Raman Spectroscopy Probe With In Vivo Applications

L.A.S.E.R.S. - Live-Action Safe Examination Raman Spectroscopy

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Senior Design 2
Final Documentation - August 1, 2017

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Sponsored by Ocean Optics

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1 Executive Summary

It is estimated that 87,110 new cases of invasive melanoma will be diagnosed in the U.S. in 2017, and melanoma only accounts for 1% of skin cancers (American Cancer Society). These patients must visit a dermatologist to have freckles, moles, or other abnormal skin lesions checked for the potential of being cancerous. This usually results in a biopsy of the skin to be sent to a pathologist who uses a microscope to check if the skin sample is cancerous or noncancerous -- a process that may take several days to receive results. A biopsy is performed by cutting out or excising an area of the skin under consideration. This can be painful for the patient and may result in scarring. The stress of waiting for results is difficult for a patient waiting to hear potentially life-altering news. Further biopsies may need to be performed if the biopsy sample did not contain the abnormal cells, if the concentration of the cells was not high enough for an accurate diagnosis, or if the skin appears to change after the biopsy. Therefore, the motivation of the Live-Action Safe Examination Raman Spectroscopy (L.A.S.E.R.S.) project is to provide a non-invasive alternative to a biopsy for skin cancer screening, all while maintaining the same standard of accuracy and reliability and reducing medical expenses.

In today's ever-evolving medical field, analysis and innovation go hand in hand. Without powerful technological advancements, it would be next to impossible to analyze cells, DNA, or gather information that could be helpful in the future. Medical breakthroughs cannot be possible with just theoretical data; tools need to be created to either prove or dismiss these theories. *In vivo* Raman spectroscopy will be the new first step in skin analysis and cancer diagnosis, making invasive biopsies the second step.

The project's focus is to gather analytical data from samples. Samples will include gemstones such as diamond, Asha simulant diamond, and an unidentified purple gem. Skin samples will not be used because of health and safety regulations and forms. The way this is done will be through the use of spectroscopy. Spectroscopy is an optical science that measures the spectra of materials resulting from the electromagnetic radiation interaction with said materials. Therefore, spectroscopy can be used to evaluate the light-matter interaction of the gemstones. In particular, Raman spectroscopy will be utilized to gather the unique Raman spectra of the gems. An excitation laser will illuminate the sample. The reflected or scattered light from the sample will have a different frequency than that of the incident laser light. This scattered light will be collected and analyzed by a spectrometer. In fact, the scattered light from the sample will manifest a spectrum that is unique to the molecular constituents of the sample. Using this sample "fingerprint" the Raman spectrum should be differentiable among gemstones. In future applications, this can be applied to differentiate between cancerous and normal skin. The spectral peaks of interest for skin will be within the range of 1000 to 1800 cm^{-1}. It is within this range that changes in the spectra of the biochemicals collagen and amide are strong indicators of the presence of cancer.

Raman spectroscopy is well known to be capable of providing data for biomedical and diagnostic applications *in vitro*, *in situ*, and *in vivo*. Raman spectroscopy *in vivo* is becoming increasingly popular for real-time, non-invasive biomedical applications. It is non-invasive because measurements are taken using light and require no cutting into the skin like a biopsy would. To implement these measurements, the team could design a probe specifically engineered to collect the Raman scatter from skin. Instead, the team will design a probe to collect the Raman scatter from gemstones. The probe will also incorporate a camera for imaging of the sample area being measured. The collected Raman signal will be directed to an Ocean Optics spectrometer designed for analyzing Raman signals, capable of measuring the necessary spectral range with high resolution. The spectral data will then be collected by a charged coupling device, which will allow the team to view data points that can be plotted utilizing a microcontroller. From there, the data can be transferred (through Wi-Fi, Bluetooth, USB, or some other means) to a display such as a desktop computer to further analyze and present the results.

The components – laser, spectrometer, and probe -- are configured in a modular format, which allows the team to prevent issues such as overheating or diagnostic frustration. Having the components in their own separate spaces gives the team the ability to detect errors in hardware and allow for quicker replacements, as well as have them preconfigured to work as stand-alone components. Modularity, however, will not render the project too large. This will be a portable, handheld device that can be utilized at the comfort of a client's home or office, so long as there is power.

This project is being sponsored by Ocean Optics, a local company specialized in designing and manufacturing spectrometers and accessories, including those for Raman applications. Ocean Optics is providing a laser source, spectrometer, and probe. The laser is a 785 nm (near-infrared) spectrum stabilized laser. The spectrometer is a high resolution spectrometer configured for the appropriate wavelength range needed for analyzing the spectra of the samples. The probe is a commercial Raman probe (InPhotonics, Inc.) to be used for testing comparisons. The team will design a handheld, Raman probe integrated with a camera that will operate in conjunction with the laser source and spectrometer. The team will also design an algorithm for analyzing the spectra.

This report describes the engineering specifications and standards behind the design of the Raman probe. A brief explanation of the Raman effect is presented, followed by research on the Raman applications for skin analysis. Electrical considerations include the printed circuit boards and microcontroller necessary for powering and controlling the many components of the system. Optical considerations include the light-matter interaction of the laser with the sample and engineering the propagation and filtering of the light information. Computer engineering considerations include programming to control the data transfer between components as well as analysis and presentation of the results.

The aim of this project is to design and build a Raman probe capable of identifying gemstone samples based on their Raman spectra. Figure 1 below illustrates the project overview. The Main Box houses the laser, spectrometer, Raspberry Pi, and power PCB. Light is delivered to the sample and collected via the probe. Spectral results are shown on a computer display. Through rigorous planning, project construction, research and development, this group will be creating a device that can be reliable, cost-effective, immediate, and most importantly, safe.

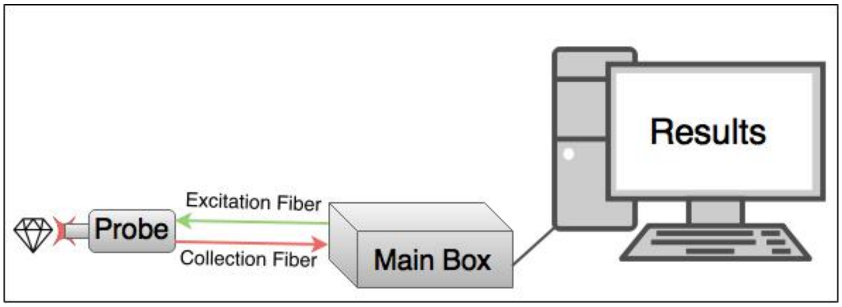


Figure 1. Overview of the completed project.

2 Project Description, Requirements, and Specifications

2.1 Project Description

The immediate goal for this project is to design and build a Raman probe for identifying gemstones including diamond, Asha simulant diamond, and an unidentified purple gem. The future goal for this project is to design and build a compact, accurate, portable Raman probe that can be used to identify cancerous versus noncancerous skin cells *in vivo* on virtually any external location on the body. It entails using a Raman spectrometer that operates at a wavelength range known to obtain the identifying features of the Raman spectra of cancerous versus normal cells. The Raman spectra are analyzed to help diagnose the possibility of skin cancer as an early detection measure. This system offers patients and medical offices a more efficient and cost effective option compared to expensive Raman spectroscopy system and traditional biopsies. Patients could be able to see day-of results, faster when compared to a biopsy that must be sent away and processed in a lab. The use of Raman spectroscopy is less invasive for patients, as a biopsy requires the removal of skin cells from the potentially impacted body part. At the center of the project, this is a Raman probe that can be combined with a Raman spectrometer and laser source for the specific application of skin cancer detection.

Research has been conducted and is currently being conducted to show that Raman spectroscopy is a feasible method for the *in vivo* diagnosis of skin cancers including basal cell carcinoma and melanoma [1-6]. A measurement method of interest includes *in vivo* spectroscopy using a fiber optic probe [3]. Using a probe would meet the design goal of portability. In a clinical setting, the use of a probe may be a comfortable method for the patient.

Medical technology is always relevant in the industry with new solutions always needed and improved upon. This project implements the range of talents across the team together and as individuals. It will use a combination of each team member's knowledge from education as well as experiences at internships and careers, tying together traditional and practical knowledge.

This compact option is less expensive than typical on-the-market Raman spectroscopy systems. A less expensive option for a doctor's office to buy and operate reduces patient fees. On average, a portable Raman spectroscopy system can cost anywhere from $2,500 to over $50,000, a range that varies with the purpose and accuracy of the system. The average biopsy without insurance costs approximately $200, which adds up for a high-risk patient that may need to have many biopsies performed. Lower fees mean that a patient may be more likely to have potentially life-saving testing done with less of a financial burden. Providing a reliable, safe, affordable, and effective option compared to current methods will prove this design as a success.

2.2 Project Requirements and Specifications

This project requires the design and construction of a Raman probe. Aesthetically the probe should be handheld and ergonomic for ease of use and comfort to both the user and patient. There are four engineering objectives to accomplish with the design of the probe. The first is to make an instrument capable of illuminating a sample area using light from an excitation laser. The second is to capture an image of the sample area using a camera to be matched with the Raman spectra of that area. The third is to design the optical components in a manner that will effectively filter the laser line and the collected Raman scatter and then deliver the collected signal to the spectrometer. The fourth is to transfer and analyze the spectral data from the spectrometer and display it to a device.

There will be several necessary components that must be added to allow for proper data flow and power flow. From a data perspective, there are few methods to get accurate readings from skin samples from scanned spectra onto a viewable interface. To accurately collect data from the spectrometer, the use of a Charged-Coupled Device (CCD) is required to convert the light collected from the sample into quantifiable data to be vetted. To securely and safely convert that data from light information, the use of microcontrollers will be necessary to guide the data onto a more viewable perspective. Once the microcontrollers can access the data and determine what has been seen via the spectrometer, there will be coding in place to provide a digital interpretation of the data that can be displayed onto a screen. Through the algorithm, this project will be able to gauge if the scanned cells show cancerous traits. These traits will allow the device to be able to provide the user with the algorithm's interpretation of the presence of cancerous cells, as well as the ability to show these results to their trusted medical professional.

2.3 System Power and Data Flow Diagram Overview

From a power perspective, the main goal is to provide power to each component in the most modular way possible. Modular electronics are beneficial because of their heat reduction, lack of interference (especially in medical devices), and for their ease of troubleshooting and identifying rogue or defective components. To provide a modular design, there will be at least two Printed Circuit Boards (PCB): one dedicated to the camera module and the LEDs, switches, and the other specifically for power distribution and power to the Raspberry Pi. Due to the sensitivity of the components being used, they will have their own PCB setup to ensure they are not tampered with. The purpose of the first board will be mainly to provide power to the rest of the device.

To summarize the overall flow of the project, it was imperative to construct a power and data diagram to indicate the corresponding functions and interconnections of the major system components, being the laser, spectrometer, probe, camera module, LEDs, Raspberry Pi, and PCBs. This representation allows for a concise and understandable overview of the project, and what is happening from a data and power perspective. Figure 2 depicts the overall flow of the power and data in the system.

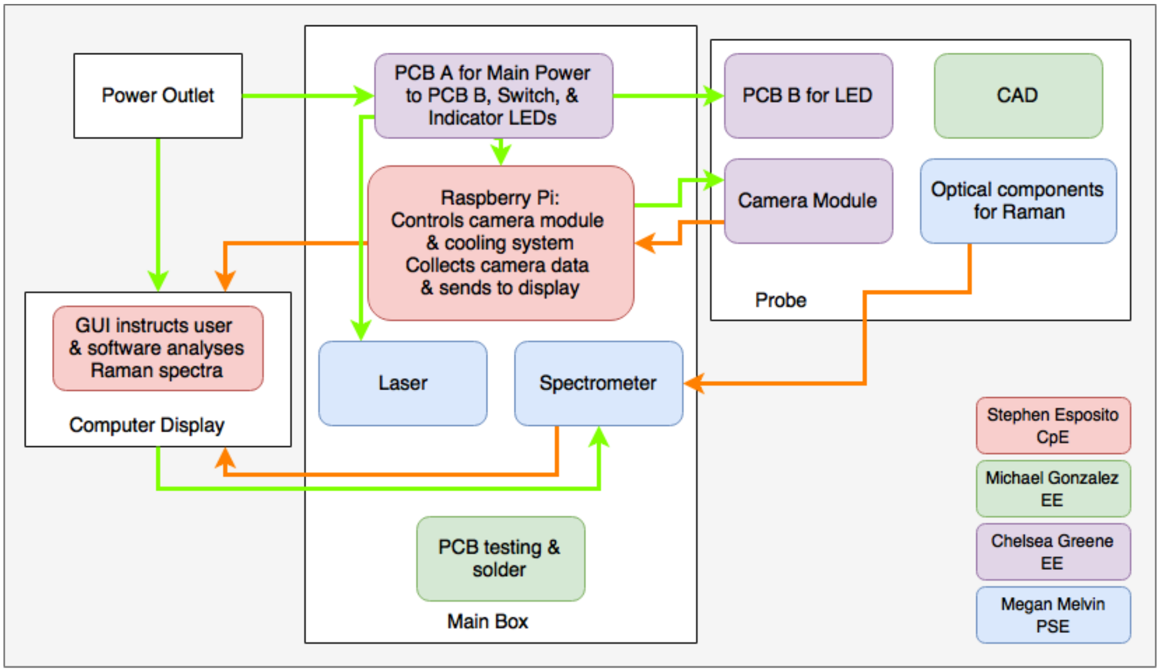


Figure 2. Data (orange arrows) and Power (green arrows) Flow Diagram

For the power representation (green arrows), start from the power outlet and navigate to the first PCB (in purple). This PCB has three responsibilities: to obtain the power from the outlet, to utilize that power onto the laser and Raspberry Pi, and to distribute that power onto the second dedicated PCB and a fan. After the power is pushed onto the second PCB, the power is delivered to the LED beam indicating the location of the probe's viewpoint. This is the most cost-effective way to maintain a modular design.

For the data representation (orange arrows), it is best to begin from the initial data collection from the sample. The laser will propagate the excitation wavelength through an optical fiber connected to a Raman probe. A camera inside the probe will capture an image of the illuminated sample area. Upon exiting the probe, the laser light will reflect off the sample and be re-collected by the probe. This reflected light will be filtered by the probe so that only the Raman signal will propagate back through an optical fiber to the spectrometer. Within the spectrometer the Raman signal will be dispersed via a diffraction grating, and the signal will be collected by a CCD. Once that is done, the light data will be sent onto the microcontroller so that they can utilize that data as input for their code to represent whether the cells have cancerous properties. It will then send that result onto a user interface to verify and show their results.

2.4 House of Quality

As demonstrated in Figure 3, this project is intended to be cost effective, safe, reliable, and provide a shorter diagnostic response time. Reliability of the system is ability for it to operate as designed and with consistency. Accuracy is the ability of the measurement system and data analysis to identify that a sample has a higher or lower level of X compared to normal skin. For example, X may be the amides present in the collagen of skin. Safety deals with the laser exposure to the skin, preventing burns, and preventing accidental exposure to the eye.

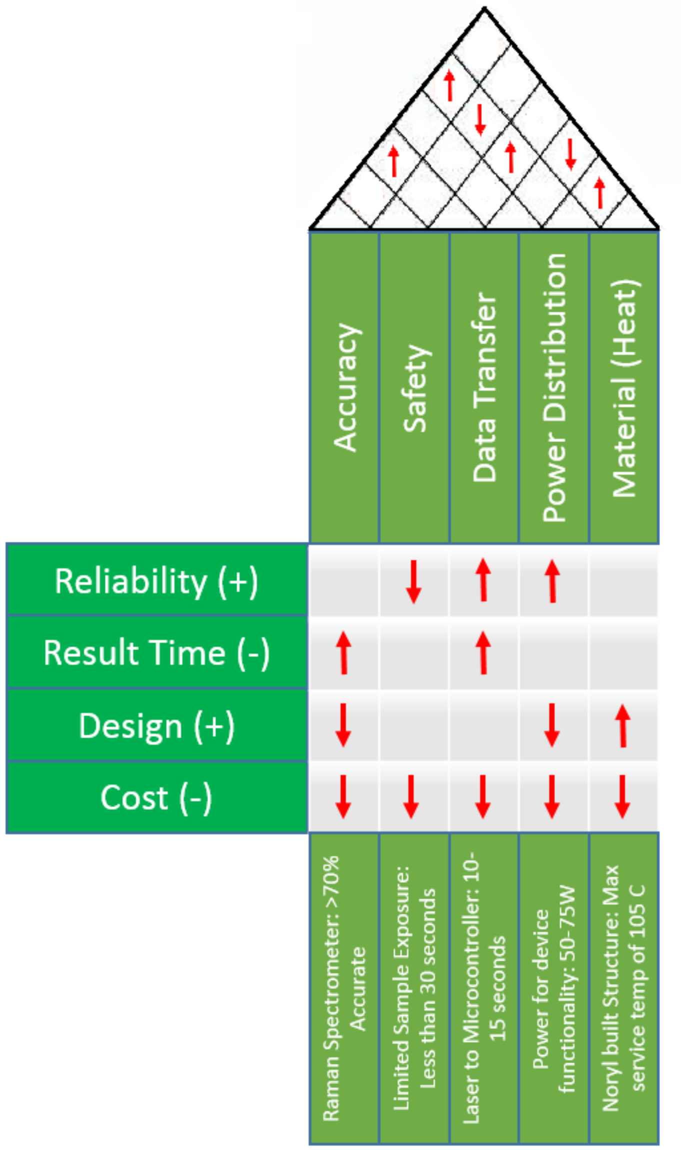


Figure 3. House of Quality

Accuracy does not impact reliability; however, it will help decrease the response time while making the cost greater and the design less simple. Safety will not impact the result time or the design complexity, but will increase the cost and make the system less reliable. Effective power transfer will increase the reliability and the cost of the system while decreasing the diagnostic response time. Efficient power distribution increases reliability and cost while improving the design. Choosing materials that can properly handle the heating of all electronic and optical parts will improve the design while increasing cost.

2.5 Block Diagram

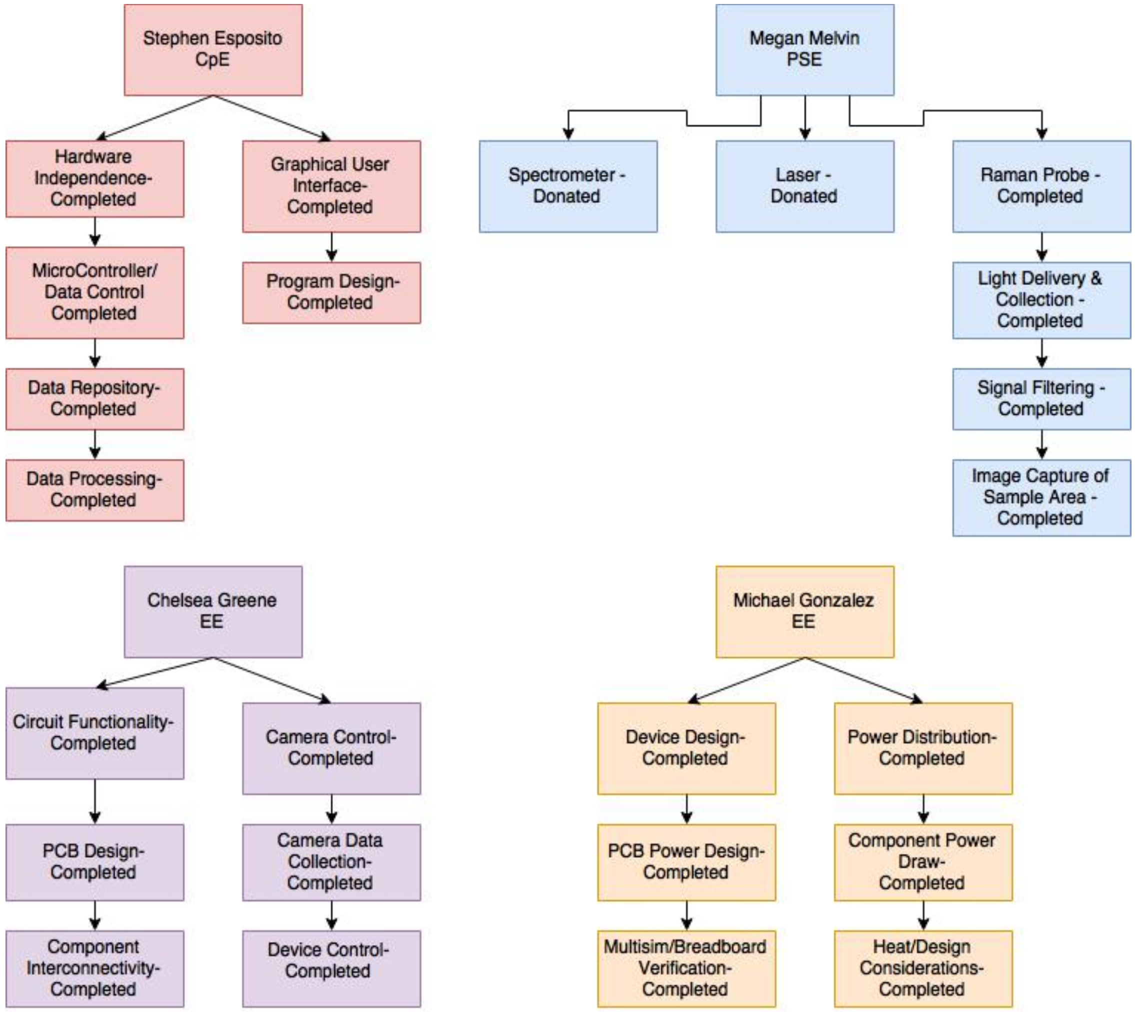


Figure 4. Block Diagram

Block Diagram Legend:

PCB: Printed Circuit Board for electronic components
Spectrometer: Optical instrument to measure light intensity as a function of wavelength; the Raman spectrum obtained will provide the Raman shift of the sample.
Laser: Near-infrared excitation source to illuminate the sample and induce Raman scattering.
Raman Probe: Optical instrument acting to deliver and collect light, filter the signal, and capture images of the sample area.
Microcontroller: Will be used to receive the optical data from the sensors and transfer the data to the main computer to be graphed and analyzed.

Per Figure 4, red blocks are the responsibility of Stephen Esposito; blue blocks are the responsibility of Megan Melvin; purple blocks are the responsibility of Chelsea Greene; and orange blocks are the responsibility of Michael Gonzalez.

2.5.1 Block Diagram Status

As of now, all components are completed, including the power consumption, heat distribution, device control, and the optics. The samples include diamond, Asha simulant diamond, and an unidentified purple gem. The peaks in the Raman spectra for these samples have been measured and defined; and the software to analyze the samples has been completed. The PCB and casing are completed. The Raman spectrometer and microcontroller are already acquired. Future improvements are discussed in the summary and conclusion section of this paper.

The Electrical Engineers are responsible for the PCB, design, power distribution, LED and switch circuit design, and sensor control. The Computer Engineer is responsible for the microcontroller and graphical user interface. The Photonic Sciences Engineer is responsible for the spectrometer and probe design. Each team member will participate in the design of all four components.

Looking back at the block diagram from the initial document in January, it’s evident that the design has gone through a massive overhaul and change from its original concept. This is one of many indicators that designs need to change their scope and focus in order to be efficient, marketable, and feasible in the time and design constraints allotted.

A major shift since the initial proposed project occurred for the Electrical Engineers. Initially, a focus was placed on utilizing and incorporating a CCD sensor into a Raman spectrometer. While a CCD sensor is still essential to the final device, the team shifted their focus to a CMOS camera. This change occurred upon gaining sponsorship from Ocean Optics and receiving a spectrometer that already incorporates a CCD sensor inside the device. The research remains relevant, however the design focus had to shift with the project itself.

From a Photonics perspective, the initial plan was to design and build both a spectrometer and probe. The laser source was to be acquired. Optical components required for these components, such as a laser, lenses, mirrors, and gratings, can quickly escalate in price. Because of cost and time constraints, the project was simplified to a Raman probe design per the advice of Ocean Optics.

3 Research

The research that was done to fully understand the concepts, components, and structure of this design is of the utmost importance. The research for this design is the first task that must be completed, especially when all the hardware and software should coexist at the most optimal level. It is imperative that every detail, from pin configurations, to PCB layout styles, to skin health, should be explored and understood. The sections below will cover several categories that have been researched such as Raman spectroscopy, skin cancer and diagnosis, as well as the electrical and data representations of the system.

3.1 Skin Cancer and Diagnosis

Per the Centers for Disease Control and Prevention (CDC), cancer is the second leading cause of death in the United States, and skin cancer is the most common form of cancer. Basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma and three of the most well-known skin cancers. According to the American Cancer Society, the most common type of skin cancer is BCC, while melanoma is the most likely cancer to metastasize.

It is paramount for the patient that skin cancer is diagnosed correctly and treated appropriately. When a dermatologist has suspicions of a lesion, a biopsy of the lesion is performed. A biopsy is the removal and examination of living tissue to ascertain the presence and extent of a disease. [7]

Biopsies are achieved through several techniques. A shave biopsy is performed using a scalpel or blade to cut with a sweeping motion into the superficial epidermis while taking care to include some dermis to detect any invasive disease such as melanoma. Care must be taken that the biopsy is not so superficial that a diagnosis could not be achieved or else a second biopsy must be done. A punch biopsy utilizes an instrument called a trephine, which is a cookie-cutter like tool to cut into the skin down to a desired depth. The skin sample must then be removed with forceps and cut free using scissors. An excisional biopsy removes the entire lesion, while an incisional biopsy removes a portion of a lesion that is too large to be completely removed. Sutures and/or bandages are often necessary at the removal site. Patients may have cosmetic concerns as temporary or permanent scarring may result from the biopsy. [7]

A pathologist or dermatopathologist will examine a skin biopsy to determine the presence and extent of diseases. Skin samples are typically inspected with a microscope. Diagnoses depend on the interpretation of “tissue reaction patterns” and “patterns of inflammation” [8]. Because biopsies are visually inspected and can present a numerous amount of various results, “[d]ermatopathology requires years of training and practice to attain an acceptable level of diagnostic skill” [8]. Furthermore, obtaining the results of a biopsy may take one to several days, especially if there are delays due to sample processing and any special stains or additional tests that must be done for accurate diagnosis.

3.2 Raman Theory

The Raman scattering effect was discovered in 1928 by C.V. Raman. Raman reported that the energy of incident photons on a material was different from the energy of the scattered light. Raman recognized that this exchange of energy corresponded “to the partial exchange of energy into atomic vibrations of the molecules” [9]. From this stemmed the development of Raman instrumentation and measurement techniques that have evolved to present day technology. [9]

Molecules of different materials have different vibrational energy modes. When a molecule absorbs incident light of a certain energy $h\nu$, the reflected or scattered light may be of equal energy $h\nu$ called Rayleigh scattering, lower energy $h\nu - \Delta h\nu$ called Stokes Raman scattering, or higher energy $h\nu + \Delta h\nu$ called Anti-Stokes Raman scattering. Figure 5 demonstrates these scattering concepts.

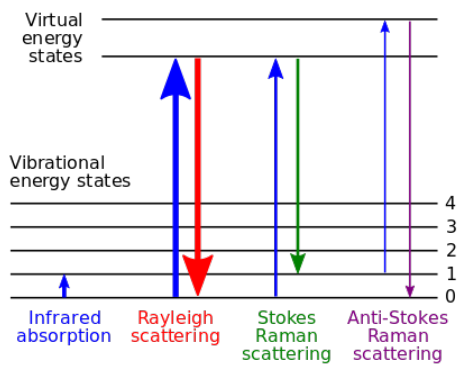


Figure 5. Representation of Rayleigh and Raman scattering.

Rayleigh scattering is a form of elastic scattering, meaning the incident and scattered light both have the same frequency. Stokes and Anti-Stokes Raman scattering are forms of inelastic scattering, meaning the incident light is of a different frequency than the scattered light. The Raman shift is what L.A.S.E.R.S. aims to measure. Our spectrometer will ideally measure the Raman shift of a skin sample, analyze the spectral data, and determine if the skin sample exhibits a Raman spectra typical of normal skin or of cancerous skin, such as basal cell carcinoma.

The Raman spectra is the key data L.A.S.E.R.S. must acquire. The peaks in the spectra are characteristic of the sample being measured, resulting in a kind of “fingerprint” unique to the sample. The samples measured will likely be a human skin analog such as pig skin. Typical wavenumber regions for skin analysis range from 400 cm^{-1} to 1800 cm^{-1}. By looking at the results of research studies, the team found that collagen is a major constituent of skin contributing to approximately 80% of the Raman signal. The most intense peaks of the Raman spectra are in the Amide I, II, and III bands. Amides are proteins that make up collagen. The amide I peak is found around 1600 to 1700 cm^{-1} (1575 to 1730 cm^{-1} for pig skin). The amide II peak is found around 1480 to 1575 cm^{-1}. The amide III peak is found around 1200 to 1300 cm^{-1}. These peaks in the collagen spectra should be sufficient for seeing differences in the Raman signals of normal skin and cancerous skin. In fact, collagen contributions to the spectra are decreased in BCC and melanoma as compared to the contribution in normal skin. Therefore, the wavenumber range of the spectrometer will be from 1000 to 1800 cm^{-1}.

Analyzing the wavelengths at which the peaks occur will provide insight into the molecular composition of the particular sample. Raman shift is typically measured in wavenumbers. To convert the wavelength (in nm) to wavenumbers (in cm^{-1}) requires the following equation: $\Delta w(cm^{-1}) = (\frac{1}{\lambda_o(nm)} - \frac{1}{\lambda_1(nm)}) \times \frac{(10^7 nm)}{(cm)}$ where λ_0 is the excitation wavelength, λ_1 is the Raman spectrum wavelength (nm), and Δw is the Raman shift in wavenumbers (cm^{-1}).

3.3 Raman in Biomedicine

Raman spectroscopy is popular in the biomedical field because the Raman signal interacts weakly with water. This is important because many biological samples, including human skin, consist mainly of water. Therefore, the Raman signal will only strongly interact with other biomarkers of interest. Raman microscopy is popular for its diffraction-limited spatial resolution, making it ideal for imaging micron-sized biological cells. [10] For example, human skin cells are an average size of 30 microns.

The Raman scattered signal is weak, therefore, optimizing the signal through design considerations is necessary. Biological materials may exhibit fluorescence, the emanation of light resulting from the absorption of incident radiation. This fluorescence is often stronger than the Raman scattering signal, making proper signal analysis difficult. There are several solutions to overcome this issue including the use of ultraviolet (UV) or near infrared (NIR) laser excitation and signal processing methods, which will be discussed later. [10] UV and NIR wavelengths are outside of the typical absorption regions of biological materials that result in fluorescence. Additionally, the water composition of the materials helps to reduce fluorescence. [11]

Lasers are an intense, monochromatic light source. Exploiting this intensity assists in increasing the Raman scattering signal and reducing acquisition time. On the other hand, this intensity may be damaging to the sample depending on the power of the laser and the excitation wavelength, particularly in the UV region. [11] Therefore L.A.S.E.R.S. will use relatively low power of less than 350 mW and a NIR excitation wavelength. A visible excitation wavelength will suffice if a NIR laser is inaccessible.

3.4 Raman Spectroscopy and Skin Cancer Detection and Diagnosis

Raman spectroscopy is appealing as a method of skin cancer detection and diagnosis. Current research [1-6] on the *in vivo* application of Raman spectroscopy promises a less invasive technique with fast results compared to a conventional skin biopsy. In one study by Gniadecka et al., the spectra of normal skin and BCC exhibited considerable differences when analyzing the spectral peaks of certain proteins and lipids present in the skin biopsy samples [1]. In another study by Schut et al., the Raman spectra was used to differentiate the border between normal skin and BCC, demonstrating "the sensitivity of Raman spectroscopy to biochemical changes in tissue accompanying malignancy, resulting in a high accuracy when discriminating between basal cell carcinoma and noncancerous tissue" [2]. Silveira et al. concluded from their study that because normal and cancerous skin consist of different biochemicals, the intensities of the Raman spectra are different even if the concentrations of biochemicals are the same [3]. This indicates that the Raman spectra accurately represents the material under inspection.

The research conducted by Silveira et al. showed that the major spectral characteristics of skin originated from actin, collagens (I and III), elastin, and triolein. Research conducted by Gniadecka et al. showed that basal cell carcinoma and melanoma, in comparison to normal skin, exhibited changes in the intensity for the bands of proteins and lipids. For skin with BCC and melanoma, there was a decrease in the intensity for proteins in the regions of 1500-1800 $cm^{-1}$and 1310-1330 cm^{-1}. On the other hand, lipids exhibited an increase in the intensity around 1300 cm^{-1}. The Raman spectrometer will observe the amide bands over a spectral range at least from 1000 to 1800 wavenumbers to measure the Raman shift between normal and cancerous skin.

3.5 On Market Raman Spectrometers

Ocean Optics currently offers four Raman bundles. Each bundle includes a laser source, spectrometer, probe, and OceanView spectral software. The lasers offered include common Raman excitation wavelengths at 532 nm, 638 nm, 785 nm, and 1064 nm. A visible wavelength at 532 nm works well for inorganic materials that do not auto-fluoresce. Of the four options, this shorter wavelength produces a stronger Raman signal due to the relation $\frac{1}{\lambda^4}$. A visible wavelength at 638 nm works well to reduce some autofluorescence of organic materials. This wavelength also reduces the risk of damage to biological materials when used for biomedical applications. A near-infrared wavelength at 785 nm is perhaps the most common wavelength for Raman spectroscopy of biological materials because it enables high resolution and a reduction in auto-fluorescence of organic materials. A short-wavelength infrared wavelength at 1064 nm is another common wavelength for Raman spectroscopy because it virtually eliminates all auto-fluorescence of organic materials. However, longer wavelengths result in a weaker Raman signal and may more easily induce thermal damage in biological samples.

The 532 nm laser has an output power greater than 50 mW and uses an FC connector. The 785 nm laser has an output power that can be adjusted greater than 350 mW and uses an FC connector. The 638 nm and 1064 nm lasers use FC connectors.

The spectrometers offered with these lasers each use a 50 μm slit width. For the 532 nm and 638 nm lasers, the gratings used in the QE *Pro* spectrometer have 1200 lines/mm, a blaze wavelength of 750 nm, a spectral range of approximately 123-170 nm covering 532-700 nm and 638-799 nm respectively, and efficiency over 30% from 500 nm to 1100 nm. The spectral resolution (FWHM) of the 532 nm spectrometer is 0.43 nm (9-15

cm^{-1}). It measures Raman shifts from 150-4000 cm^{-1}. The spectral resolution of the 638 nm spectrometer is 0.41 nm (7-10 cm^{-1}). It measures Raman shifts from 150-3150 cm^{-1}. The QE *Pro* spectrometer used with the 785 nm laser uses a grating with 900 lines/mm, a blaze wavelength of 500 nm, a spectral range covering 785-935 nm, and efficiency over 30% from 325 nm to 1225 nm. The spectral resolution of the 785 nm spectrometer is 0.48 nm (6-8 cm^{-1}). It measures Raman shifts from 150-2650 cm^{-1}. For the 1064 nm laser, the NIRQuest512-1.7 spectrometer uses a grating with 300 lines/mm, a blaze wavelength of 1200 nm, a spectral range of approximately 350-380 nm covering 1060-1446 nm, and efficiency over 30% from 750 nm to 2200 nm. The spectral resolution of the 1064 nm spectrometer is 1.73 nm (9-15 cm^{-1}). It measures Raman shifts from 150-2480 cm^{-1}.

The probes offered operate at their respective excitation wavelength. The spectral range extends from 300 cm^{-1} to 3900 cm^{-1}, but this is limited by the spectral range of the spectrometer. The probes are equipped with a safety shutter although a few probes are offered without a safety shutter. The typical probe fiber length is 1.5 meters. The numerical aperture of the optical fibers is 0.22. The probes use an FC fiber connector for the excitation light and an SMA 905 connector for collection. These particular probes have a 7.5 mm working distance, probe length of 107 mm, and probe diameter of 12.7 mm. The sampling head is made of anodized aluminum. The laser-line blocking has an OD (optical density) 6. The spot size on the sample for these probes is typically 155 μm with the 7.5 mm working distance.

Table 1 below compares potential optical parts considered for the probe and system. Included are bandpass filters, longpass filters, plano-convex lenses, mirrors, and beamsplitters.

Manufacturer	Part Number	Product	Cost (ea.)
Ocean Optics	USB2000+	Spectrometer	Donated
Ocean Optics	QE Pro-Raman	Spectrometer	$13,500
Ocean Optics	P200-1-VIS-NIR	Collection Fiber	Donated
Thorlabs	M18L01	Excitation Fiber	$75.75
Newport	KPX019AR.16	Collimating Lens	$63.00
Thorlabs	LA1540-B	Focusing Lens	$31.25
Thorlabs	DMSP805T	Beamsplitter	$174.00
Newport	10Q40BS.2	Beamsplitter	$266.00
Newport	07SD520BD.2	Mirror	$55.00
Thorlabs	BB05-E03	Mirror	$50.50
Newport	5CGA-800	Longpass Filter	$15.00
Thorlabs	NF785-33	Notch Filter	$500.00

Table 1. Potential optical parts.

3.6 Specificity, Selectivity, and Sensitivity in the Scope of Studies

In various fields of study, analytics and statistics are indicators of how well or how productive a solution is to a problem. The three common analytical terms that are used to identify the best solution are selectivity, specificity, and sensitivity. To properly gauge how well the spectrometer can detect cancerous cells when scanned, the team needs to apply these three techniques.

Specificity refers to the quality of a choice or solution that is deemed the most effective or suitable for a diagnostic test. In the case of this project, the design's specificity is the measurement or proportional value of negative results that are correctly diagnosed with the spectrometer. With this device, the goal is to properly gauge and identify the correct amount of negative results to eliminate false positives. To obtain an optimal specificity, the diagnostic should be able to properly eliminate most false positives, giving a ratio close to 1:1.

Selectivity focuses on the extent to which a specified solution is selected to a distinct problem or condition. In pharmacology, for instance, selectivity is the extent in which a drug or dose of medicine will produce the intended effect for that illness. In many cases, specificity does not always guarantee selectivity, but specificity is a requirement to have a semblance of selectivity [12]. In regards to the device the team is creating to test for skin cancer, the selectivity of this project will be to accurately test for several forms of cancer while getting a low estimate of false positives and negatives.

Sensitivity is another analytical statistic that is important, especially in the field of science and engineering. Sensitivity is the ratio in which samples or conditions are properly diagnosed under the correct criteria. In any given diagnostic test, sensitivity is also the probability that a given result is known as a true positive rate [13]. In general, if the sensitivity of a test is lowered, then the specificity of that same diagnostic test will increase. For this project, if the team tests for all types of skin cancer, the sensitivity will be higher, which decreases the specificity by nature. The main components that will provide the level of sensitivity will be the spectrometer, the CCD, and the microcontroller.

After speaking with several advisers and Ocean Optics, it was suggested that the test conducted with the spectrometer encompasses a broader agenda; to not focus solely on one form of cancer or skin condition, but rather to have a control (usually a non-affected part of the patient's skin) and test for any spikes or abnormal readings when comparing to the control itself.

3.7 Receiver Operating Characteristics Curve

A great indicator for determining whether a diagnostic is accurate is the use of a Receiver Operating Characteristics (ROC) curve [14]. The ROC curve is well known to statistically determine the four different criterion for any given test: True Negative, False Negative, False Positive, and True Positive. Below in Figure 6 is a graphical representation of an ROC curve with two different test populations:

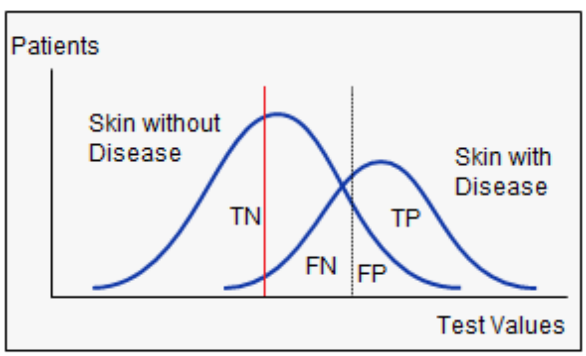


Figure 6. ROC Curve with two Skin Types

Figure Legend
TN: True Negative
FN: False Negative
TP: True Positive
FP: False Positive

The image above is a representation of two different skin types: One population with the disease, and the other population without. The dotted line at the center is the sensitivity line of the disease. Although the graphical representation has the sensitivity at approximately 50%, it is almost never the case in a real-life diagnostic. Considering that the diagnostic test the device is examining for is all forms of cancer and carcinoma, the sensitivity will increase. This is represented by the red line on this image. The left shift allows for more positive results to be passed, which effectively eliminates the possibilities of having more false negatives. The result of the shift is to eliminate uncertainty from the user; at any point the test reads positive, the patient can then go to a professional to do further extensive examinations.

3.8 ROC Curve Statistical Analysis

The following equations are utilized in the ROC curve to determine what the ROC curve looks like, and how it can be directly related to the data:

$$\text{Sensitivity} = \frac{True\ Positive}{True\ Positive + False\ Negative};$$

Sensitivity is the true Positive rate of a disease being present.

$$\text{Specificity} = \frac{True\ Negative}{False\ Positive + True\ Negative};$$

Specificity is the probability that the test will yield a negative diagnosis when the disease in not present.

Other equations can be obtained from these two formulae, such as the ratio between the true positive and false positive occurrences (Sensitivity / (1 - Specificity). Swapping the variables around, you also obtain the likelihood of a negative result ((1 - Sensitivity) / Specificity). The goal of these equations is to reconfigure the designed devices and spectra accordingly to obtain the most optimal results when diagnosing skin samples. All of the calibrations after the initial sample test from the first prototype will stem directly from the statistical analysis provided from the formulae above.

When testing, and configuring the sensitivity and specificity thresholds, the main parameter to look at is a control sample of the skin. If the scope of the project itself is to test for any abnormalities and not a specific form of cancer in the skin, then the results (specificity and sensitivity) will be changed. Once the final scope of the design can be calibrated to test for any skin abnormality, the next step is to use the equations above to hone in on a specific form of cancer or condition. It can even go as further as having a general database of what certain cancerous results would look like saved onto a display or PC. Doing this will then allow the user to layer the results over one another to see if there are matches.

3.9 Electronics Design

The electrical engineers on the project understood what major components must be included in the spectroscopy system design, however wanted to consider any research into the design process in order to be more effective and gain more insight into the system as a whole. The major components of the spectroscopy system include a sensor, microcontrollers, the optics system, a Printed Circuit Board (PCB) dedicated to power flow, a PCB dedicated to the sensor apparatus, and data connectivity. Thankfully, as with all technology, minimizing size is often a design goal. Research into ways to minimize the size of a Raman spectroscopy system will provide insight for the electrical requirements for L.A.S.E.R.S., which will be a relatively portable device. A thorough investigation into size reduction is detailed in “The research of digital circuit system for high accuracy CCD of portable Raman spectrometer,” a useful resource to pin down what exactly is needed for a Raman spectroscopy system to be both smaller and effective, particularly with respect to the electronics design. Although the described design uses a few pieces that vary from the design plans for L.A.S.E.R.S., the main component logic remains the same. In general, “The digital circuit system for high accuracy CCD is composed of multi-voltage circuit, sequential generation circuit, driving circuit, A/D transition circuit and data exchanging interface,” [15].

These elements allow the sample to be read and the data sent to the tablet or computer that will tell the device user their results, which is the goal of the spectroscopy system design. Analog to digital conversion and any associated standards will be an important element to a successful spectrometer. The signal read in from the sensor, will go through programmable gain amplifier (PGA) and an analog to digital converter in order to be processed [15]. The cleaner the signal, the more noise can be reduced. A reduced signal to noise ratio is ideal for any system, but especially while seeking the Raman shift. The research article also notes that it is important to note the clock information for the specific sensor [15]. Understanding how the specific sensor functions is essential to designing a system that is precise, efficient, and will deliver the most accurate data.

3.10 Miscellaneous Electronics Selection

Components that are smaller in size are sometimes more important than larger ones, and care must be taken in choosing the various electronics parts utilized in the final design. Items such as resistors, capacitors, transistors, light emitting diodes (LEDs), and switches must be selected.

3.10.1 Resistors and Capacitors

Resistors and capacitors are a given for any circuit that must deliver and control power. The values required will depend on the load and device components. Because current flow is a top priority for a few of the major device components, capacitors will be key in making sure a safe amount of power reaches all parts of the device. These simple elements allow for the fine tuning of the amount of voltage and current flow in more sensitive components.

3.10.2 Transistors

Transistors are useful tools for the regulation of current. Transistors can amplify or reduce the amount of current flow depending on the type and how they are configured within the circuit at hand. Several configurations utilizing the connections of the transistor exist, which enables the transistor to help or hinder the voltages and currents directed at the outputs. In the three cases below, one terminal will be common to the input and output voltages. The table below depicts the transistor configurations.

Configuration Types	**Common Base Configuration**	**Common Emitter Configuration**	**Common Collector Configuration**
Voltage Gain	Significant Gain	Moderate Gain	Little/No Gain
Current Gain	Little/No Gain	Moderate Gain	Significant Gain
Power Gain	Low Gain	Significant Gain	Moderate Gain

Table 2. Transistor Configuration Types

3.10.3 Light Emitting Diodes (LEDs)

The application of the L.A.S.E.R.S. device will allow for a simple light emitting diode to be used. Therefore, the team will utilize two LEDs, one red for indication that the laser is on and useable, one white for visibility of the tested surface. Red is a color that captures attention, and that is ultimately the goal of the indicator light. The white LED is a clear choice for a light that is to allow the user to see for clearly. The following table, Table 3, lists the important component information.

