APPLICATION OF RAMAN SPECTROSCOPY ON TEETH

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ABSTRACT

Dental caries is tooth decay caused by acid released from cariogenic bacteria attacking the tooth enamel. Phosphate anions, primarily present in tooth enamel in the form of hydroxyapatite lattice, promote tooth enamel health which leads to tooth decay prevention. The dentin of teeth, on the other hand, is mainly composed of collagen in collagen crosslinks. Visual and tactile methods used in offices limit the determination of mineral content and collagen levels resulting in failure to detect dental caries at its earliest stages. Subjective views vary depending on personal experience and judgment; thus, a quantitative approach is necessary. The objectives of this study are to determine the capability of Raman spectroscopy to measure mineral and organic content in teeth, determine the different spectra associated with different levels of carbonate, phosphate, and collagen at varying stages of decay, determine the relationship between mineral and organic content levels and stages of decay. To achieve this, we classified a group of teeth into three categories of decay (healthy, mild, and severe) and visually assessed their decay severity. We then utilized Raman spectroscopy, with an excitation level of 532 nm, to obtain spectra of each tooth and compared them to the healthy, control tooth. The results of this study demonstrated that Raman spectroscopy can be used to obtain quantitative measurements of the presence and intensities of molecular landmarks in each layer of the tooth aiding in the determination of dental caries at its early stages.

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INTRODUCTION

Tooth decay, also known as dental caries, is the most prevalent dental disease in the United States with 90% of adults above the age of 20 having had a cavity in their lifetime (Heng, 2016). If caught at the earliest stages, tooth decay may be reversible, however, if left untreated it may lead to gum disease and tooth loss. Tooth decay begins once the cariogenic bacteria, *Streptococcus mutans* (*S. mutans*), adheres to the surface of a tooth via plaque, a soft and sticky layer, formation (Heng, 2016). Over time, *S. mutans* degrade sugars and release lactic acid which leads to the demineralization of teeth. This causes the tooth tissue to soften and collapse, creating a cavity. At the stage where *S. mutans* is merely on the outermost layer of the tooth, the enamel, the damage is reversible (Heng, 2016). However, once decay reaches the dentin, the layer beneath the tooth enamel, damages require much more extensive treatments. This is especially true once the pulp, or the layer of the tooth containing nerves and blood vessels, has become infected with the bacteria (Heng, 2016). While a simple restoration using composite or porcelain would be plausible during the earliest stages of the cavity, more invasive treatments such as root canal therapy or extractions would be necessary once the infection has reached the pulp (Heng, 2016). From there, the infection could even extend beyond the mouth into other bodily systems. Evidently, cavity treatments would not only pose a physical and emotional toll on an individual but would also introduce financial burdens.

Given that tooth decay is often invisible to the eye at its earliest stages, dentists will often use a dental probe and mouth mirror to investigate the tooth in question visually and tactilely. While this may be more effective in identifying a problem area on the occlusal surface of the tooth, visual and tactile methods fall short in determining tooth decay embedded in proximal contact areas (“Tooth Decay & Cavities – The Silent Disease”). For these areas that are harder to

view with the human eye or reach with a dental probe, dental professionals will turn to x-rays for further guidance. Although x-rays may raise suspicion of decay in certain areas, clear identification of decay arising in between teeth (proximal contact areas) remains challenging as 30% loss of tissue must occur prior to the problem area becoming apparent via x-ray ("Tooth Decay & Cavities – The Silent Disease"). Meanwhile, Raman spectroscopy presents the ability to provide a quantitative approach to diagnosing tooth decay at its early stages by measuring mineral content and collagen levels in tooth enamel and dentin.

The human tooth's outermost layer, the enamel, is the highly mineralized, white layer we see when smiling. It is high in hydroxyapatite lattice which is depicted by the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. This lattice contains phosphate (PO_4^{3-}) anions, calcium, oxygen, and hydrogen molecules giving the enamel its high mineral content. In fact, the enamel is composed of 96 wt.% inorganic material and only 4 wt.% organic material and water making it the most mineralized tissue in the human body (Ionita, 2009). Its high mineral nature gives enamel its tough exterior slowing down bacterial penetration. In less mineralized layers of the tooth, such as dentin, bacteria penetrate and proliferate at a higher rate (Heng, 2016). Beneath the enamel lies the dentin, the layer of the tooth that is primarily composed of collagen. With only 70 wt.% of inorganic material, dentin is the softer and more porous tissue of the tooth consisting of many dentinal tubules (Ionita, 2009). These dentinal tubules rest amongst collagen crosslinks which are the primary source of collagen within the dentin. Its softer nature provides *S. mutans* with the capability of rapidly proliferating which leads to decay worsening at a higher rate once it has crossed the enamel (Heng, 2016). Between the enamel and dentin is the dentin-enamel junction (DEJ), shown in Figure 8. At this junction, phosphate concentration and the crystallinity nature of the enamel decreases as it crosses into the dentin where collagen crosslinks begin to takeover.

