Raman Microscope Attachment

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ABSTRACT — **This project involves designing a microscope attachment that can create and detect a Raman signal for samples exhibiting characteristics of producing strong Raman scattering. Raman microscopy allow for chemists, forensic scientist, and engineers to characterize materials given a spectrum. The sections of this project includes optics to produce Raman excitation and a spectrometer to detect the Raman signal. Electronics will be integrated into the system to produce safety measures, temperature control, lighting control, and light signal detection. Along with electronics and optics, software is included to control the system and display system characteristics through a Graphical User Interface (GUI).**

Index Terms **— Bragg Gratings, CCD Image Sensors, Notch Filters, Raman Scattering, Software.**

I. INTRODUCTION

Spectroscopy is a branch of science that involves the creation and collection of a spectrum that acts as the "fingerprint" of a sample. Raman spectroscopy is a spectroscopic technique used to obtain a spectrum induced from molecular vibrations, which can be used for sample identification and quantification [1]. When a laser is incident on a sample, Rayleigh scattering or elastic scattering is a dominant process that is the same wavelength as the excitation source. However, inelastic scattering is of interest in Raman spectroscopy and is caused when the vibrational states of a molecule causes the scattered wavelength to shift from the excitation wavelength [2, 3]. A Raman spectrum shows the Stokes and Anti-stokes scattering or shifts from the excitation wavelength [2, 3]. The spectrum is plotted in intensity of the light signal vs. wavenumber (cm^{-3}).

A microscope is a convenient device used to observe a sample with high-resolution imaging. Furthermore, sending a laser light through the objective lens of the microscope allows for strong focusing of the laser light onto a sample. Utilizing the strong focusing of a microscope can be useful for Raman spectroscopy because a strong excitation signal can be created. Having a strong excitation signal allows for particular optical signatures to be detected that can serve for distinguishable sample characterization and identification. Our customer and sponsor, Dr. Matthieu Baudelet, wants a Raman spectroscopy setup to be built as an attachment for his microscope to detect Raman spectra.

The excitation section of the project includes the laser, volume bragg grating (VGBs), photodiode, and focusing optics. For the excitation component of the project, the laser power must be controlled and measured. In terms of safety, a beam blocker has to be utilized to block the laser beam whenever a sample is being placed onto the microscope sampling stage. Additionally, the laser needs have a safety switch to turn it off in case of emergency. Temperature sensors and fans will also be placed in the excitation section to give a temperature measurement and keep air following through the system respectively, thereby preventing a buildup of heat. The detection section is a spectrometer that involves using optics (filters, mirrors, lenses, and grating) to guide light to a detector. The detector is used in a circuit to convert the Raman light signal to an electric signal for a computer to display the results. An overall user interface should show the temperature of the system, the Raman spectrum, a camera feed of the sample, and the laser power. The user interface must also allow for laser power control.

II. RAMAN EXCITATION DESIGN

The goal of the excitation section of the project is to inject a laser beam into the microscope so that the microscope objective can tightly focus to a high irradiance the laser beam onto a sample. The focusing of the laser beam will produce the Raman scattering signal that a spectrometer can analyze. Fig. 1 below shows an optical schematic of the excitation section.

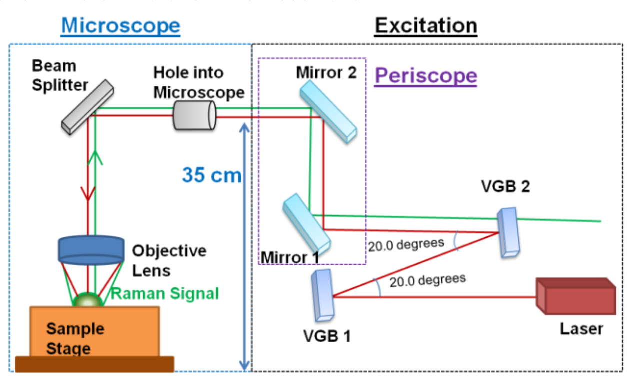

Fig. 1. Optical Schematic for Raman Excitation.