Part	**Color**	**Operating Voltage (V)**	**Operating Current (mA)**
RL5-R5015	Red	1.87 - 2.5	12 - 30
RL5-W8045	White	3.0 - 4.0	20 - 35

Table 3. LED Information from Datasheet [41]

3.10.4 Switch

The comfort of a switch may be modified with casing around it; thus, the basic switch piece does not need to be elaborate, just a simple on-off functionality switch. The team will use a simple button that was acquired in an electronics kit along with the LEDs, resistors, and capacitors. The button has four pins and registers as “on” if it is pressed in, “off” if no force is placed on the button. The simple button is rated for 12.0 V and 0.5 A [42].

A small, simple button as a safety switch is also versatile. It can easily be incorporated into a handle or other easily accessible part of the casing. The user comfort of the switch can be easily adjusted by adding a casing around the button portion. For example, a silicon piece can be placed around the button, or a physical plastic level that can hold the button into the ON position can be utilized. A simple case component allows for the ability to meet economic standards while allowing for versatility in the user-friendly design.

3.10.5 Voltage Regulator

A voltage regulator may be used in order to better control the voltage and current leading into the microcontroller. There are various methods of regulating voltage. The input voltage can be reduced by using a wall wart with as low of an input as possible for the system. Voltage regulators will absolutely be needed.

Brand	Specific Product	Price	Output Current	Max Wattage
ST Microelectronics	L7805CV 5V Regulator	$0.40	1.5 A	7.5 W
ST Microelectronics	L7809CV 9V Regulator	$3.89	1.5 A	13.5 W
Sanken	IC REG BUCK ADJ 3.5A TO263-5	$2.88	3.5 A	10 W
Texas Instruments	TPS 54302 Switching Converter	$0.60	1.0 A	5 W

Table 4. Comparison of Voltage Regulators

The LM78XX series of voltage regulators are affordable, effective, and simple to integrate into a circuit as they only have three pins, input, output, and ground. The “XX” at the end is replaced with the value of voltage that is to be regulated into the device, in this case a LM7809. While linear voltage regulators tend to give off a lot of heat, there are various components within the overall L.A.S.E.R.S. device, such as a fan and heat sinks that will help manage heat flow, thus the system will not be greatly impacted.

3.11 Sensor Selection for Spectroscopy and Imaging

This section encompasses the two different types of sensors utilized in the device. The CCD sensor is useful for spectroscopy, particularly in the non-visible spectrum, whereas the CMOS sensor is useful in applications such as a camera for photographs and video, as well as image processing. Both types of sensors show promise when discussing the

L.A.S.E.R.S. system, but considerations must be taken to determine which type of sensor would suit the application best.

3.11.1 CCD Sensor

A sensor is used to read in light and convert that information into data, which can then be read in and analyzed by a microcontroller. This piece is essential to bridge the data read in by the probe to the data that will be delivered on the user interface to the patient or doctor using the device. Such sensors are used in a variety of applications involving the processing of images or light data, from digital cameras, to astronomy applications such as telescopes, and medical imaging. Given their usefulness and versatility, it was clear that a sensor would be an important aspect of this project. In any application involving the processing of light or data from an optical apparatus will require a sensor device.

The sensor that collects the data from the probe apparatus could be either in the form of a CMOS (complementary metal-oxide semiconductor) or CCD (charge-coupled device), the two most popular and common types of sensors. Both systems take on the same task of converting the data in the form of a wavelength of light into electrical signals that can be read and processed by a signal processing system. It is important to understand the difference between CMOS and CCD sensors, as well as to understand which applications most commonly use one or the other. Compelling arguments for the use of both CMOS and CCD sensors exist; the application of the system is the most essential aspect in making the final decision of which type of sensor to utilize.

Initially, a CMOS sensor seemed more attractive. In general, CMOS are cost effective in a variety of formats and sizes. With optics parts running a typically high price point, trying to keep within budget on this part felt essential. In general applications involving medical technology, CMOS have proven successful. In a comparison of CMOS and CCD specifically for biological image applications, it was found that, "...even a mid-performance CMOS camera can extract close enough information to that of a CCD camera when applied to get information on the spatial distribution of the fluorescent signals on an image. Therefore, CMOS cameras may also be considered for quantitative investigations on cells or tissues when dealing with fluorescence microscopy," [16]. In this article, researchers compared the validity of a CMOS sensor to a comparable, and more preferred, CCD sensor. The article conveyed a sense that a CCD sensor would be the more ideal option, but with modifications the two could perform on similar levels. Thus, if the team needed to use a CMOS sensor should the price of a CCD become unreasonable for the constraints of the project, it would be possible to complete the task given the research on the topic.

Despite being typically more expensive, it was important not to ignore a CCD sensor on the basis of only a rough price estimate. If articles existed to discuss how to bridge the gap between CMOS and CCD sensors, there must be a reason why CCD sensors earned a reputation for success in the medical field. A particular focal point was placed on finding examples of CCD sensors in Raman spectrometer devices in order to justify which direction to take. In a journal article specifically discussing the biomedical applications of

Raman spectroscopy, the use of a CCD sensor is discussed in detail, along with how a CCD sensor is utilized in this system. CCD sensors are effective at picking up the Raman spectrum, and an efficient handheld spectrometer can work with just a single dimension CCD array [15]. Learning that it is possible to have an effective device that does not have a larger, more expensive array was essential to moving forward in this project. The idea of using a CCD array was attractive based on the apparent preference for using one specifically for Raman spectroscopy. The idea that using a single array would decrease the cost compared to a more square-shaped CCD, commonly used in cameras, offered hope that a CCD array that met the economic constraints of L.A.S.E.R.S. could be procured.

While the price point of the CCD became worrisome, it was found that a CCD would be more ideal for a Raman spectrometer. Dr. Han informed the team that a one-dimensional CCD array would be ideal, but could cost up to $300. In Dr. Han's professional opinion, a CCD sensor has a better quantum yield, essentially the absorption of the data from the sample, of approximately 90% compared to a CMOS sensor's approximately 50%. The quantum efficiency will vary based on the wavelength of interest; however, this general observation was yet another encouragement for a CCD array. In addition to their high quantum efficiencies, CCDs are also capable of low signal-to-noise ratios (SNRs). To reduce dark or thermal noise from the CCD, it is possible to cool the detector using liquid nitrogen or thermoelectric Peltier cooling. Additionally, the use of a deep-depletion CCD instead of a back-illuminated CCD will help to reduce noise and reflections of the near-infrared light to be used. [11] A reduced Signal-to-Noise ratio also means a potentially less rigorous signal analysis while processing the received data from the probe.

While meeting with Dr. Hagan during a review of the team's initial document, he also suggested a linear CCD array. With the professional opinion of faculty advisors behind the idea of a CCD array, this settled decision to select this type of sensor, so long as the team could find one that met project constraints. The next step, as noted in the project components section, would be to find a CCD array that fits the project budget and meets design specifications. The team was successful in finding a sensor that matched all of these goals using a single array CCD sensor.

In order to read in the data from the laser, a collector device that fits the needs of the Raman Spectrometer must be chosen. The two main options for the device included either a charge-coupled device (CCD) or complementary metal–oxide–semiconductor (CMOS) array. In general, a CMOS array is more affordable than a CCD; an attractive choice considering the price point of the optical parts of the system. While a CMOS array is easier to access and often to communicate with, research and consultation with advisors led to the decision to choose a CCD array. Ultimately, the advice of both Dr. David Hagan and Dr. Kyu Young Han solidified this choice that seemed promising from research.

To meet design specifications, the chosen CCD array must be relatively affordable and effective enough to successfully read in the wavelength of the spectra of the testing material. Ideally, the CCD will also have a low power consumption, under 5 V. Based on other Raman Spectrometer projects and suggestions, Toshiba products became a focal point for product research. Toshiba offers linear CCD image detectors from a variety of price points, including sensors with a retail value of under $50. Options considered are as follows:

Brand	Specific Product	Price	Voltage Consumption
Toshiba	TCD1304AP	$14.99	4.0 V
Toshiba	TCD1305DG	$34	5.0 V
Sony	ILX554A	$9.99	5.0 V
Sony	ILX511B	$27.99	5.0 V

Table 5. CCD Model Comparison

CCD arrays require high speed analog-digital conversion in order to deliver efficient results. Aside from the above specifications, the chosen CCD must function with a microprocessor such as a Raspberry Pi. Simple functionality that does not require many additional configuration steps is ideal, however this is a difficult task for a CCD array. High functioning CCD arrays cannot be directly connected to a Raspberry Pi due to port functionality restrictions. This issue can be bypassed by adding in an additional microcontroller, to be discussed in detail within its own section. In short, a Nucleo F401RE can bridge the communication between the Raspberry Pi and the CCD array.

With the ability to work with this selection of CCD arrays thanks to workarounds via microprocessors or specific chips, the CCD of choice is the Toshiba TCD1304AP. This specific part has 3648 pixels of size 8µm×200µm, which will be efficient for the wavelength of the laser and the spectrum of focus for the spectrometer [30]. The operating temperature ranges from -25°C to 60°C [30]. This temperature range will be within the overall temperature specifications, and therefore will be at an acceptable operating temperature. This element will also be placed in an area away from the laser and thus will be protected better than if they were housed in the same space.

The above considerations were important in moving forward with the TCD1304. To verify that this part could be successful for the application of Raman spectroscopy, the team asked other optics students and read into any other projects using this part. After seeing at least three projects that utilized the same or similar CCD array for the application of Raman spectroscopy, it was decided that the TCD1304 would be effective. The other options were explored, however it seemed that the quantum efficiency of all explored options were the same, if not similar, at the wavelengths of interest. There was little advantage found in exploring an option that had seen less of the same application of Raman spectroscopy. With a lack of supporting evidence to go with a similar but more expensive option, the decision was made to move forward with the TCD1304AP.

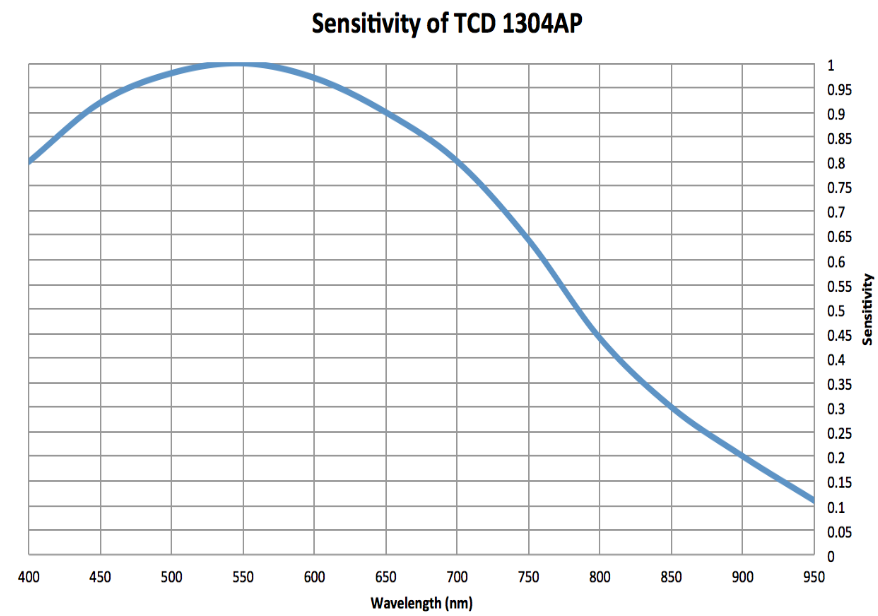


Figure 7. Plot of Sensitivity of TCD1304AP, information from datasheet [30]

An important element of the CCD array is the spectral response. The spectral response or quantum efficiency is the efficiency at which something can detect a certain spectrum of light. Given a wavelength of choice, the datasheet of the specific sensor will show the relative response at that point [30]. From the graph of the spectral response of the CCD, created using information from the TCD1304AP datasheet, one can see the response at the infrared wavelength range used [30]. These data points are taken when the ambient temperature is approximately 25°C. Around the wavelength of 785 nm, the wavelength of focus, the spectral response is around 50%, which is sufficient for this application. Around the wavelength of 635 nm or 632.8 nm, two lasers that may potentially be used in the device, the sensitivity greatly increases to above 90%. Thus, all lasers in question will be able to work with the chosen TCD1304AP CCD array and still meet specifications, so long as the wavelength is below 825 nm. Given a greater budget, it would be possible to have a higher spectral response; constraints must be considered for this project design.

A few care instructions must be considered for the TCD1304AP, especially when handling this part in the created device. The glass on top of the array must be cleaned carefully to maintain maximum functionality and avoid damage. This CCD must avoid exposure to infrared lighting to avoid reduced resolution quality. Soldering at temperatures above 350°C must be quick to avoid damage [30]. Thus, it is essential to take care when handling, testing, and implementing the TCD1304AP. The casing design must properly secure the PCB holding the TCD1304AP and protect it from moving or being hit by another part should something slide out of place.

The CCD array will be placed on a separate PCB to better isolate and protect the part in both the construction process and the typical functionality environment. This separate

PCB will contain space for the 22 pin TCD1304AP as well as power and data connectivity. The CCD array must be placed within the focal plane of the mirror in order to receive the greatest amount of light. No matter how high the quantum efficiency is at the specified wavelength, the system will not be effective if the CCD is not in an ideal location. This does not discount the importance of quantum efficiency. Quantum efficiency is the efficiency at which the sensor device can process or read in all of the light signals that are in the range of the sensor. It is essentially the measure of how effective the sensor is at its job of capturing the incoming light signals. A way to work around a lower quantum efficiency is by compensating with grating. Grating requirements, if needed, will be discussed in their own section.

The two major points of focus to operate the CCD array are power supply and data connectivity. In order to have proper communication with the microcontrollers, the pins dedicated to the signal collected must be understood, as well as the clock, pulse, and frequency information. The voltage input range for both analog and digital input is between 3.0 V and 5.5 V, with 4 V as the optimal input value [30]. When the input voltage is at the optimal value of 4 V, or greater, the data and clock information is as follows.

As the project shifted and sponsorship was gained, the CCD selection became limited to the support and donations from the team's sponsor, Ocean Optics. This support meant that the CCD apparatus, at first a focal point, would not longer need to be built by the team. The CCD in the donated spectrometer is the Sony ILX511B, a linear CCD array sensor. This specific part has 2048 pixels of size 14μm×200μm, which will work effectively with the wavelength of the laser [43]. The operating temperature ranges from -10°C to 60°C [43]. This temperature range will be within the overall temperature specifications and the overall specifications of the given spectrometer [43, 44].

While donated parts are very appreciated, it must be ensured that the parts meet project requirements. As mentioned previously, the spectral response plays a key role in the effectiveness of the spectrometer for this application. Around the wavelength of 785 nm, the wavelength of focus, the spectral response is around 45%, which is sufficient for this application. Around the wavelength of 635 nm, another wavelength that may potentially be used in the device, the sensitivity greatly increases to 90%. Thus, all lasers in question will be able to work with the ILX511B CCD array within the spectrometer and still meet specifications, so long as the wavelength is below 825 nm. Given a greater budget, it would be possible to have a higher spectral response; constraints must be considered for this project design.

3.11.2 CMOS Sensor/Camera

As per design specifications from Ocean Optics, the probe must have a camera attachment with live feed and a clear view of the patch of skin to be tested with the probe. This will allow the user to better find the area of skin of interest, but also allow medical professionals to see the exact spot that registers as an issue instead of removing cells unnecessarily. Thus, the add-on complementary metal oxide semiconductor (CMOS) camera will improve accuracy while allowing the user to see both a photo of the affected skin as well as the spectra of that skin.

The CMOS module must be relatively compact. It will sit near the lens and LED beam on the probe so that the image matches the scanned skin, but will be protected from the laser emission itself. There are a variety of camera modules that exist, especially small ones that can be utilized, however ideally one could find a CMOS sensor, design a lens, and design the PCB interface that will provide power and data connectivity as needed for this specific device. This goal depends heavily on time and economic constraints. The chosen CMOS sensor must have a resolution that allows for clear imaging and video feed of a skin sample that is within 5 inches or less. Therefore, perhaps the most important quality of the chosen camera or camera module is if it can automatically adjust for changes in light, such as those that could come with moving a camera closer to a surface. Ideally, this camera or camera module will have a resolution of at least 5 megapixels for images, 2 megapixels for video. The required voltage to operate the camera should be no more than 6 V.

Brand	Specific Product	Price Range	Megapixels
Adafruit	Raspberry Pi Camera Module V2	$29.89	8.0
Arducam	OV5647 Mini Camera Module for Raspberry Pi	$14.99	5.0
SainSmart	Camera Module Board Webcam Video for Raspberry Pi 3	$13.99	5.0

Table 6. CMOS Camera Comparison

After shopping through various options, including single cameras utilized in cell phones and tablets and full camera modules, and it became clear that a camera module would be ideal. Economic and time constraints limit the ability to build a camera that is small enough and meets requirements to be ideal for this application. The simplicity, versatility, and specifications of the Raspberry Pi Camera Module V2 were immediately appealing.

The Raspberry Pi Camera Module V2 sports specifications that meet all of the design specifications and constraints. The module costs under $50, which meets economic constraints. The focal length is 3.04 mm, in line with desirable size constraints. The camera allows for both video and still images, ideal for functionality of the probe. This allows for the “live feed” of the view of the skin, as well as the potential for still images of the skin area of interest to be taken. With an 8.0 Megapixel resolution for images, 2.0 Megapixel resolution for video, and features such as automatic exposure control, white balance, and a band filter, the camera meets the versatility desired. This will allow for simpler implementation while meeting all design considerations and specifications. The overall size of the module is a slim 23.86 x 25 x 9 mm [33,35].

At the heart of the camera module is a Sony IMX219PQ CMOS image sensor. This sensor was also a point of interest when the team considered completely building a camera. The sensor boasts a high rate of image processing as well as being highly sensitive. For DC voltage, the input voltage is a low 1.2 +0.1/-0.12 V and can be connected directly to the Raspberry Pi to further simplify the power circuit. The input clock frequency has a range of 6 to 27 MHz [34].

The Raspberry Pi camera module connects to the Raspberry Pi via a 15 pin flex cable that allows for power and data connectivity. While the team initially planned on separating the cable to attach to a PCB, this caused more problems and made this component unstable; therefore, the camera module is connected directly to the Raspberry Pi. Once the camera is connected and enabled, the fine tuning via code may begin. Aspects typical to a camera may be specifically modified in Python, such as brightness, contrast, and sharpness of the image. Using Python, the team can write an application to perform the functions needed to make the camera module useful: on the monitor connected to the Raspberry Pi, preview the view from the camera, then allow the user to capture an image of interest. The image can then be saved to allow the patient and the medical professional performing the test to see which parts of the skin were tested and potentially cancerous.

3.12 Printed Circuit Board

A Printed Circuit Board (PCB) is the main board that interconnects all of the electrical components via conductive features that are engraved from copper, and then is placed on an insulated substrate, usually plastic. In any electronic application, PCB’s are the backbone component that interlaces all other components to communicate efficiently. Not only are PCB’s important for supplying power to any application, but it is also useful in supporting mechanics. The fact that it has lasted decades in the electrical industry is telling, and its presence in the market is overwhelming; now existent in cellular devices, embedded systems, televisions and even automobiles [17]. This project will require at least two circuit boards for full functionality: one will be dedicated to the power and maintenance of the microcontrollers, and the other will be solely for the power and maintenance for the CCD and the spectroscopy system. Again, this was done as a precaution; the intent to divide the device’s system for better serviceability from the CCD and the spectrometer. This will provide a more accurate result and will pose for easier calibration once initial data has been computed. If resources were to be a constraint, the next possible option would have been a multi-layered PCB that can handle both the microcontroller and CCD/Laser applications.

For the second iteration of the L.A.S.E.R.S. system, the design will still require two circuit boards for full functionality: one for the power, the power indicator LEDs, maintenance of the microcontrollers, temperature sensing, and the other will be solely for the power and maintenance of the CMOS camera module and the beam LED.

3.12.1 General Layouts of Printed Circuit Boards

Of all the different circuit board construction templates, the three most common are the single-sided PCB's, the double-sided PCB's and the multi-layered PCB's. For single-sided PCB's, they dedicate one side of the substrate for the components. A double-sided PCB is introduced when the number of components exceeds either the physical or electrical capabilities that a single-sided PCB harbors. The way the two sides communicate with one another is by drilling into the substrate onto the other side and plating the holes with copper or any other conducting metal. The multi-layered configuration, however, will differ by having layers of insulation in between the layers of printed circuitry. The way the layers interconnect with one another is by having the components configured on the top layer connect onto the plated holes that are connected to the corresponding circuit layer [18].

In short, the simplicity and complexity of PCB configurations and schematics can be understood by observing the number of layers that are on the PCB. For instance, a simpler PCB will have 1-2 layers versus a more complex design, which can boast 7-10 layers. The more layers added, the more copper patterns and arrangements are added. This is significant because it will allow the copper to not overlap between their lines, which will evade the problem of having the electricity flow undermined [19]. Over the copper layer is the solder mask, where all the copper lines and traces roam to their components to power the system. The purpose of the solder mask is to protect the copper from contacting any other metals. Lastly, a silkscreen is also added over the solder mask to properly map the lines and components to allow for easier assembly and installation.

3.12.2 Overview of PCB Components

The PCB is capable of housing various components, all which have different functionalities and qualities intended for a particular circuit schematic. Most, if not, all of these common components have been utilized in labs such as Networks and Systems, Electronics I/II, and even Embedded Systems. Below is a list of the most common PCB Components:

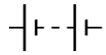
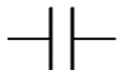
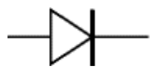
PCB Component	Electronic Symbol	Description and Use
Battery		Supplies a voltage to the circuit design.
Resistors		Controls the current throughout the circuit.
Inductor		Stores energy in magnetic field; resists changes in current.
Capacitor		Accumulates electrical energy when attached to a voltage.
Diode		Maintains current flow in one direction only.
Transistor		Used as a current amplifier; also can act as a switch in ON or OFF states.

Table 7. Common PCB components and their functions.

There are two main methods of connecting the components onto the circuit board: Through-Hole-Technology or via Surface Mount Technology (SMT). The former requires that small holes be drilled through the board itself to allow for thin leads from the component to pass through. Once the leads are passed, they are then soldered onto pads on the opposite face of the circuit. The latter method - which has been the industry standard since the 1980's - has proven to be a more feasible solution to adhering components onto the circuit boards [20]. Instead of having to drill small holes through the board and risk potential damage, or risk incorrect hole sizing, components could be soldered directly onto pads that sit on the surface of the circuit board. For the two PCB's that are being designed, the most time-efficient and cost-friendly method would be the standardized SMT.

3.12.3 Assembling the Printed Circuit Board

Now that there is a basic understanding of the PCB and its natural design structure, this next segment will introduce the assembling of the PCB itself. There are about 4-5 steps to have a completely functional PCB, starting from the design schematic, all the way to assembly and troubleshooting. For the vast majority of the project, the PCB will be in the possession of a professional designer, but the most crucial steps are taken at the very beginning, where the design has to be laid out and properly configured. Below are the procedures to creating a PCB.

Circuit Schematic Design:
To begin the process, the first portion that has to be done is to create a working circuit design. In most cases, a way to test for a viable circuit is to generate the circuit layout onto a circuit simulator on a computer, as well as by testing out that same circuit via a

breadboard. Once all the outputs are within a specified degree of freedom and the circuit is fully functional, the next step is implemented.

Board Design through Software:
Once the circuit is working and designed, the next step is to have that circuit rendered into an Electronic Design Automation (EDA) software [21]. There are several EDA software programs to choose from, such as Eagle, Allegro, and KiCAD, but the constant within the software must be the file format. The standard file formatting is the Gerber format. This format communicates with the software to outline the components onto the PCB and illustrates different dimensions of the PCB. Images can be created from the file, almost like a PDF format, to show where certain components are sitting, or where the copper wires will transport the current. When creating the PCB, be mindful that there are different configurations of boards. It is important to mention that the design has to be created in reverse on the software do to the viewpoint of the board [22]. If the project that is being implemented needs more components and more copper leads, then utilizing a single-sided layout will not be ideal. Once the software creates the Gerber files, the PCB can start the fabrication process.

Printing and Initial Fabrication of the PCB:
The Gerber file that is given to the fabricator will have several layers depicting drill hole locations, signal traces, and component layouts, making it easier to read and fabricate. The PCB will go through several stages in the fabrication before it can be ready to test. These stages include etching, drilling, component plating, and protective electrical measures. After the stages are complete in the fabrication, the board itself will be tested to ensure the fabrication and design was done correctly.

Testing the PCB:
A PCB does not only have to pass certain electrical evaluations; it must also be tested from an aspect of rigor and durability. Verifying the functionality of the PCB is arguably the most extensive procedure in the assembling process. Depending on the application it is being used for, a single PCB can be subjected to many tests, rating certifications, or industry standard examinations. For all applications, however, the PCB must go through an electrical test that verifies all the points of contact throughout the board. This ensures that the design inputs and outputs can be verified, and can also guarantee the fabrication is working. Once the leads and contact points are tested and fully functional, the last step is to mount the components for final assembly.

Final Assembly of the PCB:
After all the contact points are tested and cross-referenced in the design specification, the last stage of the PCB is to have the components mounted onto it. As stated in the earlier sections of PCB's, the type of mount is decided in the design section. If the design required the components to be installed using a through-hole mount, the leads are inserted into their respective holes and lead into their respective contact positions according to the design. However, if the design required the components to be installed on the surface of the PCB, then the components will be soldered onto pre-configured pads that match with

that specific component. Once the components are secured onto the board, the components will also go through a form of electrical troubleshooting to ensure they are working properly. After the test concludes and all of the components are tested, the PCB can be declared viable and complete for use.

3.13 Microcontroller Research

The device may use at least one microcontroller in order to process the signal read in by the sensor apparatus. Not only must the potential microcontroller(s) be considered, but also their arrangement and usefulness to the spectroscopy system. A stand-alone computer system could also be used in lieu of additional hardware.

3.13.1 Microcontroller vs Stand-Alone System

Both a microcontroller and a stand-alone computer system have similarities that can blur the decision whether the team's application would require each system. Both systems have a processor, RAM, and the necessary input and output devices the team needs in order to be able to control the spectrometer, and capture the relevant results from the completed tests. Each system has its positives and negatives for incorporation into the device itself.

Microcontroller:

- Small
- Power Efficient
- Easily Interfaceable with CCD
- Can only perform a single task
- Requires an outside computer system to then further interpret the data.

Stand Alone System:

- Too large to fit "inside" the handheld device
- Huge power draw
- Harder to interface with CCD
- Can perform all necessary tasks of both Microcontroller and Stand Alone system

In the end, the decision on whether to have a microcontroller independent of an external computer system became quite clear. To gain hardware independence, the system would incorporate a microcontroller inside the device, coupled with an external program that can run on any machine to then review the results.

3.13.2 Microcontroller Choices

Arduino Uno

The Arduino Uno is a board based on the ATmega328P. It is a great beginner microcontroller board that has 14 Digital input/output pins, 6 analog inputs, a 16Mhz quartz crystal, a USB connection, and a low power draw. This board is designed for beginners to programming to do very simple at home projects, many of which include photography and other photo sensor data. This is a very lower power system, and would handle just the aspect of relaying the sensor information to the computer system without fault, but the low power of the system could also be a bottleneck if more data throughput is eventually necessary. The cost of this microcontroller device is a non-prohibitive $20, which lends itself to creating a cheaper final product. However, with the desired real time results, the minor savings of the overall system is not worth the delay this device may cause over some of the team's other options.

Quick Computation Specifications:
Processor Speed: 16 Mhz Quartz Crystal
Flash Memory: 32 KB
SRAM: 2 KB

Quick Design Specifications:
Input Voltage: 7-12 V
Length: 68.6 mm
Width: 53.4 mm
Weight: 25 g

Texas Instruments MSP4305172

The Texas Instruments MSP4305172 is a 16-bit ultra-low microcontroller platform which is used for a wide range of low power applications. It is a great learning development device that has 29 GPIO pins, a 25Mhz Crystal, a USB connection, and a super lower power draw. The board is designed for learning and use in several types of Analog and Digital Sensor systems. Much like the Arduino Uno, this device would only be able to handle the aspect of collecting the sensor information and passing it off for collection/retention to our computer system. The system provides ton of customizability and has applications in many fields, but the ability to handle the data stream, and then further process it would be too much for this lower powered system. The low cost of $5 does provide a greater degree of freedom with budget, however it would have to include housing, as well as design a development board to provide the necessary IO ports which provides additional cost and design time.

Quick Computation Specifications:
Processor Speed: 25 Mhz Crystal
Flash Size: 32 KB Flash
SRAM: 2 KB

Quick Design Specifications:
Input Voltage: 1.8-3.6 V
Length: Depends on Custom PCB Size
Width: Depends on Custom PCB Size
Weight: Depends on Custom PCB Size

Raspberry Pi 3

The Raspberry Pi 3 is a 64-bit low-powered computer platform which is used for a huge range of applications ranging from imaging devices to a full mini-computer environment. Unlike the microcontrollers described before this, this system is capable of running a full version of Linux, and is a much more robust system, which can be capable of both relaying and storing the data gathered by the spectrometer system. Its 1.2 GHz ARMv8 CPU, 1 GB RAM, expandable HD space via MicroSD and 40 GPIO pins allow for this system to handle a larger chunk of the responsibility, along with a greater sense of independence from a full computer system external to the system. This device comes housed and has the necessary PCB to be able to control any sensors needed for the team's design, as well as the intended functionality of capturing the sensor data simultaneously.

Quick Computation Specifications:
Processor Speed: 1.2 GHz 64-bit Quad Core Armv8 CPU
Flash Size: 1 GB RAM
Storage: 32 GB (via MicroSD)

Quick Design Specifications:
Input Voltage: 5 V
Length: 4.8"
Width: 3"

Microcontroller	Arduino Uno	MSP4305172	Raspberry Pi 3
Processor	16 Mhz Quartz	25 Mhz Crystal	1.2 Ghz Quad Core
RAM	2 KB SRAM	2 KB SRAM	1 GB RAM
Drive Space	32 KB Flash	32 KB Flash	32 GB Flash
Other features	14 DIO Pins, USB	29 DIO PIns, USB	40 GPIO Pins, USB
Input Voltage	7-12 V	1.8-3.6 V	5 V
Dimensions	69 mm x 53 mm	Needs own custom PCB	121 mm x 76 mm

Table 8. Comparison of microcontrollers

3.14 Coding Research

The back-end code for this project will provide the necessary flow of data from the sensor arrays into a form that the users can understand, and thus will make the product a viable option to bring to market. Different aspects of the code will have different responsibilities, but the end goal is consistent -- providing an easy to use, reliable system to deliver accurate test results.

The aim to provide a relatively high-level program which should prove to be cross platform, without the need for lower-level system processes. This has led the team to several choices to possibly utilize to program the project in, with initial compatibility being designed for use on a Raspberry Pi 3 microcontroller.

Python

Python is a high-level programming language used mainly for general purpose programing. It was created by Guido van Rossum, and first released in 1991, and emphasizes readability and a syntax that programmers find easier to express concepts in less lines than in other programming languages. Python is a multi-platform system, and is readily available on most current operating systems. Unfortunately, Python requires a native compiler that can take the code and transform it into something the host system can handle. This simultaneously restricts the overall number of system that it can run on, but allows the overall speed of the program running to increase.

Some of the features of Python that may prove beneficial for use case are the dynamic coding approach, as well as Python's program speed. Since one of the largest design features is real-time results and accuracy, a program that runs as fast to real-time as possible is ideal. However, there is a need to also take into consideration the ability of running this on several systems, as one of the team's other intentions is for this to be an at-home device.

Java

Java is another high-level programming language originally created by Sun Microsystems in 1995, developed for general purpose computing. Java is a concurrent, class based, and object oriented language, which natively has fewer dependencies on hardware. Programs written in Java are compiled for use in the JRE (Java Runtime Environment), which are then able to be run on any hardware capable of running the JRE, regardless of computational power, operating system, or hardware.

Due to the modularity constraint of the project, Java's native platform independence is an indispensable tool, that will allow the team to take the working code and run it on any of the 3 billion devices currently running Java. That portability factor allows the implementation of the microcontroller to change as the group is able to come up with final design constraints. Java is also known for having a large database of classes that are written, to prevent users from having to re-write the same functionality. This feature set will allow the team to make changes and modifications to the source code to troubleshoot more effectively.

Data Analysis

The microcontroller will be receiving data from the CCD in two value formats. One value will be the wavelength which is being tested (measured in wavenumbers cm^{-1}), the other value will be the intensity of light (measured in A.U (arbitrary units)), which is a measure of the number of photons received by the CCD array. The combination of these values will be further translated into a dataset which the program can then work with to determine the likelihood that the patient is at risk for cancer.

For standard spectroscopy, the cancer indications would be in how the data changes from a non-cancerous skin sample versus a cancerous skin sample. The deviation in these data sets will further indicate an unstandardized cellular makeup, which may include the possibility of cancer cells. The dataset will be plotted in an (X,Y) format using (Wavelength Numbers, Arbitrary Unit).

Data analysis will be conducted for any large variations of two parameters: Intensity (change in Y-axis) and Raman Shift (change in X-axis). Though our methodology for a definitive decision is not yet decided, the team will be using two well-known algorithms to determine each one of these shifts.

Intensity Shift

Intensity shifts will be experienced when there is a large variation at a single peak. This change in peak can be attributed to the presence of a non-standard cellular makeup. To test for this, the group will be comparing individual data points for changes in intensity larger than a defined value, larger than a later defined standard deviation of normal skin comparisons.

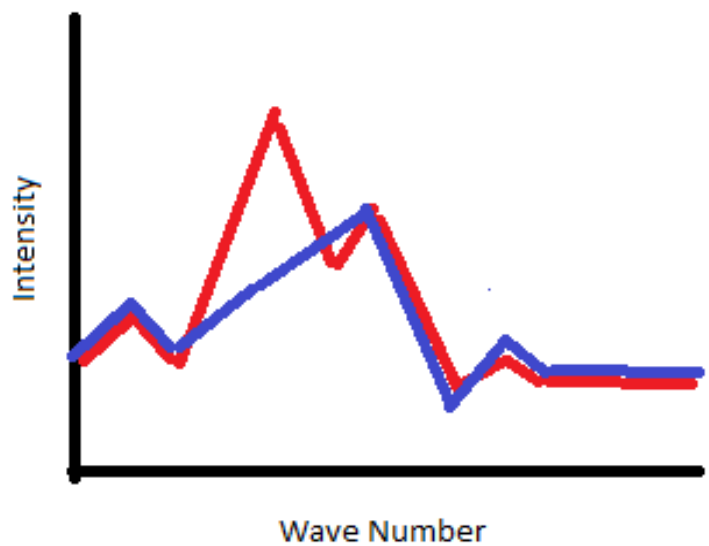


Figure 8. Intensity Shift Example

Intensity shift will also be measured looking at the Euclidian distance of the lines comparing the data values, and testing for the average change. Two standard tests on a similar piece of skin should result in a net Euclidian distance change of ~0, so this will be indicative of a large shift in intensity using the following formula. [23]

$$\left(\sum_{0}^{n} (y_{n2} - y_{n1})\right)/n$$

Phase Shift
A large variation in the wavenumber location of the peaks will be apparent by the phase shifts in the signal. This also can possibly indicate the presence of a non-standard cellular makeup. Testing for this will be more complex and will require the program to test for a shift in both directions, accounting for the possibility of the number of peaks changing due to this shift.

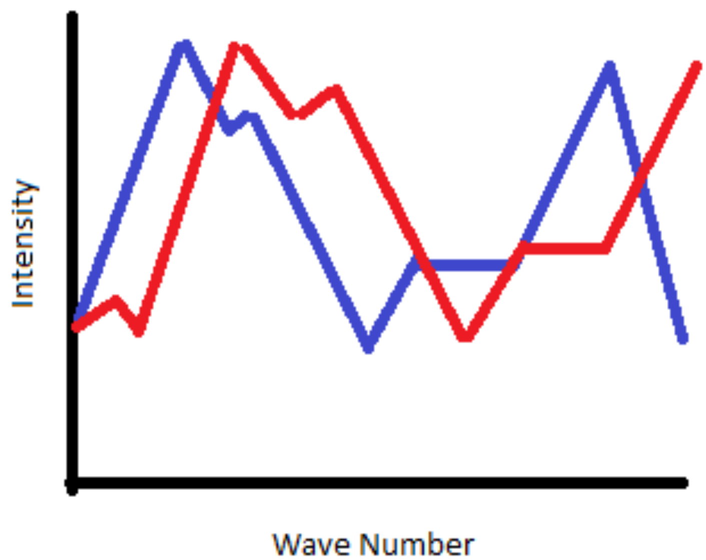


Figure 9. Phase Shift Example

3.15 Dataset Smoothing

Smoothing the dataset will allow any operations performed on the data to run into less operational errors due to extraneous information, jagged lines, and other undesired data. The process of smoothing the dataset is performed in a several steps, allowing different types of extraneous data to be removed. Once this process is completed, the data will be able to passed onto the algorithms designed to detect the cancerous spectrum.

3.15.1 Dataset Averaging

Averaging together successive takes of the same dataset will allow the team to increase the accuracy of the results taken, by averaging out the spectral analysis from several tests taken. This will help remove outliers in the data-set, which will help remove noise. The Signal-to-Noise ratio will be expected to be 10x lower after 100 tests, and each test should be able to completed quickly. After these data sets are averaged together, the result should be a graph that will be significantly more accurate than any single pass. [36]

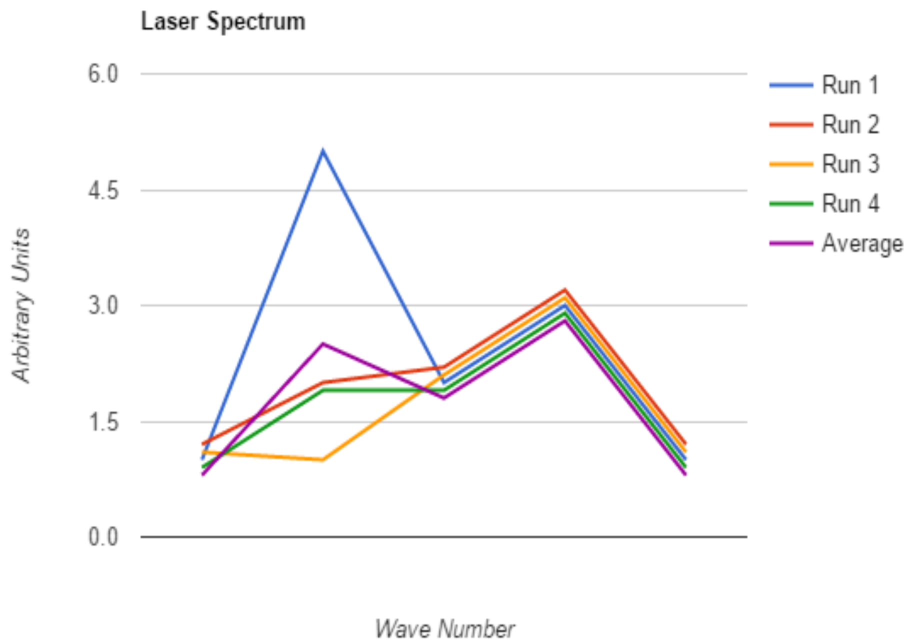


Figure 10. Example Average Noise Reduction

The above example shows how the successive runs allow the peak experienced in the first run is removed by successive runs. As each successive run is taken, it removes the data peak that is experienced, which helps remove the likelihood of false positives. This noise that is received would be perceived by the system later as a data point that can indicate cancerous cells in our patient.

3.15.2 Dark Spectrum Analysis/Subtraction

Dark Spectrum analysis is vital for removing the noise received on the CCD when there is no laser stimulus affecting the system. This removes the input of any stray light or radiation from the data, as well as the possibility of any deficiencies in the CCD array itself. Once this data is taken, then the group is able to subtract these results from the overall dataset.

Dark bias subtraction is the process by which we will subtract the Dark Spectrum analysis from the Laser Spectrum analysis. This process will remove any noise picked up on by the Dark Spectrum analysis from the overall data set, giving the team the most possible correct dataset.

As shown in the dataset below, the use of dark bias subtraction allowed for the test to consider the light pollution received from external to the system. The removal of this bias allowed for the valley located in the center of the graph to be removed, thus allowing for a more consistent dataset to be achieved.

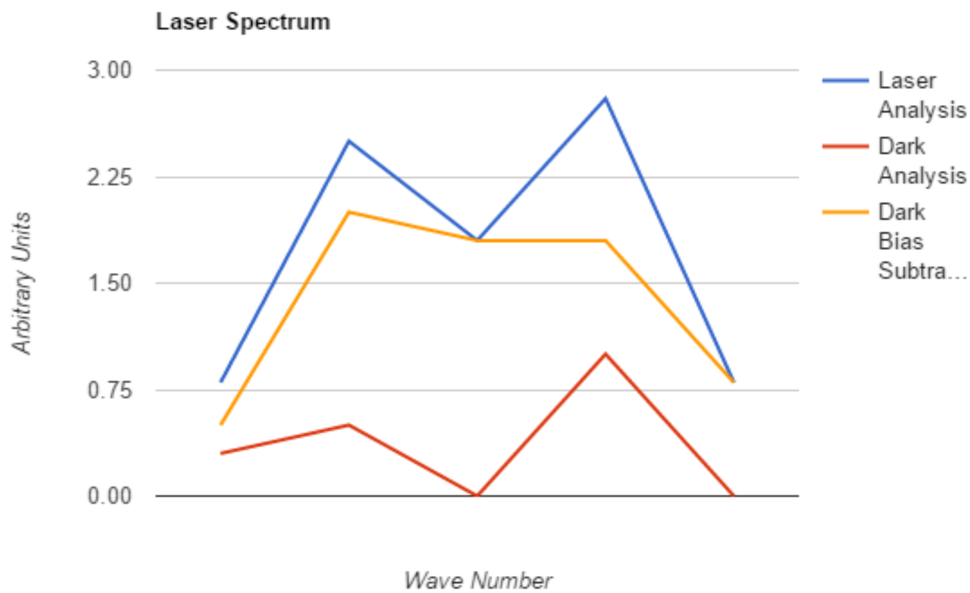


Figure 11. Dark Bias Subtraction Example

3.15.3 Non-Linearity Removal

When taking in large datasets, there is the possibility for datasets that have nonlinear characteristics. The nonlinear characteristics in the dataset will remove validity of the data, due to false peaks and valleys in the overall dataset. While the intensity shift algorithm is being used, this will result in large amounts of extraneous positive results.

The process of non-linear data removal will remove any abnormal spikes in the data set. When the graph begins to change in a nonlinear fashion, it is typically a result of sensor corruption, and can be considered extraneous. Inclusion of these nonlinear spots may lead to false peaks, as well as unprocessable data sets due to extreme and unexpected values.

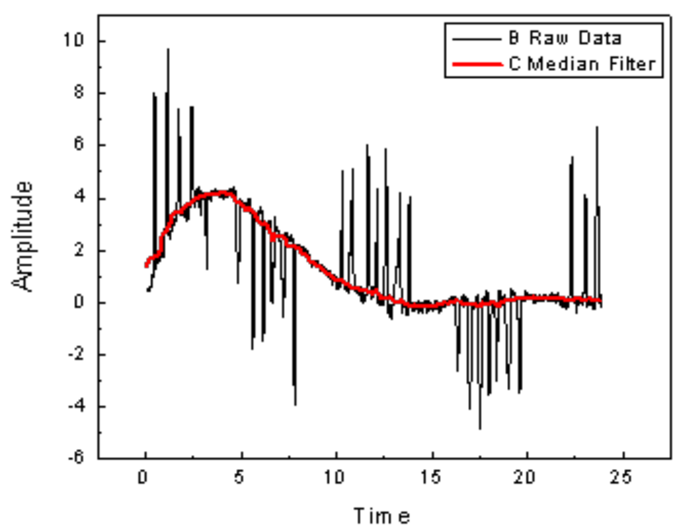


Figure 12. Non-Linearity Removal

As shown above, the dataset contained large amounts of local peaks and valleys. To remove these random peaks and valleys, we will look at the previous average slope, and see if the slope calculated for the current data point is practical. If the slope of the previous part of the section is hovering around 2-3, and suddenly increases to a slope of 15 over one data point, it is safe to assume that the increase is extraneous data. [37]

3.15.4 Smoothing Principles

Smoothing will be the last step in the process to remove short-term variations, or noise, to reveal the underlying important data. Several different smoothing principles are relevant to smoothing the data, without causing a large loss of data precision. The validity of these principles will be tested on the data sets, and we would like to allow the user to select the type of smoothing used.