Coming from the dentin, collagen crosslinks will decrease in concentration as the DEJ fades into the enamel. With the knowledge of molecular distinctions of each tissue layer, Raman spectroscopy could be used to identify differences between healthy and decayed teeth by measuring the presence and intensities of molecular landmarks of each layer.

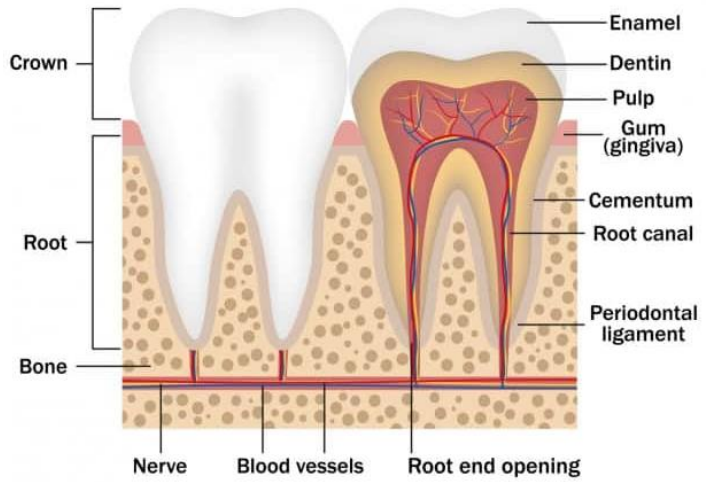


Figure 1. Image showing layers of a tooth. ("Different Parts of the Teeth")

	enamel	dentin
mineral crystallites	micrometer-sized hydroxylapatite needles	nanometer-sized hydroxylapatite platelets
mineral content (hydroxylapatite)	ca. 97 %	ca. 70 %
organic matrix content (proteins and lipids)	ca. 1.5 %	ca. 20 %
water content	ca. 1.5 %	ca. 10 %

Figure 2. Table comparing mineral content and organic content in enamel and dentin. ("The Chemistry of Dental Care")

METHODS

In Raman spectroscopy, when the laser beam targets a sample, most of the monochromatic light will pass through the sample as transmitted light ("Basics and Principle of Raman Spectroscopy"). However, some light will reflect off the sample, a phenomenon known as scattering. Rayleigh scattering is the result of the frequency of the incident light being equal to the frequency of the scattered light ("Basics and Principle of Raman Spectroscopy"). However, when the incident frequency does not equal the scattering frequency, this is known as Raman scattering. In Raman scattering, the electron in the sample will be excited to a higher energy state by the incoming monochromatic incident light ("Basics and Principle of Raman Spectroscopy"). Once the electron comes down to a different vibrational level than where it initially began, it is said to have lost a different amount of energy than the amount it absorbed from the incident photon. As a result, the photon emitted by the electron will have a different frequency from the incident photon ("Basics and Principle of Raman Spectroscopy"). This results in Raman scattering as the energy of the incident photon is higher than that of the emitted photon. Each molecule present in each layer of the tooth will have its individual Raman scattering, much like a fingerprint. Raman spectroscopy will present a unique spectrum for each molecule and by analyzing the wavelengths and intensities of the peaks, it is possible to deduce the concentration of these molecules within the tissues. By corresponding the molecule to the tissue layer, early diagnosis of decay is achievable by analyzing the quantitative measures provided by the Raman spectra. For the purposes of this study, the excitation level used was 532 nm.

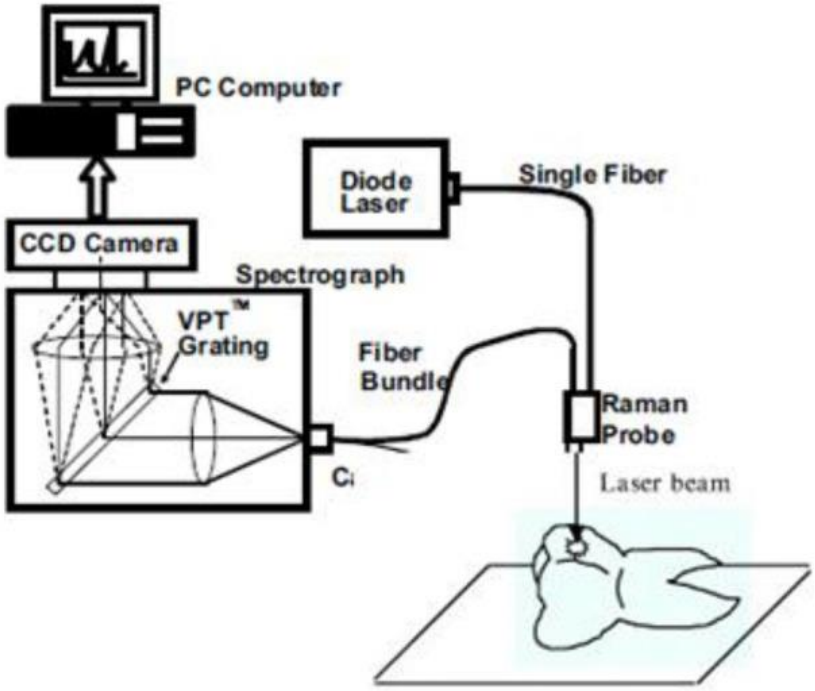


Figure 3. Schematic demonstrating of Raman spectroscopy applied to tooth sample. (El-Sharkawyi, 2019)

