A SIMPLE AND COST EFFECTIVE RAMAN-FLUORESCENCE SPECTROMETER

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ABSTRACT

Research, design, construction, and operation of a portable mixed Raman and Fluorescence type spectrometer will be presented. The spectrometer was used on a wheeled vehicle which competed in the University Rover Challenge sponsored by the Mars Society. It uses a 50 mW, 532 nm continuous wave laser to probe a sample of soil for bacteria or biomarkers. The device costs 2,000 USD, weighs 1.4 kg and is 23 cm x 23 cm x 10 cm in size. Results from the competition, complications of analyzing mixed Raman-Fluorescence spectra via digital signal processing, and future ideas and improvements will also be discussed.

KEY WORDS

Raman, Fluorescence, Spectrometer

INTRODUCTION

The Mars Rover Design Team (MRDT), based out of the Missouri University of Science and Technology, is a student led team with the goal of building Mars rover analogues to compete in the University Rover Challenge (URC) [1] that is hosted by the Mars Society. The rovers, designed primarily by undergraduates, must be capable of performing a variety of tasks that would simplify life for human operators on Mars. These tasks include traversing rough terrain and using a robotic arm to manipulate levers and pick up tools, as well as performing a science task on board the rover. The science task requires that a soil sample be collected from a geological region of biological importance and then be analyzed to determine the possibility of life within the sample.

Traditionally, the rovers on Mars have also included a variety of instruments capable of analyzing soil and rock samples for the possibility of life on almost all of their missions to Mars. Analysis using these instruments, however, can require an amount of time greater than what is allotted by the URC and they can be destructive to the sample. Because of this, the MRDT decided to explore other technologies that could perform quick, informative experiments that would preserve any prospective life.

Spectroscopy was identified as the primary candidate for noninvasive probing of soil samples because spectroscopic methods typically work on very short time scales, allowing for quick data acquisition. Additionally, there are multiple types of spectroscopy that can provide the exact molecular composition of a sample. Raman [2] and infrared [3] spectroscopy were both considered for the experiment. Raman spectroscopy was ultimately chosen because it has recently been shown to be able to detect biomolecules that are typically associated with extremophile bacteria underneath rocks and in soil samples [4]. Raman spectrometers, however, can cost upwards of 10,000 USD and the URC limits rovers to a 15,000 USD maximum budget for all on-board components. Thus, a spectrometer could not be purchased and instead, had to be designed and built by the members of the MRDT.

THEORY

Raman spectroscopy is possible because of a phenomenon known as Raman scattering [5]. Raman scattering occurs when photons scatter inelastically off of a molecule. This inelastic collision results in either the photon imparting energy on a molecule, which is called Stokes scattering, or a molecule imparting energy on a photon, which is called anti-Stokes scattering. Because the energy of a photon is proportional to frequency, and therefore inversely proportional to wavelength, Stokes scattering leads to a photon with increased wavelength. Conversely, anti-Stokes scattering leads to a photon with decreased wavelength. Raman spectroscopy measures the difference in

wavelength between the incident photon and the scattered photons, called the Raman shift. The spectra that result from this process are unique to the molecules that produce them.

In addition to Raman scattering, fluorescence and Rayleigh scattering may also occur. Rayleigh scattering is the elastic scattering of photons off of a molecule, resulting in no wavelength shift. Fluorescence is the excitation of an electron due to the absorption of a photon. Fluorescence produces a wavelength shift and is a more common phenomenon than Raman scattering. Fluorescence, through the use of complicated electronic equipment in the spectrometer, is typically filtered out because it can prevent the detection of Raman spectra by saturating the photodetector. Fluorescence, however, can provide valuable information on mineral composition in a soil sample that Raman scattering alone would not normally provide. By sacrificing part of the Raman scattering signal, it is possible to obtain more diverse information about a sample while saving money on electronics.

DESIGN

Because the spectrometer was placed on Horizon, MRDT's 2014-15 competition rover, it was subject to weight, size, and budget restrictions. The URC limits rovers to a 50 kg weight limit and a 15,000 USD on-board component limit. This resulted in the spectrometer having a weight limit of 2 kg, a budget of 2,500 USD, and a size limit of 23 cm x 23 cm x 10 cm.

The primary components of a spectrometer are the laser, the filtering optics, the monochromator, and the photodetector. The laser is used to probe a sample to elicit Raman scattering, the filtering optics are a series of filters that serve to filter out noise due to Rayleigh scattering, the monochromator is used to separate mixed color light into individual wavelengths, and the monochromatic light is then focused onto a photodetector and analyzed.