Boxcar Smoothing

Boxcar smoothing is a smoothing algorithm that is very simple to implement, and replaces each data value with the average of its neighboring values. These neighboring values can be assigned weights to change the effect each value will have, depending on its distance from the point being smoothed. The basic algorithm for this algorithm is:

$$\bar{x}[i] = \frac{1}{2M+1} \sum_{j=-M}^{M} x[i+j]$$

In the above equation, M is the total distance to each side to measure, i is the current x-axis location we are currently smoothing. This algorithm assigns an equal weight to each value, and combines them, and then divides the total by the 2M+1. [38]

Savitzky-Golay Smoothing

Savitzky-Golay smoothing is a much better procedure that averages points using a least squares fit of a small set of consecutive data points to a polynomial. The calculated central point of the fitted polynomial curve becomes the new smoothed data point. The smoothing effect of the Savitzky-Golay algorithm is not as aggressive, as seen with the boxcar smoothing shown before. This will cause less precision loss and distortion in our dataset. [39]

$$Y_j = \sum_{i=-\frac{m-1}{2}}^{\frac{m-1}{2}} C_i \, y_{j+i}, \qquad \frac{m+1}{2} \le j \le n - \frac{m-1}{2}$$

In the above equation, the point Y_j is smoothed out using m convolution coefficients, which are represented by C_i. This algorithm does not assign an equal weight to each of the values, unlike boxcar smoothing, which should cause less data distortion.

3.16 Casing Material

The elements of the Raman spectroscopy system are to be encased in a smooth, relatively slim, and heat safe material. The protective casing will be designed in AutoCAD and created using a 3D printer. As an on-market device, the casing would be made of a durable plastic of a higher, more expensive quality. However, due to economic and time constraints, a 3D printer will be used to create the casing for the prototype designed in the L.A.S.E.R.S. system. Various goals must be met for this aspect; the casing is to have an attractive design that is ergonomic and feels smooth and cool to the touch. The heat of not only the general electronic elements but also the laser must be considered. Thus, the chosen plastic must be relatively heat resistant and able to handle the functionality of the device. It is also important that the material is non-conductive or at least minimally conductive for safety. The casing should protect parts of the device and make it more user friendly and approachable.

The main types of plastic for use in 3D printers are Acrylonitrile-Butadiene Styrene (ABS) and PolyLactic Acid (PLA). PLA filament is 13s plant-based and considered to be more environmentally friendly, while ABS is oil based and more difficult to destroy [29]. As the article "PLA vs ABS: Filaments for 3D Printing Explained & Compared" describes, these characteristics define which type of filament is ideal for a certain type of project. As mentioned previously, the glass point or glass temperature is important to meet the temperature constraints of the system. The glass temperature will give the team an idea of what type of filaments is more ideal for this particular application, which will include some heating to a degree. PLA filament has a lower glass point than ABS, and the article states that, "ABS is better suited for objects that need to withstand rough usage, hot environments, that need to be weather-proof, that may be dropped or have to be bendable. It can be used for parts that are subject to mechanical stress, for interlocking parts or pin-joints," [29]. ABS filament is more durable and able to handle a variety of situations that require regular use. Thus, ABS filament appears to be the ideal material for the casing of the spectroscopy system. The table below compares various ABS filaments available by brand, price, and its specificity:

Brand	Specific Product	Price
SainSmart	1.75mm ABS Filament 1kg/2.2lb for 3D Printers	$26.99
Gizmo Dorks	RED ABS 3D Printer Filament, Dimensional Accuracy +/- 0.05 mm, 1 kg Spool, 1.75 mm, Red	$21.99
iXCC	White ABS 3D Printer Filament 1.75mm Diameter ,1kg Spool	$19.99

Table 9. Comparison of Filament

The dimensions and properties of all ABS filament options explored are roughly the same. The colors and brand specific additives vary. With regard to pricing as well as reviews found on Amazon, the SainSmart product appears to be reliable and affordable. Due to the consistent glass temperature of 100-105 degrees Celsius, pricing becomes the largest concern.

While the team has researched the brands and products above, the matter of 3D printing on a machine or by sending out the casing design is still to be determined. Services exist online where one can send out a CAD design and receive the finished product in the mail. From a service like this, the team would just have to verify that the company or individual printing the casing design has ABS filament, as well as get the product details. The University of Central Florida has a 3D printer in the Manufacturing Lab. The cost of using the Manufacturing Lab's 3D Printer is $5 per cubic inch. The team must create an STL file to of the casing design and request the ability to use the space as well as receive a quote on cost. This step cannot be considered until the casing design itself is complete. Price will be a varying factor that depends on printing location and final design.

In addition to the filament that makes up the majority of the device housing, parts of the casing will be lined with lead foil to protect the user and other device elements from the laser. The foil should be thin, easy to connect and secure to the housing, and inexpensive.

Near infrared radiation ranges from 750 nm to 1400 nm. Inside the spectroscopy system, it is important to use materials that react to infrared radiation as necessary for this specific application. For example, optical lenses, filters, and windows made of Silicon would exhibit 0% transmission at 785 nm wavelength. Other optical and non-optical properties to consider are index of refraction, coefficient of thermal expansion, thermo-optic coefficient, and hardness. Silicon is commonly used in spectroscopy applications because it is low cost and lightweight.

Optical mounts must be secure and immobile. To control costs, the optical mount for the probe was 3D printed after a custom CAD was made for the optical components. The CAD was ensured to properly align each optical component by having the centers of the optics in line with each other. The mount securely holds in place the lenses, beamsplitter, mirror, longpass filter, and fiber adaptors for the optical fibers. The encasing for the probe was also designed using CAD and 3D printed. This resulted in a lightweight, sturdy, handheld probe as shown in the figure below.

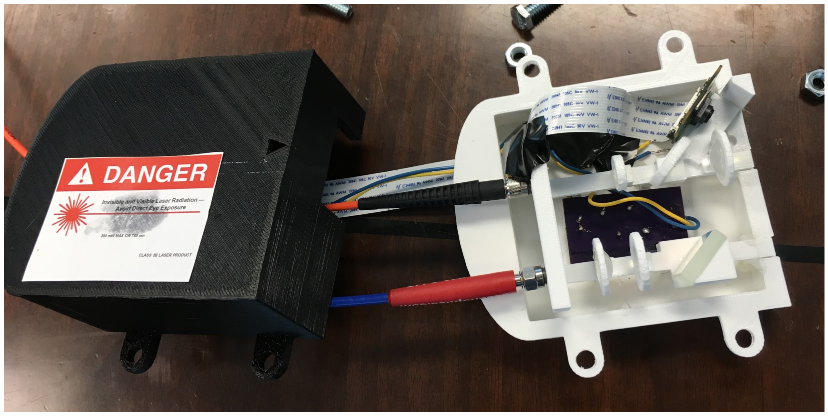


Figure 13. Final assembled handheld probe, opened to show the internal components.

3.17 Heatsink Research

Temperature plays a vital role in ensuring data accuracy and reliability in the system. For most of the device components, very little heat will be generated. However, the two most vital components, the laser and the Raspberry Pi, will be located in a small enclosure with one another. The amount of thermal radiation given off by these two devices is the majority given off by the entire system, and these two systems reliability and accuracy can be directly affected by it. To offset some of the heat given off by the system, we will be designing a heat sink which will pull the heat away from these and allow the units to operate at maximum efficiency for the duration of the tests. [26]

Heatsink design uses several important parameters:

1. Thermal Resistance
2. Airflow
3. Volumetric Resistance
4. Fin Density
5. Fin Spacing

Due to these parameters, and the given design of the system, we will only really have control over material selection, fin density and spacing, as well as the length and width constraints outlined by the design.

The overall heat sink design will be made to fit inside the casing around the Raspberry Pi and the laser. The material we will be using will be aluminum due to its low cost, easy to CNC attributes, and its light weight.

The heat sink design for this project will be similar to that that comes with the Raspberry Pi in a typical fin design. The team's intention is to use whatever space is necessary to ensure stability of the systems, while staying in a portable form factor.

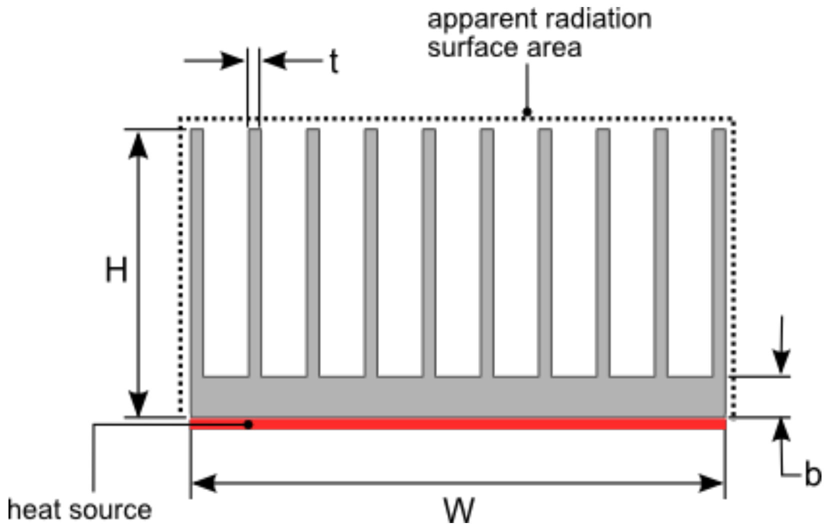


Figure 14. Heat Sink Template

3.17.1 Material Selection

Material Selection is one of the most determinant ways of thermal management of the microcontroller systems. The selection of materials for the system requires the team to consider the functional requirements for each individual component in the system. After following the Ashby method for material selection, which takes into consideration the attributes such as density, strength, cost, resistance to corrosion, and others that we may find beneficial to the project's planning. For the team's needs, there are identified two possible metals that serve the project specifications.

Material Name	Aluminum	Copper
Cost	\$1.91/kg	\$5.76/kg
Density	2.7 g/cm^3	8.96 g/cm^3
Thermal Conductivity	205	385

Table 10. Heatsink Material Choice

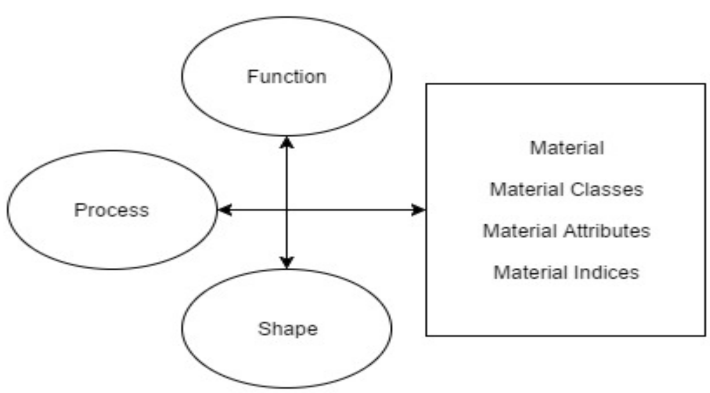


Figure 15. Heatsink Design Constraints

3.17.2 Active Cooling Design

At this time, we have no intention of an active cooling solution, but if the heat dissipation requirements end up calling for it, we will be designing a small air flow port and a place to put a small fan to be able to move air across the heatsink. If an active cooling solution is deemed necessary, the fan will be controlled by the Raspberry Pi, using temperature sensors which will be constantly monitoring the status of both the laser and the Raspberry Pi.

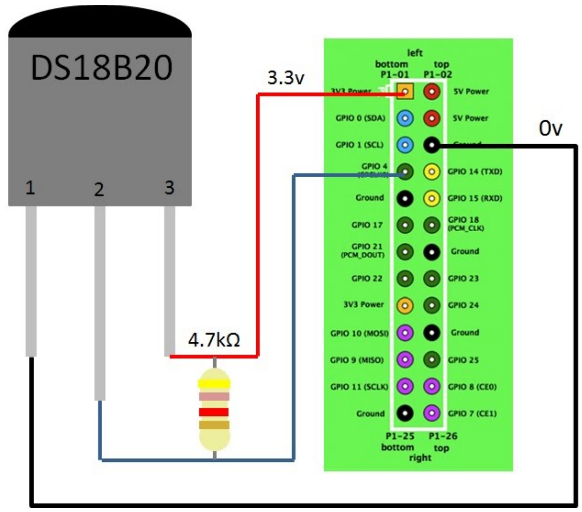


Figure 16. Temperature Sensor

Fan Selection

Though fan selection may seem like an arbitrary task, there are several ideas to take into consideration while selecting a fan. These considerations allow for optimal airflow through the system which greatly reduces the necessary run time for the active cooling fan.

Fan Size

Currently this is the largest determining factor for the fan. The larger the size of fan allows for more air movement, As the size of the diameter of the fan increases, each fan blade will increase by a factor of ½, which when extrapolated over the 4-6 fan blades will result in a vastly increased amount of air moved. The negative to a larger fan is the increased package size, which when creating a platform designed to be useable at home is not ideal. These parameters should be balanced to provide an optimal experience.

Air Movement

Fans of a similar size will indicate different varying measurements of air movement, which is largely determined by blade shape, and maximum rotations per minute (RPM). Though in a pure cooling sense more air movement is ideal, the other desired traits of the project will also play a key role in determining optimal air movement. Fan shape largely determines air pressure as explained below, and RPM greatly increases power consumption, ambient noise, and vibrational considerations of the project. It will be optimal for the team to select an RPM dependent setup as to vary the air movement as it is needed.

Air Pressure
Though a fan moving more air is typically determined by only air movement, the fans ability to move air under pressure is also a large consideration for this project. Air resists movement naturally, but when the air must be pushed past the fins of a typical heat sink array, the static pressure of the air builds up. Increasing the amount of air moved does not necessarily move this air, and it creates a buildup of heat in the system. A fan with a large resistance to static pressure will be ideal in this situation, since it will be moving the air in an enclosed space, with many objects inhibiting its flow.

3.18 Part Selection Summary

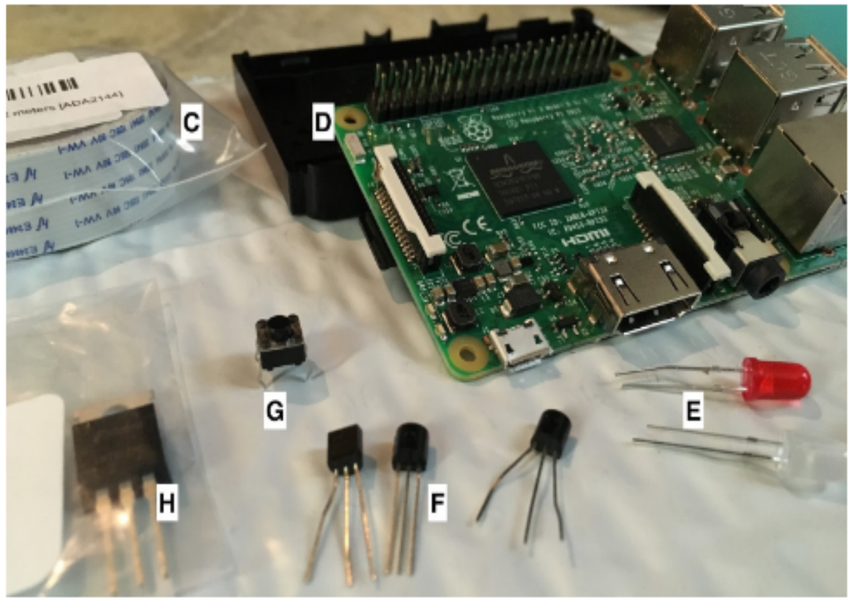
This section contains a table of all parts ordered and implemented in the device, as well as photographs of major parts.

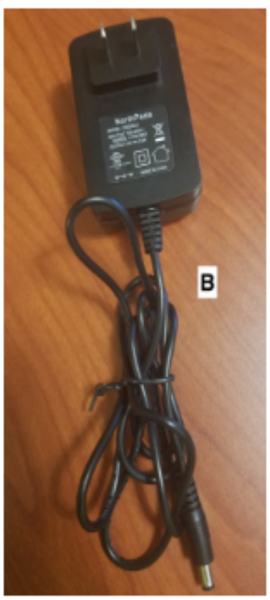
Item	Specific Model	Manufacturer	Qty	Cost per Item
Multimode Spectrum Stabilized Laser Subsystem (785 nm)	LASER-785-IP-02-1208	Ocean Optics	1	Donated by Sponsor
Raman Probe	RPM785-C	InPhotonics	1	Donated by Sponsor
Spectrometer	USB2000+	Ocean Optics	1	Donated by Sponsor
Lens	KPX019AR.16	Newport	1	Donated by CREOL
Mirror	07SD520BD.2	Newport	1	Donated by CREOL
Fiber Optic Cable	P200-1-VIS-NIR	Ocean Optics	1	Donated by sponsor
Beamsplitter	DMSP805T	Thorlabs	1	$174.00
Fiber Optic Cable	M18L01	Thorlabs	1	$75.75

Item	Specific Model	Manufacturer	Qty	Cost per Item
Fiber Adaptor Plates	SM05SMA	Thorlabs	2	`
Lenses	LA1540-B	Thorlabs	2	$31.25
Longpass Filter	5CGA-800	Newport	1	$15.00
Microcontroller	Raspberry Pi 3 Model B	Raspberry Pi	1	$35.00
Temperature Sensor	DS18B20+	Maxim Integrated	1	$3.25
CMOS Camera	Raspberry Pi Camera Module V2	Adafruit	1	$29.89
3D Printed Probe	custom	Minimalist Ventures	1	$30
Switch	Rocker Switch-SPST	Sparkfun	1	$0.95
Wall Adapter Power Supply	9V DC 2A	NorthPada	1	$8.99
PCB A V2	-	OSH Park	1	$43.00
PCB B	-	OSH Park	1	$7.23
5 V Voltage Regulator	L7805	Parts Express	2	$3.20
Flex Cable for Camera	ADA2144	Adafruit	1	$12.49
Additional Cables, Ports	DC jack, USB port, etc		1	$11.42
Additional Electronics	LEDs, transistors, etc.		1	$49.08
TOTAL				**$558.50**

Table 11. Part Selection

All purchased components are shown in the following figures.





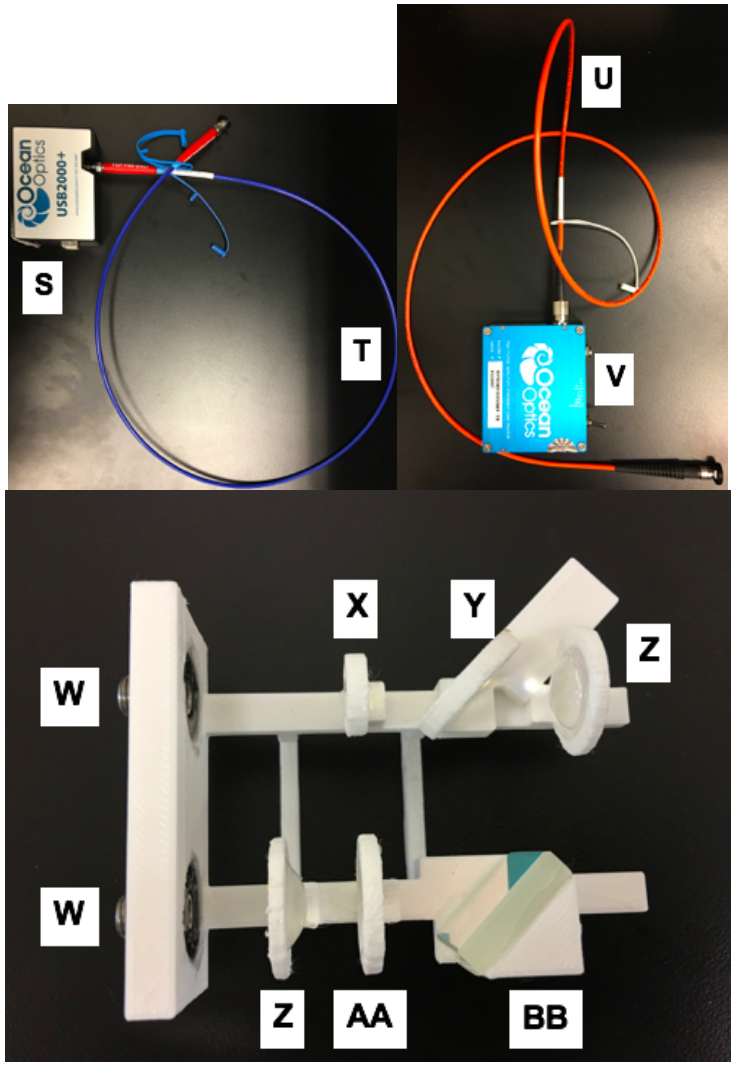


Figure 17. Various Project Components (See Legend Below)

Figure 17 Legend:
(A) Raspberry Pi Camera Module V2, (B) 9 V Wall Adapter Power Supply, (C) Flex Cable for Camera, (D) Raspberry Pi, (E) LEDs Utilized,(F) 2N3906 BJT, (G) Button, (H) L7805 Voltage Regulator, (I) Resistors of Varying Values, (J) Leads, (K) LEDs of Varying Colors, (L) Potentiometers, (M) Transistors and Operational Amps, (N) Capacitors, (O) Buttons, (P) Test Power Supply for breadboard, (Q) Diodes, (R) Capacitors, (S) Spectrometer, (T) Collection Fiber, (U) Excitation Fiber, (V) Laser, (W) Fiber Adaptors, (X) Collimating Lens, (Y) Beamsplitter, (Z) Focusing Lenses, (AA) Longpass Filter, (BB) Mirror

4 Project Specifications, Related Standards, and Constraints

Project constraints are the limits put in place by the design team to fully understand what the final device must deliver and any details surrounding the implementation of the final design. Some constraints, such as time and cost, are beyond the control of the team and add to the challenge of the final deliverable. Other constraints consider general safety and usability goals. The device must appeal to an audience, whether a patient, medical professional, or other engineers, in order to be considered a success. The Project Constraints ensure that the goal of the final L.A.S.E.R.S. device is always considered throughout the design, construction, and implementation processes.

To prove that L.A.S.E.R.S. works, it is able to achieve and has achieved the following specifications.

General:

1) Construct a handheld probe weighing less than 3 lbs (1.4 kg).
2) Construct a main box that fits in a typical office environment space of less than 2.5’ by 2.5’.
3) Capture an image of the area being sampled.
4) Analyse the Raman spectra and discriminate wavenumber differences between samples.
5) Display these results in an easy-to-interpret manner.
6) The total cost of the system should be in line with that of medical equipment.

Optical:

7) Capture a Raman signal of the samples.
8) Manipulate the camera lens to capture macro images (matching the focal length of the focusing lens).
9) Ensure a narrow laser bandwidth to detect the nearest Raman shift.
10) Deliver a laser power greater than 20 mW to the sample.
11) Minimize exposure time to the sample to less than 30 seconds.

Electrical:

12) Create a modular design to ease troubleshooting.
13) Integrate the camera module into the system.
14) Keep the voltage within the device to no more than 15 V.
15) Keep the system temperature below 70°C (158°F).

Computer:

16) Enable the user interface and spectroscopy software to run on various market devices.
17) Keep data sampling and analysis time to less than one minute.
18) Achieve a data analysis accuracy of at least 75%.

In order to design a safe, effective, and marketable device, the L.A.S.E.R.S. system should adhere to some key industry standards. Adhering to standards is beneficial not only for the design process, but also when marketing and explaining the device to other engineers or professionals. Some standards focus on safety, which is of utmost importance. Such standards should be considered a priority.

Other standards discuss basic implementation of a type of system or device, which is useful for the design process. Another standard type that comes in handy while building a device and selecting parts is related to procedures for testing a certain kind of system. There is a variety of standard types, useful guides that can be utilized in all steps of the design and construction process.

Given the nature of this project, the focus was placed on standards related to power distribution, laser safety, and other processes related to the flow of data within the device. Working with a laser was a new experience for most of the team. A full understanding of what dangers the team could face as well as what to look out for while designing for safety was an essential point of research. Other standards are useful and helpful tools, especially for beginner designers, however understanding safety is always the top concern.

4.1 ANSI Z136.1-2014 - Safe Use of Lasers

ANSI Z136.1-2014 is the American National Standards Institute's most current edition of the standard describing the Safe Use of Lasers. Z136 is a series of laser safety standards, while Z136.1 outlines the measures for safely operating lasers, classifying lasers according to their potential hazards, and controlling these hazards. The laser hazard classes are described in the following section. Technical information including calculations in determining hazard classes and the appropriate optical density of eyewear is included in Z136.1.

4.2 Laser Hazard Classes

Lasers can present unique safety hazards. Lasers are highly focused, highly directional beams of intense light. Improper handling of lasers can result in serious eye damage and burns to the skin. As such there are strict standards and regulations that must be followed in the manufacture and use of lasers. The Food and Drug Administration (FDA) identifies four major classifications of laser hazards. Other organizations with similar laser hazard classifications include the American National Standards Institute (ANSI) and International Electrotechnical Commission (IEC). The laser hazard classes are described in the following paragraph.

As defined by the FDA, there are four major laser hazard classes (I-IV) with subclasses (IIa, IIIa, IIIb) describing the dangers posed by such lasers. An increase in the class rating means the lasers are more powerful and pose a greater risk. Class I lasers are non-hazardous, meaning the laser cannot emit radiation at levels that can damage skin and eyes. This hazard increases if optical aids such as magnifiers are used for viewing. Class

II and IIa lasers are visible lasers that cannot cause skin or eye damage if viewed for a time period less than the human aversion response of 0.25 seconds (II) or maximum exposure of 1000 seconds (IIa). This hazard increases with the use of optical aids. Class IIIa lasers are Class II lasers that cannot be viewed with optical aids.

Lasers of Classes I, II, IIa, and IIIa are lower powered lasers. Class IIIb lasers are medium powered lasers, whether visible or invisible. Class IIIb lasers may cause eye damage from either intrabeam viewing or specular reflection. Higher powered IIIb lasers may cause skin damage and diffuse reflection may cause eye damage for certain wavelengths. Class IV lasers are high powered lasers, visible or invisible, that can cause immediate and serious damage to the skin and eye by either intrabeam or reflected exposure. Class IV lasers also pose a fire hazard and are capable of producing byproduct emissions, known as Laser Generated Airborne Contaminants (LGAC) from the target material.

Overexposure to ultraviolet light can cause health hazards such as sunburn, skin aging, and skin cancer. Exposure to high powered infrared light can cause thermal burning of the skin. Non-beam hazards may include electrical shock and exposure to hazardous chemicals.

4.3 ANSI Z136.3 - Safe Use of Lasers in Health Care

ANSI Z136.3 Safe Use of Lasers in Health Care provides processes to protect any person who may be exposed to non-ionizing laser radiation in healthcare applications. These applications may include instances where lasers are used for the diagnosis of disease, bodily structures or functions are altered, and any other application in which lasers are used for healthcare purposes. Operating manuals for these lasers must include instructions for assembly, calibration, operation, and maintenance. Included should also be instructions for preventing hazardous laser exposure, including protective eyewear. The control measures of ANSI Z136.3 are in place to protect the patient, public, and personnel operating the lasers.

4.4 ANSI Z136.8 - Safe Use of Lasers in Research, Development, or Testing

ANSI Z136.8 describes the Safe Use of Lasers in Research, Development, or Testing. As an addition to ANSI Z136.1, this standard details hazards of the laser beam path and beam interaction; control procedures in restricted, unrestricted, and various other locations; and information on eyewear for alignment, export controls, warnings signs, etc. This standard applies to locations such as universities and research labs where lasers are used for research, development, and testing.

4.5 ISO/TC 172 - Optics and Photonics

The International Organization for Standardization (ISO) catalogue of standards ISO/TC 172 is specifically for Optics and Photonics. The standards (ISO/TC 172/SC1, SC3, SC4, SC5, SC6, SC7, SC9) apply to fundamental standards, optical materials and components, electro-optical systems, and others. The subsets of these sets are broken down into individual standards for optics and photonics. For example, ISO 10110-10 defines how to tabulate data regarding optical elements and assemblies as preparation for optical drawings.

4.6 IEEE STD 1-2000 - Temperature Limits: Rating and Insulation

IEEE STD 1-2000 is the IEEE Recommended Practice-General Principles for Temperature Limits in the Rating of Electrical Equipment and for the Evaluation of Electrical Insulation, a series of recommendations that will help guide the design goals of reducing hot spots on the device and making sure that no system elements are overheated. These standards help to guide the design so that it is electrically safe, offering points of reference and specifics to look for while choosing a safe temperature range.

The standard first introduces general concepts to consider. Two overreaching theories should be followed: the ambient temperature is unlikely to stay at a maximum or minimum for a long period of time, and that load cycles may contain moments when the load is not at the typical rating [24]. These principles remind the engineering team to keep in mind that the system will not always be ideal, so leeway must be considered in specification designs. It would be insufficient to assume that ideal temperatures and functionality will always be achieved. The standard explains that temperature is not measured directly in one specific place. Instead, "The permissible temperature rise is generally specified, therefore, to be less than the difference between the temperature recognized in this recommended practice and the temperature of the ambient air or other cooling medium," [24].

In order to find the temperature of the system, one could employ one of the following suggested techniques: resistance, embedded temperature detector, applied thermocouple, contact thermocouple, or simply use a thermometer [24]. The temperature measurement standard suggests finding a way in the design to cool or maintain temperatures. This consideration is important in the design of the casing for the team's Raman spectroscopy system. The table in the standard includes values suggested by IEEE STD 1-2000 for potential rises in temperature in a system. Of these values, a change in temperature at a value between 30 °C and 35 °C would be ideal as a maximum change, especially considering that someone will be holding the probe for the spectrometer and that a large jump in temperature could pose a problem for safety constraints and specifications. A temperature change within these values would not be a problem for the system requirements. While system requirements are important, all safety considerations must take priority. The L.A.S.E.R.S. Raman spectroscopy system should not cause harm to anyone using the system, else the purpose of the project would be lost. For the specific application and purpose of this project, only the lowest values on the table would be acceptable.

Material temperature limits are important to consider. In choosing the filament for for casing material, it is important to note that most materials do not immediately go from a solid to liquid at the melting point. The standard states that, "In these cases, the functionally important softening temperature, which is generally known as the glass transition temperature, may relate to the mechanical stresses imposed in service and the amount of deformation and creep that can be tolerated," [24]. Thus, it is perhaps more important to be aware of the glass transition temperature than the actual melting temperature. The glass temperature is lower than the melting point, and any shift in the stability of the material used for the casing could be catastrophic for the built Raman spectroscopy system. It is essential that the fragile parts of the system are stable and protected within the casing; no decay from the solid point of the material must occur in order to meet the goals of the design specifications. Thus, the glass transition temperature will be an important feature to understand when choosing the filament for the casing of the L.A.S.E.R.S. system.

Next, within the standard, considerations to prepare to choose a design standard are discussed. In general, the purpose and functionality of the product must be considered when choosing design specifications. The standard recommends that, "In considering such factors, predominant conditions rather than extreme requirements should be used as a basis for standards," [24]. For the design of this Raman spectroscopy system, the device must be able to be used daily, potentially multiple times a day. The device will sit in a room that will have air conditioning, set to a value between a range of 68 °F (20 °C)and 78 °F (25.56 °C). Assuming the maximum allowed change in temperature, 35°C, the maximum temperature of the Raman spectrometer will be approximately 141.008°F (60.56°C). Therefore, the material chosen for the casing must have a glass transition temperature above 60.56°C in order to be acceptable. A consumer would expect the device to last for years as it is an investment. Therefore, longevity must be considered as well as how the device elements will heat and be impacted by heat with daily use.

4.7 IEEE STD 1241-2010

IEEE STD 1241-2010 is the IEEE Standard for Terminology and Test Methods for Analog-to-Digital Converters. This standard may be applicable to analyzing and processing the data received by the sensor array. The standard discusses important considerations for testing analog to digital converters as well as important concepts essential to fully utilizing a designed system. These guidelines will be useful for the part of the system that consists of the microcontrollers and the sensor apparatus. This standard is written specifically to cover signals that have already been both sampled and quantized [25]. Therefore, the team must be able to confirm conditions to follow the standard must be met.

First, IEEE STD 1241-2010 discusses important background information on the topic of analog to digital conversion. The standards illustrate the importance of understanding the designed system. It is essential to fully understand the requirements for each element of the system. As described in the standards, "The user should fully understand the manufacturer's recommendations with regard to proper signal buffering and loading, input signal connections, transmission line matching, circuit layout patterns, power supply decoupling, and operating conditions. Edge characteristics for start-convert pulse(s) and clock(s) must be carefully chosen to maintain input signal purity with sufficient margin up to the analog input pin(s)," [25]. Thus, in order to make successful design constraints, the team must turn to the datasheets for each major component to determine what is required.

Understanding how to go about troubleshooting an analog to digital conversion system is essential to verify that the system is functioning properly. There are two ways to analyze errors found within an analog to digital conversion system [25]. As with any electrical system, it is important to understand common pitfalls of a project to figure out which troubleshooting method(s) is/are most applicable. One could either analyze the code using the code center error analysis method, checking the signal at the middle, or the code edge error analysis method, checking the signal at the edges [25]. When testing devices and systems for functionality, it is important to check for any information given with those objects. If any conditions must be considered while testing, they must be followed to successfully analyze.

As stated in the standard, "Since the test condition ranges are generally specified in continuous intervals, they describe an infinite number of possible test conditions, which obviously cannot be exhaustively tested. It is up to the manufacturer or tester of an ADC to determine, from design knowledge and/or testing, the effect of the test conditions on the test result, and from there to determine the appropriate set of test conditions needed to accurately characterize the range of test results," [25]. In order to successfully follow IEEE STD 1241-2010, the team must be sure to verify any suggested conditions for device operation. The team must also fully understand any information given in the datasheet about system/device requirements and make sure that, at all times, these specifications are met during the testing and development process.

4.8 Coding Best Practices

Coding does not have the standards most other engineering discipline have established, but the need for best practices allows for improved readability of the software, which allows it to be understood more quickly. Also, as this is a part of the product, it should be something that is as well packaged as any other component of the project. These considerations allow for the maintenance of the software to be performed with more ease through the lifetime of the code.

4.8.1 File Organization

Each programming file should contain sections that should be separated by empty space, with use of a comment identifying each section. This allows anyone viewing the code to have an idea of what to expect from this piece of code, as well as which code fragments can be separated. Comments are a vital part of this, giving the user an idea of what datatype should be input to each method, what will take place and computed, and what to expect in return.

4.8.2 Declarations

Declarations are implicit in coding, and help in the spacing of the file, as well as in the safety of the data. In Java, only one variable declaration per line is recommend, as to reduce comment confusion. Also, it is recommended to keep declaration naming functional to provide a reminder as to what data or method it is performing. Method and class declarations should have no space between the method name and its parameter list, and the open brace and close brace should exist in the same virtual column for easy viewing.

4.8.3 Statements

Statements are the basis of a program, and their associated best practices allow for a user to follow the flow of the program from line to line. If there are multiple statements on a single line, the results are a program that are unreadable. Each statement should be followed by a semicolon, which indicates the completion of a single statement. For conditional and loop statements, several rules apply, which will result in a syntax error if not followed. Examples include: If must be followed by else, for statements must have initializations, condition, and update statements, and each try statement should also be followed by a catch. These will prevent logical breaks that can result in broken code with certain conditions.

4.9 Power Standards

In general, a safe amount of power must flow through each device component. The team must check the data sheet of every system component and verify the purpose of the part as well as the voltage and current rating to ensure that the design is safe and effective. In working with an on market device, one must verify that the device is operating as per the user manual so that it is running safely. It is important that in designing power supply or power supply control that the team verifies that the designed modules are compatible with the previously built on market devices.

Ideally, the power that flows through a device component will be roughly the ideal rated value; that is, that no excess power or heat will reach any component. The current and voltage values of the component should be within the middle of the acceptable values listed in their respective datasheets. Keeping these values in the mid-range, not at an outlier point, while also mean a more stable and safe circuit and overall device.

Once the datasheets of all the components are thoroughly reviewed, it is also standard to check the circuit voltage, current, and power values after each stage of the circuit schematic(s) to maintain validity throughout the design. Failure to do so could cause damage to the circuit components, or worse, lead to adverse safety hazards during the breadboard circuit configuration. To minimize/prevent this from happening, it is essential to simulate the circuit design at each stage of the schematic before proceeding to any form of breadboard testing.

4.10 Design Constraints

Working with medical technology and optics brings a challenge of specific design considerations as well as safety protocol that must be followed. Considerations must be made in order to meet the cost and design goals that allow the final product to be accurate, safe, and reliable. This project considers each design specification individually and as they contribute to the complete system. The following design, engineering, and safety goals define the focus of the research process and the overall goals of the project.

4.11 Time Constraints

While the idealistic goals of the project are to create a device of similar quality of something found on the market, there are constraints to consider that limit the final product to a device that would be considered a prototype. The total length of time to design and build a Raman spectroscopy system and probe, on little funds and experience in comparison to a corporation that regularly builds similar devices, greatly impacts the scope of the project. The final product will meet all standards, constraints, and the mission for the L.A.S.E.R.S. project; it would be unrealistic to expect a device that looks immediately ready to be sold. The heart of the project, as well as the theory and logic behind the goal of potential cancer detection, will still hold despite constraints of time and funding.

There are four major time constraints that must be considered: time to research and design, time to get the required parts, time to build, and time to demonstrate the device. The time to research device requirements, choose specifications and constraints, find applicable technical specifications, design the device, and write this document is approximately fourteen weeks. This time is utilized by each member of the team focusing on their specific area of specialty and interest. While the team works together and all members are equally present in the design and research process, it is more efficient for each member to have a focal point to be the team expert for a specific part. It is important to note that this time must also consider time spent testing potential parts for the project to see if they meet specifications.

A difficult constraint to work with is the time to procure a required piece for the Raman spectroscopy system and probe system. This challenge ties in with economic constraints. Most of the parts required, such as microcontrollers, the filament used for the casing, and the CCD array, can be purchased and delivered in a reasonable amount of time, less than two weeks, for under $70 each. Elements that are more complicated, such as the laser, grating, lenses, filters, mirrors, fiber cables, printed circuit boards (PCBs), and the 3D printed casing, take more time and money to procure. Both the PCBs and the 3D printed casing must be sent out to a facility that will realize the designs made by the team. On average, it may take up to two weeks to receive the final PCBs and two weeks to receive all 3D printed parts for the casing. Optical pieces provide the challenge of being the most expensive part of this project. Sponsorship has been obtained by working with Ocean Optics to create a probe design for one of their existing spectrometer systems. A probe system will be developed and a live-action camera sensor will be added to their existing probe sensor. Also, all necessary coding/control functionality to implement their spectrometer into the team's own system will be written with a license to their OmniDriver suite. With use of their spectrometer, and the provided 785 nm laser, much of the unnecessary design section for the spectrometer has been removed, allowing us to focus on the important pieces of the project, the coding and user devices.

The time needed to build and construct the L.A.S.E.R.S. system once all necessary parts are purchased or donated will last from the end of Senior Design 1 through Senior Design 2, approximately 13 weeks. During this time, all items required must be in hand and ready to put together. All electronic parts must be connected and soldered onto the PCBs. All optical parts must be placed in their proper arrangement. Each portion of the device will be assembled individually and verified as functioning. Once all of the physical hardware pieces are in place, then the software portion of the project can be tested and verified. This integration is the key to the success of the project. The assembly portion of the project includes the physical building of the device as well as the testing of the individual parts within the whole. Once each portion is verified, then the testing process of the L.A.S.E.R.S. system as a whole can begin.

Lastly, the final demonstration at the end of Senior Design 2 is the ultimate goal of the project, and time to demonstrate the device is a necessary constraint. The full presentation lasts a total of 20 minutes, with 10 of those minutes to demonstrate the product created. Given that the device is not automatic, that someone must scan the object or sample in question, time needs to be considered to physically demonstrate the Raman spectrometer. Thus, the actual process of scanning an object or sample and receiving data must be completed in less than 5 minutes.

4.12 Health Constraints

The health and safety of the user and the patient must be considered as priority for this device design. Thus, the focus should be placed on reducing unnecessary exposure to the laser, making sure that no hot spots exist, and no sharp edges are in the plastic casing. The plastic casing will be enclosed around all electronic and optic parts to prevent stray light from affecting results and to prevent eye damage for both the user and the patient. The casing will be a heat-resistant plastic wrapped with lead foil to prevent exposure. In order to increase the attractiveness of this option to a patient, sample time must be relatively short. Sample exposure time should be less than 30 seconds. An analysis time with a five-minute maximum is ideal to provide fast results to the patient and healthcare professional.

A major benefit of the design and application of this Raman spectroscopy system and probe is that, compared to typical biopsies, this device is a non-invasive tool to check for potential cancer cells. Therefore, this device should not leave any physical marks or cause any pain for the patient. This should be an attractive device with its improvement for the patient experience as a major selling point. There should be no risk to the patient for having this form of testing performed on them, especially in comparison to a biopsy. Biopsies are invasive and require collecting skin cells, while the L.A.S.E.R.S. system only requires the cells on the skin, or the testing sample, to be read by the probe.

4.13 Economic and Cost Constraints

The overall spectroscopy system must be cost efficient for the potential user of the device. The goal is for the device to be under the cost of five biopsies, roughly $1,500. This cost constraint is not only necessary for parts that the team must pay for, but also to justify the device to a patient or physician. In any market, an updated device should be either more effective or more cost efficient in order for consumers to justify making the purchase. This constraint allows for flexibility in the consumer of choice.

There are two potential consumers for this device: a patient with a higher risk factor for skin cancer and/or a physician's office. For a patient, the price point should cost less than the five biopsies and any other potential costs for going into a physician's office, such as a copayment or another office fee. For the physician, the price point should be less than the cost of any lab, processing, and shipping fees to send out many biopsy samples for a multitude of patients. For the team to build this device, it should meet expectations of the budget for four college students. While the team hopes to receive parts via donation, the ability to borrow, or a sponsorship, these are not guaranteed and therefore the worst-case scenario of having to pay for all parts of the system must be considered. The economic and cost constraints are vital.

4.14 Size Constraints

The Raman spectroscopy system will have a compact size to reduce its footprint, making it more attractive to potential customers. The probe will be handheld to more easily analyze skin at different locations on the body, while still attaining high throughput. The size of the overall system is not to exceed what is reasonable for a typical desktop space found in a doctor's office. The dimensions of the completed system must fit within a 2.5' by 2.5' space. The main body of the spectroscopy system will contain the spectrometer, microcontrollers, and power connectivity, therefore these parts will determine the minimum size of the device. As long as the design is streamlined and efficient, the size will fit this constraint. The size and design of the casing must also consider heat flow.

There must be an attractive and ergonomic overall design for the users of the system. The probe, the part of the device that contains the laser and would be applied to the patient's skin/the sample, must be large enough to hold the required electrical and optics pieces. This same part must be small enough for the user of the device to comfortably hold in their hand. The comfort of a potential patient must also be considered, and therefore the size must not be too large to comfortably fit on the sample.

4.15 Temperature Constraints

To ensure that the system is operating safely, the temperature of the system must be monitored. This is to ensure that that laser, optical elements, electronic elements, and the casing do not reach a temperature near the glass transition temperature, as discussed in

IEEE STD 1-200 is the IEEE Recommended Practice-General Principles for Temperature Limits in the Rating of Electrical Equipment and for the Evaluation of Electrical Insulation. Material selection for the outer casing must consider the heat distribution of all electronic and optical parts. The overall heat of the system must not exceed 90°C (194°F), a value that must be below the glass temperature of the casing filament.

4.16 Sensor Constraints

The collection optics sensor, either a CCD or CMOS array, will receive the scattered light effectively. It must be able to account for at least 40% of received light in order to be accurate and effective. The chosen sensor must be able to accurately pick up light emitted from the Raman spectrum at the wavelength of choice. Size and economic constraints greatly impact the choice of sensor. For proof of concept, receiving greater than 40% of light will suffice. With more funds to work with, ideally the sensor would take in at least 80% of the emitted spectrum. This percentage may increase depending on the wavelength of interest, which depends on the chosen laser.

Another important requirement is that the chosen sensor must be able to communicate with the rest of the system and be easily accessed. It must be a simple enough array that the collected data from the sensor can easily and quickly be taken in by attached microcontrollers, and then displayed on the user interface within a reasonable amount of time. The more simple and effective the specific sensor array, the better for this application. A smaller sensor will also be useful to reduce cost and be easier to protect within the casing of the body of the Raman spectrometer.

4.17 User Interface Constraints

To tie in all the data found, the spectroscopy system must have an attractive and user friendly user interface. Software must accurately and reliably analyze the Raman spectra of the scattered light read in with a sensor. Ideally, the designed user interface will have the ability to run on a variety of market available devices, such as a laptop or tablet, so that there is an increased accessibility for potential users of the system. It is important that the user interface offers both ease of use and a simple appearance, all while delivering information that a patient and/or medical professional can trust. It must be simple enough that someone not trained in medical sciences could interpret the results easily, and take the advice of if the trip to a doctor will be worth it, while being informative. The user interface must be simple, options must be clearly labeled, with simple guiding steps on the procedure to test presented to the user in a straightforward fashion. It is essential for the L.A.S.E.R.S. system to be informational without oversharing complicated data.