EXPERIMENTAL

Sample preparation

A total of 12 teeth were collected from Dr. Grant Jong's Oral Surgery office in Riverside, California, and the Riverside Free Clinic (RFC). Out of the 12 teeth collected, three teeth were used for experiments. Figure 4 shows all three teeth used for the experiments next to each other for a clearer comparison of decay. The (a) tooth on the left side is that with no visible decay, (b) the tooth in the middle is that with mild decay, and (c) the tooth on the right side is that with severe decay. Beneath each petri dish containing the tooth is the cap of the petri dish with the letters H, M, and S representing the healthy tooth, the tooth with mild decay, and the tooth with

severe decay, respectively (Figure 4). The healthiest tooth (a), as depicted with the letter H on the cap of the petri dish, is a mandibular first molar. The tooth with mild decay (b), as represented by the letter M on the petri dish cap, is a mandibular second molar. The tooth with severe decay (c), demonstrated by the letter S on the petri dish beneath it, is a mandibular third molar.

The 12 teeth were transported from dental offices to Dr. Bardeen's research laboratory in a securely sealed glass container with a 1:10 bleach solution. In the laboratory, the teeth were rinsed of visible debris using water and soapy detergent. All teeth were then placed in a fresh 1:10 bleach solution separate from that they were transported in. On the day the experiments were to be run, all 12 teeth were autoclaved on a dry cycle for 30 minutes at 121°C, 15 psi. To keep teeth from drying out, they were then stored in sterile, deionized (DI) water (Figure 6).

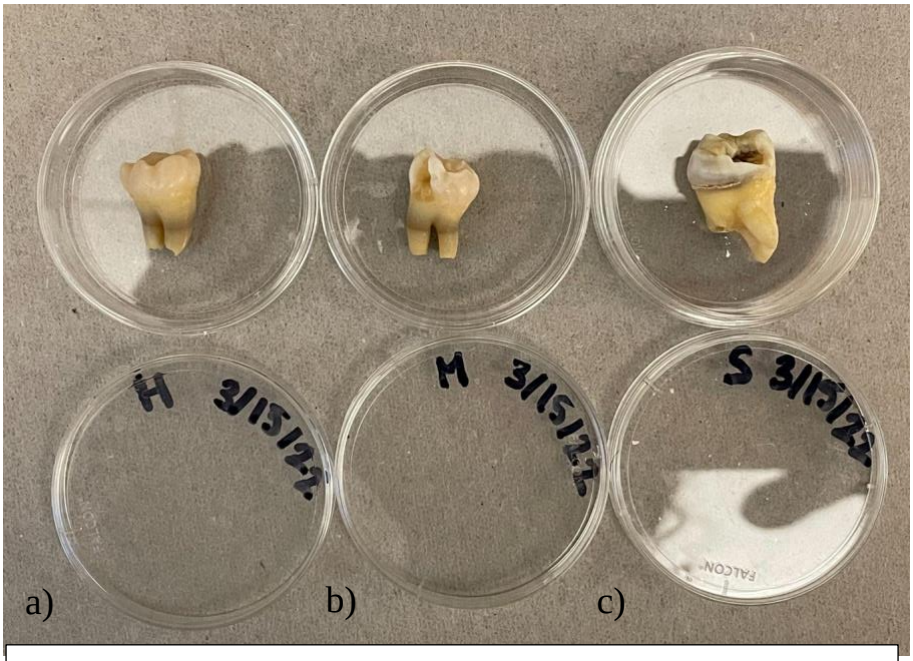


Figure 4. All three teeth next to each other with the healthiest tooth on the left (a), tooth with mild decay in the middle (b), and the tooth with severe decay on the right (c).