The laser used is a single mode 785 nm laser from the company Innovative Photonic Solutions and can output a beam that is already collimated. Included on the laser is an optical isolator that prevents light from being reflected back into the laser diode to cause damage, which can occur from reflective samples and optics. Out of the wavelengths used for Raman spectroscopy (532 nm, 785nm, and 1064 nm), the wavelength 785 nm is chosen for this project because it provides a middle ground for excitation efficiency, fluorescence, and heat absorbance [4]. The higher the excitation wavelength, the lower the excitation efficiency and fluorescence, and the higher the heat absorption. The equation below [5] shows that the power of the Raman signal is proportional to the intensity of the laser incident on a sample and is inversely proportional to the wavelength of the laser.

$$P_{scattered} \propto \frac{I_o}{\lambda^4} \quad (1)$$

In the optical setup, two Reflective Volume Bragg Gratings (VBGs) or Reflection Bragg Gratings (RBGs) are for spectral and spatial filtering of the laser beam. RBGs operate effectively by reflecting light of a particular wavelength at a particular incident angle. Once the RBG is at an angle that reflects the majority of the laser intensity, spectral and spatial filtering occurs. Spectral filtering can remove the Amplified Spontaneous Emission (ASE) that adds background to the laser spectrum, thereby narrowing the laser spectral profile to less than 5 cm^{-1} or 0.31 nm. In spatial filtering, undesirable noise or features in the laser beam intensity profile is cleaned to make it Gaussian. Having a laser beam with a Gaussian intensity allows for the microscope objective to tightly focus the laser onto a sample to create Raman scattering with a higher efficiency. The smaller the area the laser illuminates when being focused, the intensity of the laser increases and therefore increases the power of the scattering as described by equation (1). Furthermore, a laser having a spectral profile with a narrow line width will create a Raman signal with a narrow spectral profile that a spectrometer can detect. Once the laser beam reflects off the two RBGs, it is guided by two mirrors on a periscope into the microscope. A periscope is used to guide the laser beam to the hole in the microscope that is elevated by 35 cm.

The microscope is an Olympus BH2 with four objective lenses with magnification 4X, 10X, 20X, and 40X. The higher the magnification of the objective lens, the higher the numerical aperture and the shorter the focal length. Therefore, using an objective lens with a high magnification will allow for a more intense Raman signal to pass through the system since more light will be captured through the objective and the laser light is focused to a smaller area.

Fig. 2 below shows a Raman spectrum of a silicon sample taken from the Ocean Optics Spectrometer QE65000. Silicon has a strong peak that appears at around 514.5 cm^{-1} and the spectrum in Fig. 2 shows that the excitation design is capable of producing a Raman signal that is detectable by a spectrometer. The concept that the higher the magnification of the objective lens, the stronger the Raman signal is also demonstrated by Fig 2.

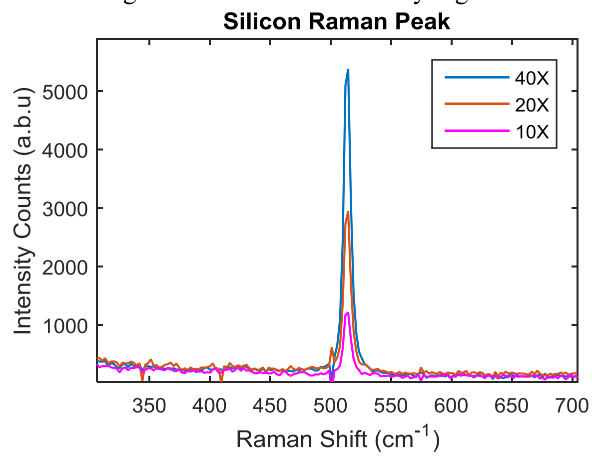

Fig. 2. Silicon Raman Peak for objective lenses 40X, 20X, and 10X.