4.18 Optics Key Design Constraints

The optical components of the system must consider both functionality and size. The focal lengths of the optics (mirrors and/or lenses) must be reasonable so that the spectroscopy system is relatively compact. The laser must be relatively stable to avoid mode hopping. Mode hopping may cause an error in measuring the Raman shift. The bandwidth of the laser must also be narrow (only a few nanometers) in order to have sharp Raman peaks. Because the intensity of the Raman signal is inversely proportional to the excitation wavelength $(1/\lambda^4)$, a shorter wavelength is generally more powerful. However, the fluorescence of organic molecules must be accounted for because it can overwhelm the Raman signal. Laser excitation wavelengths of 785 nm to 830 nm (NIR) are typical of Raman spectroscopy of biological tissues. The output power of the laser necessary to obtain Raman signal can be less than 350 mW. Spectral ranges of typical studies of normal and cancerous skin are from 400 to 1800 cm^{-1}, which corresponds to a wavelength range of approximately 810 to 915 nm. This range captures the spectral peaks of certain proteins and lipids that make up skin that will be used to classify and diagnose the sample. A resolution of 6 to 11 $cm^{-1}$is sufficient for resolving the spectral peaks for the determination of cancer. Of course a resolution smaller than this is better. The team aims for at least 70% sensitivity and 70% selectivity.

There are several important parameters and constraints to consider when designing the Raman spectrometer according to Butler et al. Considerations include identifying the purpose of the Raman system, knowing the constraints of the sample, the laser excitation source and interaction with the sample, the detector to collect the spectral data, resolution of the spectral data, and various optical elements such as filters, the aperture, and microscope objective. Furthermore, the system will require calibration and data processing. [11]

First is the need to identify the investigative aims and analytical goals of the Raman spectrometer. L.A.S.E.R.S. will be a diagnostic tool, using the "fingerprint" of the Raman spectra for spectral classification and diagnosing of the sample. [11] The samples to be measured will have characteristic peaks at certain wavelengths based on the vibrational energy modes of the material. These peaks will be used to determine the composition of the sample, specifically whether the skin sample appears to be normal or cancerous. To remain within a reasonable scope of the project, the Raman spectroscopy system will be able to identify normal skin cells versus skin cells exhibiting spectra indicative of basal cell carcinoma (BCC).

Second, the constraints of the sample must be considered. The sample will determine the laser excitation wavelength, power and intensity of the beam, beam spot size, and acquisition time. It is the goal of L.A.S.E.R.S. to examine *in vivo* the Raman spectra of human skin. Therefore, the safety of the human subject is of utmost importance. The University of Central Florida Institutional Review Board (UCF IRB) is "a committee established to protect the rights and welfare of human participants involved in research." A benefit of Raman spectroscopy is the minimal sample preparation needed. Samples can be superficially cleaned with a wipe. The optical focus of the setup simply needs to be maintained, which is simple for samples with a relatively flat surface. The sample must be suitable to withstand the incident photon energies without becoming photo-damaged. The incident photon energy of 785 nm wavelength is usually low enough to reduce the risk of damage to a sample.

Third, the excitation source plays a role in the interaction of the sample and resolution and sensitivity of the system. The type of laser source, desired excitation wavelength, and desired spot size are important factors in considering what source to use. Diode lasers are commonly used because of their efficiency and long lifetime. Their durability and compactness is of interest because L.A.S.E.R.S. is meant to be portable. Using a NIR or UV wavelength will reduce the fluorescence of the sample, increasing the spectral quality. The linewidth will also directly affect the spatial and spectral resolution of the system. The intensity of the Raman scattering signal is inversely proportional to the fourth power of the wavelength as represented by the equation $I = 1/\lambda^4$. Intensity is defined as the ratio of the power of the beam to the area of illumination. Therefore, the intensity is dependent on the beam spot size. Intensity is also dependent on the magnification of the beam. The magnification will be determined by the optical element(s) used and its numerical aperture (NA). Keep in mind that, as mentioned previously, the sample must be able to withstand the wavelength, intensity, and temperature increase resulting from the laser source. In summary, the spot size and sensitivity of the system depend on wavelength, and the spatial resolution depends on the spot size.

A fourth consideration in designing a Raman system is the optical and electrical elements to be used for measurements. L.A.S.E.R.S. will use a fiber-optic probe to act as the focusing and collecting optics of the light. A probe will allow the system to be modular, portable, and comfortable to use. Optical fibers will distribute the power of the laser beam spot size over the sample resulting in higher spectral quality. Filters will be used to select the wavelength(s) to be collected and analyzed, filtering out the Rayleigh scattering and allowing only the Raman scattering.

Holographic notch filters and dielectric edge filters are two options. Edge filters will allow the transmission of wavelengths above the laser excitation wavelength, while notch filters transmit only the excitation wavelength. The transmission wavelengths allowed determine whether Stokes and/or Anti-Stokes measurements are possible ($E = \frac{hc}{\lambda}$). A slit or pinhole, typically ranging in size on the scale of microns, can be used as an aperture to control the wavelengths allowed into the system. The size of the aperture depends on the balance between the amount of light entering the system and the spectral resolution. For instance, a larger aperture will allow more light, increasing the Raman signal intensity, but will reduce the spectral resolution.

Lastly, calibration and data processing of the Raman system will be required. Calibration involves laser beam alignment with the optics and fitting the spectral peaks of the signal to particular pixels of the detector. Butler et al. suggests silicon as one reference material because of its sharp peak at 520.5 cm^{-1}. The data processing of the spectra involves some pre-processing in addition to the spectral classification. Data pre-processing techniques, such as Principal Component Analysis (PCA) and normalization, will be utilized to remove outliers and spectral peaks caused by cosmic rays. Cosmic rays appear in spectral data as sharp peaks with very narrow linewidths. L.A.S.E.R.S. will specifically measure over the range of wavelengths containing the spectral peaks of normal and cancerous skin. This limited range of wavenumbers will help to reduce the amount of data to be processed. Then PCA or other machine-learning techniques can be utilized to classify the spectra compared to a database of typical spectra for a specific sample type.

4.19 Health and Safety Constraints

UCF Institutional Review Board

In order to use human skin samples or human subjects for testing the Raman spectroscopy system, the University of Central Florida Institutional Review Board (UCF IRB) would require the team to obtain the proper clearances and complete the appropriate documentation. The UCF IRB would require the team to request an iRIS user account and submit an application to conduct a study. Approval by the team's faculty advisors and then by a director or department chair follows. In order to meet federal regulation guidelines, the team would need to undergo CITI training. It is important to note that CITI training must be completed by all investigators involved in the study before IRB approval can be obtained.

The team contacted the UCF IRB to determine what documentation must be submitted in order to conduct research. Particularly for this project, the team would have to create a research protocol as required by the form HRP-503: Human Research Protocol Template and Instructions, and form HRP-502a: Consent - Adult. Due to the administrative clearances and time constraints, the team has decided to investigate alternatives to human skin, such as pig skin.

UCF Institutional Animal Care and Use Committee

The UCF Institutional Animal Care and Use Committee (IACUC) is a part of the Office of Animal Welfare. The IACUC requirements for using animals in research states the following: "If the study involves only tissues that are specifically acquired from a live or deceased vertebrate animal solely for research purposes or from another agency, investigator, institution, or a commercial vendor, a protocol *may* need to be filed with the Office of Animal Welfare and approved prior to implementing the study." The Office of Animal Welfare was contacted in order to determine if IACUC approval is required to carry out this project. The pig skin samples may be purchased from a local butcher shop. Because the samples would originate from a dead animal, the Office of Animal Welfare has deemed that our project will not require IACUC approval. As a contingency to pig skin, we may prepare samples at differing concentrations containing select proteins and lipids present in normal and cancerous skin.

4.20 Overall Functionality Constraints

Successful functionality means that the overall system must be reliable, deliver a faster diagnostic response time, be accurate enough to replace a biopsy, and be safe. L.A.S.E.R.S. will boast efficient design that allows for simple and effective troubleshooting with just as simple and effective use. Low and efficient power consumption and distribution is essential. In general, no power will be distributed to an element in the system that is not required. The data transfer must be fast and secure, offering quick but efficient information about the tested sample or patient. An important limit to keep in mind is that results must be collected and viewable within the time limit of the final presentation, approximately ten minutes at maximum; ideally this time period will be less than five minutes.

4.21 Printed Circuit Board Constraints

PCB constraints exist in virtually every procedure in which it is fabricated. From the initial circuit design that was simulated up until the final stages in component mounting, the PCB will have to be properly made and revised to ensure optimal consumption with little to no performance loss. If the initial designs or fabrication are done incorrectly, then there will be a delay and could alter the date of completion. In this project, there will be multiple PCB's, each with their own dedicated task to complete the primary objective. Understanding the constraints for each PCB is necessary to ensure that the design upholds its performance marks.

From a chronological view, the first constraint that is encountered when focusing on PCB's is their design schematic. Considering that this project will require two or more PCB's, the immediate concern is power flow and interconnected design. It is understood that this design needs to remain modular, which does help for troubleshooting and heat concerns, but it can also make it more difficult to interconnect and communicate between each PCB. It also will require more connections, which may end up being costlier and more fallible. The main constraint in the design schematic is time and efficiency; the circuit must be tested at every level, from the computer simulation through to the breadboard simulation. If at either of those junctions the PCB does not yield the result needed, then it will need to be redesigned and reconfigured [27].

In the following stages of the PCB design, the next constraint is related to the software in which the PCB will be created. To ensure that the PCB is done correctly, there has to be proper documentation and corresponding drawings that match with the design needed. For example, if the Gerber Files that were extrapolated from the software for the first PCB do not properly allow for the microcontrollers to mount on top of them, then the drawings need to be recreated to ensure functionality.

After creating a properly working schematic, the question that needs to be answered is which type of PCB layout is needed. Looking at the design of the project itself, the PCB's cannot be too large or too small due to size and component constraints. If the PCB's are too large, the overall spectrometer design loses its reputation for being mobile. However, if the PCB's are too small, then the surface area of the PCB decreases, meaning that the PCB loses its capability to have more components attached to its surface. The conditional constraint here is to find a layout that would enable each PCB to be small enough to maintain the overall modular-yet-compact design while maintaining its performance capabilities. The common complications in placing the components is the potential for interferences, ranging from thermal pollution, noise, and functionality. The PCB's will need to be carefully examined for the temperatures of the components, their susceptibility to noise, and potentially redesigned and rewired for better functionality.

4.22 Probe Add-On Constraints

As per the desired functionality from Ocean Optics, the probe design must integrate value-added items. First, a camera must be added. When probing a sample, it is important to see the area of the sample being scanned so that the test process if more efficient. Because the probe tip must be enclosed to prevent laser exposure to the eyes, the user must use the camera to determine where the laser spot is located on the sample. The user should be able to know if the lesion of interest is within the sample space. This image of the sample area can be related to its respective spectral scan. Additionally, an image of the lesion will be useful for the dermatopathologist to visually inspect in diagnosing whether the skin is cancerous.

The camera needs to capture still photographs as well as video, while providing a live feed. The camera must be able to function within a close range of a surface, therefore it must have a high resolution, white balance features, and be able to function as the surrounding light changes. It must provide a clear indicator that the user is approaching the skin area of interest, as well as provide medical professionals a better idea of where the skin area of impact is located.

Again because the probe tip must be enclosed to prevent laser exposure to the eyes, the sample will be dark. Without a visible light source within the probe tip, the camera will not be able to capture an image of the sample. To help with the task of clear imaging, an LED beam will also be added to the probe. This LED must be vivid enough to help illuminate the skin surface. The LED also has a usefulness in that the user of the device can better see and guide where the probe will read the skin if they can see it better with improved lighting.

This direction from Ocean Optics aligns with the initial design goals of a device that improves the patient and user experience. While the final product has shifted, the deliverables and the final goal remains the same. The insight from Ocean Optics on valuable problem additions provides concise, specific probe design elements that only strengthen the quality of the final piece.

5 Project Hardware and Software Design

The L.A.S.E.R.S. system is shown in Figure 18. The major components of the system - laser, spectrometer, and electronics - are housed in the Main Box, an area no larger than a desk space of 2.5’ by 2.5’ in order to keep the system compact and portable. An optical fiber carries the excitation light from the laser to the Raman probe. The probe is lightweight and handheld for maximum comfort of both the user and patient. Another optical fiber carries the collected light from the probe to the spectrometer. Using the GUI, results are provided on a computer display.

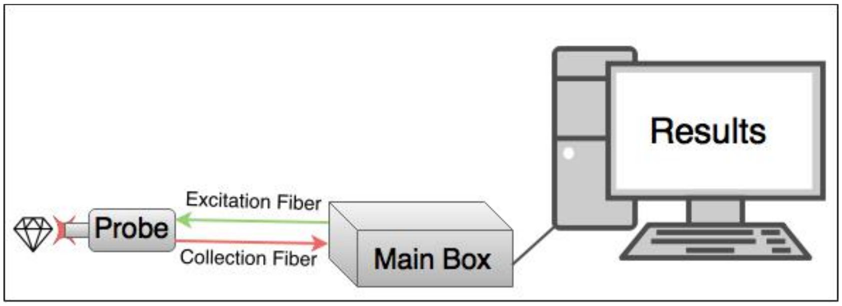


Figure 18. Design Concept for the Raman System

5.1 CMOS Camera

The CMOS camera module will sit alongside the lens and LED beam. A challenge presented is utilizing the module while saving space. The cable that connects the Raspberry Pi Camera Module to the Raspberry Pi is considerably short, too short for an easy to use probe. However, the cable is easily removed and able to be replaced to a length of up to 2 meters. This flexibility is essential to the functionality and usability of the camera on the probe. It would not fit design constraints to have a maximum probe length of under 5 inches.

When connected to the Raspberry Pi using the pinout below, the camera will have power input, data input, and data output. This will allow the camera to function and to record video or capture an image. This module does not record audio, which is not necessary for this application.

Pin Number	Name	Purpose	CCD Connection
1	Ground	Ground for CMOS	Ground
2	CAM1_DN0	Data Lane 0	-
3	CAM1_DP0	Data Lane 0	-
5	CAM1_DN1	Data Lane 1	-
6	CAM1_DP1	Data Lane 1	-
13	SCL0	PC Bus	-
14	SDA0	PC Bus	-
15	+3.3V	Power Supply	-

Table 12. Pin configurations

Since the team decided to use a camera module instead of designing a camera from scratch, the design of this component is minimally extensive. Details of implementation of the CMOS camera into the probe design can be found in the probe design section.

5.2 Probe Design

Initially, the scope of the project involved the design and construction of a spectrometer, laser source, and probe. Upon discussion with Ocean Optics on the realism of achieving such goals, Ocean Optics suggested the idea to create a value-added Raman probe, based on one of their existing probes, utilizing a camera to capture real-time images of the sample area that is being illuminated by the laser spot. In order to meet the requirements of Senior Design, the team has been tasked to design and construct a Raman probe with camera. In essence, the probe should contain the necessary optics (filters, lenses, mirrors, etc.) and a camera and source of illumination, such as a low-powered light emitting diode (LED), for illuminating the sample area. For design reference, Ocean Optics is providing an Inphotonics RPM785-C Raman probe.

According to Dr. Wei, the Department of Electrical and Computer Engineering at the University of Central Florida has no claim on any intellectual property (IP) of undergraduate students. The students have complete ownership and therefore decide how to handle IP with the sponsor. Dr. Wei mentioned that it is typical for the students to sign a non-disclosure agreement (NDA) with the sponsor to give the IP to the sponsor. The team is discussing with Ocean Optics how to handle IP.

A lightweight, handheld probe will allow measurements to be easily made at any external location on the body. The probe should be placed at an angle normal to the skin for maximum signal collection. To implement this, optical fibers can be utilized to direct both the excitation and collected light between the sample and spectrometer. More detailed information is given in the section on optical fibers.

At this time, we have two probe design concepts. The designs are based on a Raman probe from B&W Tek. One probe, illustrated in Figure 19, contains all of the optics - lenses, filters, mirror, camera, light source (i.e. LED) - within the handheld. This design would utilize two optical fibers: one fiber (single mode) for excitation, and one fiber (multimode) for collection. More on the choice of single mode versus multimode is described in the fiber optics section.

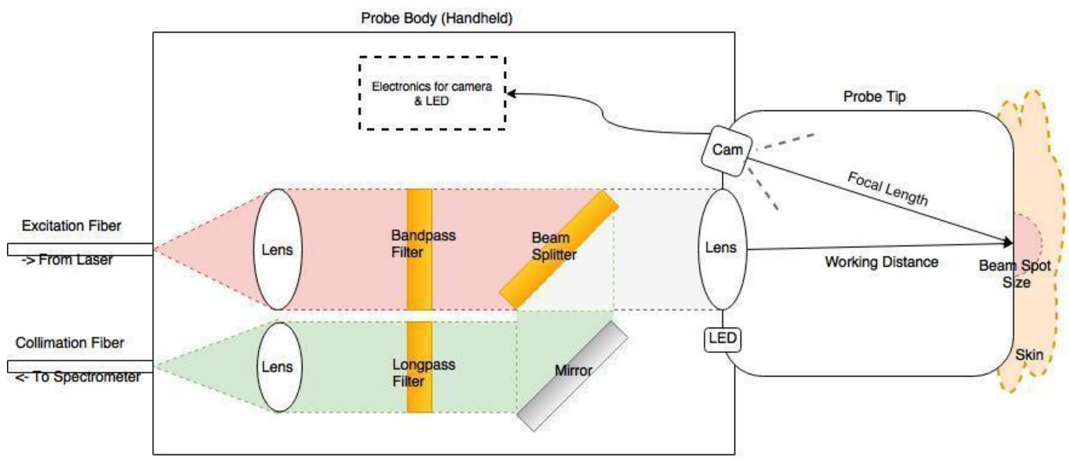


Figure 19. Probe design concept.

The excitation light from the optical fiber is collimated by a lens to be passed through a bandpass filter in order to narrow the laser line. The laser line must be narrow in order to differentiate the scattered Rayleigh signal from the Raman signal. The laser light is focused onto the skin/sample using a lens. This same lens can act as a collector of the reflected light. The beamsplitter transmits the excitation light but reflects the collected light to a mirror. The mirror directs the collected light through a long-pass filter to block the laser line (Rayleigh signal) allowing only the Stoke's Raman signal to be directed to the spectrometer. A short-pass filter would transmit the Anti-Stoke's Raman signal. A notch filter would transmit both Stoke's and Anti-Stoke's Raman signals. A third lens couples the collected signal into the collection fiber, which is connected to the spectrometer.

A second probe design, illustrated in Figure 20, separates the filters from the camera and light source. In this design, a small case would contain the lenses, filters, and mirrors necessary for directing the light through the filters. The case could be attached to the spectrometer via fiber optic connections as in the first probe design. A small handheld probe would contain the camera and light source and have a fiber optic cable (double clad) connected to the small case. This handheld would be responsible for directing the filtered laser light to the sample and for capturing images using the camera and light source.

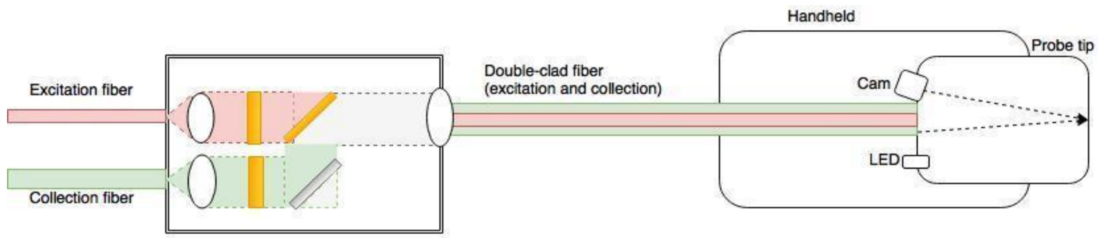


Figure 20. Probe design concept.

5.3 Spectroscopy System Design

The spectrometer is an important element to the analysis of the Raman spectrum of the sample. The spectrometer is the portion of the Raman system that disperses the Raman signal into its constituent wavelengths in order to measure the Raman shift. Ocean Optics, as the team's sponsor and partner, has agreed to provide the spectrometer and laser source for this project. Ocean Optics is providing a spectrometer for the Raman measurements.

The Ocean Optics USB2000+VIS-NIR is a miniature, modular spectrometer that is capable of taking visible and near infrared spectroscopy measurements. It covers the wavelength range of 350 nm to 1000 nm and has an optical resolution of approximately 1.5 nm FWHM. The SNR is 250:1. A wavelength calibration sheet should be provided with the spectrometer.

The spectrometer will be connected to the team's Raman probe via an optical fiber. The fiber optic connector on the spectrometer is made for use with a SMA 905 to 0.22 numerical aperture single strand fiber.

The spectrometer has an asymmetric crossed Czerny Turner configuration. The Czerny Turner configuration consists of a concave collimation mirror, planar diffraction grating, and concave focusing mirror. The diffraction grating used in this spectrometer has a grating density of 600 lines/mm, spectral range of 350 - 1000 nm, and a blaze wavelength of 500 nm. It has a 25 µm slit width. There is low stray light of less than 0.05% at 600 nm.

The detector used is a Sony ILX511B, 2048 element linear silicon CCD array. The pixel size is 14 µm x 200 µm with a pixel pitch of 14 µm. The relative sensitivity of this detector is over 30%, making it fitting for the team's Raman application. For a single spectral acquisition, the detector has a dynamic range of 1300:1.

The spectrometer has a rapid integration time, typically 20 seconds but ranging from 1 millisecond to 65 seconds. It is capable of storing into memory 1,000 spectra every second. The USB interface is a USB 2.0 with a 480 Mbps data transfer rate. The USB cable can be connected to a computer for spectral analysis by the OceanView software and by the team's own software design. This spectrometer is also configured for serial port communication RS-232 which can transfer data to other devices such as a microcontroller, if need be.

This spectrometer operates without the need for external power. The packaging dimensions of the spectrometer are 89.1 mm x 63.3 mm x 34.4 mm, making it compact and portable, weighing only 190 grams, greatly reducing its footprint on a desktop space.

The Federal Communications Commission (FCC) rules and regulations for CFR Title 47 Part 15 classifies this spectrometer as a Class A digital device. The user manual for the spectrometer states the following: "These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which the user will be required to correct the interference at his own expense."

The laser, spectrometer, and probe were located at the Ocean Optics facility in Orlando. The team is scheduled a meeting with Ocean Optics to discuss the design plans and picked up the components in order to begin to testing and prototype process, the start of Senior Design 2.

In its simplest form, the spectrometer will be comprised of an entrance slit, collimating and focusing mirrors or lenses, a diffraction grating, and the detector. The size of the entrance slit determines the divergence of the light into the spectrometer to be mapped onto the detector, which in turn determines the optical resolution of the spectrometer. If using an optical fiber to couple the input light into the slit, then stacking or bundling fibers will help to match the dimensions of the slit. The image of the entrance slit should be larger than the pixel width of the detector for high resolution. A collimating lens or mirror will collimate the light onto a diffraction grating. The diffraction grating disperses the light into its constituent wavelengths, determining the spectral range of the system. Then a focusing lens or mirror will focus the dispersed light onto the detector.

The spatial resolution that can theoretically be achieved is diffraction limited. This diffraction limited spatial resolution, or essentially the laser spot diameter, is found through the equation $R_S = 0.61\frac{\lambda}{NA}$. Using an excitation wavelength at 635 nm and an objective in the probe with a numerical aperture of 0.22, the diffraction limited laser spot diameter would be 1.76 μm. Of course this is the theoretical diffraction limit and is difficult to achieve experimentally.

The spectral resolution of the spectrometer should ideally be about 6 cm^-1. A resolution between 8 cm^{-1} and 11 cm^{-1}, however, is sufficient for good spectral analysis. A spectral resolution of 6 cm^{-1} corresponds to a resolution of 0.24 nm.

The longest wavelength diffracted by the grating is equal to twice the groove period. The groove period is the reciprocal of groove density. For a maximum wavelength of 717 nm, the grating should have a groove period of 359 nm. This corresponds to a groove density of 2,789.4 grooves per mm. The minimum number of grooves required to achieve a spectral resolution of 0.24 nm is found by dividing the wavelength of 635 nm by the spectral resolution. This results in a minimum of 2,645.8 grooves.

The blaze wavelength of a grating is the wavelength at which the diffraction grating is most efficient. The efficiency will decrease by roughly 50% around 0.6 times the blaze wavelength and again around 1.8 times the blaze wavelength. For a grating with a blaze wavelength at 635 nm, the efficiency will decrease by half at 381 nm and 1143 nm. The device's wavelength range of 678 nm to 717 nm will be well within the efficient range for this grating.

5.3.1 Laser Excitation Source

Near infrared wavelengths of 785 nm and 830 nm are commonly used when working with biological materials because of low IR absorption and reduced fluorescence interference of the materials at these wavelengths.

Ocean Optics is providing a 785 nm multimode spectrum stabilized laser. This laser produces an adjustable high output power up to 400 mW. The laser is designed to be fiber coupled, optically terminated with a FC/PC connection. The fiber will be connected to the Raman probe to deliver the excitation laser to the sample. As mentioned, this laser has a stabilized peak wavelength of 785 nm over a temperature range from -10□ to +55□ with changes less than 0.007 nm/□. A stabilized laser is important for Raman spectroscopy; mode hopping would compromise the accuracy of the Raman shift measurements. The narrow spectral bandwidth of less than 0.15 nm is ideal for Raman spectroscopy that requires the laser linewidth to be smaller than the linewidths in the Raman spectrum in order to have high resolution.

This 785 nm laser has an extremely high signal to noise ratio is perfectly suited for Raman applications. The side mode suppression ratio (SMSR) of this laser is greater than 40 decibels. The SMSR is the ratio of the amplitude of the peak longitudinal mode with the amplitude of the nearest higher order mode. An SMSR of 40 dB equates to an amplitude ratio of 100:1. The laser has a quick warm-up time of 10 seconds from a cold start or 1.5 seconds from a warm start, minimizing wait time for the operator and patient.

The laser packaging is integrated with a high-performance laser driver and temperature controller. The dimensions of the anodized aluminum packaging are 7.6 cm x 6.4 cm x 1.8 cm (length x width x height) and weighs less than 113.4 grams (approximately 0.25 lbs). This compact and lightweight laser is fitting for a portable Raman spectroscopy system. The laser has an on/off switch, a 10-pin connector, a power input, and a laser output. The on/off switch will be used to turn the laser on and off. The laser has a low power consumption typically 3.5 Watts (maximum of 5.5 W). The operating current of the photodiode is 30 μA. A separate 5 V DC power supply is provided.

Not provided is a heat sink for the laser, therefore a suitable heat sink will need to be acquired and mounted to the laser. The 10-pin connector can be used to remotely operate the laser instead of manual operation, if desired. In remote operation, the power supply will be provided via the 10-pin connector. Instructions for the correct pin connections are given in the laser user manual. The FC/PC fiber connection for the laser output is designed for a fiber with a 100-105/125 micrometer core/cladding ratio and numerical aperture of 0.22.

Safe operation of the laser is important to prevent accidents. Danger signage, shown in the figure below, should indicate the output power of the laser (typically 350 mW) and the laser hazard class (Class IIIb). Warnings should be posted in the vicinity of the laser operation stating that the infrared laser radiation is dangerous to eyes and skin from both direct (intrabeam viewing) and scattered exposure (from reflective surfaces). Safety glasses rated for the infrared wavelengths should be worn by all operators and others in the vicinity. Unauthorized personnel should not operate the laser or be in the vicinity. Good safety practices are to keep the experimental setup below eye-level to help prevent exposure to the eyes and to mechanically block the laser when not in use.

The user manual provided with this laser states, "This laser module is designed for use as a component (or replacement) part and is thereby exempt from 21 CFR1040.10 and 1040.11 provisions." The provisions are a part of the FDA's Code of Federal Regulations Title 21 (U.S. Food & Drug Administration). CFR1040.10 and 1040.11 deal with radiological health, specifically laser products (1040.10) and the specific purpose of laser products (1040.11).

Figure 21. Laser warning sign describing the risk of the Class IIIb laser.

5.3.2 Optical Fibers

Optical fibers can propagate light information from the laser source to the probe and from the probe to the spectrometer. The fibers used in the system must have high throughput and a large signal-to-noise ratio. As light propagates through a fiber, the fiber generates a background signal that can reduce the SNR making Raman analysis difficult. The use of filters in the probe, as described in the section on the probe design, will filter the background noise generated by the fiber as well as block the Rayleigh scattering. The optical fibers chosen must maximize the collection efficiency of the signal from the sample. [28]

A single mode fiber is great for transmitting laser light to a tissue, while a multimode fiber is great for collecting signal from a tissue and for coupling the light into the fiber for maximal power. A double clad fiber (fiber with two cladding layers) can be used as both the excitation and collection fiber, with the excitation delivered by the fiber core and the reflected signal collected by the larger inner cladding. The NA of the fiber describes the acceptance angle over which light can enter the fiber. In choosing an optical fiber for a specific application, the signal attenuation for a given wavelength is important. As the light propagates through the fiber, some power is lost due to absorption, scattering, and bending loss. Therefore, a fiber must be chosen that has low signal attenuation in the NIR wavelengths. For example, silica fiber typically has a low loss region from 700 nm to 1600 nm making it an excellent choice for NIR diagnostic applications. Plastic fibers are also available, which may have a lower cost than glass fibers. [32]

Losses due to bending in the fiber should not be a major concern for the team's application as the fibers connecting the probe to the spectrometer and laser will not need to be bent excessively. The optical power-handling capability of the selected excitation fiber should work optimally for the power delivered from the laser to the tissue, and that of the collection fiber should carry relatively weaker powers without great losses.

A great advantage of using fibers is the elimination of the need for alignment. When optical fibers are manufactured, they are doped with OH^- ions due to the plasma torches used to soften the silica to be drawn. Fibers with a high OH^-concentration exhibit water absorption peaks in the NIR. Therefore, for our application, low OH^- fibers should be chosen to mitigate water absorption peaks. To couple light into the fibers requires lenses with certain focal lengths. Knowing the mode field diameter (ω) of the fiber, the focal length of the lens is calculated as $f = D(\pi\omega/4\lambda)$, where $D$is the laser beam diameter and $\lambda$is the excitation wavelength.

5.3.3 Diffraction Gratings

Diffraction gratings can be transmissive or reflective, allowing for variations in the setup of the spectrometer. Reflective gratings consist of ruled and holographic gratings. Ruled gratings have physical grooves etched onto the substrate. Holographic gratings have a sinusoidal variation in refractive index of the substrate. Each grating has its benefits. Ruled gratings are less expensive but may result in more stray light due to groove imperfections. Holographic gratings can correct for stray light, improving the efficiency of the spectrometer, but are more expensive. However, holographic gratings can also be designed directly onto a concave focusing mirror to both disperse and focus the light onto a detector. Because Raman spectroscopy requires a high signal-to-noise ratio, a holographic grating may be the more suitable option. Transmissive gratings, on the other hand, can make alignment simpler and are insensitive to the polarization of the incident light. An important specification in choosing the correct grating will be based on the diffraction efficiency at the specified wavelength, or blaze wavelength.

Following are some equations for determining the dispersion, spectral range, and groove density of the grating. The angular dispersion of the grating can be calculated as $\frac{d\beta}{d\lambda} = \frac{m}{10^6 d\,cos(\beta)}$. Here β is the diffraction angle of the light from the grating, m is the diffraction order, and $d$is the groove period, which is the reciprocal of the groove density. The linear dispersion, assuming a small angle approximation, can be calculated as $\frac{d\lambda}{dL} = \frac{10^6 d\,cos(\beta)}{m\,F}$, where $L$is the length of the grating and $F$is the focal length of the focusing mirror or lens. The maximum spectral range covered by the detector will be $(\lambda_{max} - \lambda_{min}) = L_D \frac{d\lambda}{dL}$. The detector length L_D is the product of the total number of pixels on the detector with the pixel width. The minimum resolvable wavelength difference by the grating is $\frac{d\lambda}{\lambda} = \frac{d}{m\,L_g} = \frac{1}{m\,N}$ with L_g as the length of the grating and Nas the total number of grooves on the grating. The upper limit on the spectral range, or longest wavelength diffracted by the grating, is twice the groove period.

5.3.4 Wavelength Filters

Optical filters will be required as part of the probe design. Figure 22 below demonstrates the functionality of the filters. Basic filter types include bandpass, short-pass, long-pass, and notch. The bandpass filter, also known as the laser line filter, allows the transmission of a narrow range of wavelengths. A bandpass filter may be used in the probe to clean up the laser line of the excitation light if the spectral width of the light is more than a few nanometers. Short-pass and long-pass filters are types of edge-pass filters. The short-pass filter allows the transmission of wavelengths smaller than the desired cut-off wavelength. The long-pass filter allows the transmission of wavelengths greater than the desired cut-on wavelength. These filters are used to isolate portions of a spectrum. A long-pass and/or short-pass filter may be used within the probe to block the Rayleigh scattered signal, allowing only the transmission of Stokes and/or Anti-Stokes Raman scattering, respectively. Notch filters block the transmission of the laser line, allowing the transmission of the Stokes and Anti-Stokes Raman scattering. A short-pass filter and long-pass filter can be combined to act as a notch filter.

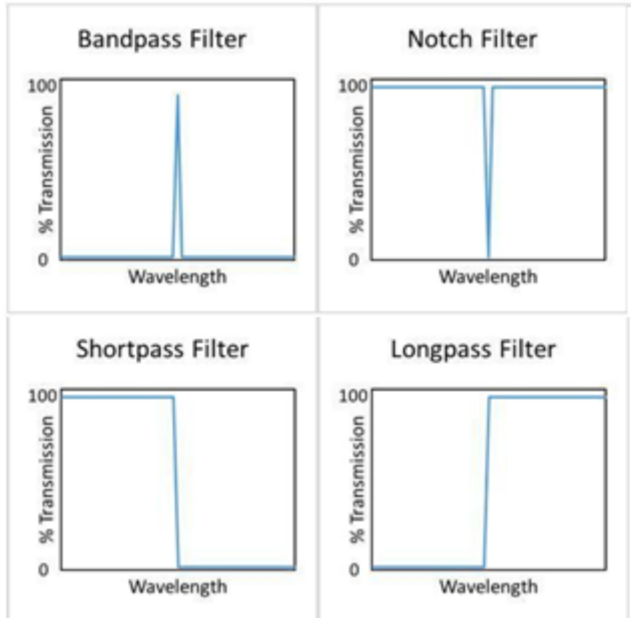


Figure 22. Different types of optical filters and the wavelengths transmitted.

Another type of optical filter is the fiber Bragg grating (FBG). These gratings are etched directly into the optical fiber. The grating is a variation in refractive index, acting as a wavelength selector. The FBG can be used to reflect certain wavelengths. Some advantages of this built-in grating are its narrow bandwidth, low optical loss, and low production cost.

Using the equation mentioned earlier for finding the wavenumber shift, a 785 nm laser scanning wavenumbers in this range from 1000 to 18000 cm^{-1} means the Raman signal should be found from 851 nm to 915 nm. The CCD in the spectrometer should have high efficiency in the wavelength range of 851 nm to 915 nm. Using a long-pass filter with a cut-on wavelength between 785 nm (laser line) and 851 nm (lower bound of Raman signal) will block the Rayleigh signal and transmit the wanted Raman signals. A long-pass filter will transmit the Stoke's Raman signal, which has a higher probability of occurrence. A short-pass filter is not the proper choice because it would transmit the Anti-Stoke's Raman signal, which has a lower probability of occurring. It would also measure a wavenumber range we are not interested in. A notch filter would capture both Stoke's and Anti-Stoke's Raman signals, but the Anti-Stoke's signal is not necessary for the wavenumber range we want to observe.

5.3.5 Lenses and Mirrors

The Raman spectroscopy system and probe will require the use of lenses and/or mirrors. Lenses within the probe will be used for focusing, collimation, and coupling into the optical fibers. A lens or mirror within the spectrometer will collimate the light from the slit onto the diffraction grating. Light from the grating will then be focused by a lens or mirror onto the detector array. There are concave mirrors available with dielectric coatings for NIR and visible wavelengths. Plano-convex lenses can collimate light from a point source or converge collimated light.

5.3.6 Optics Handling

On a technical note, optical elements such as mirrors, lenses, gratings, and optical fiber are sensitive and may be damaged easily. Manipulation of the optics must be gentle. It is imperative to handle the optics in a way as to not add fingerprints, oils, scratches, or other imperfections because the performance of the optics will be negatively affected. Additionally, keeping the optics clean prevents scattering and damage upon incident laser light.

There are best practices when handling and cleaning optics. Gloves or finger cots should be worn to prevent damage. If an optic is dusty, the best first step is to clean it with a canned air duster. Wiping a dusty optic may scratch it. A solvent such as acetone or methanol can be used in combination with a lens tissue to wipe an optic. Lens tissues used to clean the optics should never be reused as leftover dust and dirt may damage the optics. In order to keep optics safe, they should be wrapped in lens tissue and stored in an appropriate container when not in use.

5.4 Initial Optical Testing

Raman scattering is a weak process. Only 10^{-7} of the incident photons are scattered compared to 10^{-4} photons scattered by Rayleigh scattering. Therefore, it is imperative that the optical components are perfectly aligned to both deliver maximum power to the sample and more importantly collect the Raman scattering from the sample and deliver it to the spectrometer. All initial testing was conducted with the optics mounted on an optical breadboard, as pictured in the figure below.

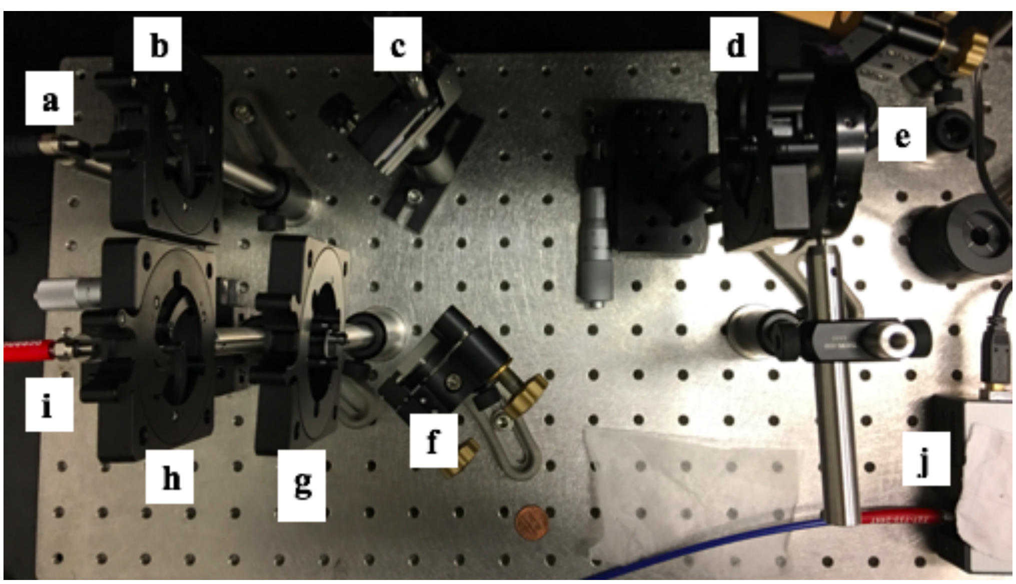


Figure 23. Probe setup for initial testing on the optical breadboard. a) Excitation fiber from the laser (not pictured), b) collimating lens, c) beamsplitter, d) focusing lens, e) sample holder, f) mirror, g) longpass filter, h) focusing lens, i) collection fiber connected to j) the spectrometer.

Painstaking measures were taken to properly align the setup. The first section involved the alignment from the excitation fiber to the sample. The excitation fiber was positioned so that the fiber end was in line with the holes on the optical breadboard. These holes were used as a guide for mounting the rest of the optical components. The height of the fiber core from where the laser light diverged was measured and placed to be at 17 cm. All components were then mounted to be centered at a height of 17 cm. The collimating lens was then positioned a distance of approximately 2.3 cm (23.16 mm is the focal length of this lens). Collimation of the light by this first lens was verified at a distance greater than 2 meters. Using a ruler and the holes on the optical breadboard, the light path was verified to be straight and remain at a height of 17 cm. Using a ruler and the optical breadboard, the beamsplitter was positioned at an angle of 45 degrees relative to the beam path. It was ensured that the collimated light was not clipped by the beamsplitter. The focusing lens before the sample was centered to the beam path. It was verified that the collimated light was now focused to a point approximately 1.1 cm from the lens (11.6 mm is the focal length of this lens).

The second section involved the alignment from the sample, back through the system, and to the spectrometer. The excitation fiber was temporarily mounted at the position of the sample. The light propagated through the sample lens and to the beamsplitter, where it was partially reflected by 90 degrees to a mirror. The mirror was position at a height of 17 cm and and angle of 45 degrees relative to the incident beam using the same process as for the beamsplitter. The longpass filter, second focusing lens, and core of the collection fiber were positioned to be at heights of 17 cm. The beam path continued to follow the holes on the optical breadboard. The core of the collection fiber was positioned a distance of approximately 1.6 cm (11.6 mm is the focal length of this lens).

Once alignment was achieved, Raman signals of diamond and Asha simulant diamond were detectable. Before proper alignment, only Rayleigh signals were detectable. The figure below contains the spectra of diamond and Asha simulant diamond as measured by the setup on the optical breadboard.

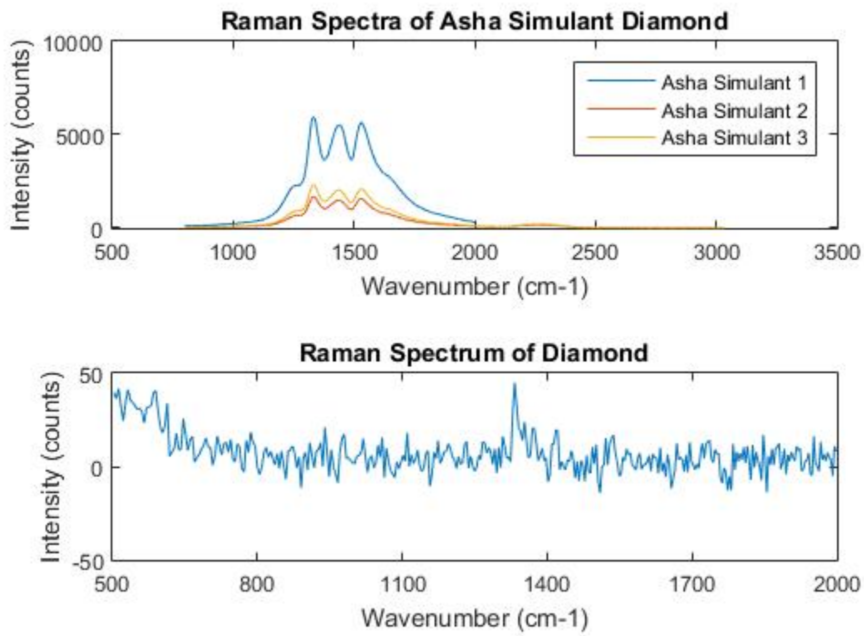


Figure 24. Test results from the probe setup on the optical breadboard. The top plot is the Raman spectra of three Asha simulant diamond samples. The bottom plot is the Raman spectrum of a diamond sample.

The following paragraphs are a discussion of the final optical elements chosen and used in the probe.

Fibers

The excitation fiber (Thorlabs M18L01) is used to deliver the laser light to the optics within the probe. The laser aperture is an FC/PC connection, so the chosen excitation fiber an FC/PC to SMA patch cable. This multimode fiber has a 105 μm core, matching that of the laser aperture. It is a low OH fiber optimized to operate from 400 to 2400 nm.

The collection fiber (Ocean Optics P200-1-VIS-NIR) is used to deliver the collected scattering signal to the spectrometer. It has a 200 μm core, almost double that of the excitation fiber to capture as much collected light as possible. Both ends of the fiber have SMA connectors. This fiber is optimized to operate from 400 to 2100 nm.

The one meter length of each fiber provides sufficient reach for the handheld probe. Both fibers have numerical apertures of 0.22. Threaded SMA fiber adaptors (Thorlabs SM05SMA) help to integrate the fibers into the probe.

Lenses

The probe consists of three N-BK7 plano-convex lenses. The first lens (Newport KPX019AR.16) has an anti-reflection coating for 650 to 1000 nm. It has a 6.35 mm diameter, back focal length of 23.16 mm, and numerical aperture of approximately 0.137. This first lens collimates the light diverging from the excitation fiber. The second and third lenses (Thorlabs LA1540-B) have an anti-reflection coating for 650 to 1050 nm. They have 12.7 mm diameters, back focal lengths of 11.6 mm, and numerical apertures of approximately 0.547.

Originally, all three lenses were KPX019AR.16. The second and third lenses were replaced with LA1540-B because of its larger diameter and larger NA. Both of these factors collect more of the light scattered from the sample.

Beamsplitter

The probe utilizes a beamsplitter (Thorlabs DMSP805T) to transmit the laser beam to the sample and reflect the Raman scatter for collection. It is a 12.7 mm diameter, shortpass dichroic mirror with an 805 nm cutoff wavelength. It is designed to transmit wavelengths from 400 to 788 nm and to reflect wavelengths from 823 to 1300 nm. It successfully transmits the laser light (785 nm) to the sample at full power and reflects the Stokes Raman scattering (above 785 nm) to the spectrometer. It sits at a 45 degree angle of incidence.

The original beamsplitter (Newport 10Q40BS.2) used was a broadband (700 to 950 nm) dielectric 50/50 beamsplitter. It had a 25.4 mm diameter. This beamsplitter was replaced with the DMSP805T because, although sufficient power was still delivered to the sample, the already weak Raman scattering was also being reduced by half upon reflection from the beamsplitter. No Raman signal was detected using this beamsplitter.