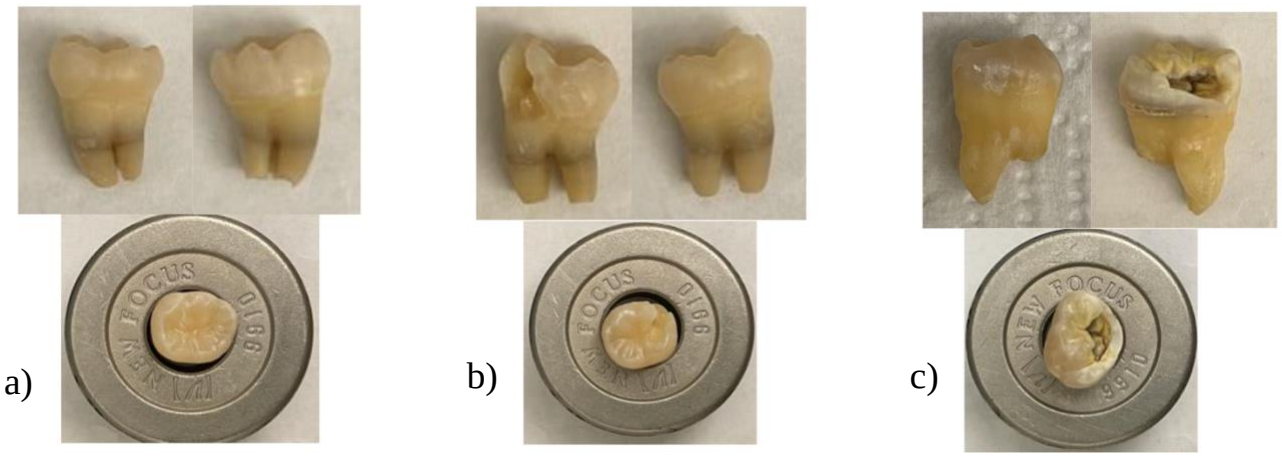


Figure 5. A closer look at each tooth. a) shows the healthy tooth from lingual, buccal, and occlusal surfaces. b) shows the tooth with mild decay from lingual, buccal, and occlusal surfaces. c) shows the tooth with severe decay from lingual, buccal, and occlusal surfaces.

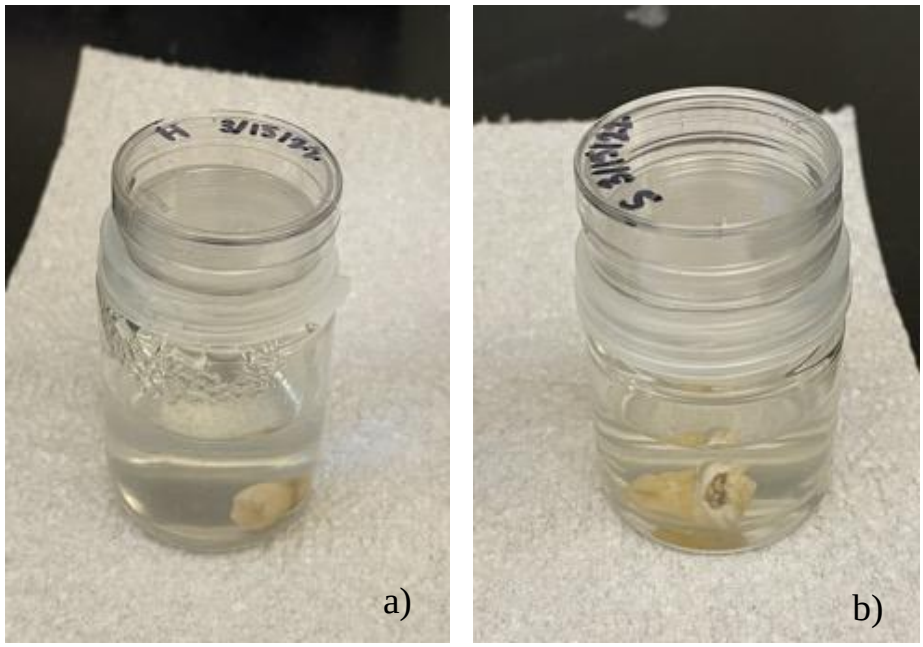


Figure 6. a) Tooth with no visible decay (healthy tooth) stored in DI water. b) Tooth with severe decay stored in DI water.

Collecting Data

Once the specific set of teeth was chosen, data were collected using a Raman spectrometer with an excitation level of 532 nm. Each tooth was placed on a round piece of metal with a hole in its center (Figure 7). This acted as a stabilizer for the tooth so that the laser beam could be directed toward the occlusal surface of the tooth rather than its side. For each sample, spectra were collected from at least one spot on the tooth. For the healthy tooth, data was collected from the occlusal surface which was made achievable by stabilizing the tooth on the metal piece. Data was collected on the grading of 1800 with an average of 10 seconds for 6 times at 10% power. Measurements were taken from when the laser was directed towards only the surface level and also while it was focused on the tissue. It was found that data from the surface and deeper into the tissue were similar, so the average was taken. These spectra are represented by Tooth Healthy (TH). For the tooth with mild decay, data was collected from the center of the caries lesion as well as from a spot slightly away from the caries lesion. Data was collected on the grading of 1800 with an average of 10 seconds for 6 times at 10% power using a 10x objective. Spectra from the spot on the tooth containing mild decay that was off to the side of the caries lesion are denoted by Tooth Mild Side (TMS). Similarly, the spot on the tooth with mild decay which lies in the center of the caries lesion is denoted by Tooth Mild Center (TMC). Data was collected from the tooth with severe decay at two spots: the first being in the center of the decayed area and the second being off to the side of the infected area on a relatively healthier section of the same tooth. The spectrum for the location of the tooth that lies in the center of the decayed area was collected on an 1800 grading with an average time of 1 second for 60 times using 10% power and a 10x objective. The location that was on the severely decayed tooth in the center of the affected area is denoted as Tooth Severe Surface (TSS). Spectra were also collected

from the same tooth but at a relatively healthier spot, this was denoted as Tooth Severe Surface Healthy (TSSH). These data were collected at 10% power for an average time of 10 seconds for 6 times on a grading of 1800.