Another observation with the objective lenses is that the higher the magnification, the less laser power will be passing through the objective lens. For instance, for an input power of 77 mW entering into the microscope, the laser power loss for the 40X, 20X, 10X, and 4X objective are 1.46 dB, 1.16 dB, 1.08 dB, and 0.60 dB.

The path of the Raman signal travels through a similar path as the excitation laser as shown in Fig 1 until it is incident on VBG 2. VBG 2 functions as a notch filter, which transmits Raman signals that have a wavelength that is not 785 nm. After the VBG 2, filters and optics will guide the Raman signal into the spectrometer.

III. Calibration

The spectrum obtained from the CCD requires calibration because the each pixel on the CCD is not assigned to a particular wavelength or wavenumber shift. Therefore, a spectrum is plotted in terms of intensity counts with respect to pixel number when calibration is not preformed. Since a fixed diffraction grating disperses a light signal into its wavelength components, different wavelengths will be incident on a certain pixel on the pixel array. By knowing the wavelength that will illuminate a particular location on the pixel array, a spectrum can be calibrated.

Fig 3. shows a spectrum taken from the CCD in our spectrometer design that can be used for calibration. An argon lamp is used because it has known peaks that show up within the designed spectral window. The laser signal is aligned so that it is incident around the center of the pixel array and this can be seen in Fig 3. By using the 785 nm laser signal as a reference, the peaks of Argon can be determined. Fig 3. shows the assigned wavelength for the argon peaks.

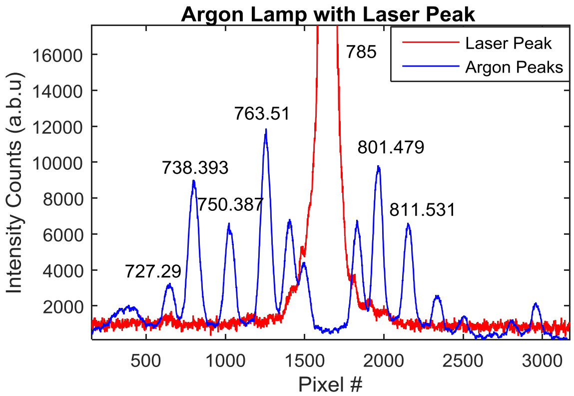

Fig. 3. Argon Lamp and Laser spectrum with assigned lines.

Once the wavelength of the Argon peaks are known in the spectrum, each wavelength can be assigned to its corresponding pixel location. By making a graph of wavelength vs. pixel and fitting the points to a linear or quadratic fit, this fitted function can be used to assign each pixel in the spectrum to a wavelength. After converting each pixel's wavelength value to Raman shift in cm^{-1}, a calibrated spectrum can be displayed that shows Intensity Count vs. Raman shift.

IV. Filtering

After the scattered light has been collected and transmitted from the excitation phase, it must be processed optically before it can be analyzed with a spectrometer. This scattered light has two main components: Rayleigh scattering and Raman scattering. The Rayleigh scattering, which has the same wavelength as the excitation laser, must be removed from the signal, because it is several orders of magnitude more intense than the Raman scattering. If it is not removed, it will saturate the spectrometer and make it impossible to analyze the signal.

The optical processing has four components: a collimator, a spectral filter, an aperture, and a pinhole. Each of these will be described in detail in the following sections.

A. Collimator

The light that is passed from the excitation phase is not coherent or collimated, with a very slight but noticeable divergence. This divergence must be eliminated in order for spectral filtering to work properly (this will be discussed in the next section).

The light is collimated using a simple two-lens telescope. The first lens is a diverging lens, with a focal length of 25 mm, while the second converging lens has a focal length of 50 mm. These lenses are placed so that their focal points overlap. The distance between the lenses, approximately 25 mm, is then experimentally manipulated until the beam is collimated. Both of the lenses have an AR coating to reduce reflection at the operating wavelength (785 nm).