Mirror

The mirror (Newport 07SD520BD.2) is a broadband dielectric NIR (700 to 950 nm) square mirror with dimensions of 19.05 by 19.05 mm.

Filter

Raman spectroscopy requires the use of either a notch or a longpass filter to reduce the intensity of the Rayleigh scattering, which should allow for the Raman scattering to be detectable. A longpass filter (Newport 5CGA-800) was chosen instead of a notch filter because only the Stokes (longer wavelengths) Raman scattering is of interest. This is a 12.7 mm diameter longpass filter with a 800 nm cut-on wavelength. This means the intensity of the Rayleigh scattering at 785 nm will be reduced while transmitting the Raman signals above 800 nm (~239 cm^{-1}).

Depending on the type of light source used, a bandpass filter may be required for Raman spectroscopy. The Rayleigh scattering at the laser wavelength from the sample will be considerably more intense than the Raman scattering. Therefore, a narrow linewidth will allow for Raman shifts near the laser wavelength to be detectable and not overpowered by the Rayleigh scattering. The laser used in this project has a narrow linewidth and is spectrum stabilized to prevent mode hopping. The Lorentzian lineshape function was used to determine that, given our laser source, a bandpass filter was not necessary.

The Lorentzian function is a spectral lineshape function used to describe spectral broadening and for spectroscopic curve fitting. It takes the form of $L(x) = \frac{1}{\pi}\frac{\frac{1}{2}\Gamma}{(x-x_0)^2+(\frac{1}{2}\Gamma)^2}$. Here Γ represents the linewidth (FWHM) of the laser, given in the user manual as 0.12 nm typical. The x_0 represents the laser line of 785 nm. Then x represents the range of wavelengths of interest in relation to x_0.

As stated earlier in this report, the nearest Raman signal is 1000 cm^{-1} from the laser line, corresponding to a Raman wavelength of approximately 850 nm. Therefore, when plotting the Lorentzian function, the intensity relation between the laser line lineshape and this nearest Raman wavelength will mathematically determine if a bandpass filter to spectrally narrow the laser line is necessary.

Evaluating the Lorentzian function in Matlab results in the below graph. The peak at the left is due to the laser line at 785 nm. The selected data point at 850 nm shows a corresponding intensity of 4.52e-06 arbitrary units. Taking the log of this value, as 10 log(4.52Ee-06) yields a value of -53.4 dB. This means that the intensity (amplitude) of the laser wavelength drops by 53.4 dB, a considerable amount. It may then be concluded that a bandpass filter is unnecessary for this application.

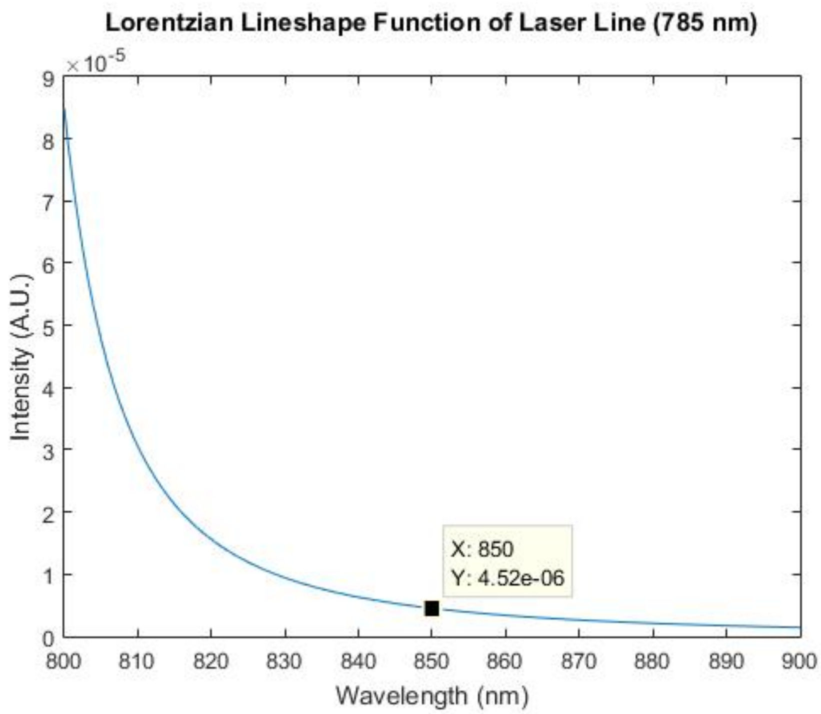


Figure 25. Plot illustrating that the nearest Raman shift of interest for a skin sample is far enough to not be overpowered by the laser line at 785 nm, concluding that a bandpass filter is not necessary.

5.5 Casing Design

The purpose of the plastic casing is to safely house the Raman spectroscopy system and all individual parts to protect the electronic parts. Another important purpose for the casing is that it will protect the user from the laser and make the device more comfortable to use. The cables to deliver power and provide data connectivity will also run through the casing. Thus, the design of the overall casing will be efficient from the perspective of the engineering team while ergonomic for the device user.

First, the parts made from filament for use in the 3D printer must be considered. These pieces include:

1. Housing apparatus for probe/CMOS sensor
2. Housing for PCBs, microcontrollers

The housing apparatus for the laser must be able to securely hold the laser in place, be easy to hold, be cool to the touch, and be protected with lead foil. It must also securely hold buttons to control power and PCB functionality. This piece will contain the probe apparatus and most of the optical pieces. Thus, the dimensions of the laser, heat sink, and optical pieces will determine the dimensions of this portion of the casing; the goal being to have as minimal a size as possible in order to be user friendly.

The casing of the main body of the device must house the majority of the device components. Thus, not only must the size be considered, but also the heat distribution and accessibility to data and power connectivity. It will contain PCB 1, power control, supply, and switch, as well the Raspberry Pi and a fan to moderate airflow. It is important that the casing has holes to allow for port access: a power supply cable must be able to reach PCB 1, data and power connectivity must be able to reach the probe. The casing should also be able to connect as two half pieces: on base that will securely hold the device components, and an easily removable top piece that will allow for device troubleshooting if needed.

Although the goal is to create a small, reduced box that would allow for proper air flow and space for the components to function at an optimal level, there still needs to be space left for the components in case the initial seating during the prototype phases does not meet the temperature and size constraints. It would also behoove the team to make a larger case design to allow for other components to be added at the request of Ocean Optics. Thus, while the casing will be streamlined and a portable size, it will also be flexible in use at the request of a sponsor if needed. The figure below shows a box with dimensions of 15” (L) x 11” (W) x 6.5” (H). The other important note is the face of the box has a 40 mm fan at the center to help with airflow within the encasing.

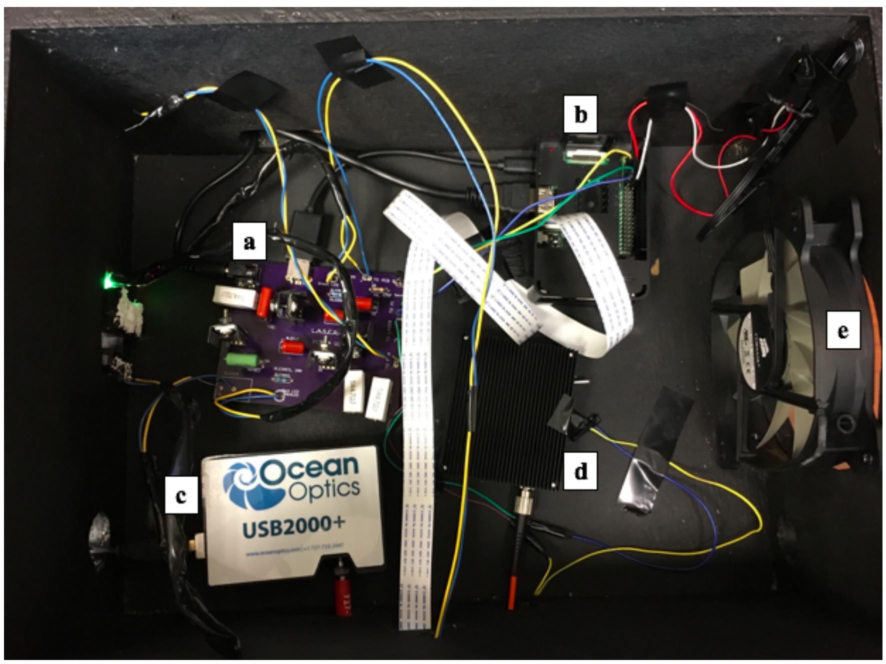


Figure 26. Final design of the Main Body box, containing a) the main power PCB, b) Raspberry Pi microcontroller, c) spectrometer, d) laser, and e) cooling fan.

Thermal Design

The overall design for the main casing includes a small form factor, and an insulated housing material. This a recipe for components that will be outside of their standard operating temperature window in a very short period of time.

To counteract this, we will be utilizing a temperature sensor, and a 40 mm fan placed on the top center of the design casing. The temperature sensor will engage the fan as necessary. The fan, due to its central location on the top of the case, will help remove the excess heat buildup inside the system. As the warm air rises, the fan will push the air outside of the case, allowing for cooler air to be substituted in its place.

The housing apparatus for the electronic elements must be able to fasten all elements as well as maintain an acceptable temperature range. A main focus will be placed on securing and protecting the CMOS sensor to avoid damage to the glass. This major component will be on a PCB connected to the two microcontrollers. Thus, the dimensions of the microcontrollers will determine the dimensions of this portion of the casing. To allow for simple troubleshooting, the CMOS array holding place should be secure and protected, while the microcontrollers will be secure but able to be removed easily. Another element placed within this main house is the power control PCB with various electronic elements connected to a cable to plug into a wall outlet as a power source.

5.6 Light Emitting Diodes (LEDs)

In order to simplify the device for the user, the team decided that adding LEDs would greatly improve the system. LEDs are relatively simple to add to existing device plans. The circuits for all LEDs consist of a power source, connected to a pre-existing voltage input, a resistor, and the LED. The LEDs also work as a quick glance method of checking if the device is ready to be used. Unless all LEDs are illuminated, the user will know that the system is not yet ready to be used and they must check that all steps were followed to operate the device.

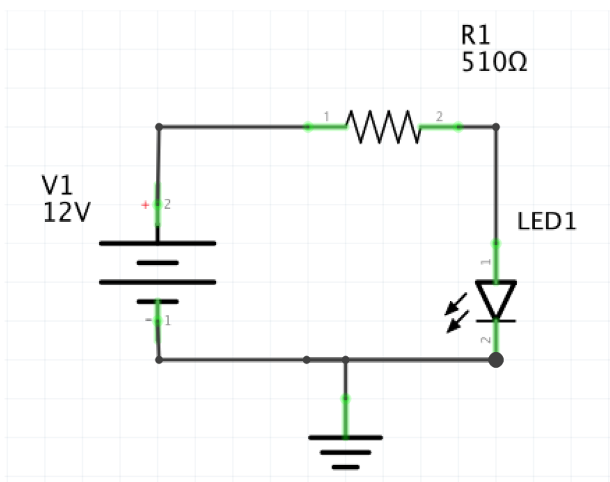


Figure 27. Basic Circuit for LED Implementation

LEDs 1 and 2: Power Indicator

The first LED in the system will be a red LED that indicates if the laser is fully powered. It will only be on or off in order to simplify the usability and reduce any confusion. This LED will be on the PCB alongside the power control module and the microcontroller connectivity on the large PCB. If power can flow to the laser, then power will flow to this LED and allow it to illuminate. If power is flowing to the laser, the red LED indicates that it can be used. If power does not flow to the laser, the red LED will not light up, indicating to the user that the laser is not ready for use. This same logic applies to the green LED that will be used as a power indicator for the microcontroller.

LED 2: Beam
The beam LED is a white LED that will guide the user when placing the probe on the skin/skin sample as well as illuminate the sample area for the camera to detect. It will be on a PCB next to the camera module. Between the two parts there will be a space for lens access. If power can flow to the main PCB, even with the switch for the microcontroller and laser power off, then the LED beam will illuminate.

5.7 Power Flow Design

Power distribution is essential to not only operate the spectroscopy system, but also to ensure that the laser operates at a most safe state possible, one with the proper amount of power. The main source of power will be a typical US wall outlet that delivers 120 V AC. Power must run to the following major components: the PCB that will manage the power distribution for the microcontroller system, laser, and LED beam PCB. As per the respective sections for each element, these components require the following power supplies:

Part Name	Minimum Power Required (W)
Laser (785-IP-02-1208)	3.5
Spectrometer	1.25
Raspberry Pi	2.5
Fan	1.8
Camera Module	1.75
LEDs	0.036

Table 13. Power supply required of the components.

Laser
The laser is to be provided by Ocean Optics. The laser, under the 785 nm spectrum, will be used to scan the skin to read the Raman signal of the sample. The laser will obtain the power from the main power PCB, with an output at that port being around 5 V, 0.8 A. The power to the laser is arguably the most essential, considering that it is the foundation to providing an accurate reading; the power must be kept within the range specified in the table above. The connection inside the laser will allow for powering as well as potentially transmitting data.

Spectrometer
Once the laser has scanned the sample completely, the spectrometer will begin to vet through the light sensitive elements by digitally mapping the photo responses into data.

This is done using the filament connected to the probe from the spectrometer which is located on the board. The Spectrometer is powered by the USB that connects to the monitor to transmit data.

Raspberry Pi

The Raspberry Pi will run and display the view from the camera, control the fan via a temperature sensor, and potentially run the spectral analysis, displaying all data for the user to handheld computer or other HDMI compatible monitor. To ensure the full functionality of the device, the Raspberry Pi needs at least 2.5 W to turn on and about 7.25 W to power everything connected to it.

In order to distribute this amount from a typical wall socket, the first element must be the power control PCB. This circuit will take in the electricity from the wall source and into a circuit that regulates the amount of voltage allowed into the spectroscopy system. The power control circuit will regulate current and voltage within the system. It will safely distribute power to all major elements of the spectroscopy system that require the power source. The power will flow following the pattern seen in the Power Flow diagram figure below.

The Raspberry Pi requires 5 V and anywhere from 0.7 to 2.5 A, depending on peripherals, to operate its GPIO pins. The Raspberry Pi's power requirements are considered to be of utmost importance in the design of the power control system due to the importance of the functionality of the Raspberry Pi in the device. The Raspberry Pi acts as the main orchestrator of the entire project. Failure of this component results in the failure of the other components.

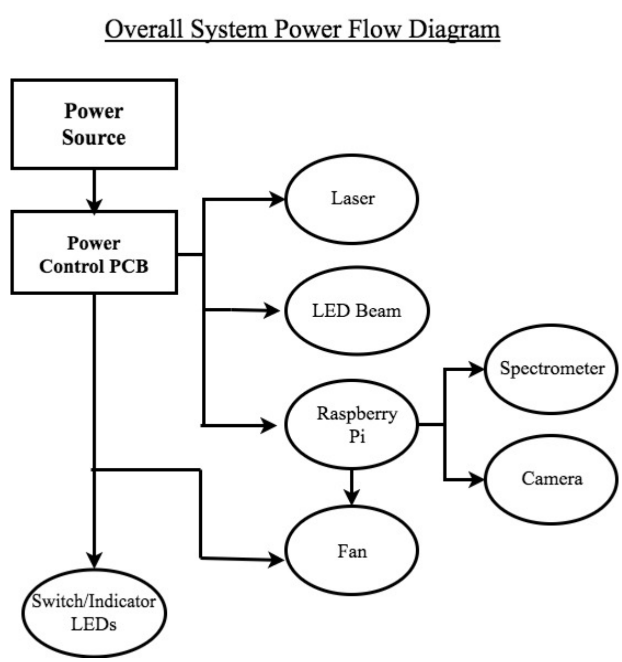


Figure 28. Power Flow Diagram

5.8 Data Flow Design

The data flow of the system is complex, and there are several pieces that must work simultaneously for the team to ensure that reliability, safety, and accurate results are able to be processed. Each data stream below will be outlined in terms of what data we expect to be transmitted, how it will be transmitted, and what we intend to use that data for. The team will work its way from the highest level to the lowest level hardware dependencies.

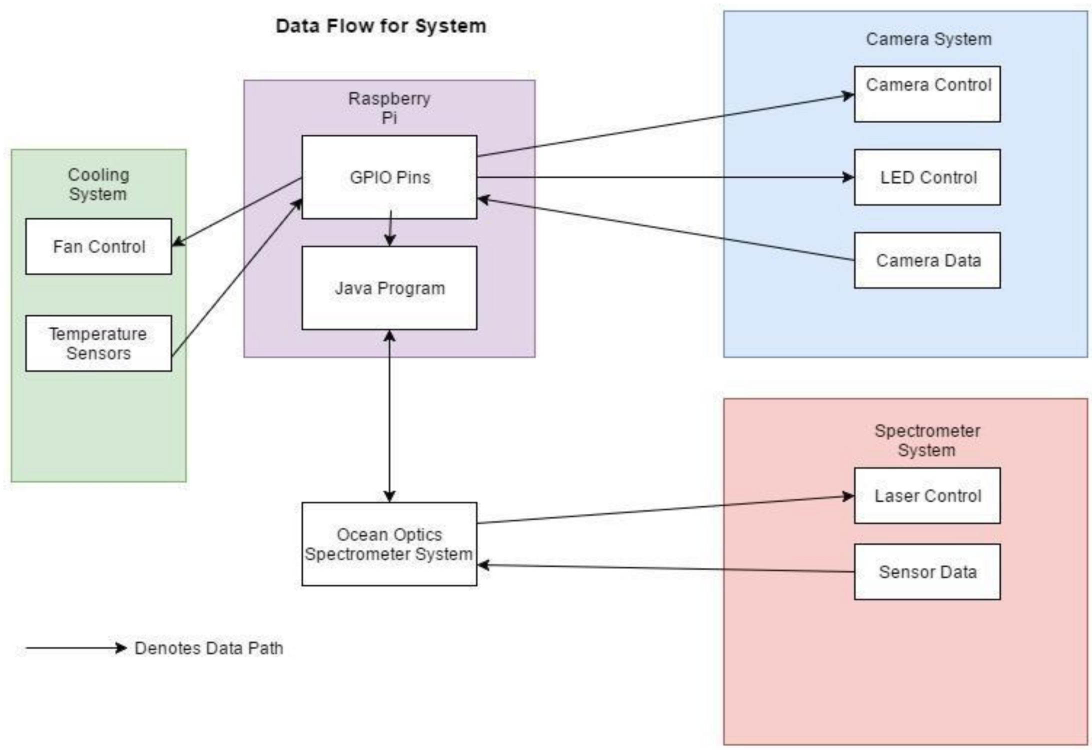


Figure 29. Data Flow Diagram

5.8.1 Raspberry Pi Data Flow

Java Program to GPIO Pins

The Raspberry Pi has 40 GPIO pins built into the system that allows anyone using the system to be able to control hardware natively. The program itself will have direct access to these pins, to allow the running program the ability to control these devices as it needs, to allow for full functionality of the system. Both the fan and camera system will be controlled off these pins, as well as the Raspberry Pi will be able to read data flow in as needed.

5.8.2 Cooling System Data Flow

Temperature Sensors to GPIO Pins

As referenced in Section X.X-Active Cooling, the system will use temperature sensors designed to monitor the temperature of vital components that need monitoring. This sensor will then report these temperatures to the GPIO pins, to be processed by the active cooling program, to activate the fan as needed. This should be able to allow the fans to react quickly to temperature changes, allowing for system stability.

GPIO Pins to Fan Control

As the temperature sensors detect the system is above the optimal temperature, the Raspberry Pi will be able to activate the fans by modulating the voltage on several pins which will be powering the fans. As the Pi modulates the voltage, the fans will increase and decrease in speed, allowing for an active cooling approach.

5.8.3 Camera System Data Flow

GPIO Pins to Camera Control

The system control program will be activating the camera when the input is needed. When the test is begun, we are encouraging the users to be able to use the live feed to aim the laser system. To reduce the overall powered on time of the system, the camera will only be active during this phase. The GPIO pins will send the necessary signal to the camera module when it needs to be powered on. When the system detects the laser is being activated, the camera will shut-off to protect the camera system.

GPIO Pins to LED Control

Due to the current design of the system, the camera will not have enough light for the user to be able to see what they are currently targeting as the probe gets closer to the skin of the subject. Therefore, as the light of the image drops, we will have to activate the LED's to illuminate the target. This will be achieved by using one of the voltage pins from the Raspberry Pi to send the power to the LED's. When the system detects the laser is being activated, the LED's will shut-off to reduce the amount of noise from the visible light of the LED's.

Camera Data to GPIO Pins

In order to be able to give the user a real-time view from the tip of the probe, the camera data will be directly transmitted to the Raspberry Pi. Minimum resolution to be expected for this application will be 480x360x16 bit video. This resolution should allow for the user to be able to see enough detail of their skin, while also allowing us to process the video in real-time. At the current time, this will be a non-customizable option that we intend to allow users to define at a later date.

Before the camera does close, we would also like to have the system take one full resolution image so when the information is processed, it can also be passed with a full image for consideration.

5.8.4 Spectrometer System Data Flow

The spectrometer system is the only fully external system, and will not be controlled via the GPIO pins. Using a provided software set, OmniDriver, we will be able to creatCCD sense a fully custom program to handle the spectrometer analysis. OmniDriver operates through the onboard USB port of the external computer system. Using this combination, we should have full independent control over the Ocean Optics Spectrometer System.

Ocean Optics Spectrometer to Laser Control

Controlling the firing of the laser is a vital step in ensuring the viability of the system. Anytime the laser is fired without the necessary precautions are taken, we run the risk of extraneous results, as well as the safety of the users. The laser will be operated in short bursts, over several passes. However, when we are using the system, we want to ensure the laser is only firing when the proper safety steps are taken, including:

1. The user has indicated via software the test should begin
2. The dead-man switch is depressed, ensuring minimal unintended exposure.
3. The pre-defined system configuration calls for the laser to be fired.

Sensor Data to Ocean Optics Spectrometer

The sensor data is the information that propagates about the Raman Spectra from the CCD sensor, which then needs to be passed back to the spectrometer. This data will consist of data points (wavenumber, intensity) which then will be processed and handed back to the Raspberry Pi system.

5.8.5 Hardware Control Flow

While the PCB system will be controlling the voltage via an analog channel to each of the devices, having software defined hardware control will allow us to have a greater degree of control over the system at all times. Using the GPIO pins on the Raspberry Pi, we will be able to control each device in the cooling/camera system powering on and off, which should help increase product life, as well as overall customer safety.

Cooling Hardware System Control

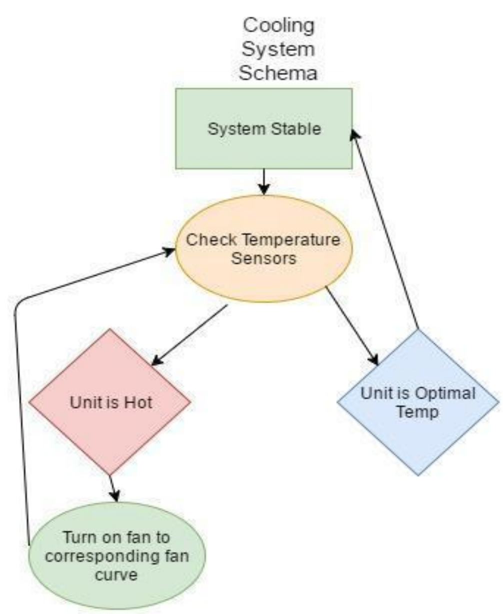


Figure 30. Cooling System Control Schema

The cooling system will be a separate program running simultaneously, when the overall system is powered on. It is independent to the overall system, as to ensure that no errors received in this system affect the overall test results, but the overall system will receive a notification that the cooling system has errored out.

The Raspberry Pi will use the temperature sensors implemented to monitor the temperature of each individual component. The Pi will then have two options on how to proceed, either to declare the system stable, and check again, or to decide the component is above the allowed temperature. When this happens, the Pi will activate the fan until such a time that its temperature falls to its usual threshold. When this happens, the system will revert to its stable status.

Spectroscopy Hardware System Control

The diagram below outlines the Spectroscopy hardware control system we intend to implement using Ocean Optics OmniDriver API and an external computer system. The triggers, highlighted in green, will cause the change in hardware state as shown. We intend to control these triggers using the same java program that will be running the tests, as these systems will need to be activated in sequence with the tests.

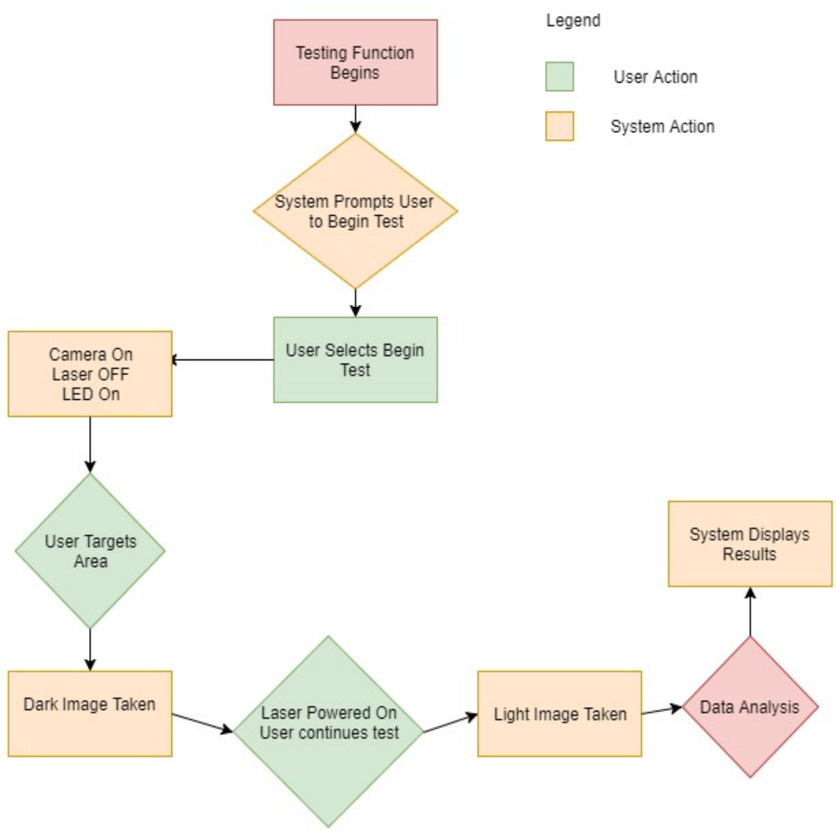


Figure 31. Spectroscopy hardware control schema

5.9 Spectroscopy Software

The spectrometer we are using was built by Ocean Optics, and has several ways of being operated. Ocean Optics has provided us with access to their spectroscopy software, Ocean View, which is a much more open system allowing the user to analyze the spectra and other tests using their spectrometers. However, the Ocean View software is for a much more advanced user, and gives the user much more data than they will need for the team's purposes. Therefore, the design will implement the team's own spectroscopy software, using the native controls given to us by OmniDriver, Ocean Optics native control API.

5.9.1 Graphical User Interface Flow

The design philosophy behind the GUI for this project will be ease of use for the customers. This project sets out to be an at-home solution to a very complex problem, at home skin-cancer testing. With the complexity and emotion that may arise in the customer during use, the team wants the app to be intuitive with very few possibilities for the user to get lost during use.

There is very little need for an extra functionality, as the device should be fully contained, the software is more of a stand-alone piece so that the user can view the results easily. There are plans to add extra functionality, such as the ability to directly upload results to doctors, as well any functionality we decide may further help the customer have a pleasant experience using the product.

GUI Flow:

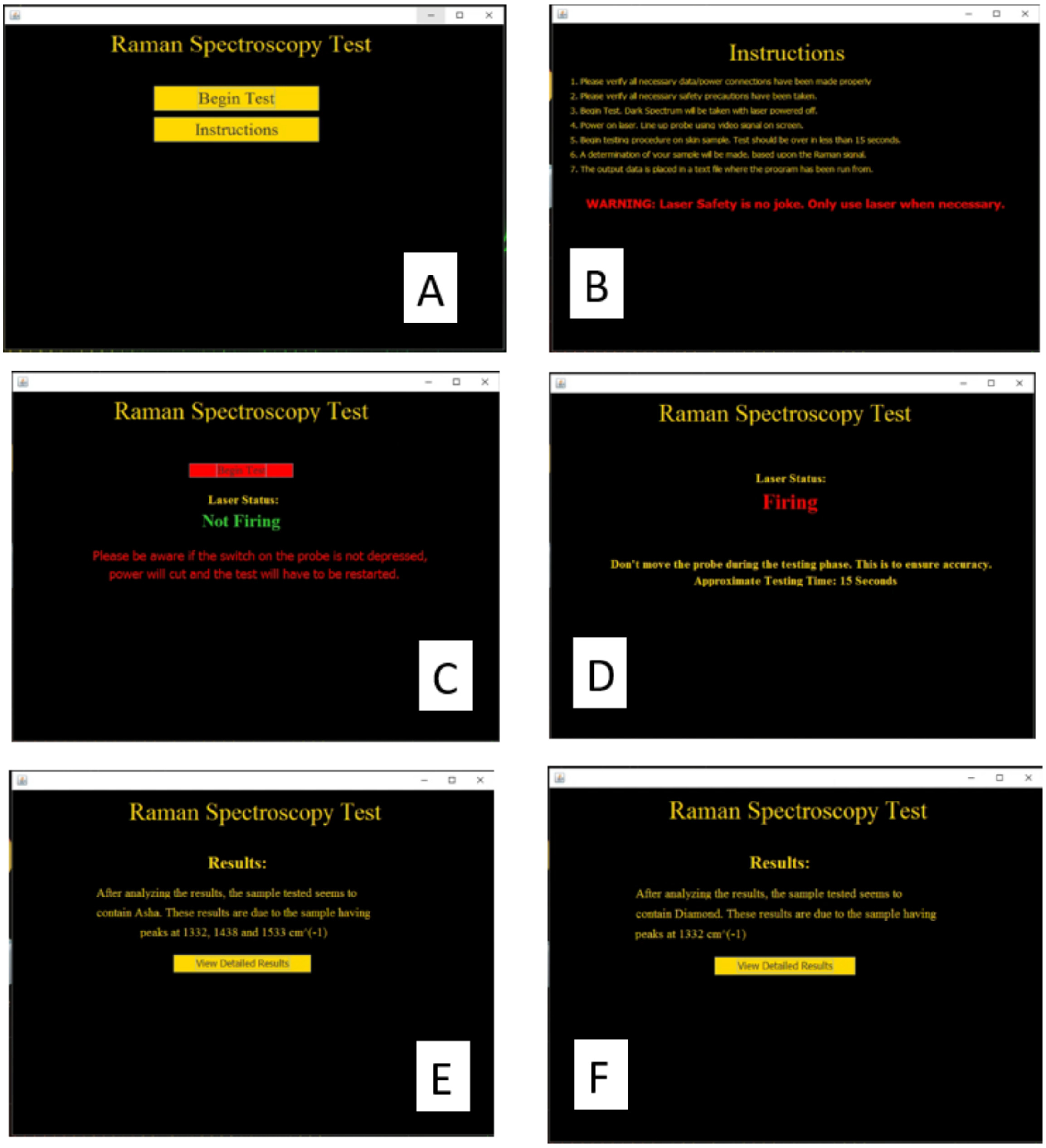


Figure 32. GUI Screens

Screen A:
This page is designed to allow the user to have several options on how to begin the test. Currently, the two buttons only either begin the test for those users who are familiar with the procedure, or to view the instructions. Note: In the future, we would like to add a settings function from this page, to allow the user the make any configuration changes.

Screen B:
The instruction page will present a user who is unfamiliar with the system an easy way to understand how to manipulate the device. Though the goal of the design of the overall

project was for ease of use, some customers will surely review instructions before use. Note: In the future, we would like to add a series of quick video instructions for each step, allowing the user to see how the developers perform each step.

Screen C:
The testing page should provide the user with a notification that the dark spectrum has begun, and to not have the laser firing at this point. It provides the user with instructions on what to do if the test fails, and prompts the user to power on the laser, and begin the test..

Screen D:
The second testing page will provide the user with the laser firing status, and notify the user of the approximate time table to complete the results.

Screen E-H:
The Quick results pages is the page in which the program will deliver the basic "Yes" or "No" message, further providing the user with the possibility of viewing the results in a more detailed manner. It provides the user with the basic facts of the diagnosis, and allows them a decisive decision on the presence of the gemstones. Each gemstone has its own page, outlining these facts for the user.

Screen I and J:
The detailed results page will provide the user with a line graph representation of the data the test has collected. This information will be exportable to several formats, so that the user can use the data in a variety of third-party programs.

Having this format for the GUI's will not only make the device easier to use, but it will also eliminate technological red tape that could deter the user from utilizing the device. This GUI is curtailed to the user that does not have much experience with computers or touch screen displays.

These graphs are updated based upon the data taken in from the system, and are only as good as the test being run. This provides the user with intensity data among other key points, that will allow a more informed user of the status of the test.

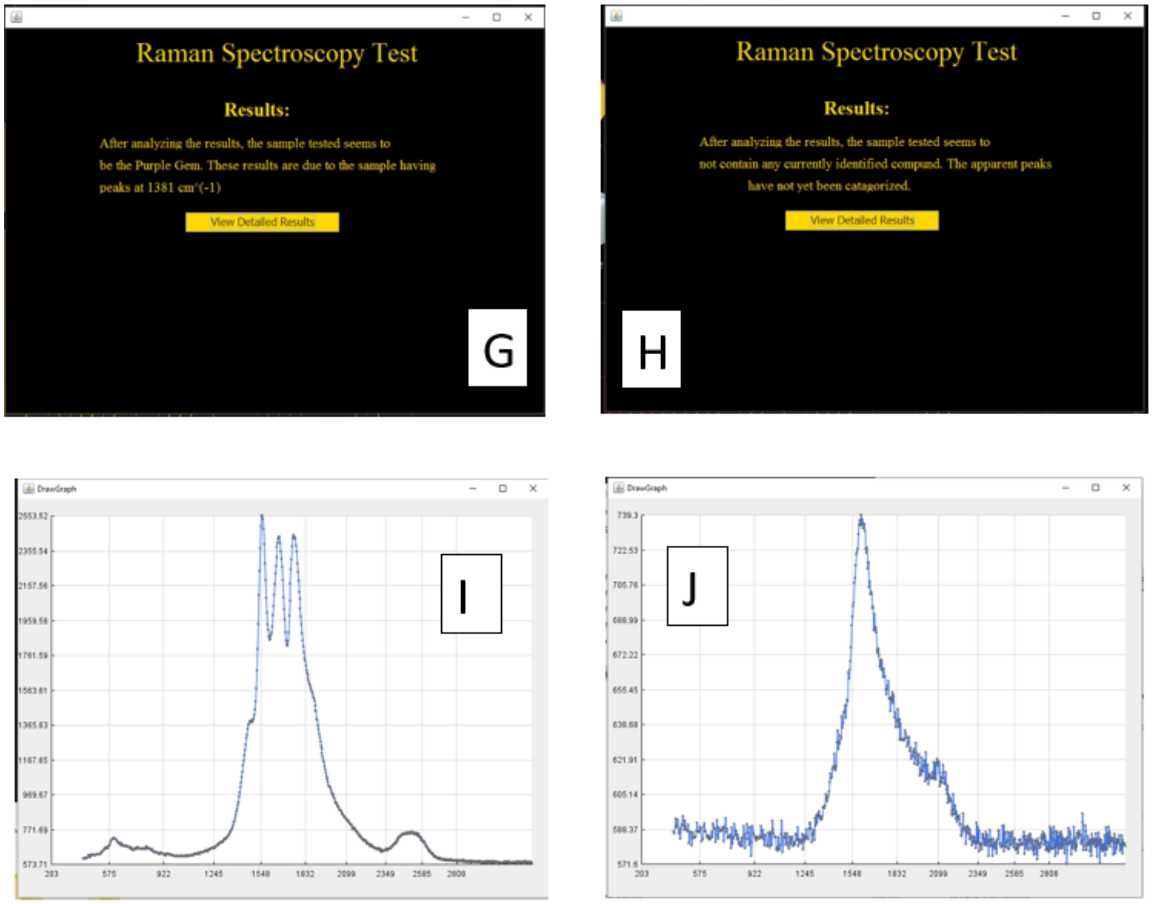


Figure 33. GUI Screens Continued

5.9.2 Spectral Testing Algorithm

In order to maximize accuracy of results, the spectrometer data will be collected several times, as well as processed using noise reduction techniques. The steps taken will ensure that the user has maximum confidence in the product's result.

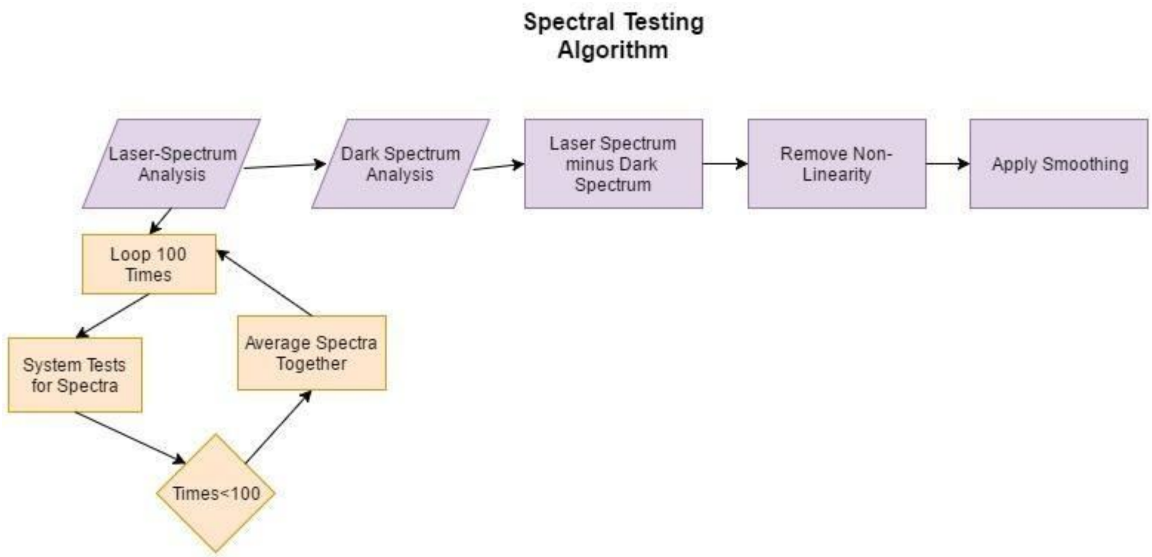


Figure 34. Laser Spectrum Algorithm

Laser Spectrum Analysis
The Laser Spectrum analysis loop shown in the algorithm example above will allow us to remove the data outliers received in the system. Currently, the team expects to be taking around one hundred analysis runs per test, to reduce the Signal to Noise ratio from 1:1 to 1:10. One hundred analysis runs is a realistic value taking into consideration the health and safety of the user, the system run time, and the reduction of SNR.

This value will be tested more extensively once the system is built, to increase the possibility of further SNR reduction. This will take into consideration total exposure time, testing area temperature, and system reliability during prolonged run-time.

Dark Spectrum Analysis
The Dark Spectrum analysis loop will help the team by taking the average of the noise received on the CCD with no laser stimulus. The team will be conducting these tests staggered around the laser spectrum analysis loop. This will provide the team with a consistent average amount of the radiation received by the CCD array with no laser stimulus.

Through research the team expects to conduct one dark spectrum analysis per ten laser spectrum analysis, so the team will plan on taking five dark spectrum analysis samples before, and another five after the laser analysis.

Dark Bias Subtraction
Dark bias subtraction is the process by which one will remove the dark spectrum from the laser spectrum. Using basic subtraction of these two data sets, one can remove the noise that is coming in from external to the overall system. This will help remove false positives from the team’s overall dataset, allowing only the stimuli of the laser to be present, which should greatly improve accuracy.

Non-Linearity Removal
Non-Linear graphs will be impossible for the algorithm to interpret. Large spikes caused by the noise not removed by the previous two steps will cause the intensities to vary greatly, which can lead to inaccurate results for the users. Removing non-linear sections, where the change in intensity changes to exponential growth, will allow for smoother transitions and a much more interpretable dataset.

Using known data points from previous increases, the team will be able to interpret large jumps in intensity, and be able to remove these outliers from the dataset in a sliding fashion. Taking into account the change on either side of the data that will be currently interpreted.

Smoothing Principals
The last step before being able to process the data with the algorithm will be to smooth the dataset to make it more interpretable. The team will initially plan on using a Savitzky-Golay algorithm with a convolution depth of 2 on either side of the dataset. This will allow for the data to be smoothed, while not removing the important data points.

5.9.3 Data Interpretation

The external computer system will be receiving a data stream from the Ocean Optics Spectrometer with information that it will be populating to a two-dimensional array list with corresponding values of wavenumber and intensity respectively. The array list will store the data for both the operation and documentation feature. To ensure the data set is not corrupted during these processes, independent copies will be made for each function, so that neither can affect the original dataset directly. Providing read-only access to these datasets will be important so that we can ensure the data is not lost, so the test does not have to be repeated by the user. Therefore, when the program is completed, the data can be written to a file so the user can take the data as they please, whether to send to a medical professional, or for repository in another location.

Current Plans: Gemstone Testing
The current algorithm uses a different subset of principles in order to determine which gemstone is present during the testing procedure. It locates key data points, primarily local maxima, and logs these key data points. Comparing these data points to known online databases of Raman emission spectra, our algorithm then uses basic comparison logic to determine if the subset of maxima are representative of a known compound. If any of them are present, the test will return that it is present. If not, it prompts the user that the emission spectra is not known, but provides the data plot anyways.

Future Plans: Cancer Sensing Algorithm
The algorithm is one of the most vital pieces of project, which should look at all results from data collection, and then should provide the user with a binary result of their likelihood of having possible cancerous cells present in the test area.

Composition/Shifting Test
The test we will use is the composition/shifting test. Cancerous cells are known to contain chemicals in differing concentrations from a normal cell. While analyzing the composition of the test subject's cells, the algorithm will be able to perform an analysis of the composition of the cells in question, which will then be able to compare directly to the peaks of these cancer-indicating chemicals. From this composition, it will give the algorithm an indication of whether or not the cells contain these chemicals in differing concentrations than expected.

To test these differences, the team will be using a combination of Intensity Shift, Phase shift, and distinct important data point analysis. The team intends on finalizing an equation that will have the necessary considerations to accurately predict cancerous cells in the test site. When the results are above a certain threshold of probability, the device will indicate to the user that they should get themselves checked for skin cancer through a binary test by a medical professional.

```
PsuedoCode:
Arraylist Operational=Arraylist Master
Operational.IntensityChange(arraylist intensityvalues)
Operational.ShiftChange(arraylist shiftvalues)
for(all intensity values)
        Check for large peak changes

for (all shiftvalues)
        Check for large shifts
if(intensity || shift ==true)
        Return true
Else
        Return false
```

5.9.4 Data Exporting/Storage Design

Due to the nature of the device testing, sharing old and current data, as well as viewing old data is vital for a user to be able to track their progress. The team's program will allow users to not only save the past results to a file for viewing later, but we also intend for them to be able to export the data into another program altogether. Since the data will be in plain double values, the data will be stored as plain text. This data type can be transferred to a number of commonly used data-types, or imported to programs for other types of graphing and manipulation. These file types will allow the user to share their results with others without the need for specialized software, which will allow the user to share the results with medical professionals as necessary.

All data will be stored in a database on the storage of the computer system, and will be easily accessible from outside the program. The file is stored using the current Time/Date and this practice will allow the user to pull up old data results, if they would like to be able to compare old results to new results.

5.10 Thermal Flow Design

Since maintaining standard operating temperatures is vital for the reliability in both the short and long term of the project, the thermal flow is a design that is vital to the device's success. The team intends to keep all devices in the system well within their standard operating temperature, at the possibly cost of increased weight, size, and noise. The team intends to achieve this using both passive and active cooling solutions, located in strategic positions as to most efficiently cool all components of the spectroscopy system.

Device Name	Standard Operating Temperature Range
Laser	-10 to 55 □
Raspberry Pi	0 to 70 □
CCD	-25 to 60 □
PCB	Varies with components
CMOS Camera Module	-20 to 60 □
Spectrometer	-10 to 50 □

Table 14. Operating temperatures of the devices

5.10.1 Device Main Body Thermal Design

The main body is the computational center of the project, and will also be the piece requiring the most cooling. In this situation, the team will be implementing both active and passive cooling solutions, which are respectively, a temperature sensor controlled fan, and heat sinks attached to all devices.

Heat Sinks

The heat sinks will be attached to the laser, all TO-220 modules (voltage regulators, transistors), and the Raspberry Pi as shown below in Figure . These heat sinks will pull the heat off of these temperature sensitive devices, and allow the fan to cool them more efficiently. We have selected a typical staggered fin design with two axes of ridges, which should be easily cut from a solid piece of Aluminum, which will optimize weight, thermal conductivity, and cost in a very effective package.

Figure 35. Heatsink on a TO-220 module

The heat sinks will be attached to the devices with thermal compound as necessary, which will allow for increased thermal conduction between the devices and the heat sink. From there, the heat sink will have to be held down with a retaining clip, which will be attached to mounting spaces in the device main body.