The laser is one of the most important components of Raman spectroscopy. Frequency hopping in the laser must be minimal to ensure the filtering of Rayleigh scattered light. If frequency hopping occurs and is not filtered out, it will cause noise in the data. Additionally, the wavelength of the laser is also important as different wavelengths excite different types of molecules. A 532 nm laser was chosen as this frequency is ideal for detecting organic molecules via Raman scattering and exciting fluorescence in various minerals. The Roithner LaserTechnik GLP-III-532-50 laser was chosen. This is a 50 mW constant waveform handheld laser with only +/- 1nm variance in the wavelength and costs only 341 USD. The laser is powerful enough to provide a large number of photons for a strong Raman signal, but it is not powerful enough to burn organic molecules. The laser was modified to be remotely controlled using transistor-transistor logic (TTL). Raman spectroscopy requires the upper range of detection to be at least 4,000 wavenumbers

(1 wavenumber = 1 cm^{-1}) past the wavelength of the laser. This makes the range of the spectrometer 532nm – 675nm.

The filtering optics consist of a laser clean up filter, a dichroic mirror, and an edge filter. The laser passes through the laser clean up filter, is reflected off of a dichroic mirror and onto the sample. The Raman and fluorescence signals then are collected and pass through the dichroic mirror, through an edge filter, and into the monochromator. A diagram for this can be seen in Figure 1.

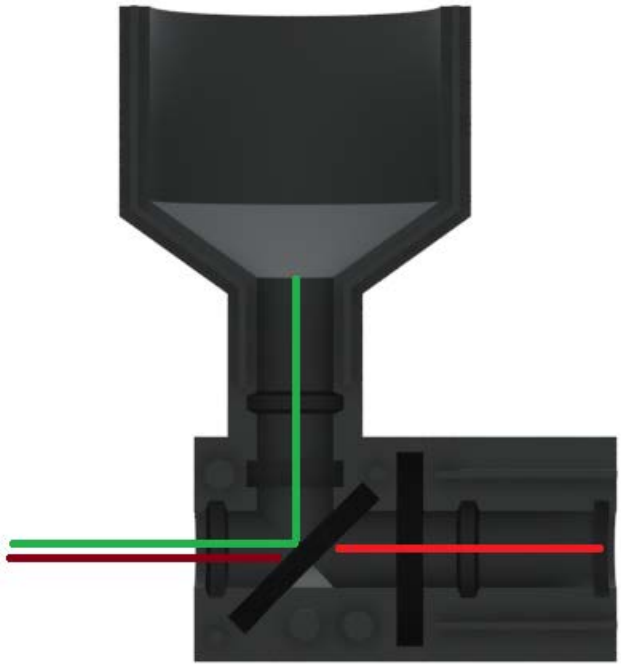

Figure 1: The Filtering Optics holder.

The laser clean up filter (Semrock LL01-532-12.5, 300 USD) allows only 532nm light to pass through. It transmits >90% of light at 532nm, but reflects all other wavelengths. The dichroic mirror (Thor Labs DMLP550, 165 USD) is >90% reflective for wavelengths less than 535nm, >85% transmissive for wavelengths greater than 565nm, and a cutoff wavelength of 550nm. The edge filter (Semrock BLP01-532R-25, 350 USD) is >93% transmissive for wavelengths above 547nm. These filters eliminate any Rayleigh scattered light that could cause extraneous noise. One consequence of this, however, is that anti-Stokes scattering is effectively eliminated from detection as only wavelengths greater than the original 532nm can be detected. Anti-Stokes scattering is much weaker in intensity as compared to Stokes scattering. This has to do with the density of states for each type of scattering. There are also 3 focusing lenses (Thor Labs LB1092, focal length = 15mm, 23 USD per lens) that are used to focus laser light onto the sample, focus laser light onto the aperture of the monochromator, as well as disperse the laser light to lengthen the lifetime of

the filters. The container for these filters was designed in Siemens NX and 3D printed. A fully composed picture can be seen in Figure 2.