B. Spectral Filtering

The main component of optical processing is the spectral filtering. The Rayleigh scattering, which is the same wavelength as the laser, must be reduced significantly in order for the spectrum to be analyzed. However, this must be done without reducing the intensity of the Raman scattering, which is already very weak.

Traditionally, this filtering is done with either a long pass filter or a notch filter. Most Raman spectrometers only measure the spectrum on one side of the excitation, so the filter is designed to have a sharp edge right after the laser. For low shift Raman, the notch must be very narrow in order to remove the Rayleigh scattering without touching the rest of the spectrum.

OptiGrate offers a Bragg Notch Filter (BNF) that is ultra-narrow. Instead of relying on carefully designed reflection/anti-reflection coatings, BNFs use Bragg gratings to remove very specific wavelengths. This is done by carefully angling the BNF so that the wavelength being filtered exactly matches the periodicity of the grating. This results in a very narrow bandwidth of less than 10 cm^{-1} and an Optical Density of at least 3. [optigrate website]

In order for the BNF to work properly, the light being filtered must be collimated. These BNFs are extremely angle-dependent, so any divergence of the beam greatly reduces the effectiveness of the filter.

C. Aperture

When the BNFs filter the Rayleigh scattering, most of it is removed via reflection. However, some is scattered during transmission. When this light is scattered, it will not be filtered by any other BNF. This reduces the effectiveness of the BNFs. However, this is easily countered using an aperture stop.

The aperture stop is an adjustable iris placed after the last BNF. A large aperture allows more Rayleigh scattering into the spectrometer, but a small aperture reduces the Raman signal. A middle ground is determined experimentally so that the Rayleigh scattering is reduced as much as possible while only slightly reducing the Raman signal.

D. Pinhole

A pinhole is necessary to increase the resolution of a spectrometer. Thus, the final stage of optical processing is to focus the signal into a pinhole so that it can enter into the spectrometer.

The pinhole we used is actually an optical fiber. The signal is focused into the fiber using a 25 mm focal length lens. The fiber then carries the signal to the spectrometer for analysis.

A fiber was chosen for several reasons. As already mentioned, a fiber has a narrow core, which acts as a small pinhole. Additionally, it allows the spectrometer to be built elsewhere on the platform, with the fiber linking the filtering phase to the spectrometer, which allows for more efficient conservation of space. However, one of the most important reasons a fiber is used is that it makes the entire system modular. Either end of the fiber can be connected to an auxiliary device. For example, much of our testing was done with an Ocean Optics spectrometer. Using a fiber allowed us to quickly switch between the two spectrometers without having to realign the entire system. Another example is that the input end of the fiber can be attached to an alternate input. Our calibration (which will be discussed in detail in a later section) was done with an Argon-Mercury lamp. An optical fiber allowed us to easily attach the lamp into our system, which allows for easy calibration.

V. SPECTROMETER

The spectrometer designed for this system is essentially a Czerny-Turner spectrometer. This type of spectrometer is one of the simplest to design, and is more than adequate for the purpose of Raman spectroscopy.

Czerny-Turner spectrometers consist of 5 major components: a pinhole, a collimating element, a grating, a focusing element, and a detector. Each of these components will be discussed in its own section, except for the pinhole, which has already been covered in the previous section.

A. Collimating

A traditional Czerny-Turner spectrometer uses a mirror to collimate the light from the pinhole. However, a lens was chosen for this spectrometer because it was easier to attach a lens to the pinhole fiber.

As a standard glass fiber, the pinhole has a numerical aperture of 0.22. A lens with a 50 mm focal length and 25.4 mm diameter was chosen to collimate the signal. As a result, the collimated light has a diameter of approximately 22 mm, which is then passed on to the grating.

B. Grating

The grating is generally what the spectrometer is built around, for several reasons. Primarily, it is the component that actually disperses the light, and is the main thing that makes the spectrometer work. However, the other major reason is that there is usually a small selection of gratings available. Thus, the grating is initially chosen, and the rest of the components are designed around the grating.