Table 1. Abbreviations for each sample.

TH	Tooth Healthy
TMC	Tooth Mild Center
TMS	Tooth Mild Side
TSS	Tooth Severe Surface
TSSH	Tooth Severe Surface Healthy

Figure 7. Tooth Mild Center (TMC) stabilized on metal piece to collect spectra.

RESULTS AND DISCUSSION

After collecting spectra from each sample and plotting them on the same graph, the data was normalized at 917 cm^{-1}. Upon normalization, a general trend appeared. With intensity being on the y-axis and wavenumbers being on the x-axis, increased fluorescence was made apparent for the TMC and TSS samples as compared to the relatively healthier samples: TH, TSSH, and TMS (Figure 9). The less healthy samples (TMC and TSS) showed nearly 288x more fluorescence than the relatively healthier samples (TH, TSSH, and TMS). From this, it is possible to deduce that an increase in fluorescence correlates with an increase in decay. This increase in fluorescence shown in Figure 9 could be due to bacterial porphyrins on the surface of the tooth, organic compounds in dental plaque, or due to the excitation levels of the Raman spectrometer (Buchwald, 2021). However, even after subtracting the background, high fluorescence was still present, so it is less likely that differences in excitation levels are what caused the significant difference between TH, TSSH, and TMS samples and TMC and TSS samples.

Taking a closer look at the spectra, a dominant peak at 960 cm^{-1} is apparent in Figure 10. It is evident that TH demonstrated the strongest intensity for that wavenumber, followed by TSSH and TMS. TMC and TSS show no peak at 960 cm^{-1}. In literature, this peak is assigned to a PO_4^{3-} vibration (Slimani, 2017). Since PO_4^{3-} is present in the hydroxyapatite lattice, it is deducible that this phosphate vibration is indicative of enamel presence. The decrease of phosphate in the dentin region also supports the logic that this 960 cm^{-1} peak correlates to enamel. These spectra shown in Figure 10 is then expected as the tooth with no decay, thus a healthy enamel, shows the strongest intensity at 960 cm^{-1}. As decay increases in tooth samples,

phosphate levels decrease. It is then expected to find that in the most decayed tooth, TSS, there is no phosphate peak.

Dominant peaks at 1044 cm^{-1} and 1071 cm^{-1}have associated with phosphate and carbonate vibrations, respectively (Ionita, 2009). Figure 11 shows the high intensity of the phosphate peak for the healthy tooth (TH). While this peak is present in TMS and TMC, it has visibly broadened. In Raman spectroscopy, a broadened peak is indicative of weaker intensity. From this, it is possible to conclude that phosphate levels will decrease with increased tooth decay. Similarly, carbonate peak seems to be present in TH, TMS, and TMC but visibly broadens moving from the healthiest sample to the most decayed. These findings follow the logic as TH contains the healthiest enamel when compared to the teeth containing mild decay. Since enamel consists of higher mineral content than dentin, it is expected to have higher concentrations of phosphate and carbonate in healthy enamel. Once the enamel is deteriorated by decay, peaks at 1044 cm^{-1} and 1071 $cm^{-1}$are less anticipated.

Just as enamel has a higher mineral content than dentin, dentin contains more collagen in the form of collagen crosslinks than enamel. Slimani et al. have indicated that contents of the dentin layer of teeth will fall within ~ 1525 - 1550 cm^{-1} range. Peaks in Figure 12 show a presence at 1525 cm^{-1}, 1555 cm^{-1}, and 1565 cm^{-1}, with the most prominent being at 1525 cm^{-1}. Specifically, TSSH shows the most intense peak at 1525 $cm^{-1}$with TMS following it in terms of intensity, and then TH. Since the ~ 1525 - 1550 cm^{-1} range is said to be associated with exposed dentin, we can deduce that the more intense peaks indicate more exposed dentin. This would follow the logic of the spectra presented in Figure 12 as it is evident that TSSH, which is relatively more decayed than TMS, shows the strongest intensity peak at 1525 cm^{-1}. The tooth with most decay is also the tooth with the most exposed dentin and by extension, the most

exposed dentin. Given that TMS is mildly decayed, the dentin of the tooth will be less exposed than that of the severely decayed tooth.

