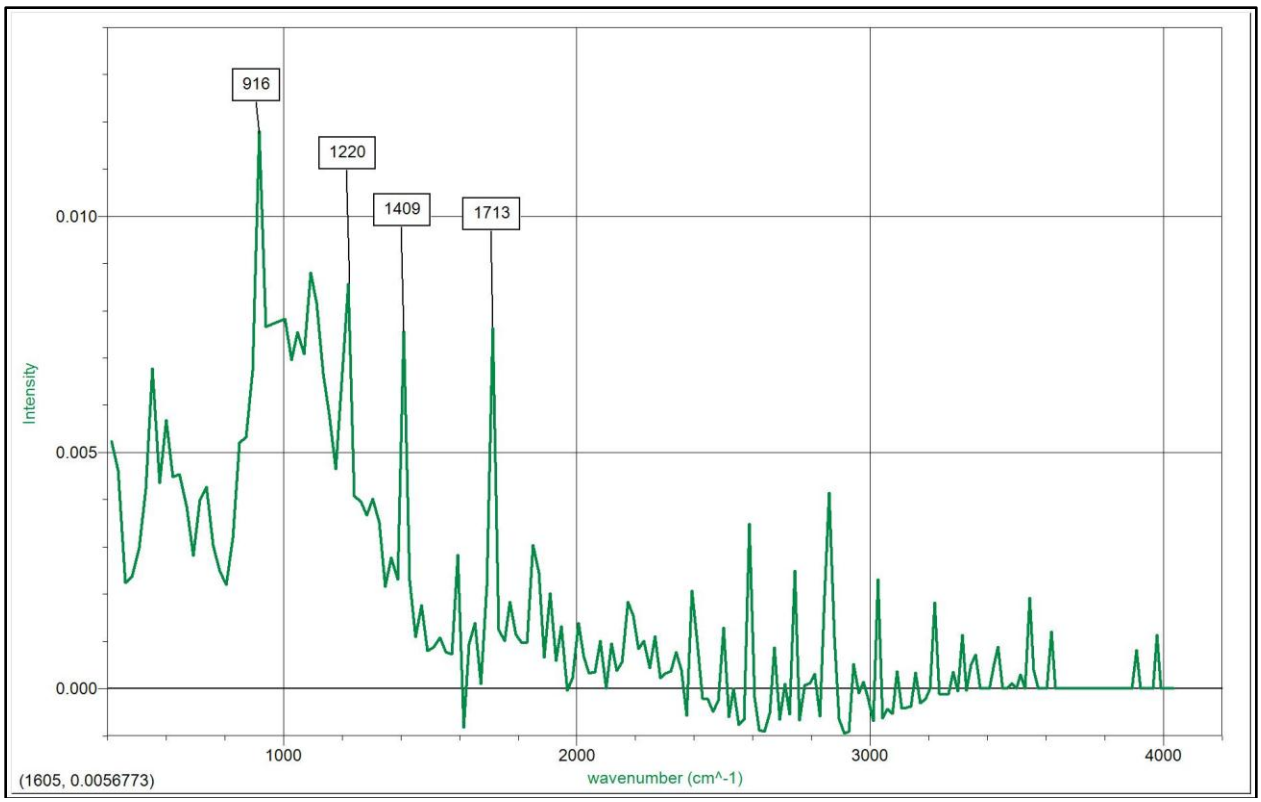

Figure 20: Spectrum of sample taken from Long Pond and concentrated from 20mL to 5mL which was taken on the Ocean Optics Spectrometer using the Argon Ion laser.

There were some initial difficulties with the samples of pond water. One of the primary troubles was aligning the detector and lenses to obtain the most signal. The sample in Figure 20 shows a strong fluorescence curve which underlies many peaks. All of the peaks which appear in this spectrum were also found when there was nothing in the sample holder. These peaks are recorded in Table 1. It is believed that the peaks in Figure 20 are simply due to noise in the Ocean Optics spectrometer.

Surface Enhanced Raman Spectroscopy Slides

In order to test the SERS slide, pyridine was used. This was because the surface enhancement effect was first discovered using pyridine, so there is a known spectrum for pyridine. The slides used were purchased from Q-SERS. The spectra from these runs always

revealed a pair of strong peaks (1440 and 1606 cm^{-1}) which do not correspond to pyridine peaks in either the literature or in the previous runs involving liquid pyridine.