Fan

The size of the fan will be determined by the final size of the laser and microcontroller systems, but the team currently plans on running one fan with a size of between 80-120mm. The fan we select will be optimized for static pressure which will allow for the fan to be able to move the air through the heatsink as desired. Current plans will be around a Corsair SP120 which will be controlled through voltage modulation based on temperature readings.

The temperature readings will be taken from a temperature probe attached to both the laser housing, and the Raspberry Pi. These readings will be interpreted in a stand-alone program that will monitor the temperatures of these components several times a second, and then use voltage modulation to increase and decrease the fan speed as necessary.

5.11 Handheld Design

The first component that needs to be created has to be the handheld laser probe module that gathers the initial data from the skin samples. According to the design constraints, the handheld has to be designed in a way that allows for the probe lenses and filters to fit in comfortably and provide space for the camera module that is to record the procedure, all while being a handheld device that is ergonomically functional. In terms of the ergonomics, this handheld has to be able to provide accuracy through its physical characteristics, as well as feel comfortable to the touch. Several factors to consider are the design's weight, physical size, as well as its ease of use. Any handheld device, especially related to medical devices, has to be created to ensure stability. From an ergonomic standpoint, that would entail measures for front-end weight distribution, as well as a design shape that can be easy to grab at various angles. This can prove challenging since the probe has to be perpendicular to the test sample for an accurate reading.To design the probe laser module, AutoCAD was used to further illustrate just how the device's form factor was designed the way that it is presented.

Originally, the device's side view was supposed to have dimensions of 152 mm (L) x 50.8 mm (W), or 6 in x 2 in. This did not account for the probe section that fits into the center of the handheld encasing itself. However, once the specifications of the probes and the optical components were issued to the team, the next step was to edit the original layout to ensure that all the components fit comfortably in the handheld encasing. Since the original length and width were not enough to meet the size requirements, the second revision for the conceptual handheld lengths became 203.54 mm (L) x 67.847 mm (W), or roughly 8 in x 2.67 in.

After the second revision and the completion of the optical mount structure inside the probe, the team discovered that the dimensions still needed to be edited. The optical mounts were created to ensure compactness as well as leniency when mounting the optical components. This led to another overhaul of the probe dimensions; the compact nature of the mounts resulted in a shorter length, but the probe mounts needed enough clearance laterally, so the probe width was made larger. Figure 36 is the CAD for the optical mount within the handheld probe. Figure 37 is the CAD for the encasing around the optical mount. This encasing is the portion that is held by the user.

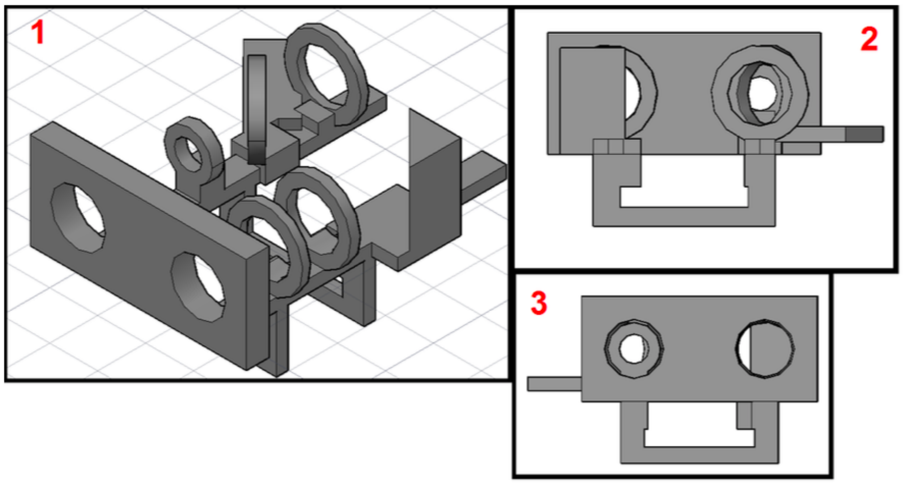


Figure 36. Isometric, Front, and Back views of the optical mount within the probe.

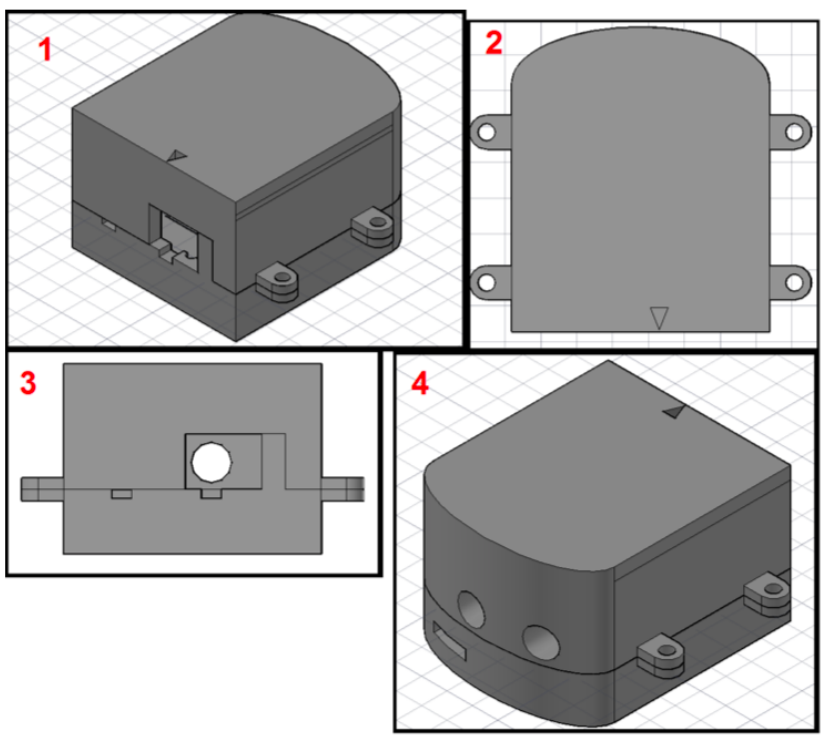


Figure 37. Isometric Front, Top-Down, Front, and Isometric Back views of the probe casing.

Below is a listed table of all essential measurements and dimensions of the final device:

Measured Distance	Dimensions (mm)	Dimensions (in)
Encasing Length (L)	10.8	4.25
Encasing Width (W)	12.1	4.75
Encasing Depth (D)	6.7	2.65
Optical Mount Length (L)	7.9	3.1
Optical Mount Width (W)	7.1	2.8
Optical Mount Depth (D)	4.0	1.59

Table 15. Probe and Optical Mount Dimensions

5.12 Analog Replacements for Skin

Due to the inherent difficulties of performing human dermal testing, finding an analog that is the most equivalent to human skin is vital. To try and find an analog that would give us results similar to what the team may experience while actually using the spectrometer on human skin. To determine which analog may be the best, the team has looked at the analogs used in other dermatological experiments.

Human Skin Equivalent (Graftskin LSE)
Graftskin LSE is a product produced by Organogenisis and is generally used for skin graft procedures in high risk patients. In these patients, removing the amount of skin necessary to cover the wound may do more harm to the patient, and instead Graftskin is used.

Graftskin is a living-cell based product and would allow us to directly test the results of the laser system on fake skin that uses living cells. However, at this time the team cannot source a cost for these samples, and since the cost would not be the insurance reduced price patients receive it at, the team is confident that this solution would be unnecessary for the project at this time.

Human Reconstructed Epidermis: (Skinethic HRE)
Skinethic RHE is an in vitro reconstructed epidermis created by culturing human keratinocytes. This is biologically very similar to actual human skin, and this is a product used to test:

1. Skin irritation/corrosion
2. UV exposure
3. DNA damage
4. Bacterial Adhesion

5. Omics
6. Permeability

This would be an ideal solution for the team's needs, however this product is a medical research grade material, and to this time the team has not found a way to attain a sample of this product for the tests. Skinethic HRE would allow us to test almost all facets of the safety of this product.

Pig Skin

Pig skin is a commonly used substitute for products intended for future human use. It is commonly used in a variety of situations, from ballistic tests on popular television shows, to use for practice for aspiring tattoo artists. The reason it is a commonly used substitute is due to the ease of procurement, the relative low cost, and the similarity to human skin.

This will allow the team to perform basic safety tests of the spectroscopy system, including skin irritation, UV exposure, heat transfer, and any other unforeseen results of this system. This is a low cost option that does not require the approval of a review board at the current time.

As mentioned in the research section of this paper, major spectral features of human skin (normal and cancerous) were due to the proteins and lipids collagen, actin, elastin, and triolein. According to a study done by Vardaxis et al., pig skin is the closest animal analog to human skin "for all types of dermatological and surgical wound investigation." [31] For example, some similarities between human and pig skin are the thicknesses (30-140 $\mu m$for pig and 50-120 $\mu m$for human) and regeneration rate (approximately 30 days for pig and 27 days for human). Interestingly, pig skin also contains collagen and elastin.

5.13 Optical Properties of Skin

Optical properties of tissue, such as the absorption and scattering coefficients, are of interest when designing the Raman system. It is important to understand how the laser light will be absorbed. The laser light will penetrate a short distance into the tissue and increase in temperature. The scattering of this light is important to consider when designing the collection optics, such as the numerical aperture of the collection fibers.

Optical wavelengths used for biophotonic applications range from UV to IR. In considering the light interaction with tissues such as skin, scattering and absorption are important. Light penetrates tissue at depths depending on the wavelength of the light. Light travels in vacuum at the speed c of 3 x 10^8 m/s, or 30 cm/ns. Through a material, however, light becomes slowed by a factor known as the index of refraction. For example, in air with a refractive index of 1.00, light travels at approximately 30 cm/ns as it does in vacuum. In a material, the speed s of the light slows according to the equation s=c/n where n is the refractive index of the material.

In tissues such as epidermis with a refractive index between 1.34 and 1.43, light slows to speeds ranging from 22.4 cm/ns to 21.0 cm/ns. For melanin with a refractive index of

1.60 to 1.70, light slows to speeds of 18.8 cm/ns to 17.6 cm/ns. [32] A material with a higher refractive index slows light more. This is because the material may absorb, scatter, and reflect the light. Consequently, the light absorption, scattering, and reflecting properties of tissues must be considered when choosing the wavelength of the laser source.

The wavelength of the light is related to its photon energy through the equation $E = h\nu = \frac{hc}{\lambda}$ where Planck's constant $h = 6.626\times10^{-34}\ J \cdot s$, ν is the frequency of the light, and λ is the wavelength. It is apparent from this equation that smaller wavelengths have larger photon energies. The NIR wavelengths range from 700 nm to 1400 nm, so the associated photon energies range from 1.63 eV to 0.89 eV. [32]

Biological tissues such as skin are inhomogeneous with varying refractive indices. These changes in index are the cause of light scattering. "Thus, although light can penetrate several centimeters into a tissue, strong scattering of light can prevent observers from getting a clear image of tissue characteristics beyond a few millimeters in depth." [32]

Tissues absorb incident light, thus also determining the penetration depth of the light. The penetration depth of light into a material can generally be described as the inverse of the absorption coefficient. As shown in Figure 38, there is a "diagnostic window" or range of wavelengths with the least amount of absorption by biological tissues. This window encompasses wavelengths from about 500 nm to 1500 nm, or from the visible to the NIR. [32] Thus these wavelengths are suitable for diagnostic applications, such as analyzing skin, which is why a NIR laser excitation source will be utilized.

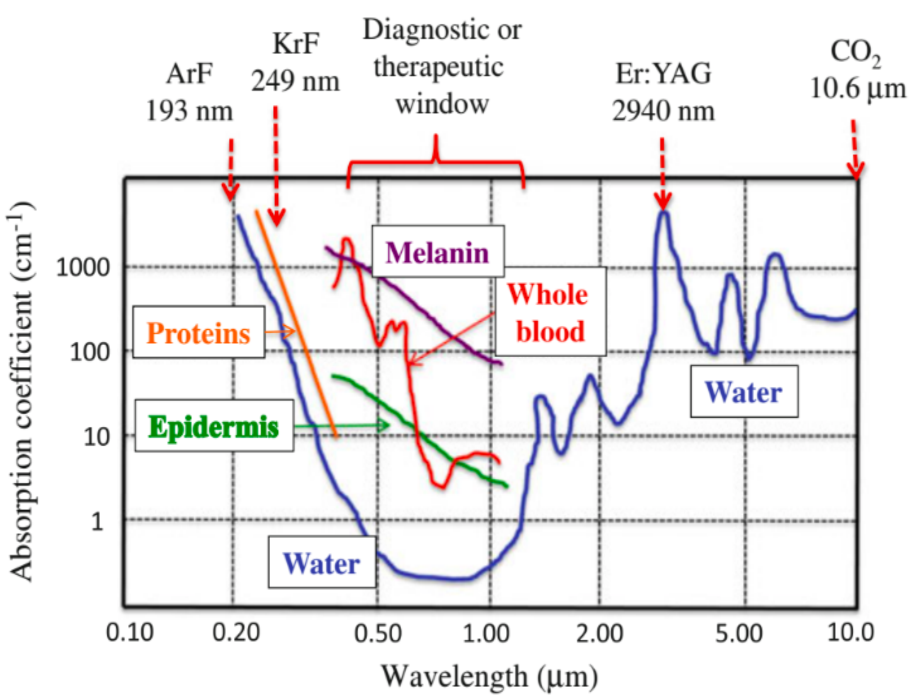


Figure 38. Absorption coefficients of selected tissues as a function of wavelength. [32]

The absorption coefficients of the components of the tissues, such as water, epidermis, and melanin in skin, factor into the damage threshold of the tissue. This tissue damage threshold is the intensity (power per unit area) of the laser beam at which the eyes or skin can become damaged. In the NIR wavelengths this would mean thermal heating and burning of the tissue.

The minimum irradiance on the eye or skin without causing damage is known as the maximum permissible exposure (MPE). The MPE depends on the wavelength, power or intensity, and time of exposure to the beam. Using an MPE calculator, the MPE for skin at a wavelength of 785 nm is approximately 0.3 W/cm^2 for a CW laser, and the MPE ranges from 0.03 J/cm^2 to 1.6 J/cm^2 for a pulsed laser with pulse times ranging from 1 ns to 1 s, respectively. Using this information allows the team to choose what optical components are necessary for the diagnostic application to skin.

5.14 Note on Sample Acquisition

Samples for calibration and testing of the spectrometer setup will need to be acquired. As part of the proof-of-concept testing phase of the project, the team will also need to acquire known cancerous and noncancerous skin samples. A second sponsor, even in a non-financial sense, will need to be a dermatology/skin cancer research center. Possible sponsors include the UCF Health Research facility at Lake Nona, or a large cancer research center such as the Moffitt Center in Tampa. Once a sponsor is confirmed, the team can then provide a gauge as to how many samples are needed, their cost, and a solution to create a more time effective scan.

5.15 Breadboard Testing and Circuit Design

The L.A.S.E.R.S. system cannot be configured properly with just one PCB due to the modular nature of the design. One PCB could be designed and utilized to control all major aspects of the system, however this setup may be less beneficial for this system. First, multiple PCBs will allow for the design team to focus on specific elements and keep like parts together. It will also help secure certain circuit elements that have special considerations, such as giving off heat or contain fragile pieces.

Another benefit of keeping like parts together is more security in connectivity. It would not be wise to have long leading wires to connect to the PCB since this wall could increase the probability of a disconnect, causing a fire hazard or malfunction. Keeping fragile elements on a separate PCB will allow for a certain space to be designated for it in the casing of the device, reducing the chances of damage to the device. A modular, streamlined design will also improve the troubleshooting process. Should an element of the system fail, it would be more difficult to quickly and effectively troubleshoot a PCB connected to every element of the system. If a problem exists only in one portion of the device, the team can more easily pinpoint that something must be wrong within that element. A focus on modular design is key to simplifying design and functionality.

The PCBs will be placed close to edges of casing, useful for port functionality and power connection, as well as easy access for troubleshooting. An insulation layer between the PCBs and casing may be necessary to protect from electrostatic discharge between the surfaces. It will be mounted on the surface of the inner casing, near a port or plug on the outside of casing that that PCB module will be connected to. The overall design for all parts of the device is first drawn and tested in MultiSim, then tested on breadboards, and finalized for the fabrication of the PCBs that will be in the final deliverable.

There will be one PCB for each major section of the device. The first PCB, PCB A will maintain and deliver power to the system, including the laser and the Raspberry Pi. This PCB will take in power from a wall outlet via a wall wart and connect into the first board. The second board will be powered through the first PCB and will specifically power the beam LED module that attaches onto the probe. Not only does the modular configuration help with component sensitivity in this case, but it also allows the user to properly troubleshoot the components on the board and inside the probe.

After all the components are known and inserted to the finalized design, the main task is to create PCB's that can interconnect with one another and provide enough power throughout the system all while meeting size, cost, heat, and component constraints. Since the system will be modular and have two PCBs, having multiple layers may not be necessary to effectively run the system. In effectively cutting out PCB layers, the cost will decrease as well as the heat within the PCB. Two layer PCBs will be plenty for this application.

After planning the configurations and observing the full spec that Ocean Optics has provided us, it has been determined that a two layer PCB will be beneficial due to its ease of fabrication and manufacturing, which saves the team time. Although the type of PCB has been identified, the next challenge was whether or not the project would be better off with Through Hole Technology or Surface Mount Technology. As previously explained in section 3.11 Printed Circuit Board, the PCB field has evolved to developing their hardware via the SMT format. Having looked at all the data, the consensus seemed to be that the through hole technology would be the main way that the components should be implemented. The amount of wattage in the system would be a bit high at some points, and ratings that provided ample room for the needs of the project were found in many through hole components.

The temperature regulation module was also a part of the PCB . This relatively simple setup will require a 4.7 $k\Omega$ resistor to bridge between the temperature sensor and the Raspberry Pi. The temperature sensor itself was also be placed on the PCB. The design for this circuit is discussed in 4.15.3 Active Cooling Design.

Verifying the functionality of the PCB will require a few tests that will happen at different levels of the PCB fabrication. Since the fabrication will be done by a third party company, most of the testing that can be done will derive from the early stages of the PCB designs: on breadboards, circuit simulators and electronic computer aided design software. Using the breadboard and circuit simulators will be the first true test of the circuits viability. After the breadboard testing passes, the next test will come from the Eagle CAD software that will be used to design the PCBs using the previously tested configuration. If that schematic also holds, the schematic will be sent to a PCB fabricator to fabricate and assembly the PCB using the gerber files outputted from the ECAD software. The third party fabricator, OSH Park, will also check to see if the circuit schematic is indeed viable, and will perform their own set of stress tests during the fabrication processes. When the PCB is finally returned, the last test that will have to be done is to check if all the contact points on the board produce a value close to the theoretical and experimental results from the previous examinations.

Breadboard testing and simulations lack some essential physical elements that must be considered when designing the PCBs. The safety switch is a physical switch, so the placement of it must be accessible to the user while still remaining connected to the rest of the related circuit. The switch, in this case a button, has four pins that can be place in the PCB, in a location as close to the edge as possible. Other items needed for the final device must also be placed on the edge, accessible to the device user. Ports for data and power connectivity for the wall wart and the USB connection to the Raspberry Pi must be included on the PCB. Like the switch, these two ports will have pins connected to the PCB. They must be placed along the edge of the PCB and casing in order to be accessible. The casing and PCB designs must work together in order to contribute to the ergonomic design of the device.

Another challenge with the PCB design is size constraints. PCB B, which holds the beam LED and related components, must be relatively small; this PCB will be placed in the probe alongside the camera module and lenses. The PCBs will also have places for data and voltage connectivity that will connect to the pins of the circuit elements.

In Figure 39 below, the layout of the PCBs is given. A is the casing for this portion of the device. B refers to PCB A, the major PCB of the device, with the power conversion, power control, and switch circuit portion. C is the Raspberry Pi. D is the location of the manual safety switch and related LED. E is the port for power connectivity, which connects the power from the wall wart to the main power control PCB. F is the port connection of components attached to the Raspberry Pi leading to the probe. Lastly, G is the PCB that contains the beam LED and related components.

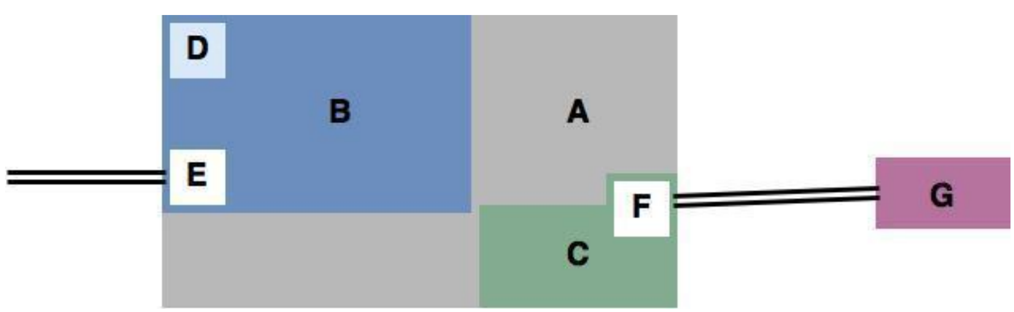


Figure 39. PCB Layout

PCB A - Power Controller & Microcontroller

The system will be powered with a power cord connected to a 120 V wall power socket. The voltage must drop from the 120 V into something reasonable for the L.A.S.E.R.S. device, such as 12 V. The first portion of the power control module must include a transformer or transistor system. In order to regulate voltage, a diode bridge may need to be incorporated. A main point of concern is not voltage regulation, but current regulation. A surge in current could be disastrous for the system, and is the most likely culprit for overheating and overpowering a part of the system.

Therefore, current regulation is a main focal point and priority for the power control system PCB. The power control circuit must also include an AC to DC voltage converter. The conversion from 120 V AC to a lower voltage will be accomplished using a full wave rectifier circuit with a diode bridge. After adjusting and converting the power to fit the needs of the system, the power control module that will match the individual components, such as the microcontrollers, camera, and LEDs, is designed. This will consist of various voltage divider circuits that deliver the required DC voltage to each module of the system.

Many system components need energy and data connectivity, including the microcontrollers that will implements the data received within the user interface. The microcontrollers are the bridges between the CMOS camera data into interfaceable data. The microcontroller module requires a PCB for power connectivity and data connectivity, as well as voltage regulation. If power is provided to the main system, power must be supplied to the microcontroller. It will remain on as long as power is provided to the overall system in order to process data. Power is provided to the microcontrollers so long as power is supplied to the overall system.

This PCB will have two parts related to the functionality of the laser: two LEDs that light up when power is supplied to the laser and the microcontroller, and a switch to allow power to reach these major components, thus allowing any related parts to also have power flow. The LEDs will be simple red (laser power) and green (microcontroller power) LEDs that will turn on if enough power reaches its circuit, which is connected to the respective power circuit. Thus, if power is supplied to the laser and microcontroller at the proper value, then the LEDs will illuminate. The LED module will consist of a resistors and the LED itself. The switch module is placed after the power input but before the rest of the components to support the microcontroller and laser.

PCB B - Beam

A PCB must be fabricated that can safely and securely hold and allow for the operation of the LED beam with ample space for the CMOS camera module. This PCB will contain the power and data connectivity required to run the LED beam. This PCB must allow for power flow and data connectivity. The CMOS camera provides image processing to allow the user to see that they are holding the probe on the correct skin samples area. This PCB will be in the probe of the Raman spectroscopy system. It must hold the beam around the lens apparatus, while each part cannot interfere with the functionality of another part. The camera must always be able to communicate with the microcontrollers while it has power. If the system is powered, the camera will receive power. Power will remain on. If the system is no longer powered, the camera will lose power and turn off.

The beam is a single white LED. The beam allows the user to better see that they are holding the probe on the correct area of skin. The circuit of the beam will consist of two resistors and the LED. If the main PCB is powered, the LED beam will receive power and turn on. The LED will stay on. If the main power PCB is no longer powered, the beam LED will turn off and lose power.

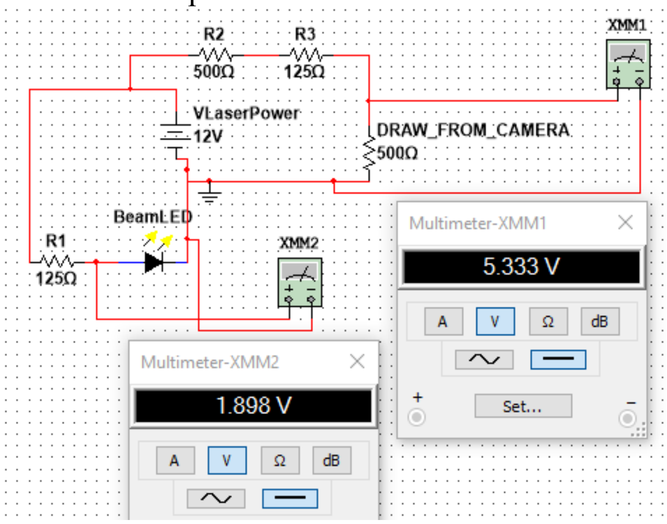


Figure 40. Early Test Schematic of PCB 2

Breadboard testing is essential to prove that the concept of the design will work on the final product. It is also essential to make sure that the potential design will work before ordering any PCBs. This test includes all major elements as well as any supplemental electronics parts, such as transistors, diodes, capacitors, and resistors. One portion of a breadboard will correspond to one PCB, theoretically. Once the design is proven both via computer simulation and in the lab on a breadboard, then it may be considered the final proof of concept and the PCBs can be designed and sent to a manufacturer.

First, each breadboard is tested separately. It is essential to verify that each module of the design works separately before implementing the complete system. For parts that have been delayed in shipment, a load resistor will be tested in order to ensure that the required voltage to power that part will be possible in the circuit. Parts implemented in breadboard testing chosen for the final PCB circuit will be listed in a table at the end of the document.

A major challenge in the breadboard testing timeline was the change in the scope of the project. Since some elements are awaiting deliverables from a sponsor, some of the circuits could not be immediately finalized. Because of this, the focus was placed on simulating the circuits to verify that the design itself will work, even if some component values change.

Once the layout is confirmed, the circuit is built. Initially, it was possible the camera module may be placed on the Raspberry Pi or directly on a PCB. To test how well a system would work with the camera on the same PCB as the LED beam, the following circuit was tested on a breadboard. A load resistor was used as a placeholder for the camera, verifying that the correct amount of voltage is drawn from each circuit element. This design for the smaller PCB is compact and verified to be effective, a perfect match for the size constraints of the probe design.

The most crucial step before sending out the circuit design to be implemented on a PCB is testing the simulated schematic on a breadboard. Although there were some minor changes to the passive component values, such as adjusting resistor values, the circuit design and topography was validated on the breadboard throughout the stages of the design. Throughout the breadboard testing session, the outputs of each section were examined before continuing onto the next phases. The circuit, much like the simulated design, was created from left to right, and then tested at the final output, the Raspberry Pi load resistance.

Looking at the simulated design, the first test that has to be done is to ensure the white LED that helps the user illuminate what area of the skin is being tested. Using a 470Ω resistor in series with the LED, the measured voltage and current outputs for the LED were very close to the simulated voltage and current outputs. The table below reveals that the percent errors between the currents and voltages were well within the acceptable range:

Outputs	**Simulated**	**Measured**	**Percent Error**
Voltage (V)	1.829V	1.816V	0.716%
Current (A)	25mA	24.155mA	3.38%

Table 16. White LED Simulated vs. Measured Outputs

The next stage that needs to be verified are the two BJT's that are configured in series (Q1 base feeds into the collector of Q2). Again, due to the current output being roughly half of what was needed in order to power the Raspberry Pi, the first BJT raises the current substantially via its common collector configuration while the second BJT acts as the short circuit protection due to its common base configuration.

The following table will show the voltage and current outputs for the after circuit goes through the first two transistors and into the voltage regulator; note that the errors were a bit higher, possibly due to passive elements needing to be reconfigured or the leads on the voltage regulator not properly sitting well in the breadboard:

Outputs	Simulated	Measured	Percent Error
Voltage (V)	7.989V	8.316V	3.932%
Current (A)	5.439A	5.135A	5.920%

Table 17. Voltage Regulator Simulated vs. Measured Outputs

This final table will have two voltages and two current outputs at those two levels. Remember, the final stage has to have a voltage at the Raspberry Pi of 5V and a current of 2A, and the LED must have the same ratings as the white LED from the first stage. Looking at the results and their percent error, it is well within the acceptable range and has been cleared for the testing session.

Outputs	Simulated	Measured	Percent Error
Voltage at Pi(V)	5.163V	5.234V	1.356%
Voltage at LED (V)	1.817V	1.922V	5.463%
Current at Pi (A)	2.334A	2.212A	5.513%
Current at LED (A)	25.719mA	25.821mA	0.395%

Table 18. Raspberry Pi and Laser Switch Simulated vs. Measured Outputs

After seeing each stage and their simulated results coincide with their measured results, it is safe to say the breadboard testing went better than the team expected. At first, some of the resistances were slightly off from what was expected, but after swapping them out and reconfiguring the leads properly, the rest of the breadboard testing turned out well. It helped a great deal to have the proper components, as well as a proper data sheet for each of these components to allow the team to edit and configure them at their discretion.

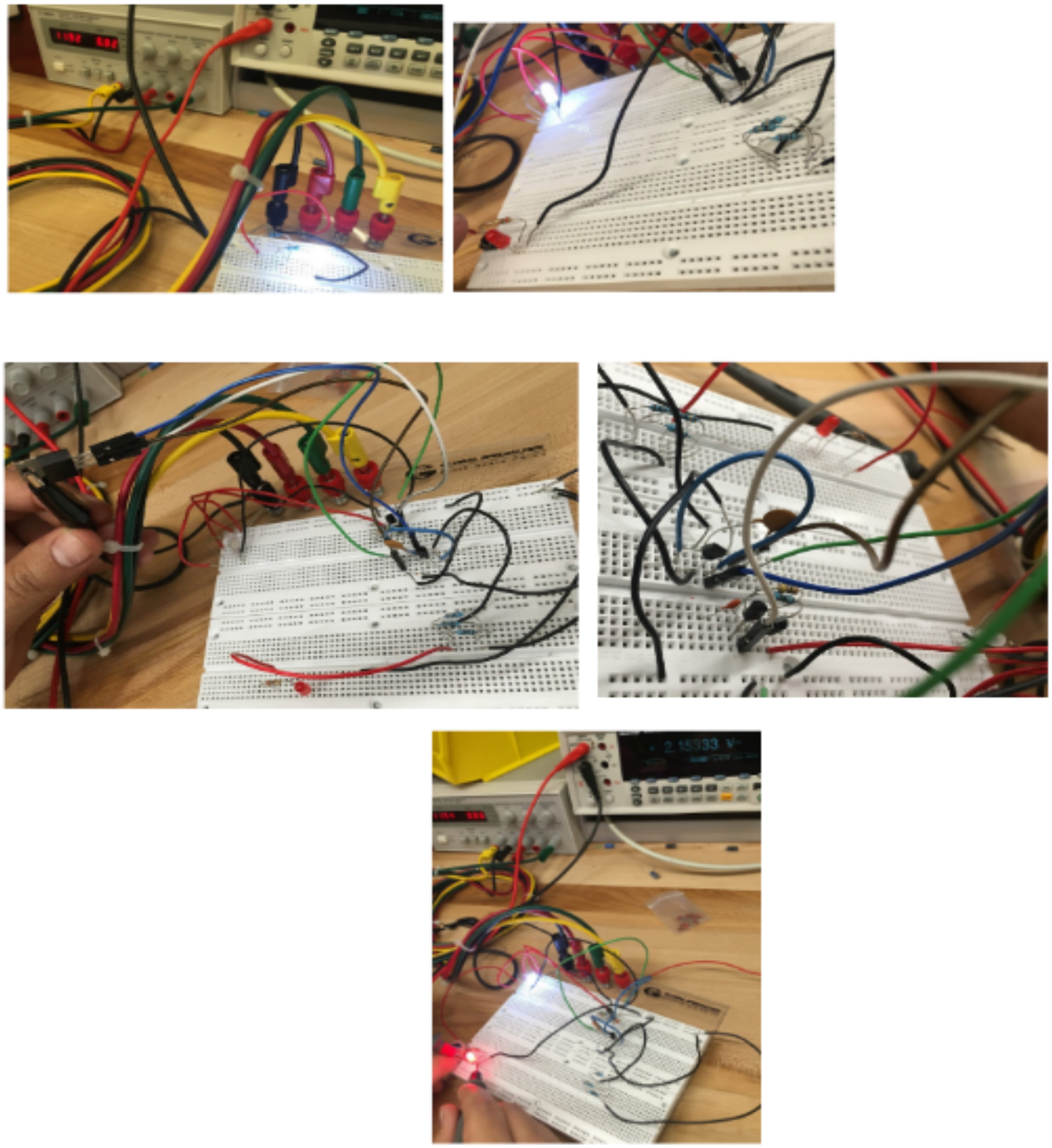


Figure 41. Overall Breadboard Configuration Tests

5.16 Printed Circuit Board (PCB) and Related Electronics Design

For the design, it was essential to note much power the system needed as well as where the system was going to get the power from. There are several solutions that included powering the circuit using a 9 V to 15 V battery, which could have been done simply by dropping the source anywhere inside the circuit configuration. The issue with this solution, however, is that medical devices need to be more reliable and safe; even if the battery will hold up while performing multiple tests, it seemed too risky and potentially unstable for the system. Instead, the team decided it was best to use an AC to DC converter that comes from the wall to the power distributor board.

5.16.1 AC to DC Conversion Solution

In order to use the power from a wall socket, the first step of the power control system is to convert the 120 V AC from the typical wall outlet to a reasonable DC voltage, such as 12 V or 15 V. A full wave rectifier is able to achieve this task, made up of a four-diode bridge along with capacitors and resistors to stabilize the DC voltage output. Figure 42 shows the circuit schematic layout of the full wave rectifier outputting 12 V DC from 120 V AC. Of course, a rectifier that meets the needs of the system, with a clean DC output, is successful in theory. The problem is that typical full wave rectifier elements cannot handle the amount of power flowing through them and will cause damage to the system, most likely melting some components.

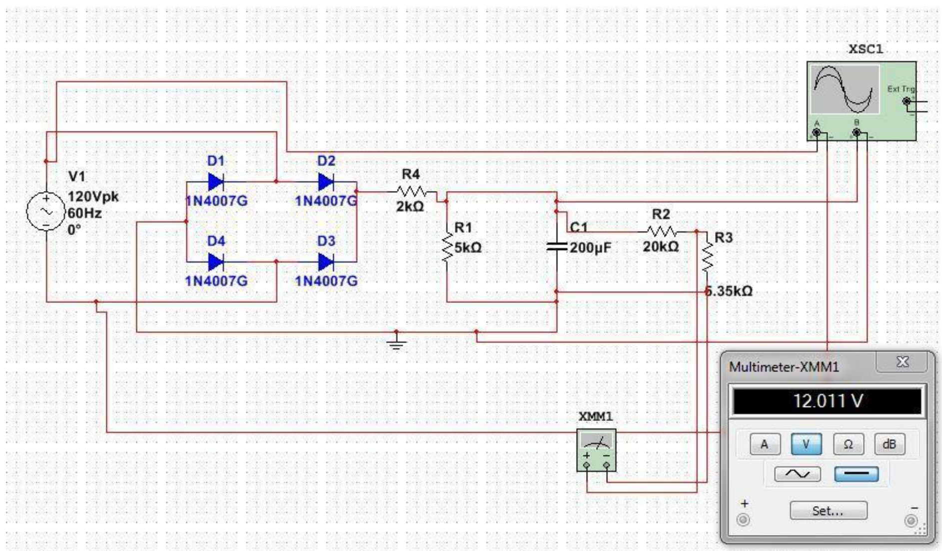


Figure 42. Designed Full Wave Rectifier for AC-DC conversion

It is essential to understand that while a simulation may deliver near perfect results for a designed system, one must read all datasheets and understand the parts of the design before making a potentially dangerous implementation. To bypass the cost and size of adding a transformer to the power control circuit, the team decided it would be best to purchase a power connector that plugs into a wall outlet and performs the conversion from AC to DC. Thus, a power cable was chosen that converts the 120 V AC voltage to 9 V DC with 2 A.

5.16.2 Power Distribution

The main challenge and focus of the design for the power system was stabilizing the voltage while regulating current. It was recommended to use a voltage regulator to stabilize and remove noise from the power supply. The voltage regulators had to regulate to 5 V in order to match the requirements of the overall device. The regulator used allows for a cleaner DC power delivery to the rest of the circuit when compared to a simple voltage divider. Considering the nature of the device and its use, it is imperative to have stable power flowing through the device at all times. In looking for a voltage regulator, the seemingly ideal choice was for 12 V, the LM7812; however, the output current is limited to 1 A. This voltage regulator has three pins, as shown in Figure 43 below. Pin A is for the input, pin B is the ground, and pin C is the output. For this particular voltage regulator has an output of 12 V and 1 A at pin C.

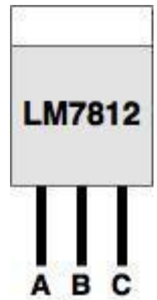


Figure 43. Pin Out Diagram of the LM7812

The Raspberry Pi may require 2 A depending on peripherals attached, and making use of the microcontroller is the top priority. To increase the current flow to the Raspberry Pi, a couple of options existed. One fix involved placing two of the voltage regulators in parallel, allowing for 12 V and 2 A to enter the power control circuit. In theory, this is sound and had evidence of being successfully utilized. However, two pitfalls to this method were important and ultimately led to abandoning this path. The first of those being the heat that could build up in the circuit would pose serious concerns. The other issue was the chance that the regulators will not allow a perfect 1 A to flow through, meaning that it was possible the circuit would not reach the needed 2 A. Not only must the proper amount of current and voltage reach the proper elements; it must also be verified that a safe amount of power flows through each component. The datasheet for a LM7812 offered a circuit to increase the current output. This circuit offered two ideal properties: increase in current while maintaining protection from short circuiting. Ultimately, the L7805, which has the same TO-220 package, was utilized to be more efficient, logical, and to reduce the wattage in the device. The L7805 allows for a 5 V output and up to 1.5 A. This transistor can also work with the configuration needed with the additional transistors to ensure that the Raspberry Pi will have enough current in order to function properly.

The final design incorporates various capacitors and resistors to provide steady power flow to various components, as well as two TIP 42C transistors, which are rated for a higher current than smaller transistor packages and were recommended in the datasheet. The transistors used in this case are pnp bipolar junction transistors (BJT). In the case of the design specs, the transistors are useful when the current in a circuit must be regulated or amplified; this allows for proper calibration at each component end. Looking closely at the topography of the BJT's (Q1 and Q2), the main idea to understand is that one is a common collector configuration; meaning that it will raise the current while lowering the voltage being supplied to it. In theory, this is what is necessary to obtain a high enough amperage, but it may cause a surge in current to reach the attached load in a worst case scenario. To combat this high current, another transistor is inserted in a common base configuration. This type of configuration allows the voltage to rise while lowering the current as it goes into the voltage regulator stage of the circuit. This is the short circuit protection that is implemented into the system. Once the circuit flows into and around the voltage regulator and outputs towards the Raspberry Pi, the current values will be effective for the Raspberry Pi, allowing it to pull on two TO-220 packages that can allow up to 1.5 A each. While linear regulators can become warm, the device has multiple heat regulating components to be discussed later in this document. An important piece to the heat regulation is to have a heatsink directly connected to every element in the device in the TO-220 family, including the voltage regulators and transistors used. An LED is included to indicate the proper amount of power is reaching the port for the Raspberry Pi

Another use of the 9 V power supply in this schematic is to power the LED beam that is used to illuminate the area of skin that is being tested. This white LED will be in series with two resistor to allow the proper current and voltage to operate across the LED. The current ratings for most LED's is about 25 mA while providing voltages of around 1.8V to work effectively. Once ohm's law was used to calculate the resistance needed, the resistors were attached, and then the circuit was closed at the ground under the voltage source. In this particular case, the LED will always be on when the circuit is powered with 9 V coming from the AC to DC converter. The 9 V will also power the fan if the Raspberry Pi does not work out for this.

On the bottom portion of the power module schematic lies the laser circuit. This power circuit utilizes the L7805 voltage regulator as well. Since the rated current for the laser is lower than the Raspberry Pi the added transistors were not needed. The circuit also contains a red LED and resistors to maintain the proper current through the LED. As with the Raspberry Pi power circuit, when enough power reaches the laser so it may operate, the LED illuminates. When the switch is open (the laser is off), the adequate voltage to turn on the LED is not met, which does not allow the LED to shine. However, when the switch is closed (the laser is on), the voltage through the power system will allow the LED to shine, indicating device usage. This quick check is primarily for the user of the device to have the laser in the ready position to function as expected.

These individual circuits will be configured together to work fully. The 9 V DC source is in series with the switch. For safety, a switch is installed so that power is not delivered to the laser and microcontroller immediately when the device is plugged into a wall outlet. Additionally, this PCB contains a temperature sensor, the DS18B20. This temperature sensor is small and relatively simple to interface with the microcontroller. A temperature sensor is necessary to read real-time device temperatures to ensure safety and to ensure that the fan is working properly. The fan will be powered by the Raspberry Pi and turn on when the temperature reaches a specific value. The fan will work with heatsinks in order to ensure that the safest device temperature possible will be maintained. As a contingency plan, there is also a port for power to the fan on the PCB.

5.16.3 Overall Schematic

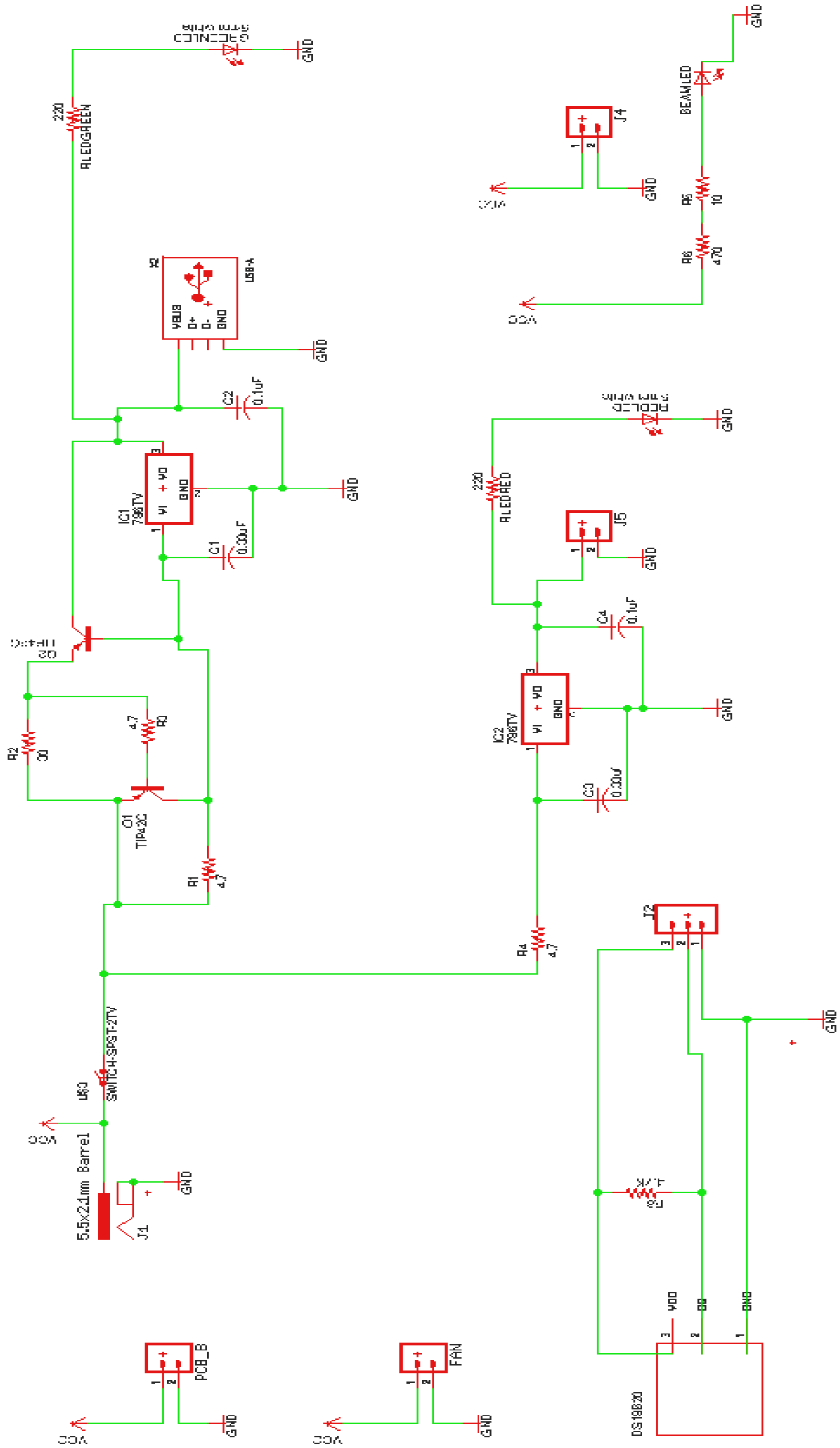


Figure 44. Overall Schematic of both PCBs

6 Project Construction

This space discusses the construction of individual modules of the device, such as the probe, main body, PCBs, and optical mounts, as well as how every piece interconnects. All power and data connections will be made, and the casing pieces will be carefully put together to surround the device components. Great care will especially be taken when working with the optical components. The probe connection to the spectrometer and laser is dependent on the spectrometer and laser that have been assigned by Ocean Optics.