Exposed dentin is also indicated by 1660/1690 cm^{-1} ratio which corresponds to collagen crosslinking (Slimani, 2017). Starting from the enamel, crossing the dentin-enamel junction (DEJ), and leading into the dentin, collagen crosslinks will increase in concentration. In this area, collagen is highly concentrated and phosphate levels are greatly decreased. As a tooth develops more severe decay, the collagen crosslinks of the dentin become exposed. This is evident as a peak at 1678 cm^{-1} is demonstrated by TSS in Figure 13, the most severely decayed tooth of the sample. Interestingly, TMS shows a peak at 1698 cm^{-1}which also falls within the 1660/1690 cm^{-1} ratio (Figure 13). TMS represents the data collected from the mildly decayed tooth but at a position on the side of the caries lesion, slightly away from its center. A peak at 1698 cm^{-1} may indicate that an area where dentin is directly exposed could have been targeted by the laser beam. It is also possible that the DEJ could have been exposed due to the caries lesion and the laser beam landed upon it. It is relevant to note that in a healthy tooth, the collagen crosslinks and DEJ will not be exposed as they will be covered by healthy enamel.

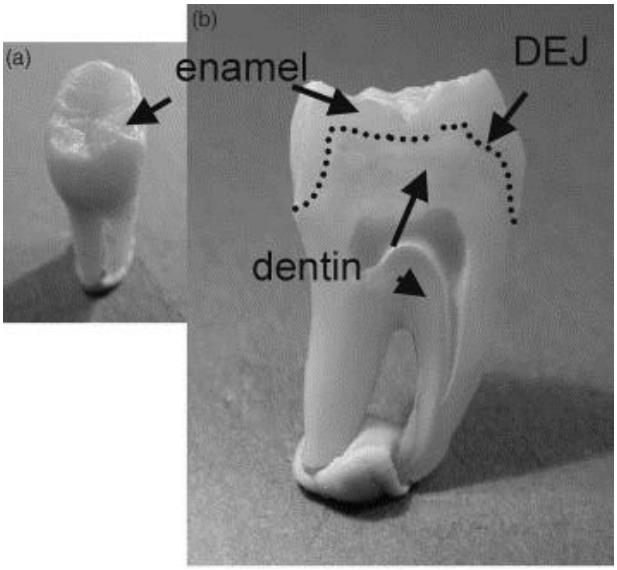


Figure 8. Image showing DEJ at bisection of tooth. (Marshall et al. 2003)

Figure 9. Graph showing overlapped spectra of each sample.

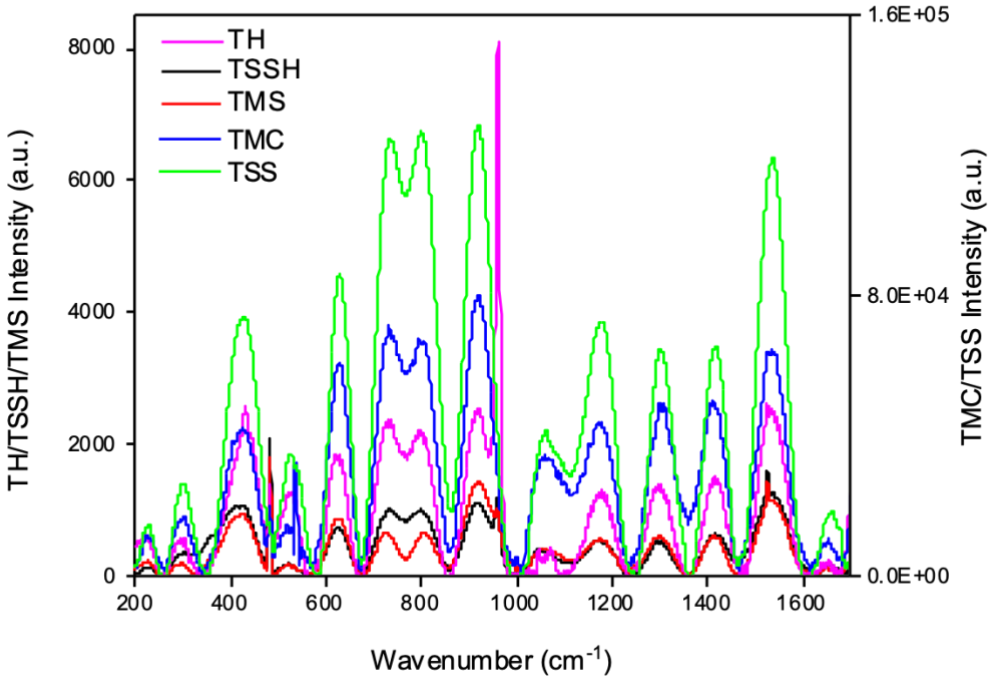


Figure 10. Graph showing overlapped spectra with dominant peak at 960 cm^{-1}.