Given that there was now an unexplained peak, a spectrum was taken from a blank slide. This also revealed the peak in the same location and with the same amplitude. In further studies, focusing on the larger peak at 1440 cm^{-1}, it was shown that this peak has some interesting properties:

1. As seen in Figure 21, it is always obtained at 1440 cm^{-1}.

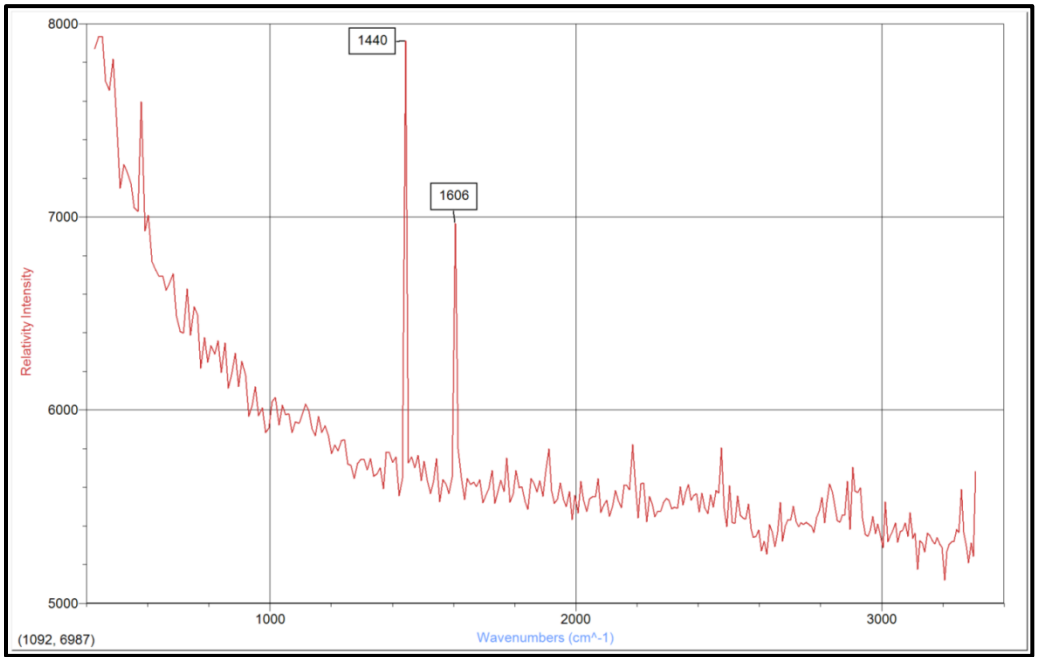

Figure 21: Peaks in the Q-SERS pyridine spectrum taken on the Acton spectrometer using the HeNe laser.

2. It can be used to align the laser as the amplitude changes based on the location of the detector, the collecting lens, and where the laser hits the sample. The intensity of the peak also increases relative to the noise.
3. It may be polarization dependent. The amplitude of this peak changed as the wave plate was rotated from 0 to 90 degrees. It reached a maximum at 0^0 went to a minimum at 45^0. A Raman signal would be expected to minimize at 45^0, and to be a maximum at 0^0. The polarization dependence is displayed in Figure 22.