6.1 Electronics

In order to verify the prototype electrical components, the team must have access to an electronics laboratory space with a multimeter, oscilloscope, and power supply. The room should have air conditioning and space to place all device components on a sturdy, level surface. The multimeter and oscilloscope are essential to read the voltage and current values in each electronic element of the device.

The team submitted their final PCB designs early in order to have the required PCBs fabricated. The team also had to submit final casing designs to be manufactured or 3D printed. Once the PCBs and casing arrived, the team had to verify that these essential components meet the given design and appear to be of suitable quality. The casing must be sturdy and non-pliable, able to securely hold all device components. The PCBs must be able to hold the dimensions of the circuit elements, especially larger components such as the voltage regulator with a heat sink. All electronic components must be soldered into their respective PCBs. Care must be taken while soldering components that are fragile. The team must make sure that the solder is cool before placing the PCBs into the casing shell. All port locations on the PCB must match up with the specified areas on the casing designed to allow access for power and data connectivity.

The main PCB of the device, PCB A, is dedicated to power and the switching module, power indicator LEDs the microcontroller, along with power and data connectivity for the temperature sensor. All components must first be placed on the bottom of the casing and secured. The main PCB, PCB A, had design errors immediately. The transistors used on the PCB were below the rating needed and began to smoke as soon as power was applied to the circuit. Thankfully, the problem was immediately clear to the electrical engineer in charge of the PCB design and within a day a new circuit and PCB were designed, tested, and send out for fabrication. This final PCB A worked properly and as expected. PCB B was straightforward and had no functionality issues.

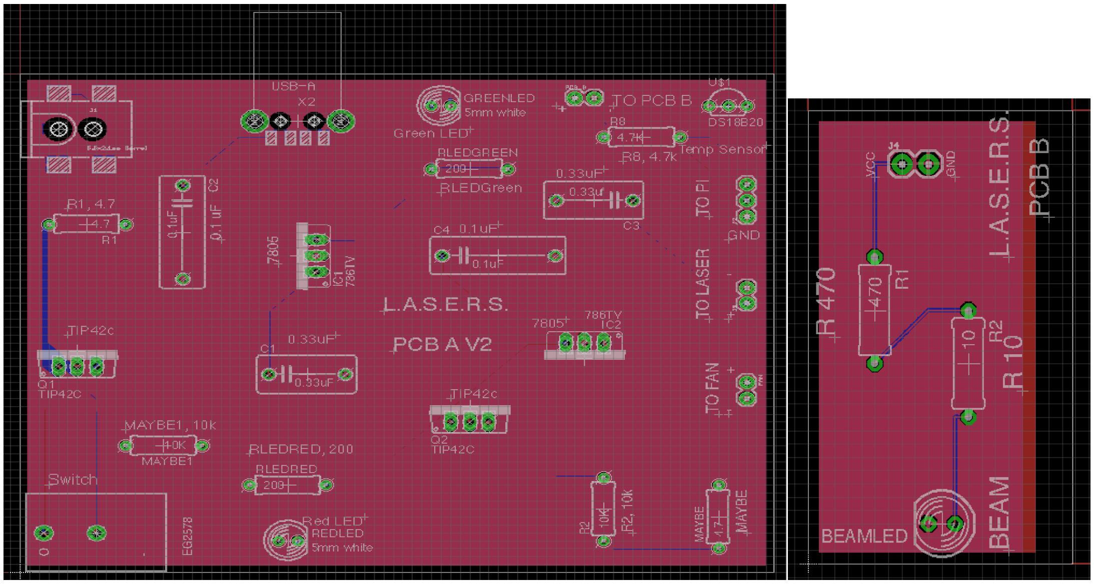


Figure 45. PCB A (left) and PCB B (right) Layout in Eagle

After both PCBs were confirmed to be functioning properly, they were placed in their respective locations within the final casing. PCB A was placed in the main body in a location convenient to all devices connected to it. Particular attention was paid to PCB components more likely to give off heat so that they were placed away from each other and in a location in the main box that allowed for ample air flow. It was important that no wires rested on any PCB components or heat sinks, as some component have up to 6 W flowing through them.

PCB B was more of a challenge to place, as the optical mount is fragile and relatively small. A shelf to hold the PCB in place was made on the mount. The PCB was carefully placed on the shelf and the very edges were glued in place with hot glue, which did not impact the electronics or the lenses. Since PCB B did not have as much current flow, the concern for heat was not as critical as with PCB A.

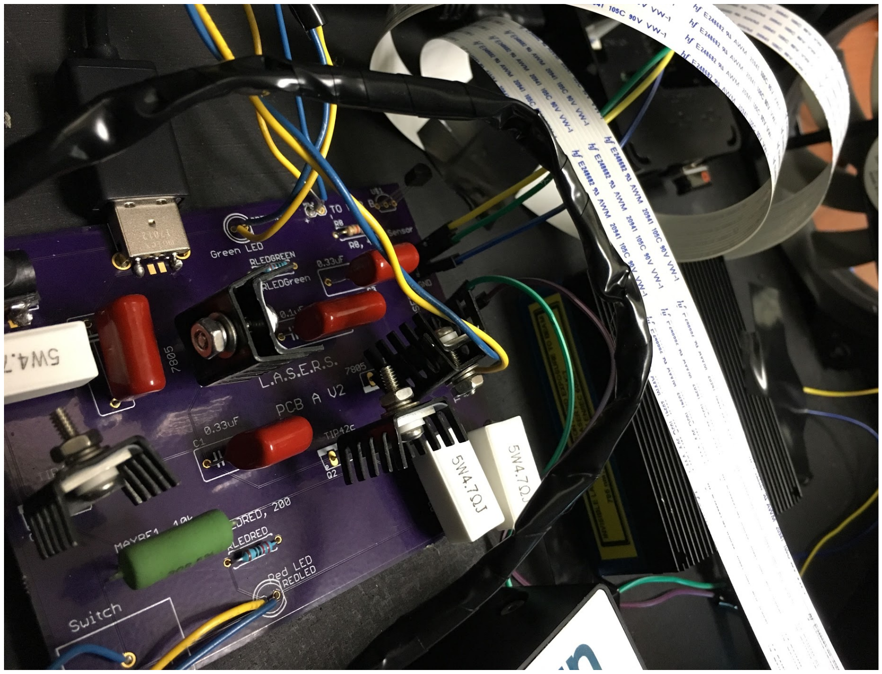


Figure 46. PCB A V2 (far left) connected with al other Main Body elements

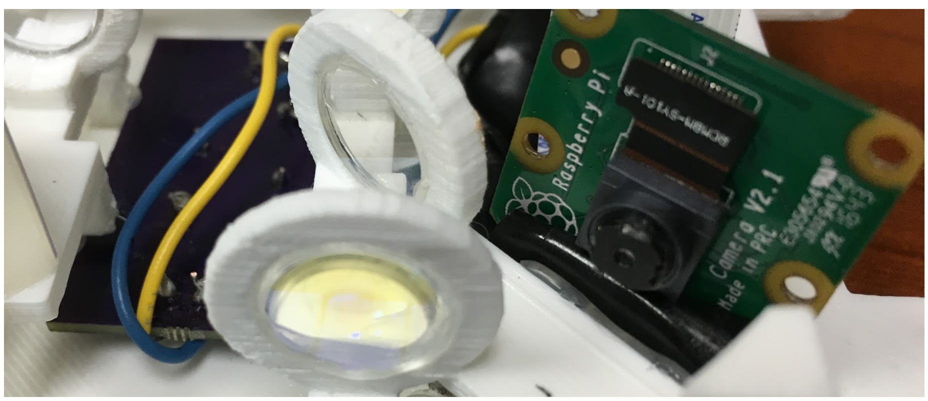


Figure 47. PCB B sits alongside the lenses and camera

6.2 Optics

In order to verify prototype optical equipment, the team must have access to an optics laboratory space with a multimeter, oscilloscope, and power supply. The room should have air conditioning and space to place all device components on a sturdy, level surface. The Senior Design Lab in the CREOL building is a space dedicated for such testing. The optical elements that must be tested are the laser, spectrometer, and optics that will be contained inside the probe.

Please Note: The laser used is near-infrared. The appropriate safety glasses with attenuation at 785 nm must be worn to prevent eye damage. Also note that nothing should be placed in the beam path that does not belong there. This includes objects that may ignite and hands or arms that can be burned.

The laser will be tested for power output, spectral linewidth, and spectral stability over a range of temperatures and a certain extended time period. The power output should be a maximum of 500 mW as given in the user manual provided with the laser. The spectral linewidth can be measured using the spectrometer provided by Ocean Optics or by another spectrometer available at the school. The linewidth should be less than 0.2 nm, as given in the user manual. The output power and spectral linewidth should be measured over a range of temperatures that may be encountered in the environment in which it is used, typically around room temperature. For example, test temperatures may range from 20°C to 27°C. The same measurements should be taken over an extended period of time that is equivalent to how the laser may be used in a clinical setting, such as a period of 20 minutes.

The wavelength accuracy of the spectrometer will be tested using calibration samples, such as a mercury argon calibration light source that produces well known spectral lines at specific wavelengths. If there are inconsistencies, the spectrometer can be recalibrated. Additionally, any offset in the spectrometer can be accounted for using either the OceanView software or software designed as a part of this project.

The individual optical elements will be tested. Laser light through the bandpass filter can be measured by a spectrometer. It should narrow the spectral linewidth (FWHM) around the laser line wavelength of 785 nm. Laser light through the longpass filter should block the laser line wavelength and transmit only wavelengths above the cut-on wavelength of the filter. A spectrometer can verify this. The beamsplitter has an anti-reflection (AR) coating on one side and a reflective coating on the other. For example, the beamsplitter may have an 80% reflection and 20% transmission ratio (80R/20T) on the reflective coated side. Light incident on the AR coated side should exhibit between 90% and 100% transmission. A power meter on the exit side should read a power level 90 – 100% of the incident optical power. Light incident on the 80R/20T side should exhibit 20% transmission. A power meter on the exit side should verify this. The lenses used for collimation and focusing can be tested by shining laser light through them. At NIR wavelengths, the laser cannot be seen. A simple piece of thermal paper can be used as a screen to view the output beam. Note that this should be done at low output powers to avoid igniting the thermal paper. To test collimation, the beam spot size on the thermal paper should remain constant as the paper is moved further distances from the lens.

As all optics elements are completed, the probe construction may begin. The lenses, camera, LED beam, and all other probe components must be carefully placed in their spaces within the probe casing. Optics such as the lenses, filters, and mirrors will need to mounted in some fashion inside the probe. It is imperative that the optics components do not shift when the probe is moved. All optical parts and lenses should be handled with gloves to preserve the integrity of the parts. All cords for power and data connectivity must be able to exit the back of the probe casing with few bends in the wires.

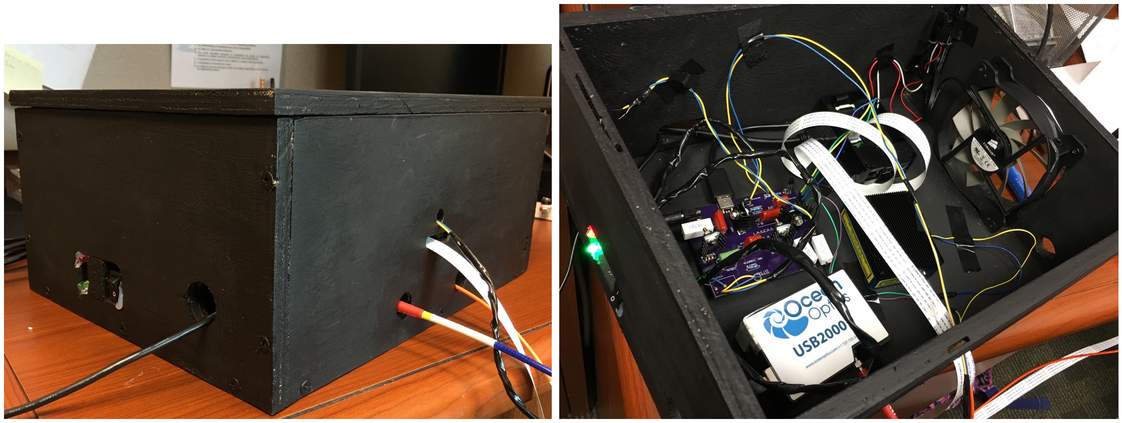
6.3 Overall Device Construction

Before the device could be completely constructed, the team had be sure that both the optics and the electronics inside the probe and cables are verified to begin constructing the device. Focusing on the design of the probe, there are four cables running from the end of the probe. Two are the excitation and collection optical fibers that are connected to the laser source and spectrometer respectively. One cable is connected to the Raspberry Pi for the camera module. Lastly, one is the power cable connected to the main power PCB A to power the device. Once the cables are relayed, the next step was to encase the probe using a custom CAD.

Once all individual components were put together, tested, and verified, construction of the overall device could begin. This process consists of two main components, the main body and the probe.

6.3.1 Device Main Body Construction

The first step was to build the box for the main casing. In order to focus on other priority items, the team decided to simply construct this piece out of wood. It secures all electronics and has holes for access to cables, ports, the switch, the fan, and power indicator LEDs. The box features a hinged top so that the team can easily get in to troubleshoot as well as to allow the laser to turn on with its individual switch. Once all holes were cut, the box was painted black. A laser indicator warning was glued to the top in an easy to see location. The fan was screwed in place, LEDs and the switch were hot glue gunned in place, and components were placed inside.



Figures 48. Main Body Box construction process

6.3.2 Device Probe Construction

By far the most challenging component to build and integrate was the probe. Proper alignment was essential to success, and the number of fragile components made for an interesting challenge. A task for the group was to figure out how the optical components will be housed and secured in the handheld probe. The original idea was to shy away from 3D printing and provide a drawing to a machine shop to construct and make the mount for the project. This, although practical, was a difficult solution due to the unknown factors and the time constraint placed. What was done, however, was revisit the 3D printing solution and see how it can be made better. The issues with 3D printing arise when there are errors in the CAD files or errors in the printing itself. However, the team designed the mounts according to the focal lengths and sizes of the lenses and mirrors, and ensured each measurement was checked and rechecked again.

The first trial run from the 3D printer was effective, but had to be more robust. It was not enough that the components were aligned; they had to have enough space to sit flush onto the 3D created mounts. It also became evident why making 3D CAD mounts was difficult: some of the sizes for the mounts needed to be slightly larger than the components themselves to allow for better seating. For instance, the laser lens was of a diameter of 6.35 mm (0.25 in), but needed about 8 mm (0.32 in) clearance to sit and adhered to the mount itself. Once the group recalculated each mount to accommodate for the lenses and mirrors, the mounts sat precisely where they were supposed to sit. Below is the image of the optical mounts that are to sit within the probe body.

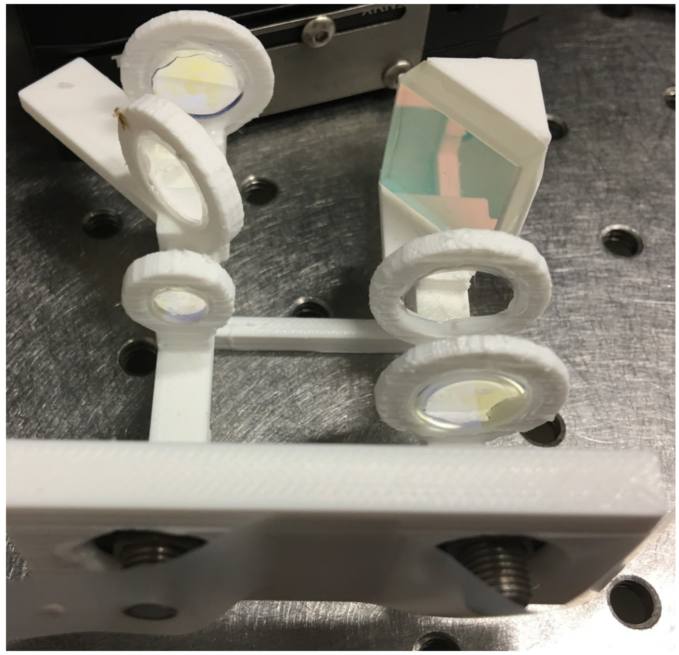


Figure 49. Optical components placed in the inner skeleton of the probe

After the mounts proved to be properly aligned and allowed the laser to travel without error, the next task was to recraft the mount to provide a seating location for both the PCB and camera module that are to be housed inside the probe. Since the Probe PCB dimensions are relatively small (0.99 in x 1.7 in), the idea from the group was to consolidate space and utilize the probe mount to also mount the PCB. Since the mounts also needed to be secured from the underside of the probe, the consensus was to use the bottom of the mount to slide the PCB underneath the optical components.

Similarly, the camera module also had to be placed in the probe securely while ensuring that it does not interfere with the optical components. The solution was to utilize the optical mount structure to create a pad for the camera to sit at about a 45 degree angle. This allows for the camera to not only get a clear image at the focal length, but it eliminates the fear of any interference. Below is an image of both the PCB and camera module mounted using the optical mount structure:

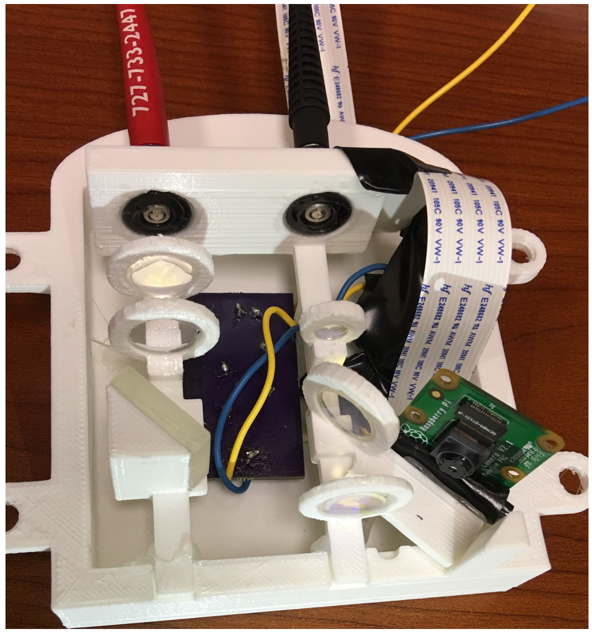


Figure 50. Mounted optics, PCB B, and camera module secured to bottom of probe encasing

Once the optical mounts were created inside the probe, the next challenge was to create a large enough case that could house the mounts and camera without interfering with the viewport of the laser and camera lens. The bottom of the probe body (in white) was created first to ensure that the optical components within were functioning at the measured lengths provided. When the testing for the probe was confirmed at the proper focal length for the samples, the next step was to created the top of the probe body (in black) to secure the components. The probe height needed to be large enough to not allow for the casing to interfere or damage the components within. Lastly, the two pieces were joined together with 4 nuts and bolts to secure the device.

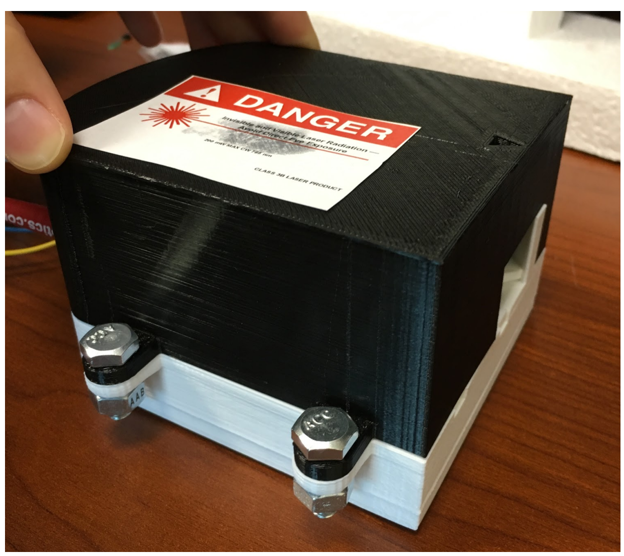


Figure 51. Completed encased probe

6.4 Programming

6.4.1 Cooling Program

The cooling system program, while considered an accessory program, was vital to the completion of the rest of the project. Without proper and adequate cooling, the rest of the project could easily overheat and have issues remaining reliable.

The first iteration of this program, was to simply take in the temperature sensor data from the DS18B20, and output it to the screen. This allowed us to monitor the temperature sensor of key componentry during the testing phase.

The second iteration of the program, was to have the temperature sensor data output, while the Raspberry Pi powers the fan. Using the GPIO pins for 5V, GND, and GPIO pin 18, we were able to power the fan system while the temperature was output. At this time, the fan system was not temperature dependant, and simply has the temperature sensor and fan going as long as the Pi was powered.

The final iteration of the program, was to use this temperature sensor data to modulate the power to this fan. Since the fan speed is voltage dependent, the Pi was able to control the fan from between 0 and 1500RPM by alternating the input signal. We tested this by measuring the voltage flowing through the power circuit, at a given temperature range and logging the results.

Temperature	Voltage	Approx Fan Speed
24 C	0V	0RPM
40C	3.2V	600RPM
50C	4.1V	1000RPM
>60C	4.9V	1500RPM

Table 19. Fan Speed Voltage

6.4.2 Camera System Program

While interfacing the hardware of the camera module is relatively simple with the Raspberry Pi, the functionality still must be defined and written in code. First, the written Python code reads the camera. From there, design considerations found in a typical camera were set, such as sharpness, contrast, brightness, and like settings. When the camera program runs, the preview begins to appear on the screen. the words, “Tested Sample Image” appear in white across the top of the screen.

The camera preview can end one of two ways. First, it can be set on a timer. In this version, the preview is set to a certain amount of time and at the end of the time limit it takes a photo, saves it to the desktop, and closes the preview. This is ideal if the user would know roughly how long the process would take. However, since the user is testing a sample it may take a bit of time to line up in the proper position. Thus, the better option that was chosen for the final deliverable was to close the preview and take a picture when ctrl + c is pressed. This option allows the user as much time as they need to get into the ready position to complete the test of the sample.

6.4.3 Spectroscopy System Program

The spectroscopy system was the software based upon the Ocean Optics OmniDriver suite, and needed to be run on the external computer for compatibility purposes. Using a basic tutorial provided to us with the OmniDriver download, we had been able to begin a spectrometer session, and log 2048 data points at various wavelengths. The intensities were logged and displayed to the console.

The first developmental step for this system was to the incorporate the data being taken in and plotting it, and then outputting all the necessary data to an identifiable file system. Making use of JSwing and JDraw functionality, we were able to have the system plotting these data intensities in a line graph format as shown in the figure below. The data points were all output to a .txt file instead of the console, to allow for readable data results even after the program has finished.

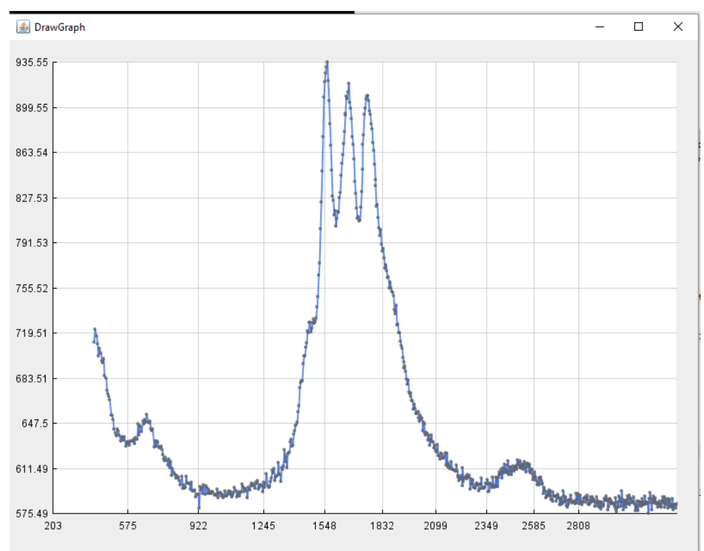


Figure 52. First Step Data Intensity Graph

The second step for this program was to then begin to strip the useless data (below 300 wavenumbers) which are considered anti-stokes. Our spectrometer system was not interested in this data, and it needed to be stripped as to reduce confusion of the system. We also removed non-linearity and began to average together several runs using the spectrometer. This reduced the noise from our system, and allowed the data to be more meaningful to the user.

The final step for this branch of the program was to implement the search for local maxima in the dataset, and correct the plotting errors we were experiencing. Before this run, the system was not able to make an interpretation about the dataset, and instead relied on the eyes of the tester to compare this to known datapoint values. We began to take the wavelength values that the spectrometer provided us with, and converted them to the understood Raman shift wavenumbers, which could then be plotted on the X-axis of the graph as shown below. With the Raman shift wavenumbers now known, we were then able to capture the local maxima and keep track of these data points. These data points could then be compared to known data points and this information could be extrapolated to the identity of the unknown gemstone being tested.

6.4.4 GUI Development

Though the GUI styling was finalized even before programming had begun, because it was being programmed using JSwing and JFrames, there would be serious limitations to the functionality and design aspects.

The initial iteration of the GUI was creating the basic design using the components natively found in the JFrame class, and positioning them and sizing them correctly in a way as to meet the specifications of minimalistic, intuitive, and professional. All colors were synchronized between each screen, and the size and positions were standardized as much as possible. Each screen was merely independent at this point, and the flow had not yet been implemented.

The second iteration of the GUI development process was to have each screen open in the correct sequence. following the design of the system. Each screen has a button which should lead you forward through the GUI, with no real way of returning to a previous screen. The philosophy behind this approach was due to the volatile nature of the components and data, the user should have no reason to return to a previous screen, as the test would be compromised anyways. With the GUI flow completed, the control functionality could be married to the GUI, and the button presses could control actual hardware functionality.

The final developmental iteration, as mentioned previously, was to marry the GUI buttons with hardware control functionality. With each button press, a testing process should be completed, as outlined in the hardware control schema earlier in this report. At this point, the GUI was ready to be tested by other users, and bugs would need to be corrected and documented as to resolve the possibility of hardware/software control mishaps.

7 Project Prototype Testing Plan

In order to be sure that the L.A.S.E.R.S. system is completely functional, first the team checked that each of the major components worked properly. Once each component was confirmed to be operating properly, then the device as a whole was checked. Testing this device for the application of identifying gemstones involved mounting the samples and using the live camera feed as a guide to place the probe in the proper position for testing.

7.1 Project Technical Testing

It was verified that each individual part meets its own design specifications, related standards, and constraints. If the individual components did not function properly, or as expected during the design process, then it was either replaced or re-evaluated for the requirements of the project. During Senior Design 2, alongside the building process, parts were tested within their respective module as well as in relation to the the overall device functionality. The final deliverable was fine-tuned as the behavior of the final design was learned through the testing process. It is essential that all components work so that the final project integration goes as expected.

7.1.1 Optics Testing

The optical components were tested independently and assembled. This section briefly describes how to test the functionalities of these components. The components discussed are the laser, fibers, filters, beamsplitter, lenses, and mirrors.

Power

The laser must be powered on. The characteristic wavelength of the laser was verified to be 785 nm using the Ocean Optics USB2000+ spectrometer. Because Raman scattering is weak, adequate power should be delivered to the sample in order to create a significant Raman signal. It is typical practice to use a power of 350 mW or less depending on the sample. Because of the weak signal, it is desired to deliver at least 20 mW to the sample. Maximum output power directly from the laser aperture was measured to be 470 mW. The output power of the laser was measured using an optical power meter. The laser light was successfully coupled into the excitation fiber. A power meter at the exit of the excitation fiber measured a maximum output power to be 260 mW, indicating loss through the fiber.

With the setup on the optical breadboard, maximum output power to the sample was 170 mW. For the final handheld probe, maximum output power to the sample was approximately 55 mW. This drop is most likely due to a slight misalignment of the excitation fiber in the probe. The excitation fiber adaptor was accidentally glued into the probe at a very small angle. This small offset causes a fraction of the light to be clipped by the beamsplitter mount, preventing that light from reaching the sample. However, 55 mW is above our specification of at least 20 mW and is plenty sufficient for stimulating a Raman effect from the sample.

Laser
The linewidth of the laser was measured with the Ocean Optics spectrometer. According to the laser's user manual, the linewidth should be 0.2 nm at full-width half-maximum (FWHM). The spectrometer measured a FWHM of 2.45 nm. This may be different because of limitations of the spectrometer's resolution. However, the specification of a narrow laser bandwidth to detect the nearest Raman shift of interest is still met.

Longpass Filter
Via the probe, the light must be focused onto the sample at an appropriate working distance. The reflected light must be collected. The collected light must be filtered to block the laser line (Rayleigh signal). A longpass filter was used for this purpose. On the optical breadboard, it was confirmed that the intensity (counts) of the laser power was reduced when the longpass filter was in place.

Contingency Plan
Dr. Richardson's research group has access to a Raman spectrometer in CREOL. This spectrometer can operate at 785 nm excitation with wavenumber shifts of 90 to 1500 cm^{-1}. If necessary, the team can use this Raman spectrometer for initial testing of samples for comparison to the data obtained with the probe design and the Ocean Optics spectrometer.

7.1.2 PCB Functionality Testing

Once the PCBs were soldered and appeared to work properly, they had to be tested within the system to make sure the proper amount of power reached each component. First, plug in the wall wart into the DC jack. At this point, the LED in the beam illuminates as it should. Full power is received at points of connection to PCB B, the fan port; no other components should have power. Then, turn the switch on. When the switch is on, all device components should receive power. Components should be warm, but not overheating. The table below indicates that all aspects of the PCB design met specifications.

Component	Voltage w/Switch Off	Voltage w/Switch On	Current w/Switch Off	Current w/Switch On	Meets Ratings?
Laser Port	0 V	4.921 V	0 A	0.827 A	Yes
PCB B Port	8.98 V	8.91 V	30.8 mA	30.22 mA	Yes
Raspberry Pi Port	0 V	5.02 V	0 A	1.78 A	Yes
Fan Port	8.98 V	8.91 V	0.79 A	0.801 A	Yes

Table 20. Major Electronics Tests

All device components turn on and function as expected with connectivity to PCB A. Nothing gets overheated, even after the entire system is plugged in and running tests for 40 minutes. Proof of full functionality will be discussed in a later section.

7.1.3 GUI Programming Testing

After the programming functionality was finalized, testing over the programming system was more or less completed during development. However, to ensure the GUI section met its requirements,the system was stress tested to ensure that the full specification list had been met.

For the GUI section, the design needed to be professional, intuitive and minimalistic. Each piece of the code would only be operable in one direction, and each button press should correctly trigger the necessary software/hardware programs as designed. Each team member took several tries in causing the GUI to glitch, however the lack of extra functionality made the system very stable, thus confirming minimalistic and intuitive. As for professional, this was a more subjective specification, but the design was clean and standardized, which we determined was at a professional level.

7.1.4 Data Collection/Analysis Testing

The data collection and analysis programming had several specifications that needed to be verified. The data should be relevant 90% of the time during the collection process, and the determination of the relevant compound should be correct 75% or more of the time. The total processes should not take more than 30 seconds for collection, and 1 minute for analysis.

To be sure the data is relevant 90% or more of the time, step had to be taken to reduce noise, extraneous data, and collection bugs in the collection process. After product testing, we were well above the 90% data collection specification, with all inconclusive data sets able to be attributed to improper testing procedure instead of a programming failure. The challenge with this was to reduce the testing time to less than 30 seconds to reduce patient exposure. The ideal testing conditions ended up occurring over 100 passes of both the light and dark spectrum, which allowed for a total runtime of around 10 seconds, which helped reduce the SNR by a factor of 10.

To verify that the relevant compound was detected more than 75% of the time, disregarding improper imaging failures, we tested each test subject dozens of times, and when proper imaging samples were isolated, the system was correct at identification more than 83% of the time. The compound with the least issue to identify was asha, with diamond being the most difficult due to its light scattering properties.To minimize the runtime of the analysis, only the necessary data points were analyzed. Analysis runtime was typically less than 5 seconds, however, at a later date with a better spectrometer, the resolution could be increased and this analysis step should remain the same under the 1 minute mark.

7.1.5 CMOS Camera Testing

It must be verified that the camera module functions in a way that meets the design specifications. To test this, the camera module will be inserted into the Raspberry Pi. Then, once it is enabled and operating, it will be tested in a variety of functionality tests. It will be verified that it can take still photographs as well as show a preview until a keypress occurs. Additional tests will verify the angle of view. Because the camera will be imaging the sample at the distal end of the probe tip, the camera must be able to see the sample area given a certain length of the probe tip. The images show that nothing is blocking the field of view. The sample, located 1.2 cm away, appears clear and crisp. The initial focal length was manually adjusted by carefully unscrewing the lens, allowing for the probe to be closer to the sample and have a better, clear and in focus image appear on the screen. The laser on the sample is visible. Camera is functional to meet specifications.

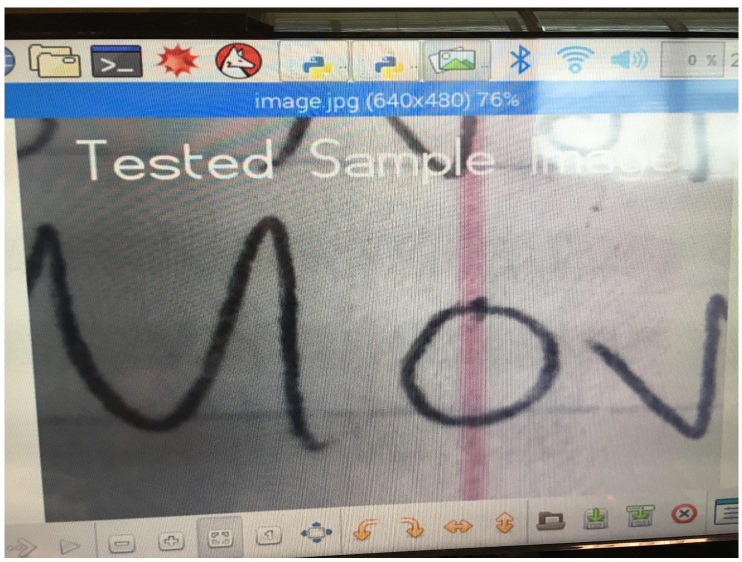


Figure 53. Image from camera taken during testing

In this example image from testing, the details of the lead pencil on paper were sharp and visible. A few additions were made in the code to enhance the image quality. The top of all saved images includes text reading, "Tested Sample Image". The contrast, brightness, sharpness, and other typical camera settings were also defined in the Python code that controls the camera on the Raspberry Pi.

7.1.6 Cooling System Testing

The cooling system had the main specification of monitoring the temperature in real time and controlling the fan as necessary. After the Raspberry Pi was connected to the appropriate temperature sensors, each was monitored 5 times a second for any temperature changes. When any of the temperature sensors read above 45 Celsius, the fan would turn on at the given voltage according to the fan curve. This was verified by monitoring the program during initial team testing, and ensuring the fan was only on after this critical point was passed, and ensuring the fan was RPM dependant. After this program was verified, it was tasked run in the background at all times, to ensure the user never had to implement this feature upon startup.

7.2 Sample Testing

The overall reliability of the spectroscopy system depends on its ability to be able to accurately take measurements from, and analyze the data from the test subject in a timely and non-harmful manner. With the future of the project being identified as *in vivo* cancer detection, proof of concept of object recognition via our Raman Spectroscopy methods first had to be planned.

As an alternative to actually testing known cancerous skin samples, it is possible to create simulated results using reflective films that can simulate the cancerous and noncancerous skin samples the team intends to receive. This will help remove some of the hurdles that are present in testing on human skin samples, and allow us to be able to test accurately without possibility of corrupting or ruining our acquired skin samples.

This would provide the significant improvement to the time constraint for this project. Testing on human skin requires training, for the whole team, which could significantly hinder the time needed to design and build the L.A.S.E.R.S. system. Any way to streamline the testing process and allow the team to focus on the technical product are ideal.

7.2.1 Gemstone Testing

To overcome the obstacles presented related to human skin testing, new samples were chosen for testing and for the demonstration. The Raman spectra of gemstones such as diamond, Asha simulant diamond, and a purple gem were measured. The graphs below are test results from the handheld probe, showing the spectra of Asha simulant diamond (left) and the purple gem (right). Asha has peaks at 1332, 1438, and 1533 wavenumbers. The purple gem has a peak at 1381 wavenumbers. These data agree with that found in other Raman spectral databases.

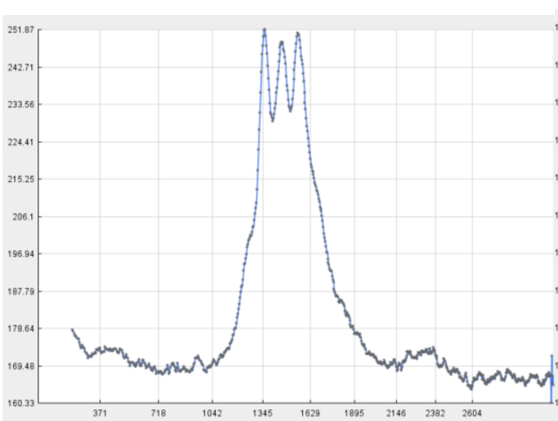


Figure 54. Raman spectral results for Asha simulant diamond (left) and the purple gem (right) using the team's constructed probe. Asha has peaks at 1332, 1438, and 1533 wavenumbers. The purple gem has a peak at 1381 wavenumbers.

Samples were obtained from jewelry items: diamond from a ring, Asha from an earring, and the purple gem from a ring. Each of these gemstones has a unique Raman spectrum with characteristic peaks.

7.2.2 Human Tissue Testing

The overall future functionality of the spectroscopy system depends on us being able to accurately scan the skin of the patient, and be able to accurately classify the skin as cancerous or noncancerous. To be able to accurately test the team's system, samples for calibration and testing of the spectroscopy system setup will need to be acquired. As part of the team's proof-of-concept testing phase of the project, the team will also need to acquire known cancerous and noncancerous skin samples. A second sponsor, even in a non-financial sense, will need to be a dermatology/skin cancer research center.

Possible sponsors include the UCF Health Research facility at Lake Nona, or a large cancer research center such as the Moffitt Center in Tampa. Once a sponsor is confirmed, the team can then provide a gauge as to how many samples are needed, their cost, and a solution to create a more time effective scan.

7.3 Our System vs Raman Probe calibration

Due to the simultaneous system development requirements of this project, the initial software calibration was done for the Ocean Optics provided commercial spectroscopy probe (InPhotonics). It was understood that this would allow us a baseline for our probe, which could then be adjusted for our probe upon completion.

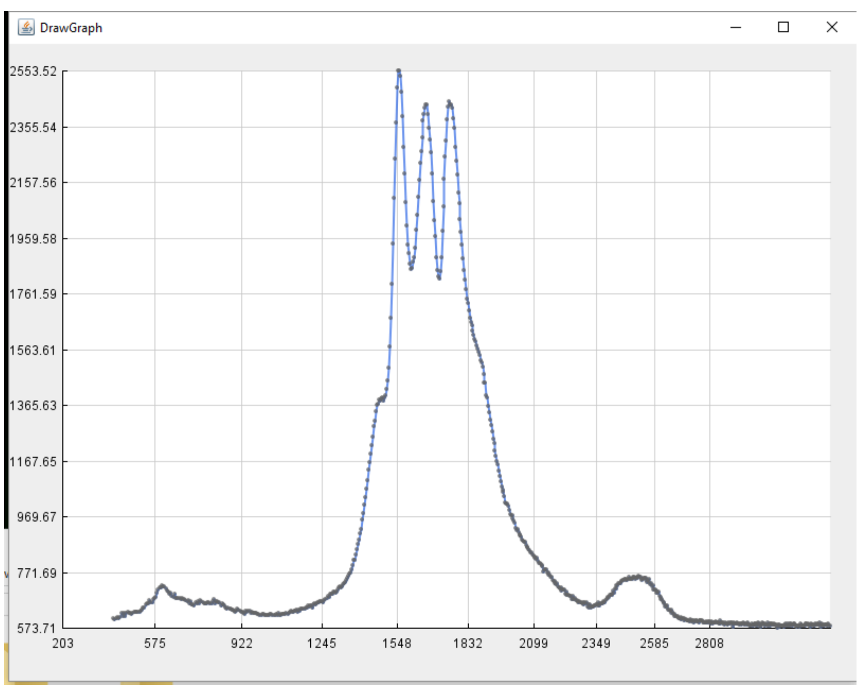


Figure 55. InPhotonics Raman Probe using Asha simulant diamond - Raman Emission

As shown in the figure above, the InPhotonics probe was able to collect the Raman emission spectra of our Asha simulant diamond sample. The boxcar width, linear correction factor, and integration time were all set according to our tests. Their probe, which is built to work their spectrometers, is highly calibrated for the system, but does very little in way of reducing laser power, and in general making it safe for a user to use the probe on their skin.

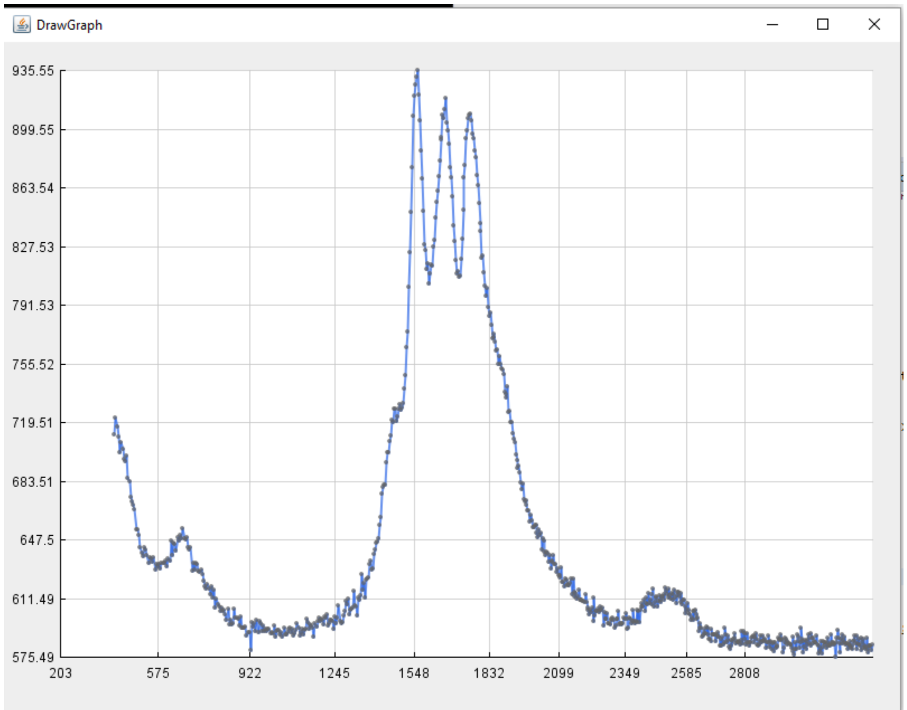


Figure 56. Our Raman Probe using Asha Simulant Diamond- Raman Emission

As shown in the figure above, our probe was also able to collect the Raman emission spectra of the Asha simulant diamond sample. The boxcar width was calibrated to 5, the linear correction factor was enabled at a factor of 1, and the integration time was virtually the same as with the InPhotonics probe. The only indicative factor of our probe vs InPhotonics is the reduction in intensity of the peaks of the graph. This is indicative of the laser power that is removed via our probe, to make the entire system safer for the user. These correction factors were gathered by testing on the same gemstone, and making the graph as ideal as possible.

This shows that our probe is as effective as the InPhotonics probe for our system. The program has no issue being able to identify any of the gemstones in our testing, using a much lower intensity peak. This allows us to move towards our goal of using the system on a living subject with a much safer laser power level being used.

7.4 Project Operation

Due to the nature of the project that has been created, it is crucial to have an all-encompassing manual that ensures the safety and well-being of the user. This section will explain how the device is to be maintained, what the individual components do, as well as ensure the safety of the customer throughout the testing process. The last section will encompass an FAQ document that has been surveyed over the course of the semester.

7.4.1 User

The typical L.A.S.E.R.S. user is expected to be either a medical professional with experience in skin cancer testing, such as a dermatopathologist or a nurse in the same field. However, the device is intended to be easy to use and could be used by any non-technical user in the comfort of their own home as desired.

The L.A.S.E.R system has been designed with the idea that any user should need no experience to be able to use the system. The system will be able to instruct the user on proper testing procedures, and all testing is to be performed with several fail safes in place to ensure the safety of even the most inexperienced user.

The L.A.S.E.R system is an *in vivo* Raman spectroscopy system which is a device which has the ability to test the Raman spectra of a given material. The Raman spectra will allow the system to analyze the makeup of the samples being tested. We are using this particular Raman spectroscopy system to allow users to be able to perform real-time skin cancer tests, with the ability to give the user an accurate result report immediately following the test.

Currently, a user of the system must go into a dermatologist to have a biopsy performed to be able to test a skin sample for the possibility of cancerous tissue. This process is an expensive process, and typically has a 2-3 day result return time. The system aims to remove the time delay from the equation, and be able to provide the same functionality at a much lower cost. Whether the user is receiving the test in a doctor's office, or at home, the overall cost is a one-time charge instead of charging per test. The L.A.S.E.R.S. system is designed with modularity in mind, to make the longevity and reliability of the system far superior to other products.