This spectrometer is designed to analyze a small spectral range with high resolution. In order to accomplish this, the grating chosen must very strongly disperse the signal. The most powerful grating readily available had 1200 lines/mm, which is adequate for our purposes.

Generally speaking, the grating should be as covered as possible without too much light falling outside the grating. Since the collimated light has a diameter of 22 mm, the best grating for this is a 25.4 mm square grating. However, our initial designs for this spectrometer used a 12.7 mm grating, which is what was purchased. At a later time, it might be beneficial to upgrade the grating to a larger size, however at this time it was not feasible to purchase the larger grating.

The grating is blazed at 750 nm. The blazing increases the efficiency of the grating by increasing the intensity of the first order of diffraction. Thus, more light reaches the detector, resulting in better detection.

C. Focusing

After the light is diffracted by the grating, it is collected by the focusing element onto the detector. The focal length of the mirror depends on the spectral range desired and the size of the detector. Initial calculations called for a mirror with a focal length of over 800 mm; a mirror with this focal length was difficult to find at a reasonable cost. The closest we could reasonably obtain was a mirror with a focal length of 500 mm. Using a shorter focal length results in a larger spectral range at the expense of a slight loss in resolution. This was a trade-off that was both acceptable and necessary.

In order for the spectrometer to work properly, the mirror must be large enough to collect the entire spectrum being analyzed. Even though the spectral range is relatively small, the grating has high dispersion, so a large mirror is required to collect the spectrum. Thus, a mirror with a diameter of 3 inches was chosen.

D. Detector

The detector is a linear array of light-sensitive sensors. The detector selected for the spectrometer is the Toshiba TCD1304AP CCD detector. This CCD has 3648 pixels, which is more than enough to ensure the resolution is limited by the optics of the spectrometer. Each pixel on the detector corresponds to a particular wavelength, so the reading of each pixel shows the intensity of the light at that wavelength.

One of the major issues encountered with this detector was the presence of thermal noise. At room temperature, the detector was reading significant amounts of noise even when the spectrometer was completely dark. The noise level was high enough that it prevented any accurate reading of the signal. In order to remove the noise, a thermoelectric controller (TEC) was attached to the CCD. Cooling the CCD reduced the noise level by almost an order of magnitude. Once the TEC was installed, the spectrums were easily distinguished from the noise level.

VI. OVERALL HARDWARE DESIGN

The hardware design of the Raman system is robust and allows full integration of multiple sub circuit systems for the overall design of the Raman system. The sub-systems are the following: Spectrometer CCD circuit system, laser power display and control, temperature sensing and control, class 1 laser blocking system, backlight control, and CCD cooling. To achieve all these requirements, 2 sub-systems were created. One system is the spectrometer CCD circuit, which is specific to powering the cod, driving the CCD, and taking the spectrum that the CCD collects, while the second electrical system is used for controlling the other design specifications.

Each sub-system is critical in the overall design of the Raman system for having a complete system that is dynamic and allows for a user to have an easy to use system, but also a system that does not require much maintenance or tweaking. Electrical system 1 is being powered by a 5v, 2A source wall plug switching power supply while electrical system 2 is being powered by an 12v, 2A power supply which is then being voltage regulated to 5V system.

VII. ELECTRICAL SYSTEM 1

A. Microcontroller

To control the electrical system 1 which is defined as the system that includes laser power sensing and control, temperature sensing and control, backlight control, and class 1 laser blocking, a microcontroller was used to achieve input and output sensing and control. For this project an Atmega328p microcontroller was used. For breadboard testing, the Arduino Uno R3 was used over developing the system. This microcontroller allowed for easy manipulation of peripherals as well as easy software integration with Matlab. Software integration is discussed in later sections.

B. Temperature Sensing and Control

Temperature sensing and control is used in the Raman system because it allows for the user to verify if enclosure temperature is affecting the Raman signal. For example, heat being generated in the enclosure can cause electronic components to not only cause damage, but can also affect the spectrum that is being generated. Because of this, temperature sensing and control is needed. Though any temperature sensor can be used, a "One-Wire" temperature sensor (DS18B20) was used because it allows for multiple sensors to be connected to a single bus, but also allows for temperature to be read with higher accuracy.