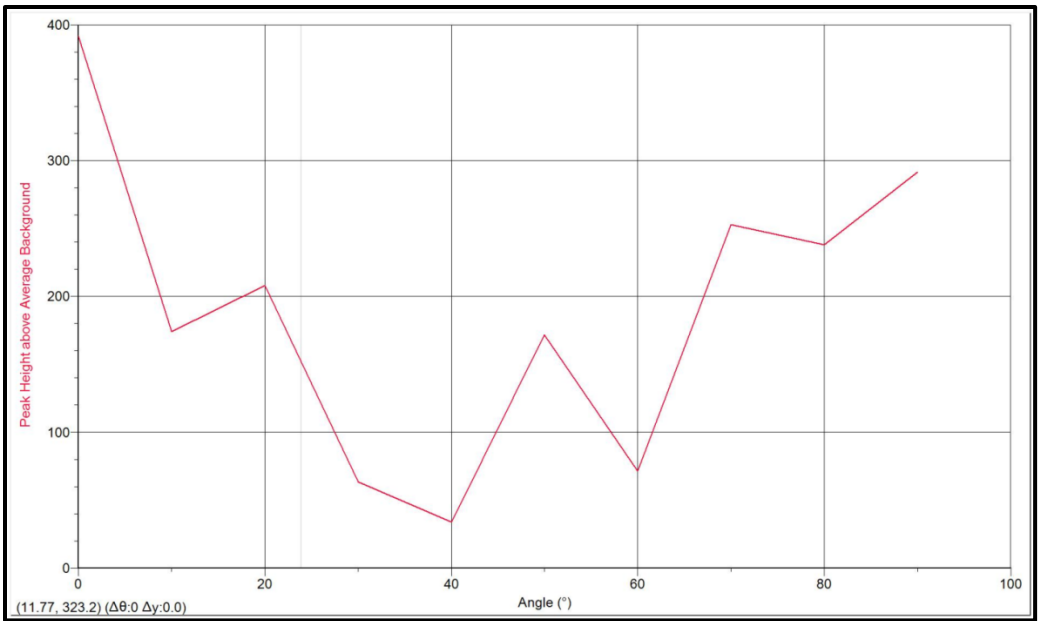

Figure 22: The height of the peak at 1440 cm-1 above the average of the background signal recorded every 10^0 between 0^0 and 90^0.

4. It is equally strong compared to background on blank and pyridine slides.

In order to see if there was a spectrum which was being masked by the background signal, we attempted to divide the spectrum obtained with a pyridine slide by the spectrum obtained from a blank slide. This did not give peaks. The Q-SERS company reported that they had had reports of background peaks when their product was used with 633nm lasers (they recommend a 785 nm laser), but that these peaks we report were different from those about which that they had previously received reports.

The next step was attempting to use a different brand of substrates. This time, Klarite Substrates were used. These also use gold nanoparticles on a chip mounted to a glass slide. However, Q-SERS claims their product to be suited to the 633 nm laser so it was hoped that it will be possible to obtain a signal from the pyridine. Klarite slides did not appear to have mystery peaks in the same region as the Q-SERS slides (that is, no peaks were detected between 872 and 1512 cm^{-1}) however; there were also no pyridine peaks.

In order to get more signal, the fiber was taken out. This was done because some of the light is reflected off the surface or is lost in the length of the fiber. In order to take a spectrum, the spectrometer itself was moved to collect the light. The 650 nm long pass filter was removed for early aligning of the spectrometer. The collecting lens and focusing lens had to be readjusted in order to focus the light on the opening of the spectrometer. The beam was also initially too high, meaning that the focusing lens had to be adjusted slightly up in order to direct the beam into the spectrometer. The spectrometer then had to be adjusted left and right in order to maximize the signal at 633 nm. Then the long pass filter was replaced and the spectrometer was adjusted again to maximize the 696.5 nm line from the aspirin. After this adjustment, the signal to noise ratio was raised from 4 to 5. A Q-SERS substrate was then tested. As the alignment and focus changed due to the change of position and size of the sample, the focusing and collecting lens were realigned to maximize the 633nm line. The spectrum of the Q-SERS and pyridine was taken and inspected for possible peaks. A line appeared at 677.1 nm which is 1028 cm^{-1}. This may correspond to the 1037 cm^{-1} line of pyridine. This peak was not very pronounced as the signal to noise ratio was only 1.25. In order to align the detector to this peak, the single point intensity versus time setting in SpectraSuite was utilized. This allowed the spectrometer to remain at a specific wavelength (in this case, the peak at 677.1 nm) and record the intensity as a function of time. This made it possible to see the effects of adjustments on the height of the peak. In adjusting the angle of the sample holder and the collecting lens, there were changes seen in the height of the peak. However, when the set-up was left alone to take data without any changes being made, the intensity dropped. When a spectrum was taken, there were the peaks at 1440 and 1606 cm^{-1} but no other clear peaks.