7.4.2 Product Design

As mentioned before, modularity is a key component of the L.A.S.E.R.S. system. This design approach allows for increased longevity and reliability. Each component of the L.A.S.E.R's system is able to be added/removed as needed whether for portability or for repairs. It also allows for simpler assembly and identification of products. The overall system will contain 3 main components:

Raman System

The Raman system is the portion of the product that houses the Raman spectrometer, the microcontroller, the external computer system, and has the probe leading out of the encased system. This section of the system is what allows the laser to scan the skin sample at the indicated wavelength to test for skin abnormalities. The external computer will then take the information and process the results before outputting the determination.

Raman Probe

The Raman probe is the handheld device that is used to test the skin samples for their abnormalities. Its design allows for easy maneuverability and functionality for the user to control the probe efficiently. The probe leads out into the spectrometer to for data collection to output onto the screen.

Power Brick (Wall Wart)

The wall wart is specifically for the power for the entire device. It converts the standard 120 V AC that you get in your house outlet into 9 V DC power that is required to power the components.

7.5 Overall Device Test

All components are integrated at this point. The main power PCB, Raspberry Pi, laser, spectrometer, and cooling fan are secured within the Main Box. The optical elements, camera module, and LED and accompanying PCB are assembled within the handheld probe. The PCB powers the Raspberry Pi, laser, and probe. The spectrometer is connected to a computer from which it is supplied power. The following paragraphs describe the process used to test the entire system.

The Raspberry Pi is plugged into a wall wart for power. The LED in the probe turns on. Once the switch on the Main Box is flipped, two LEDs indicate power to the Raspberry Pi and to the laser. The camera in the probe draws power from the Raspberry Pi. The laser is not on unless the switch on the laser itself is flipped. Samples are mounted for testing.

Figure 57. Assembled project showing the Main Box, Probe, and mounted samples.

The GUI prompts the user to begin the test. The camera provides a live feed for targeting the probe at the sample. When performed correctly, the probe should be in contact with the sample with the laser beam directly hitting the sample.

Figure 58. The probe is directed at the sample and in direct contact with the sample.

A dark spectrum is measured to subtract the background noise from the final spectrum. Then the laser is powered on. With the laser beam hitting the sample, the Raman spectrum is measured, and an image of the sample is taken. This test takes only a few seconds. The laser can be powered off after this spectral results are provided by the GUI. For example, below is the Raman spectrum of the purple gem, as well as results from the

GUI stating that the sample measured is the purple gem. Thus, the system has correctly identified the sample. This process was repeated for diamond and Asha simulant diamond.

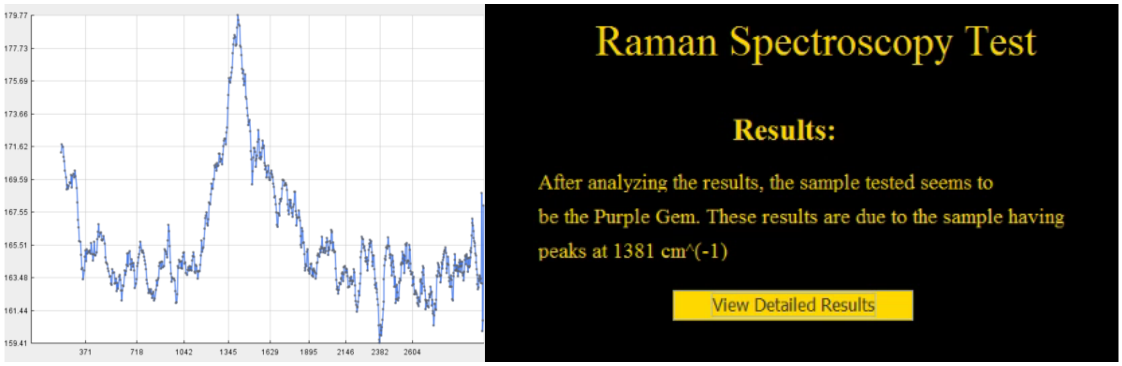


Figure 59. (Left) Measured Raman spectrum of the purple gem sample; (Right) GUI results stating the sample measured is the purple gem.

7.6 Safety Cautions

Laser Safety
This product contains a Class IIIa laser, and as such is considered dangerous without taking the incorrect handling procedures. Never look directly at the laser, or aim the laser at an unintended target, as this can cause health and safety risks.

LED Safety
This product contains several LED’s used to illuminate subject material, or indicate the status of the laser in the probe system. None of the LED’s should not be stared at directly, and can cause eyesight problems if proper safety precautions are not taken.

Electrical Hazard Safety
This product uses wall output power to provide the needed electrical connection to all of the components. Use proper handling procedures when handling all wires in the system. Including but not limited to: System power connection, Raman probe connection, and any component connections. Any frayed/exposed wires are to be considered unsafe until a technician is able to verify they are safe.

Temperature Safety
This product contains several components that are able to build up large amounts of heat and these components are kept in relatively tight quarters. Therefore, before touching any piece of the system, test the temperature by quickly touching the surface before grabbing it. This will allow you to get a quick idea of the overall temperature of the system you are picking up.

If the temperature is not safe to the touch at any part of the system (probe, controller encasing, etc.), please consider powering off the device at a natural stopping point. Do not attempt to continue to use until the system has returned to standard operating temperature.

7.7 Product Assembly/Disassembly and Power On/Off

Assembly

1. Set the spectroscopy system on a flat surface.
2. Plug the Raman probe into the spectrometer using the connector. The connector will only fit one way, so be sure to not force the connector in.
3. Attach the laser fiber connection to the Raman probe.
4. Plug the necessary components into the microcontroller (display and input device).
5. Once the Raman system is assembled, power can safely be attached.

Power Up

1. Press the power button into the "on" position.
2. The internal microcontroller and spectrometer will power on with no input from the user.
3. Flip the on/off switch on the laser to the on position.
4. The Raman probe will show no signs of power until the user begins the test. When the test begins, the user will have time with the camera targeting system before the laser actually fires.

Power Off

1. There is no native "Power off" functionality on the spectrometer. This device will power off by pressing the power button into the "off" position and unplugging the power cord.
2. When you are ready to power off the microcontroller, there will be another program available on the desktop called shutdown that will run a pseudo command to power off the Raspberry Pi.
3. Flip the on/off switch on the laser to the off position.

Disassembly

1. Remove the power connection from the system.
2. With the power connection removed, it is now safe to remove the Raman probe connection.
3. Remove the necessary components from the microcontroller.
4. Place the components of the Raman system in a safe place, with little humidity and temperature fluctuation. Preferably in a static free container.

7.8 Testing Procedure Walkthrough

The testing procedure steps outlined below should be completed in order to ensure the system receives all of the data it needs.

1. Please ensure all devices are connected and powered on. The provided power brick should be plugged into the Raman system, and the spectrometer probe

should be plugged into the Raman system also. The power button on the Raman system should be pressed, powering on the embedded microcontroller. Running the LASER.jar file will begin the program.

2. If you are a new user, please select the clickable "Instructions" button as shown. This screen will provide new users with a step-by-step tutorial on how to use the product, which will include how-to videos.

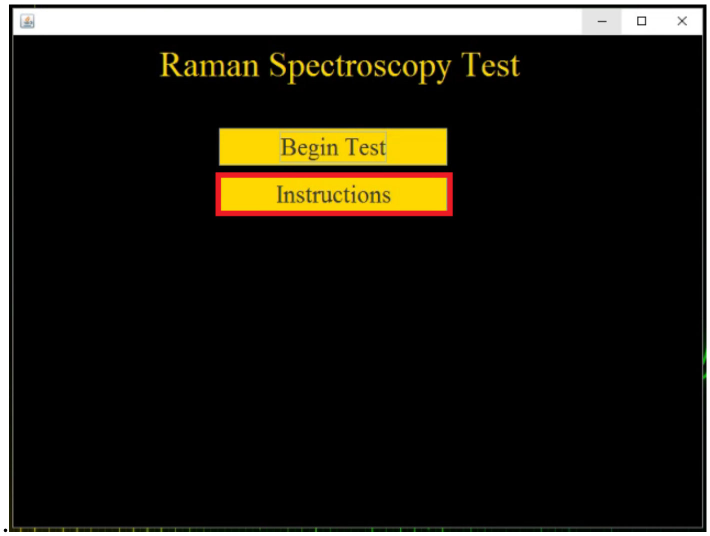


Figure 60. Instructions

3. If you are an experienced user, please select the clickable "Begin Test" button which will begin the necessary on-screen prompts.

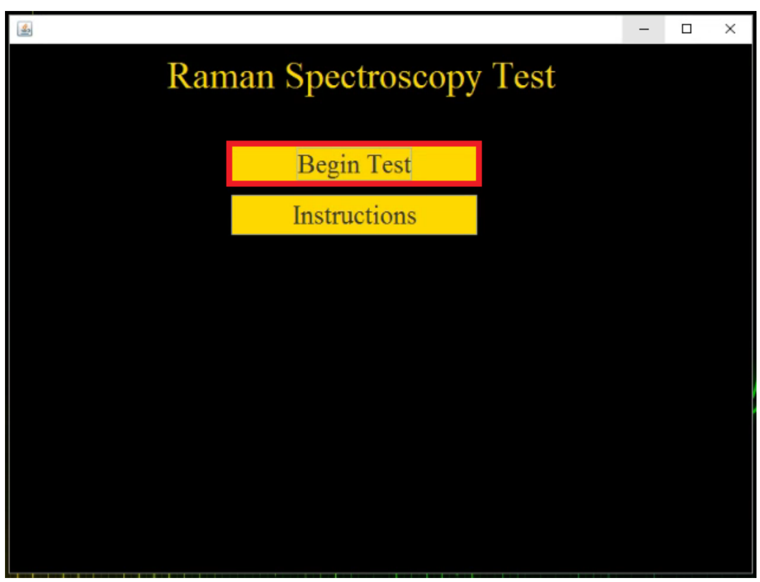


Figure 61. Begin Test

4. The first screen will ask for the user to hold the probe on the testing area, without

the laser powered on. This measurement provides the system with necessary data for interpretation for later.

5. The second screen will ask for the user to power on the laser, and hold the probe over the testing area. The measurement here provides the system with the Raman emission spectra which will be interpreted. Please use the camera system to ensure the laser is targeting the proper area.

   PLEASE NOTE- For all scans use the camera attached to the probe as a secondary viewfinder to ensure that the intended area is indeed the targeted area.

6. Once all scans have been completed, please allow between 30 seconds to 1 minute for all data to be processed as necessary.
7. Once all data has been processed, a results page will appear indicating the systems results. Four possible results are to be expected:
   a. After analyzing the results, the sample you tested seems to contain one of the following: Asha Simulant Diamond, Diamond, or Purple Gem, Each has its own landing screen which will instruct the user as to which gem the system beleives it is.

      -or-

   b. After analyzing the results, the sample you tested seems to have no maximums consistent with known data values.
8. You can then select to view the detailed results by selecting the "View Detailed Results" button on the same page.

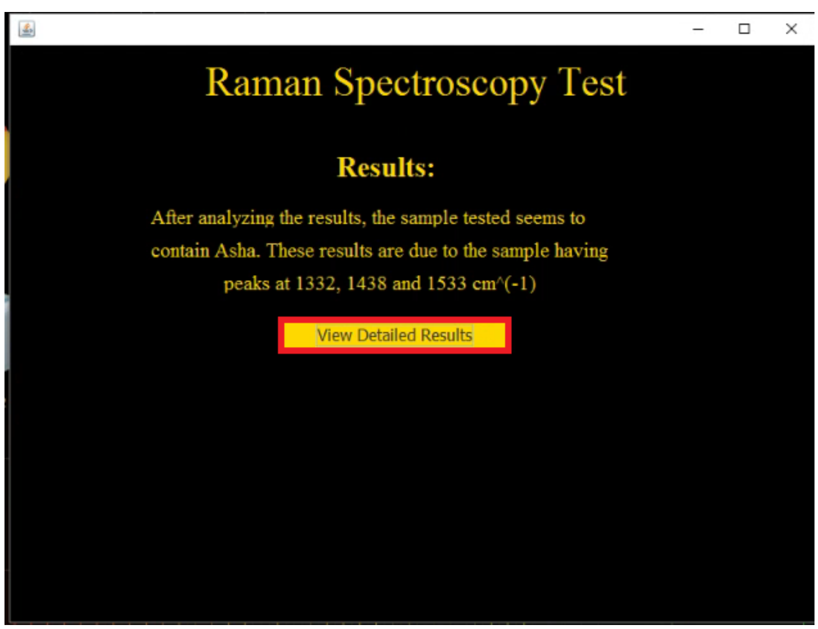


Figure 62. Results

9. If you have selected to view the detailed results, a graph will appear showing your emission spectra graph. If the data is not consistent with known peaks, this graph can be compared to databases of Raman Emission spectra for further analysis.

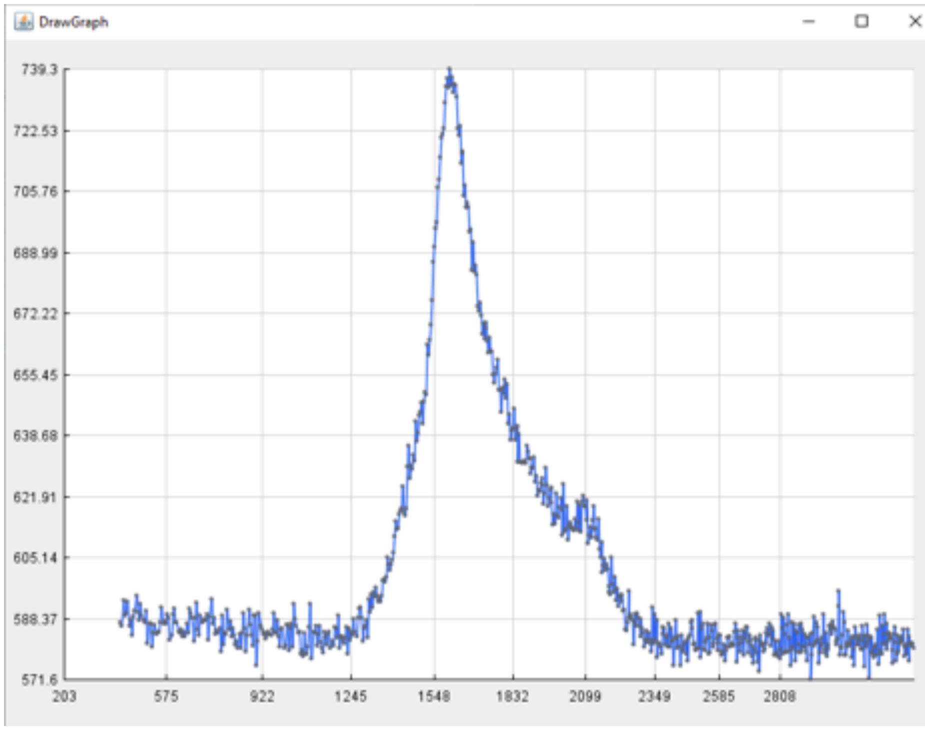


Figure 63. Detailed Results

7.8.1 Quick Questions and Answers

1. **Question**: I have begun the testing process, and the screen is on, but nothing is happening. How do I know the device is working?

   Answer: The program should dictate which steps are currently in progress, but it is possible the device is out of sync. If your test says the laser is firing, please verify the red LED is illuminated. If these two do not match, please close the program and begin the test again.

2. **Question:** What components will I need to provide to be able to use this system?

   Answer: The only external components you will need are a HDMI enabled display device for the Raspberry Pi, and a USB keyboard and mouse The external computer system needs to be Intel x86 based with Java installed..

3. **Question:** Is the laser safe? And if so, how long can I scan before it becomes unsafe?

Answer: The laser is low-power unit and is safe. A safe exposure time is less than 10 minutes in the same day. It is not advisable use more than once a week on the same skin sample.

4. **Question:** Do I need to prepare the test area before the test?

   Answer: It is advisable to have the test area free of hair, and we recommend preparing the area with an Isopropyl Alcohol pad before use to ensure no foreign substances are introduced to the test.

5. **Question:** How long will the test take?

   Answer: The testing process should take at most 5-10 minutes, with only 2-3 minutes being allotted for the actual Raman spectroscopy section. Increased exposure is considered unsafe, and therefore this time has been minimized with your safety in mind.

6. **Question:** How reliable is the test?

   Answer: The test is considered to be 90% accurate at determining the Raman spectrum of the analyzed skin sample. The determination of results are at best to be considered 75% accurate. These results can be interpreted by a medical professional, or by comparison to changes in the Raman spectrum over time. One test is **NOT TO BE** considered conclusive evidence of cancerous tissue.

7. **Question:** If I control the laser with the program, why is there a switch on the device also?

   Answer: The switch on the device is what the industry refers to as a kill switch. So long as the switch is activated, the test is allowed to function as intended. However, if the button is released, it is within reason that the testing procedure has been compromised. It will kill the operation of the test and notify you as such.

8 Administrative Content

The Administrative Content includes a look at the financial planning and schedule for the project, as well as plans for crucial parts of Senior Design 2, the final demonstration of the device.

8.1 Project Budget and Financing

The initial estimate for this project was approximately $915.00. After conducting further research, the new estimate for this project is approximately $1,465.00, of which the majority of the cost stems from the optical components. This value would cost less than the average price point of five biopsies. The breakdown of this budget is shown in the table below.

Project Budget		
Item Description	**Estimated Cost ($)**	**Actual Cost ($)**
Microcontroller	$40.00	Owned
Wire and Encasing	$30.00	$60.94
3D Printing Filament/Printing	$175.00	$30.00
Laser	$3,000.00	Donated
Spectrometer	$3,393.00	Donated
Probe Parts	$900	$466.27
PCBs	$60.00	$50.23
Total Estimated Cost	**$7,658**	**$607.44**

Table 21. Project Budget

Ocean Optics provided a laser source, spectrometer, and a commercial probe for testing comparisons for the project.

Currently the team is working with Ocean Optics to develop objectives, outcomes, and deliverables of L.A.S.E.R.S. due to them graciously providing advising, funding, and donations. With the most expensive aspect of the system being the spectrometer, and laser, it has been essential in allowing the team to focus on the user interface pieces, such as the overall design, the Raman probe, and the programming aspects which will differentiate the delivered product from those currently available.

In return for Ocean Optics giving the team this advising, funding, and donations the team is to implement a live-action camera module with specialized functionality over the current offerings from Ocean Optics. This specialized product will provide Ocean Optics with a product that can be expanded to their entire spectrometer lineup.

8.2 Project Milestones

Milestone	Milestone Date
Divide and Conquer V.1	02/03/2017
Overall design finalized	Mid February 2017
Divide and Conquer V.2	02/17/2017
Parts and materials finalized	Mid March 2017
Initial draft of documentation	03/31/2017
Sponsors contacted	March 2017
CAD, optics system, PCB, pseudocode, user interface design	Late March 2017
Vendors Contacted	March-April 2017
Parts ordered	April-May 2017
120 Page Final Document	04/27/2017
Begin building	Late April-May 2017
Conference paper	June 2017
Midterm demo	Late June 2017
Final presentation and demo	July 26, 2017

Table 22. Project Milestones

8.3 Demonstration Plans

This project was proven a success during the final project demonstration. The demo showed that the elements of the system worked by proving it can illuminate and measure a sample and accurately analyze the resulting spectra. It accurately identified the Asha simulant diamond and purple gem, while also achieving the optical, computer, and electrical specifications.

8.4 Monthly Progress

January 2017

Team assembled first week of classes. Per instruction, we were asked to form several ideas that we could use for the project. The team introduced several projects upon meeting with one another. After a week of discussing and narrowing down possible test results, the team was left with two feasible projects. After determining the tooling and access to standards required to modify a current generation car system would be too intricate, and require more knowledge of mechanical engineering, the team decided on the Raman spectrometer design.

The same day the initial project idea was decided upon, the team immediately began the research process. The team spent the rest of the month considering what would be needed to build a Raman spectrometer for the specific application of detecting skin cancer. Taking into consideration the budget, technical knowledge, and obstacles that would be faced in attempting this project, the team decided this project would incorporate the skills of each team member individually, but complement the team as a unit.

Teams were formed to begin focusing of such research included studies of the Raman shift and changes in skin and Raman spectroscopy. The use of sensors in biomedicine, selectivity and specialty, statistical and spectral analysis, and related technologies were also important points of study. The goal at this point was to design and build a Raman spectrometer from scratch.

February 2017

First document created to plan project scope. The team narrowed down specific parts of interest and spent time individually learning about potential parts related to their field of specialty.

The team members attended the Senior Design Bootcamp to learn tools to be successful in Senior Design. At the Bootcamp, topics covered were actions to take and not to take to be successful, the hopes and fears of the team members, core values of the team, and positive and negative team behaviors. After getting to learn more about each other, the team discussed our true problem statement, brainstorming ideas to solve the problem, including some sketches of the ideas. The team then evaluated their individual strengths, weaknesses, personal constraints, and expected contributions.

Together, the overall design as well as all considerations and specifications were settled. During this time, progress was made on the 60-page document and ABET quizzes.

March 2017

March consisted of finalizing the 60-page document, ABET quizzes, and making final selections for parts to be used in the L.A.S.E.R.S. device. All major elements of the device were settled during this month. The biggest challenge was acquiring a laser to meet the team’s needs that was also affordable. While an ideal laser was not found, ones that could still meet design specifications were accessible. During this time, contact was made with Optigrate and Ocean Optics to discuss any feedback or guidance they could offer for the design process.

The team's focus was placed especially on finding relevant standards. Major device components such as microcontrollers, lens design, sensor type, and casing ideas were focused on while writing the 60 page paper and while planning the overall device design. As more details were discovered about major component parts, it became easier to write about and understand functionality testing and any hurdles that could occur when using device components in a practical sense in comparison with a simulated design.

April 2017

April was, without a doubt, the most stressful and exhilarating month of Senior Design 1. The team met with Dr. Hagen and Dr. Wei to discuss modifications needed to better the design document. With their advice, the team immediately set off to better specify what exactly the focus of the project was aiming for and specifics of what would be studied.

A meeting with Ocean Optics led not only to very realistic project deadline discussions and a design challenge, but also a sponsorship. With this, the physical goals of the device changed, but the overall goal remained the same. While this led to some scrapping of previously designed parts, including some that previously were the key components, this allowed the team to focus on a more feasible goal that would also help meet time and economic constraints.

The first task was to redesign the circuit configuration; the original circuit was not going to function properly, and the added components that Ocean Optics requested would cause issues to the initial circuit. That being said, the next week (April 7th - 14th) was spent mostly reconstructing a circuit design on Multisim that met the requirements. The following week, after confirming the Multisim schematic, the next course of action was to quickly go into the Senior Design Lab to test the stages of the circuit (April 15th - 20th). After several hours in the lab, the team got a functioning breadboard design that matched the Multisim outputs.

With limited time to modify existing project plans and the final Senior Design 1 paper deadline approaching, the team focused on their areas of interest and were able to modify and in some cases, completely redesign parts of the system while still meeting the deadlines of the course.

May 2017

By the end of May, the team began prototyping the design aspects of the project. The first PCB was designed this month, which had a bit of a learning curve since Eagle was new software for the team. Once Eagle became more familiar, the easier the process became. Basic implementations of a working Raman system were assembled from the parts given to the team by Ocean Optics, such as using their donated probe with the spectrometer to test the spectral analysis. The probe design was prototyped, and all optics were further fine-tuned in the optics lab to ensure precision. Software control of the Raman system was implemented using the OmniDriver software, to be able to provide the team with the necessary controls to test the functionality of the system.

At this time, individual component testing began. This process should encapsulate the reliability of each individual system, as well as that each section adheres to the relevant standards and constraints outlined in the paper. The process of creating the critical design review began.

June 2017

During the month of June, the final stages of prototyping began. All individual systems should be assembled so that we have a fully functioning spectrometer design, using the provided spectrometer and laser from Ocean Optics, and all other components should be those designed by the team as per the scope of this project. Working hardware control began to be implemented into the graphical user interface.

Peer reviews began around halfway into June. Listening to the feedback of colleagues provided the team with insight into what changes can be made to iron out any concerns some may have with the product.

The first version of the main PCB A had some trouble, so the team began troubleshooting and was able to immediately figure out all the problems and design a new one. A strong understanding of the circuit helped reduce the amount of turn around time needed to get a new PCB in. This month also revolved around the painstaking process of perfecting the optical arrangement in order to best capture the Raman signal.

Safety and reliability testing began during this month. Using the testing methodologies outlined, these tests should place strain on the system to ensure that during normal use the system maintains reliability and safety. Any adjustments to the final individual components needed to be addressed this month or else the team would risk not finishing on time.

July 2017

Being the final month of the project timeline, July was a very hectic and rewarding month. By this time, a fully working Raman spectroscopy system was successfully implemented, and should be fully controllable via the final rendition of the graphical user interface. Testing for safety and reliability were completed for every individual part as well as all components interconnected in the completed device.

By the last couple of weeks of the semester everything came together. All systems worked about as perfect as they could separately and the integration process went surprisingly smooth for this reason. Once everything was connected and placed in their encasing, the device worked as the team hoped immediately.

The large step for July was subjecting the device to a professional review panel made up of UCF faculty. The team's final review took place on July 26 with no issues or delays in the demo. The team received positive feedback and the faculty seemed to enjoy the demo process, which even left spare time to test the jewelry that faculty members happened to have.

9 Project Summary and Conclusions

The project described within these pages is a Raman spectroscopy system with a probe capable of in vivo analysis of skin. The final functionality test for Senior Design was if the spectroscopy system could identify multiple types of gems in jewelry and tell the difference between multiple objects. The purpose of this project is to develop a working instrument capable of identifying cancerous traits in the spectra of skin. Research was conducted on all aspects of the project from basic theory to design and applications. There are multiple disciplines that needed to be researched and analyzed, from Raman spectroscopy, spectroscopy of human skin and pig skin, spectral analysis of various gemstones, to electronic components, PCB configurations and software, and microcontroller functions. This was necessary to ensure that the project in the end would have a fully functioning spectroscopy system that can detect and analyze a particular sample at a high success rate.

At the end stage for the L.A.S.E.R.S. project, the team obtained all of the components needed to prototype and build the team's full spectroscopy system. Two PCBs were implemented successfully, one optical mount was designed, two encasings were made using different methods, and a microcontroller interfaced with a fan and camera. With the spectrometer, 785 nm laser, probe, and software functionality received from the project sponsor, Ocean Optics, all unattainable components were received to test the probe design that was implemented to complete the spectroscopy system.

In regards to the electronics design of the project, it was imperative that the design complies with the standards found on the various components, as well meets the size, time, power, and safety constraints and standards discussed in this document. The design layout was made specifically to eliminate risk of component fallibility by enlisting a modular system with PCBs dedicated to specific tasks. The CMOS camera module provides live footage of the sample being tested, while the LED beam improves visibility. The main PCB is used for power control and distribution amongst the other components in the system, as well as to allow for safety features for the device.

Once the electronics design was compliant with all its standards, the next step was to ensure that the hardware design and encasing of the laser probe was compliant with safety standards. The overall material shape and design of the probe would be configured to handle the heat constraints, as well as allow the probe to function without damage. The other important characteristic that had to be considered was the design's ergonomics; its ease of use to the consumer. After finalizing the design, the next step was to speak to Ocean Optics to comply with their requirements in having a CMOS camera attached to the probe, which would fit comfortably at the face of the handheld device. The main body casing securely held all other major device elements.

In regards to the optics involved for Raman spectroscopy, guidance from faculty and rigorous research helped to define the needs and specifications of this project. Laser excitation was delivered to the probe via an optical fiber. With proper coupling using plano-convex lenses, the laser light of 55 mW was focused onto the sample at an appropriate working distance equal to the focal length of the focusing lens. The resultant Raman scattering from the sample was recollected by this lens and redirected through the probe by a dichroic beamsplitter and mirror. The intensity of the laser line at 785 nm was reduced using a longpass filter meant to pass the Raman wavelengths. Finally, another lens successfully coupled the collected light into an optical fiber that delivered the Raman scattering to a spectrometer. It was ensured that all of the optical components were coated for high performance in the near-infrared wavelengths. The optics successfully captured and detected Raman signals from the gemstone samples.

For the software design, the overall design will be intended to be easy to user-friendly, fast, and in control of the electronics at all times. The graphical user interface design will be the visual representation of the product to the user, and what will give the user their results. A professional and concise GUI is necessary to convey a professional yet intuitive design. Also, the algorithm for data collection will need to adhere to the proper testing protocols outlined in the project. Total runtime should be reasonable for the user, to reduce the possibility of harm and the ease of results. Lastly, the software will have full control over the operation of the spectrometer, and all componentry of the electronics. These devices will need to be orchestrated to provide the experience users should have with the product. When this device control fails, data integrity is forfeited, safety concerns arise, or ease of use will be diminished, which can in turn reduce the customer's faith in the product.

Once the prototypes for the hardware, electronics, and software design were completed, the combination of all elements, hardware and software, were combined and tested. Testing was completed using all standards outlined, including IEEE standards, health and safety standards, as well as peer review. The project was fully constructed and functional by the demonstration deadline in July.

This project is a prototype and has room for future improvements. Improvements include using non-linear voltage regulators to step-down the voltage in the PCB. Another is for Ocean Optics to allow for Omnidriver compatibility with ARM-based processors to allow for one-system functionality. Adding a crosshair to the camera feed will more easily indicate to the user where the laser is focused. The probe can be redesigned to be smaller, more ergonomic, and have a more intuitive way of indicating how to target a sample. The casing of the Main Box can be redesigned to be more efficient and aesthetically pleasing.

10 References

1. Gniadecka, M., H. C. Wulf, N. Nymark Mortensen, O. Faurskov Nielsen, and D. H. Christensen. "Diagnosis of Basal Cell Carcinoma by Raman Spectroscopy." *Journal of Raman Spectroscopy* 28.23 (1997): 125-29.
2. Schut, Tom C. Bakker, Peter J. Caspers, Gerwin J. Puppels, Annieke Nijssen, Freerk Heule, Martino H.a. Neumann, and Donal P. Hayes. "Discriminating Basal Cell Carcinoma from its Surrounding Tissue by Raman Spectroscopy." *Journal of Investigative Dermatology* 119.1 (2002): 64-69.
3. Silveira, Landulfo, Fabrício Luiz Silveira, Benito Bodanese, Renato Amaro Zângaro, and Marcos Tadeu T. Pacheco. "Discriminating model for diagnosis of basal cell carcinoma and melanoma in vitro based on the Raman spectra of selected biochemicals." *Journal of Biomedical Optics* 17.7 (2012): 077003.
4. Eikje, Natalja Skrebova, Katsuo Aizawa, Takayuki Sota, Yukihiro Ozaki, and Seiji Arase. "Identification and Characterization of Skin Biomolecules for Drug Targeting and Monitoring by Vibrational Spectroscopy." *The Open Medicinal Chemistry Journal* 2.1 (2008): 38-48.
5. Zhao, Jianhua, Harvey Lui, David I. Mclean, and Haishan Zeng. "Real-time raman spectroscopy for non-invasive skin cancer detection - preliminary results." *2008 30th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*(2008): n. pag.
6. Kourkoumelis, Nikolaos, Ioannis Balatsoukas, Violetta Moulia, Aspasia Elka, Georgios Gaitanis, and Ioannis Bassukas. "Advances in the in Vivo Raman Spectroscopy of Malignant Skin Tumors Using Portable Instrumentation." *International Journal of Molecular Sciences* 16.7 (2015): 14554-4570.
7. Hendi, Ali, and Juan Carlos. Martinez. *Atlas of skin cancers: practical guide to diagnosis and treatment*. Heidelberg: Springer, 2011. Springer.
8. Patterson, James W. "An Approach to the Interpretation of Skin Biopsies." *Weedon's Skin Pathology*. 4th ed. N.p.: Elsevier Limited, 2016. 3-18.
9. Lewis, Ian R., and Howell G. M. Edwards. *Handbook of Raman Spectroscopy from the Research Laboratory to the Process Line*. New York: Marcel Dekker, 2001.
10. McGarvey, J. J., and J. Renwick Beattie. "Raman Microscopy: A Versatile Approach to Bio-Imaging." *Raman Imaging: Techniques and Applications*. Vol. 168. N.p.: Springer, 2012. 219-42. Springer Ser. in Optical Sciences.
11. Butler, Holly J., Lorna Ashton, Benjamin Bird, Gianfelice Cinque, Kelly Curtis, Jennifer Dorney, Karen Esmonde-White, Nigel J. Fullwood, Benjamin Gardner, Pierre L. Martin-Hirsch, Michael J. Walsh, Martin R. McAinsh, Nicholas Stone, and Francis L. Martin. "Using Raman Spectroscopy to Characterize Biological Materials." *Nature Protocols* 11.4 (2016): 664-87. *Nature*. 10 Mar. 2016.
12. Dr. Weibo Cai, "Engineering Multivalent and Multispecific Protein Therapeutics." *Engineering in Translational Medicine* 14.3 379. (2014)
13. "Sensitivity and Specificity." Sensitivity and Specificity. N.p., n.d.
14. Schoonjans, Frank. "ROC curve analysis with MedCalc." *MedCalc. MedCalc Software*, 31 Jan. 2017.

15. Krafft, C., Schie, I. W., Meyer, T., Schmitt, M., & Popp, J. (2016). Developments in spontaneous and coherent Raman scattering microscopic imaging for biomedical applications. *Chemical Society Reviews, 45*(7), 1819-1849. doi:10.1039/c5cs00564g
16. G. Köklü, J. Ghaye, R. Beuchat, G. De Micheli, Y. Leblebici and S. Carrara, "Quantitative comparison of commercial CCD and custom-designed CMOS camera for biological applications," *2012 IEEE International Symposium on Circuits and Systems*, Seoul, 2012, pp. 2063-2066. doi: 10.1109/ISCAS.2012.6271688
17. Wodehouse, Carey. "Basics of Printed Circuit Board (PCB) Design." Hiring | Upwork. N.p., 10 Jan. 2017.
18. "Printed Circuit Board." How Products Are Made. N.p., n.d. Web. 28 Mar. 2017.
19. "The Basics Of PCB Design: Components & Construction." PCB Train Blog. N.p., 16 Feb. 2017.
20. "What is SMT Surface Mount Technology - Tutorial." What is SMT | Surface Mount Technology | Devices SMD | Radio-Electronics.com. N.p., n.d. Web. 29 Mar. 2017.
21. "Where Electronics Begins™." *About the EDA Industry | The Electronic System Design Alliance*. N.p., n.d.
22. "PCB Manufacturing Process-How to make PCB, Fabrication, Design, Guidelines." *Electronic Circuits and Diagram-Electronics Projects and Design*. N.p., 21 Nov. 2011.
23. Cha, Sung-Hyuk. "Comprehensive Survey on Distance/Similarity Measures between Probability Density Functions." *INTERNATIONAL JOURNAL OF MATHEMATICAL MODELS AND METHODS IN APPLIED SCIENCES* 2007th ser. 1.4 (2007): 300-07.*Users.uom.gr*.
24. *IEEE Recommended Practice-General Principles for Temperature Limits in the Rating of Electrical Equipment and for the Evaluation of Electrical Insulation*, IEEE Standard 1-2000, 2005.
25. *IEEE Standard for Terminology and Test methods for Analog-to-Digital Converters*, IEEE Standard 1241-2010, 2011.
26. Seshasayee, Nikhil. "Understanding Thermal Dissipation and Design of a Heatsink." (n.d.): n. pag. *Www.ti.com/lit/an/slva462/slva462.pdf*. Texas Instruments, May 2011.
27. Jul 17, 2013 Nicholaus Smith | Electronic Design. "The Engineer's Guide To High-Quality PCB Design." *Embedded content from Electronic Design*. N.p., n.d.
28. Motz, Jason T., Martin Hunter, Luis H. Galindo, Joseph A. Gardecki, John R. Kramer, Ramachandra R. Dasari, and Michael S. Feld. "Optical Fiber Probe for Biomedical Raman Spectroscopy." Applied Optics 43.3 (2004): 542.
29. Grieser, Franz. "PLA vs ABS: Filaments for 3D Printing Explained & Compared." *All3DP*. N.p., 14 Jan. 2016.
30. Toshiba, "TOSHIBA CCD LINEAR IMAGE SENSOR CCD(Charge Coupled Device)," TCD1304AP datasheet, Oct. 2001.
31. Vardaxis, N. J., T. A. Brans, M. E. Boon, R. W. Kreis, and L. M. Marres. "Confocal laser scanning microscopy of porcine skin: implications for human

wound healing studies." *Journal of Anatomy* 190.4 (1997): 601-11.

32. Keiser, Gerd. *Biophotonics: Concepts to Applications*. Singapore: Springer Science Business Media, 2016.
33. Raspberry Pi Foundation. "Camera Module." *Camera Module - Raspberry Pi Documentation*. Web. 11 Apr. 2017. <https://www.raspberrypi.org/documentation/hardware/camera/>.
34. Sony. "IMX219PQ." *Sony Semiconductor Solutions Corporation*. Web. 8 Apr. 2017. <http://www.sony-semicon.co.jp/products_en/new_pro/april_2014/imx219_e.html>.
35. Raspberry Pi Foundation, "Raspberry Pi Camera Module" Raspberry Pi Camera Module V2 datasheet. <http://cdn.sparkfun.com/datasheets/Dev/RaspberryPi/RPiCamMod2.pdf>
36. Dodd, S. "Roadside Noise Reduction – the French Approach." *Noise Notes* 1.1 (2002): 3-4. *Www.owlnet.rice.edu*. Rice University. Web.
37. Marie-Sainte, Souad Larabi. "Detection and Visualization of Non-linear Structures in Large Datasets Using Exploratory Projection Pursuit Laboratory (EPP-Lab) Software Souad Larabi Marie-Sainte." *Detection and Visualization of Non-linear Structures in Large Datasets Using Exploratory Projection Pursuit Laboratory (EPP-Lab) Software*. King Saud University, 1 Jan. 2017. Web. 25 Apr. 2017.
38. "Smoothing." *Smoothing*. WaveMetrics, Inc., n.d. Web. 25 Apr. 2017. <https://www.wavemetrics.com/products/igorpro/dataanalysis/signalprocessing/smoothing.htm>.
39. Schafer, Ronald W. "What Is a Savitzky-Golay Filter?" *IEEE Signal Processing Magazine*2011th ser. July (2011): 111-17. 15 June 2011. Web. 25 Apr. 2017.
40. Fairchild Semiconductor Corporation, "LM78XX / LM78XXA 3-Terminal 1 A Positive Voltage Regulator," LM78XX datasheet, Sept. 2014.
41. Super Bright LEDS, "5mm LED RL5-XXXXX Specifications," RL5-XXXXX Datasheets, Oct. 2009.
42. HDK, "Tactile Switches," Top Push Button Datasheet, May 2007.
43. Sony, "2048-pixel CCD Linear Sensor (B/W) for Single 5V Power Supply Bar-code Reader ILX511B," ILX511B datasheet, Dec. 2003.
44. Ocean Optics, "USB2000+ Data Sheet," USB 2000+ Spectrometer datasheet, May 2016.

11 Appendices

1. Email correspondence with the UCF Institutional Review Board.

Institutional Review Board March 15, 2017 at 8:39 AM
To: mmelvin Details

IB

TO: Megan Melvin

Thank you for contacting the IRB. Please visit our website at http://www.research.ucf.edu/Compliance/IRB/Submissions/index.html. This site has all the information about getting started, creating an iRIS account, and CITI training, as well as submitting to our iRIS system.

The forms can be found on the following webpage : http://www.research.ucf.edu/Compliance/IRB/Investigators/forms.html. Based on your description below, you would need to fill out the HRP-503 - Protocol and HRP-502 either a or b depending on your population.

Please work with your faculty advisor to complete the documents and submit.

If you have any further questions, please let me know.

Regards,

Gillian Morien
IRB Coordinator
Office of Research and Commercialization
University of Central Florida
407-823-2508
Gillian.morien@ucf.edu

See More from mmelvin

Found in Sent – knights.ucf.edu Mailbox

Megan Melvin March 22, 2017 at 9:35 PM
To: Institutional Review Board Details

MM

Hi Gillian,

What forms, if any, will need to be submitted if we only use skin samples/biopsies instead of live human subjects for our study?

Thank you,
Megan Melvin

See More from Institutional Review Board

Institutional Review Board March 23, 2017 at 8:07 AM

To: mmelvin Details

IB

To: Megan Melvin

You will need to complete the IRB protocol - HRP-503. If you plan to have interaction with human subjects to get the samples, you will need to complete the HRP-502a Adult Consent. If you cannot consent the participants, you will need to justify why in the protocol.

Regards,

Gillian Morien
IRB Coordinator
Office of Research and Commercialization
University of Central Florida
407-823-2508
Gillian.morien@ucf.edu

See More from mmelvin

Found in Sent - knights.ucf.edu Mailbox

☆ **Megan Melvin** March 23, 2017 at 10:55 AM

To: Institutional Review Board Details

MM

Hi Gillian,

Is there a separate IRB for using samples of dead animals?

Our Senior Design project is already a 120 page paper, so we're trying to figure out our options to tweak our project in order to make things easier on ourselves.

Thank you for your quick responses.

-Megan Melvin

See More from Institutional Review Board

RE: Senior Design Project Involving Human Subjects

Institutional Review Board March 23, 2017 at 10:58 AM

To: mmelvin Details

To: Megan Melvin

That would be handled by IACUC. Please feel free to email them for more information at iacuc@ucf.edu.
We work separately- people and animals.

Regards,

Gillian Morien
IRB Coordinator
Office of Research and Commercialization
University of Central Florida
407-823-2508
Gillian.morien@ucf.edu

See More from mmelvin

2. Email correspondence to Ocean Optics

Sweep Move to Categories ••• Undo

mmelvin <mmelvin@knights.ucf.edu> Reply all
Yesterday, 4:10 PM
Ty Olmstead; Steve Frey; espo92; chelseag; Michael Gonzalez; Carly Burleson <carly.burleson@oceanoptics.com>

Dr. Olmstead,

Great, thank you. We want to start specifying our design of the probe right away. Do you have a spec sheet for the spectrometer, or part number information so that we might find a spec sheet?

We would like to schedule a meeting during the week of May 8 to pick up the components and discuss our design plans.

Best,
Megan Melvin

Ty Olmstead <ty.olmstead@oceanoptics.com>
Yesterday, 3:32 PM

> Megan,
>
> Good Afternoon. I found a spectrometer for you. It is a USB style spectrometer It operates from 350 nm to 1050 nm. It should be ok for proof of principle. The system does have a 1 nm offset that will have to be accounted for. i.e. 546 nm reads 545 nm. You should be able to use OceanView with the software package with the spectrometer.
>
> Cheers,
>
> Ty

mmelvin <mmelvin@knights.ucf.edu>
Mon 4/24, 2:41 PM

Hello Mr. Frey and Dr. Olmstead,

Thank you so much for your updated parts information. This information is incredibly valuable for our paper. As we finalize our probe and software design, we were also wondering if we could get the part number for the spectrometer we are adding on to. Thank you for your help and support.

Regards,
Megan Melvin

TO Ty Olmstead <ty.olmstead@oceanoptics.com>
Mon 4/24, 1:24 PM

Multimode-Spectrum-St...
2 MB

Download Save to OneDrive - Knights - University of Central Florida

Attached you will find the manual for the laser. The laser is a 785 nm multimode laser. It is optically terminated with a FC/PC connection. We additionally have a raman probe: Inphotonics RPM785-C

...

M mmelvin <mmelvin@knights.ucf.edu>
Mon 4/24, 12:21 PM
Steve Frey; Ty Olmstead; espo92; chelseag; Michael Gonzalez; Carly Burleson <carly.burleson@oceanoptics.com>

Reply

Hi Mr. Frey,

Can you please provide an update on the surplus availability? Would it be possible to get spec sheets today?

Thank you,
Megan Melvin

...

SF Steven Frey <steven.frey@oceanoptics.com>
Mon 4/17, 7:51 PM

Megan,

I'm trying to get an answer on the hardware. There is potentially some perfect surplus that we can provide, but I need to verify that it is available. I will get you an answer this week. If it's not surplus, we will select something.

Regards,
Steve

Steven R. Frey

VP of Engineering & Technology

M mmelvin <mmelvin@knights.ucf.edu>
Mon 4/17, 12:42 PM

Good afternoon Mr. Frey,

Can you please provide an update on the equipment availability? Is it possible to get the design specs for them? We need specifics for our final paper due next Thursday, and it will help us to design the probe and electronics needed.

Thank you,
Megan Melvin

...

M mmelvin <mmelvin@knights.ucf.edu>
Wed 4/12, 12:22 PM

Mr. Frey,

We appreciate the efforts of you and your team to provide these components in a timely manner. We are excited to learn of the design specs and continue with the probe design head-on.

Regards,
Megan Melvin

...

SF Steven Frey <steven.frey@oceanoptics.com>
Tue 4/11, 3:55 PM
mmelvin; Ty Olmstead; espo92; chelseag; Michael Gonzalez; Carly Burleson <carly.burleson@oceanoptics.com>

Reply all

This message has a digital signature, but it wasn't verified because the S/MIME control isn't currently supported for your browser or platform.

Megan and team,

We met this afternoon. We think we have identified a probe and laser. I just need to make sure they are available. We will get you design specs, etc. as soon as we clear that they are available.

Regards,
Steve

Steven R. Frey