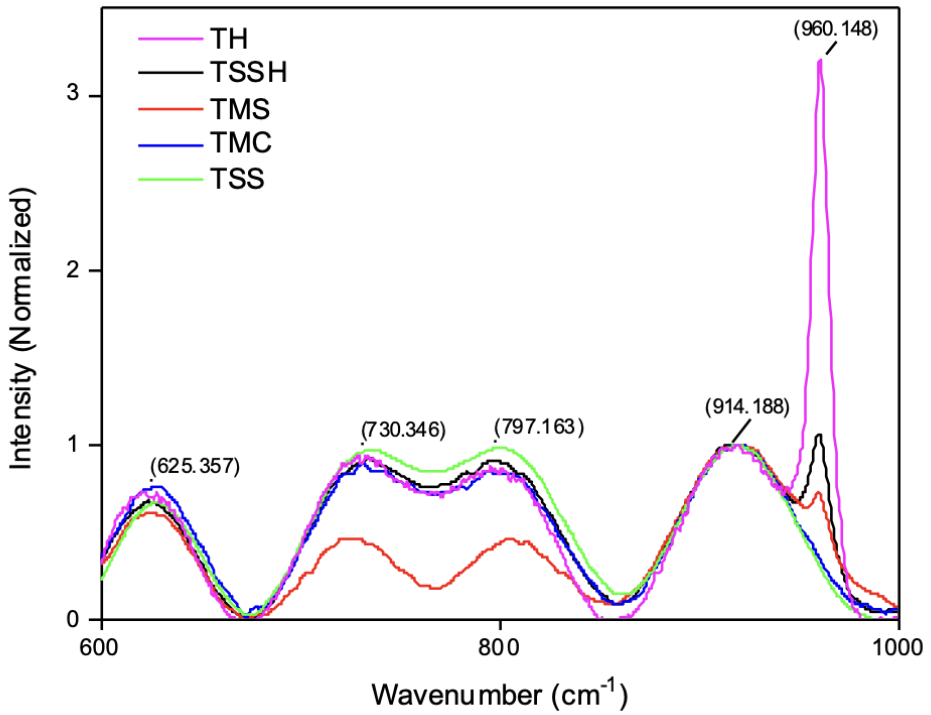
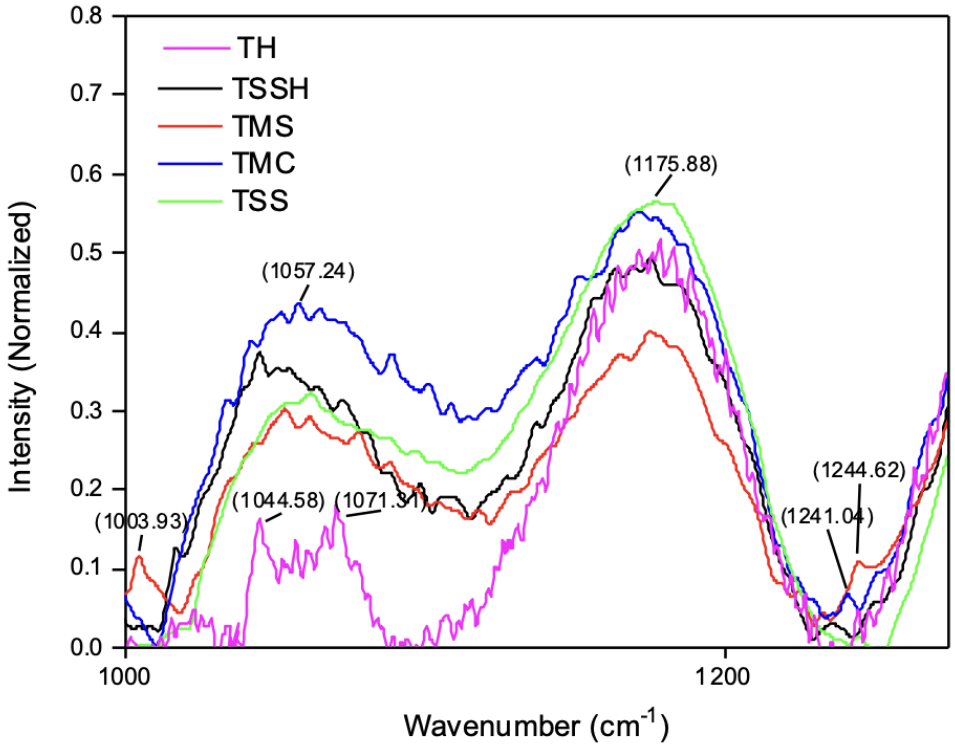


Figure 11. Graph showing overlapped spectra with dominant peaks at 1044 cm^{-1} and 1071 cm^{-1}.