Temperature control is necessary to regulate temperature within the enclosure. Fans were used to regulate temperature. These fans are pulse-width modulated within the microcontroller's software to spin at a speed dependent on the temperature. For example, if the temperature of the enclose began to rise, then the fan speed would increase and adjust accordingly. A fan is placed behind the CCD cooling heatsink while another fan is being placed by the electrical system one's PCB for cool air to pass over the electronics. To pulse width, modulate the fans, a transistor was used as a switch which allows for a voltage to be supplied to the base of the transistor from the microcontroller which dictates the speed at which the fans will spin. A diode was also placed parallel with the fans to protect the circuit against any extra current flow. Table 1 shows the relationship between temperature and the fan speed in regards to pulse width modulation.

Table 1. Temperature to PWM relationship.

Duty Cycle	Voltage	Temperature Range	PWM Value	Fan Speed
0	23mV	Less than 70 F °	0	0
30%	.778 V	70 F °	72	30%
40%	1.123 V	74 F °	102	40%
60%	2.553 V	78 F °	153	60%
80%	3.753V	82 F °	204	80%
100%	4.42 V	86 F °	255	100%

C. Class One Laser Blocking System

To achieve a class 1 laser system, the laser cannot be seen by the user regardless of the state of the system. This means that the laser cannot be seen or come in contact with the user when the user is loading a sample onto the sampling stage. To achieve this, the laser must be blocked while a sample is being loaded onto the stage. To achieve this, a solenoid was used to block the laser. Though any solenoid can be used, a small 5V, 1A solenoid is used in this Raman system. A solenoid compared to other devices, allows for quick blocking of the laser. This solenoid will be triggered when the sampling door is opened or closed. A magnetic contact switch is used for solenoid triggering which is programmed in the microcontroller software.

To use the solenoid in the microcontroller, a transistor was used as a switch which allows for a voltage to be supplied to the base of the transistor from the microcontroller which allows the main power supply to supply the solenoid as needed. A diode was also placed parallel with the solenoid to protect the circuit against any extra current flow. To use the magnetic switch with the microcontroller and solenoid, an internal pull-up resistor must be declared to remove any debouncing of the magnetic contact switch.

D. Backlight Control

Backlight control is needed for the Raman system because it is essential to the overall microscope system. A backlight can be used to help see the sample on the stage from the camera. In order to use the backlight in the system, the microcontroller must be able to control when the backlight can be turned on or off. This means that the user should be able to turn on and off the backlight as needed, but the microcontroller should be able to turn off the backlight when taking a spectrum to reduce background ambient light noise. To achieve controlling the backlight, a transistor was used as a switch which allows for a voltage to be supplied to the base of the transistor from the microcontroller which dictates when the backlight will turn on. A diode was also placed parallel with the fans to protect the circuit against any extra current flow.

E. Laser Power Control

Typically, laser power is not essential in optical systems because usually the user wants the maximum power. In this system, laser power display and control is implemented which allows the user to dictate the amount of power being used. As discussed earlier, the laser being used as a 100mW power rating. This means that the laser power control being implemented will allow the user to adjust the laser power from 0 to 100mW. The user can also not only start, but also stop the laser from the electrical one system. Laser power is controlled by supplying voltage to the main laser power system, and then pulse-width modulating the laser power enabled pin. This allows the system to still be supplied voltage to the internal electrical system, while also modulating the overall power of the system. Because the laser has maximum power rating of 100mW, the user can know what the power is at any given time, by knowing the pulse-width modulation percentage. The microcontroller being used allows for direct power manipulation from one of its pulse-width modulation pins.