As these peaks were present on blank slides, it seems to be that these peaks are somehow related to the slide itself. This is supported by the fact that the peaks found on the Q-SERS slide were not the same as the peaks found on the Klarite slides. In addition, it is interesting that changing the alignment of the system changes the amplitude of the peak in relationship to the background. This fact, combined with the fact that changing the wave plate also changes the amplitude seems to indicate that this may be Raman scattering. However, Q-SERS reported that while their slide did have a background signal with peaks, that the peaks obtained on this spectrometer did not match those in their records. This may be because the Q-SERS substrate is designed to work with a 785 nm laser while the one used in this spectrometer is a 633 nm. The manufacturer did explain that they have had customers use 633 nm lasers successfully and none of those customers had either of the 1440 or 1606 cm^{-1} peaks. Finally, Q-SERS stated that they did not believe it was possible for the peaks on these slides to have come from contamination in the room as it would require a very high concentration in the air to have a detectable amount adsorb onto the slide. The slides were also stored in plastic containers until they were used which limited their exposure to contaminants.

Conclusion

A Raman spectrometer was constructed from parts in the laboratory. This Raman spectrometer set-up incorporated a HeNe or Argon Ion laser which acted as the excitation source. This beam was then steered by mirrors through various optical elements which filtered, altered the polarization of and focused the light onto a sample. The light was then scattered and this scattered light was collected by a collecting lens. The collecting lens was placed such that the sample was at its focal point so that the beam of scattered light leaving the collecting lens was parallel. The parallel beam was then focused by a focusing lens onto a fiber optic cable. This

cable connects to the detector which recorded the intensity of the wavelengths of the scattered light.

This set-up was tested with several samples having well-known Raman spectra. These samples were both solids and liquids. The solid samples, aspirin and naphthalene were analyzed using the HeNe laser. These samples had high fluorescent backgrounds which were reduced through photo bleaching. The background was removed mathematically after the data was acquired. The spectra acquired for both of these samples showed Raman peaks which were in agreement with the peaks which have been reported in the literature.

The liquid samples, pyridine, benzene and pond water provided more mixed results. Since the signal from liquids was weaker with the HeNe laser, the more powerful argon ion laser was used instead. This brought out stronger signals from the liquids. Spectra were obtained for both pyridine and benzene which were in agreement with the spectra in literature within the resolution of the spectrometer. The pond water was more difficult as the samples had unknown composition so there were not known spectra with which to compare. This sample also had a very high fluorescent background due to the particles found in the water.

The SERS substrates had inconclusive results. The first brand of SERS substrates which was utilized was Q-SERS. These substrates were gold nanoparticles of 60 nm and 15 nm sizes deposited on a silicon chip [31]. These substrates were found to exhibit two strong peaks at 1440 cm^{-1} and 1606 cm^{-1}. These peaks were seen on both blank slides and on slides on which pyridine had been adsorbed however, pyridine Raman lines were never observed. This observation was investigated more thoroughly and it was found that these mystery peaks were found consistently on more than one of the Q-SERS slides. These peaks also varied with the alignment of the detector and the polarization of the laser. The 1440 cm^{-1} and the 1606 cm^{-1} peaks were not found

on a second brand of SERS substrates called Klarite. This substrate also used gold nanoparticles, however in the case of the Klarite substrate; the gold took the form of 20 μm diameter pyramids. This substrate did not display the mystery peaks and it did not show pyridine peaks. Therefore, the SERS results are inconclusive and this spectrometer has not been shown to be capable of performing SERS. Nonetheless, the spectrometer obtained spectra from both liquids and solids which were comparable to the previously reported spectra. This shows that this spectrometer is capable of performing Raman spectroscopy.

This spectrometer has the advantage of being less expensive than Raman spectroscopy systems which are available for purchase. These systems come with all of the parts necessary to perform Raman spectroscopy contained together in a single instrument. The price of these instruments ($24000 to $35000 [23]) may prove to be a cost barrier for some laboratories.

Raman spectroscopy is an important tool in analyzing materials as it works with very little sample preparation which makes allows results to be obtained quickly. It also is non-destructive which means that samples can be saved for further analysis. Raman spectroscopy is also capable of obtaining spectra of materials dissolved in water. These qualities make Raman useful in a number of applications. This spectrometer was shown to be capable of obtaining the spectrum of aspirin. This could prove to be useful as chemistry courses must sometimes create aspirin as a part of the laboratory experience and this spectrometer could be used to analyze the results of this laboratory.

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