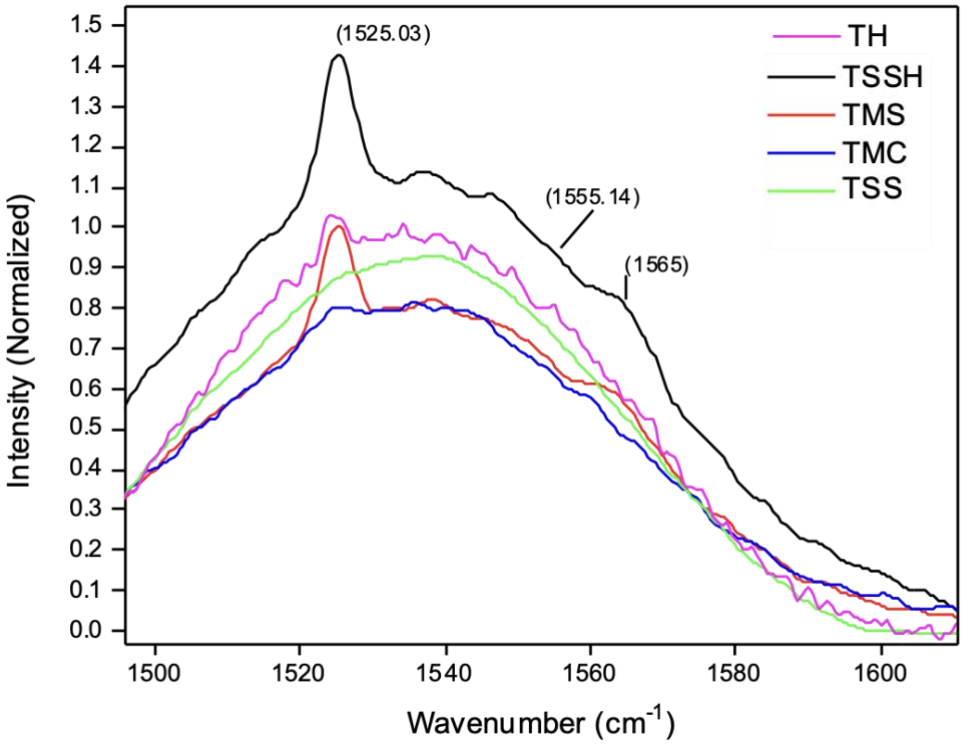


Figure 12. Graph showing overlapped spectra with dominant peak at 1525 cm^{-1}.

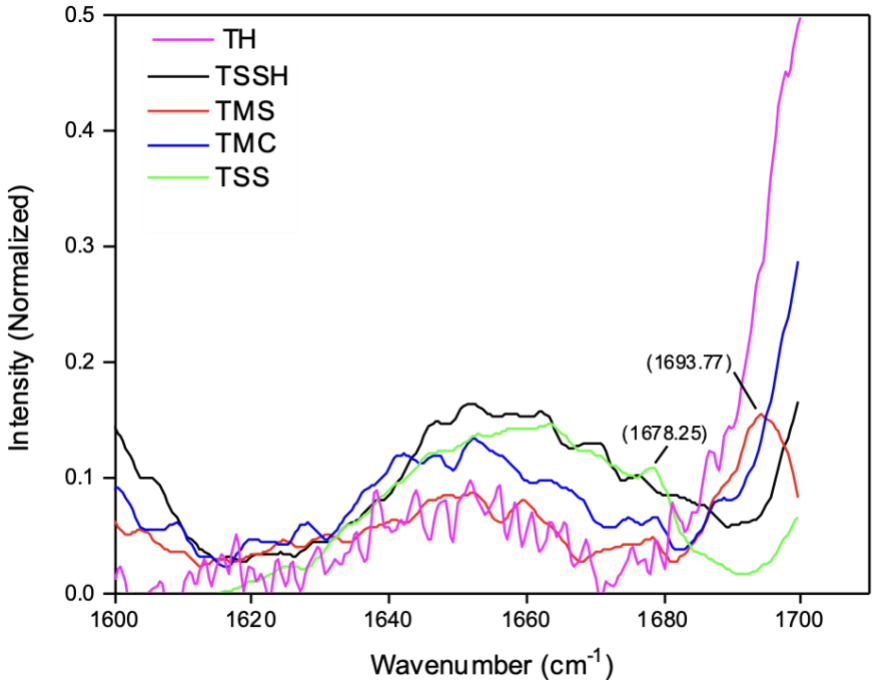


Figure 13. Graph showing overlapped spectra with dominant peaks at 1678 cm^{-1} and 1693 cm^{-1}.

CONCLUSIONS

This study has proven that Raman spectroscopy can in fact be used to identify differences between healthy and decayed teeth at a molecular level. In future experiments, it could be beneficial to use a mapping technique as opposed to collecting spectra from individual spots on the tooth surface. Enamel thickness depends on the type of tooth as well as which surface of the tooth is looked at (Stroud, 1998). By picking individual spots on the teeth, the varying thickness of enamel was not considered. Creating a map of the entire crown surface of the tooth using Raman spectroscopy would consider the different thicknesses of enamel alongside the edges and the surface of the tooth. Data from the map could then be averaged to give a single value.

Aside from using a mapping technique, it would also be interesting to include an x-ray in this study to assess for sensitivity changes in the teeth. Since Raman spectroscopy is a phenomenon that could be introduced to dental offices, it is important to investigate whether the continuous exposure to laser will alter the sensitivity levels of the patients' teeth. Studying these effects with an x-ray prior to testing Raman spectroscopy in vivo is beneficial.

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