Table 2. Laser power to PWM Relationship

Duty Cycle	Voltage	Power Prior to Calibration	PWM Value
10%	.5	10 mW	26
20%	1	20 mW	51
30%	1.5	30 mW	77
40%	2	40 mW	102
50	2.5	50 mW	128
60	3	60 mW	153
70	3.5	70 mW	179
80	4	80 mW	204
90	4.5	90 mW	230
100%	5 V	100 mW	255

VIII. ELECTRICAL SYSTEM 2

A. Spectrometer CCD Circuit

In order to achieve Raman spectrum, an accurate CCD must be used. Both the resolution and the pixel count must be considered when choosing a CCD to use in the system. As discussed early, the CCD that was used in this project was a Toshiba TCD1304ap. This CCD has a pixel count of 3648 pixels. Another consideration is the resolution to build for when creating a spectrometer. Initial resting resulted in using an 8-bit resolution. This means that a maximum of 256 values is what a pixel can equate to in terms of intensity of light being converted to a voltage. Unfortunately, 8-bits was simply not enough to capture Raman signals. A more precise resolution was needed. The actual resolution that the Raman system now uses is 16-bits. This means that there are over 65,000 possible values that the light intensity that each pixel picks up can be converted to any number of these values. This indicates that a 16-bit resolution CCD circuit allows for the user to see mill volt changes in the light intensity of the sample. This is perfect for seeing Raman signals. In reference, most company made spectrometers uses a resolution of 16-bits, along with a pixel count of anywhere from 1000 to 4000 pixels.

In order to achieve, designing and building a 16-bit spectrometer, this system was separated from the other microcontroller and electronic system because of the complexity of the CCD driving circuit. For the CCD to read the pixels being passed over the pixel stream on the CCD, the CCD needed to be driven by a microcontroller with a fast MHz clock speed. The ATmega1284p was used to drive the circuit. In order to capture the values of each pixel, 3 clocks are needed to be driven, along with clocks that needed to be drive on the ADC being used. The CCD clocks are the MCLK, the ICG, and the SH. The CCD is always clocking the shift registers when the MCLK is running, but once a combination of ICG LOW and SH High, following LOW again is when the photodiodes are dumped onto the shift registers. This allows for the data to be captured which then allows a spectrum to be generated.

B. CCD Cooling

One problem with the CCD circuit is the fact that there is too much thermal noise for picking up spectrums with such little intensity. In order to reduce the thermal noise, a temperature cooling system was built for the CCD. This system consisted of a 5V, 2A TEC plate which allowed one side of the plate to cool the CCD, while the other side of the plate warming up a heatsink to remove any thermal heat coming from the CCD. The CCD was mounted to an aluminum block which was mounted to the cold side of the TEC plate. Finally, a heatsink and a fan were used on the opposite side of the TEC to push out the heat. This allowed the spectrums being generated to have little to no thermal noise which allows low intensity light like low-shift Raman signals to be shown picked up by the CCD circuit.

IX. INTRODUCTION GRAPHICAL USER INTERFACE POWER BUTTON

The Graphical User interface is used to control all the components that are used to interact with the laser. This is very important as without precision controlling the laser can be damaged or do damage to the users The GUI has multiple buttons for the user to have complete control at all times as well as many background safety features that will automatically shut the whole system down in case of any emergency.

The GUI that was chosen was MATlab. The MAtlab GUI interface is very well made and has many different options for user interface with arduino. The I2c serial communication makes it very easy to upload a sketch have multiple programs communication at once. There are multiple libraries that are predesigned for connecting and simplifying almost all the arduino command. Therefore the hard code can be sketched onto the arduino and then matlab can easily use libraries with simple commands to call all the components and upload them to the GUI in real time.

The GUI system is comprised of two parts the frontend panel which initiates the power, buttons and all the background monitoring systems and the back end which continually updates and relays information to the front end. The frontend is very user friendly, easily implemented and is built to shut down all power to any device if any errors are prompted via the back end. The back end is comprised of multiple sets of loops that continually monitor all parts of the system to make sure that the system is in compliance with all measures of the laser safety guidelines.

X. FRONT END GRAPHICAL USER INTERFACE

The front end of the GUI consists of many buttons and textboxes that are continually updated via the backend. The whole system is initiated and then controlled by each individual button. The system has nine buttons each having its own unique code to engage or disengage something in the system. All of the buttons have been directly coded into the main power button to be disengaged if for any point the system needs to be shut down for emergency purposes.

A. Power Button

The main function of the GUI is the Power Button. The power button initiates the whole system. This sets up everything by calling the arduino and uploading the sketch after opening up a direct port for continuous serial communication. This is the heart of the program if anything is flagged this sketch will immediately shut down power to the laser and the CCD before even letting the front end know. Once the sketch is uploaded the Arduino code continually runs on the back end. The front end then pings the arduino-coded arrays and ports for updates. The temperature sensors are initiated by the power buttons and then the MATlab interface continually pings the arduino pins to get an update every few milliseconds to see if the temperature has changed. This is important as when the temperature of the box is raised the fans are initiated for cooling purpose. The power on and off displays works in the exact same manner as temperature sensor. The door status display works in the same way continually pinging the arduino ports to see if the voltage circuit has been broken which would then send a flag to the Matlab display and turns off power. This continues to check the circuit to see if it is once again but does not re engage power only allows power to be re initiated by the user via the power button.

B. Dark Button

The dark button initiates the background code to call the arduino array with all the pixel values and stores them in a variable in Matlab. The dark represents all the light that is already in the signal before the signal from the laser is recorded and stored.

C. Exposure Button

The exposure button changes the exposure time that has already been hard coded on the arduino for the CCD board.

D. Backlight Button

The Backlight button sends a PWM to the correct pin which gives power to the backlight.

E. Laser Power Button

The laser button calls the input typed into the box below and plugs into an equation to control the power with respect to duty cycle and then updates the power to the laser. Therefore if the user types in 50 the duty cycle will initiate 50% of lasers full power.

F. Create Raman Button

The Create Raman Spectrum initiates the background code to call the arduino array with all the pixel arrays and stores the values in a variable in Matlab. This then takes the pixel array from the dark button and subtracts them out to get rid of the background noise of the full system.

G. Take Picture

The take picture initiates the drivers to create a live stream update of the thorlabs camera that is in focus of the specimen we are trying to take the Raman spectrum from.

H. Save

There are two save buttons one for the camera and one for the spectrum graph. The spectrum save button saves the array to an excel file where the user can view it later. The picture file saves the picture in a word documents and stores it within a special file.

XI. THE BACKEND GRAPHICAL USER INTERFACE

For the back end of the graphical user interface we used two Arduinos. One arduino Uno and one arduino atmega1284. We call them separately and upload to different sketches that support completely different operations. The sketches uploaded on the systems run different code continuously to monitor the system. This code does all the heavy lifting. For the arduino Uno everything is directly called from matlab which makes things very simple. The arduino Atmega sketch works monitoring the system to pull and update all the specified measurements into an array. The 16 Hz Atmega is programmed with an arduino bootloader. This system is always clocking the shift registers until the system copies 3694 pixels of data. The line read out comes directly from the CCD and then to the ADC. The clock cycle can record every 4.5us this is only important with respect to the timing of the ADC as it could not handle this for the arduino Uno hardware. Once the data is stored in an array it can then be uploaded and called by matlab to display in a graph of pixel vs intensity.

A. Fan Control

The fans are part of the back end code that is initiated by the power button that communicates with arduino Uno. The fans are set to certain speeds so that once the box starts to increase above room temperature they automatically turn on. The fans also increase with speed as the temperature rises to higher levels. There is four different ranges at which the fan speeds will increase at intervals of 25% duty cycle of 5 volts.

XII. CONCLUSION

Building a Raman spectroscopy system from scratch is challenging since parts are expensive, sensitive in alignment, and requires high-resolution detection. With a powerful excitation design, and sensitive spectrometer and electronic design, a Raman signal can be detected.

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