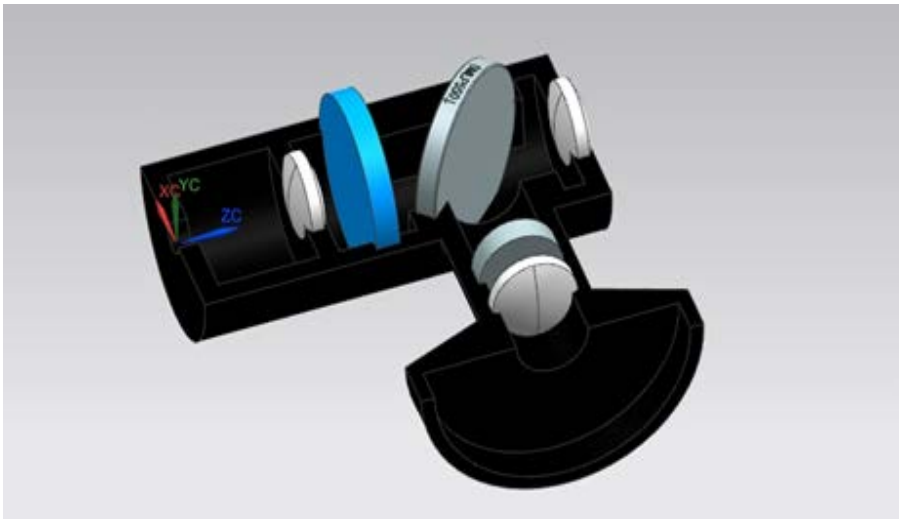

Figure 2: The full filtering assembly

Proper design of the monochromator is crucial. If the focal lengths are off, alignment incorrect, or the magnification is not properly handled then the image on the photodetector will be blurry and potentially imprecise. To counter this, it is useful to build the monochromator around the photodetector and diffraction grating [6]. This will ensure clear focus and proper wavelength mapping. Starting with the diffraction grating groove density, it is possible to calculate angles α and β seen in Figure 3

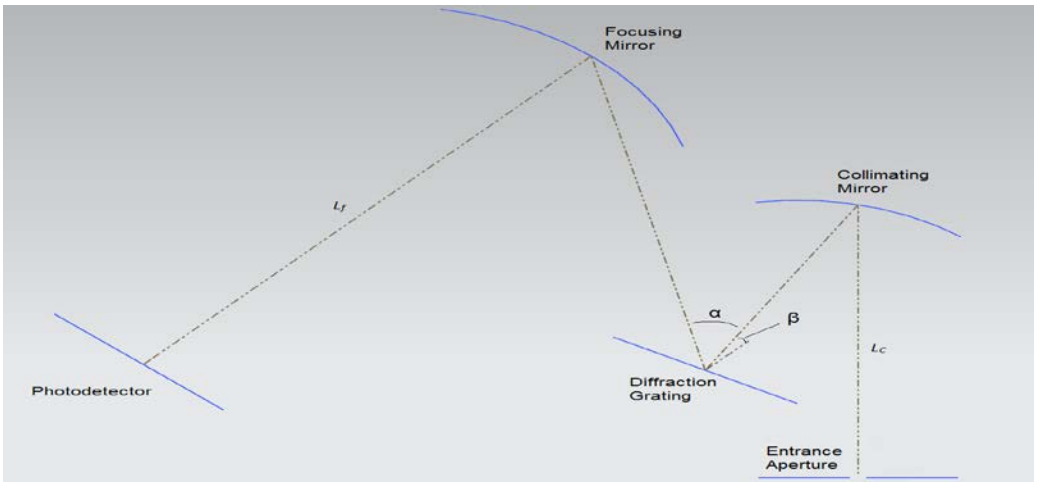

Figure 3: A Czerny-Turner Spectrometer

$$\alpha = \sin^{-1}\left(\frac{\lambda_c G}{2\cos\left(\frac{\varphi}{2}\right)}\right) - \frac{\varphi}{2} \quad (1)$$

$$\beta = \varphi - \alpha \quad (2)$$

$$\lambda_c = \frac{\lambda_f - \lambda_i}{2} \quad (3)$$

For these equations λ_c is the center frequency, G is the diffraction grating groove density, and φ is the diffraction angle (typically 30 degrees for Czerny-Turner spectrometers). Next, using the detector length, equation (1), and equation (2) the focal length for the focusing and collimating mirrors can be calculated.

$$L_f = \frac{L_D \cos\beta}{G(\lambda_f - \lambda_i)} \quad (4)$$

$$L_c = \frac{L_f \cos(\alpha)}{M \cos(\beta)} \quad (5)$$

In these equations L_f is the focal length for the Focusing mirror, L_c is the focal length for the collimating mirror, L_D is the length of the detector, and *M* is the magnification of the system. Using λ_f = 675nm, λ_i = 532nm, and φ = 30 degrees, $L_D = 30$mm, G = 1200 grooves/mm, *M* =1, and equations (1)-(5) the following is obtained:

α = -12.85º

β = 42.85º

L_f = 75mm

L_c = 98 mm

Figure 4 shows the monochromator in a Czerny-Turner type configuration, complete with the optics filters. The filtering optics are on the bottom of the picture for reference. The collimating mirror is a 25.4mm diameter spherical concave mirror (Thor Labs CM254-100-G01, Focal length = 100mm, 55 USD), the focusing mirror a 50.8mm diameter spherical concave mirror (Thor Labs CM508-150-G01, Focal length = 150mm, 82 USD), and a 25.4 square blazed diffraction grating (Thor Labs GR25-1205, 102 USD). The collimating mirror is placed exactly one focal length away from the filtering optics aperture. The light from the collimating mirror is then focused on the diffraction grating where it is separated into individual wavelengths. From there, the light is then

reflected on to the 50.8mm diameter focusing mirror. The diffracted light is the focused on the focusing mirror, which is placed exactly one focal length away from the detector. The focusing mirror focal length, L_f, was doubled from 75mm to 150mm in order to ease design and construction. This resulted in the increase of M from $M = 1$ to $M = 2$ to keep L_c the same.

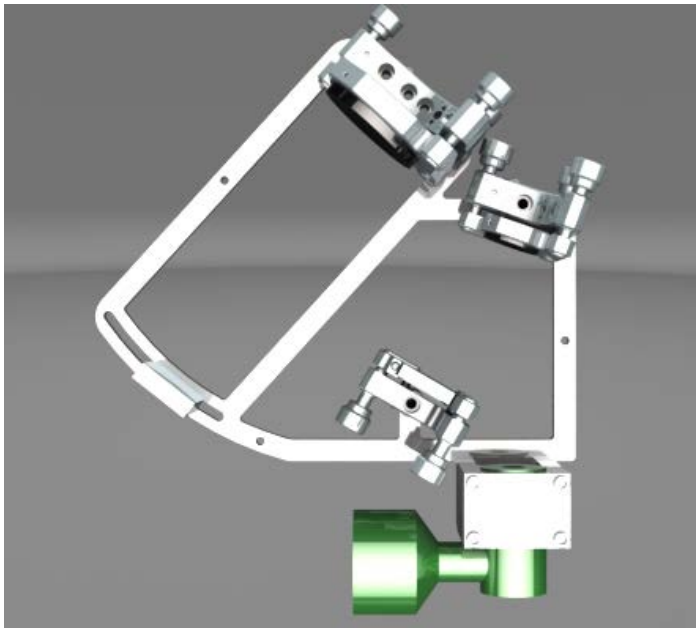

Figure 4: The Czerny-Turner Monochromator design.

Each of the mirrors is placed in a kinematic mount that allows for 4 degrees of adjustment in each direction. The collimating mirror is mounted in the Thor Labs KS1D (144 USD), the focusing mirror in the Thor Labs KS2D (148 USD), and the diffraction grading in the Thor Labs KM100S (75 USD). This yields a total cost for the spectrometer at approximately 1820 USD. Materials costs such as the printed plastics are not included.

The detector is the Toshiba TCD1304DG Linear CCD array. It is approximately 30mm long with 3648 pixels and costs 22.50 USD. This CCD cannot sense colors, and is only capable of sensing intensity. The monochromator, however, maps the 532nm-675nm range onto the 30mm long CCD. The 532nm light will begin at one end of the CCD and gradually increase until it ends at 675nm at the other end of CCD. This yields a camera resolution of 0.025 nm/pixel. It is controlled using an Arduino Due (50 USD) and communicates to Horizon's motherboard via UART. The total cost to this point is approximately 1900 USD.

The signal received by the CCD must then be analyzed. The CCD will receive both Raman and fluorescence signals simultaneously. Analysis of each spectra requires numerical differentiation to

separate the Raman and fluorescence signals. From there, more numerical analysis is required to determine the center point for any spectral lines available. These center points can then be compared to a data base of materials for matching results. This process can also be done using a neural network to allow the spectrometer to "learn" the spectra of various compounds. This process can be completed by iteratively running spectra on various samples.

RESULTS

The spectrometer design began in November 2014, construction began in February 2015 and was completed roughly one week before the URC in mid-May 2015. The CCD array is still in the process of being perfected as of June 2015, however it is working well enough to receive data. Figure 5 shows the baseline measurement of the spectrometer. This is the measurement taken when there is no sample being probed, but the laser is turned on.

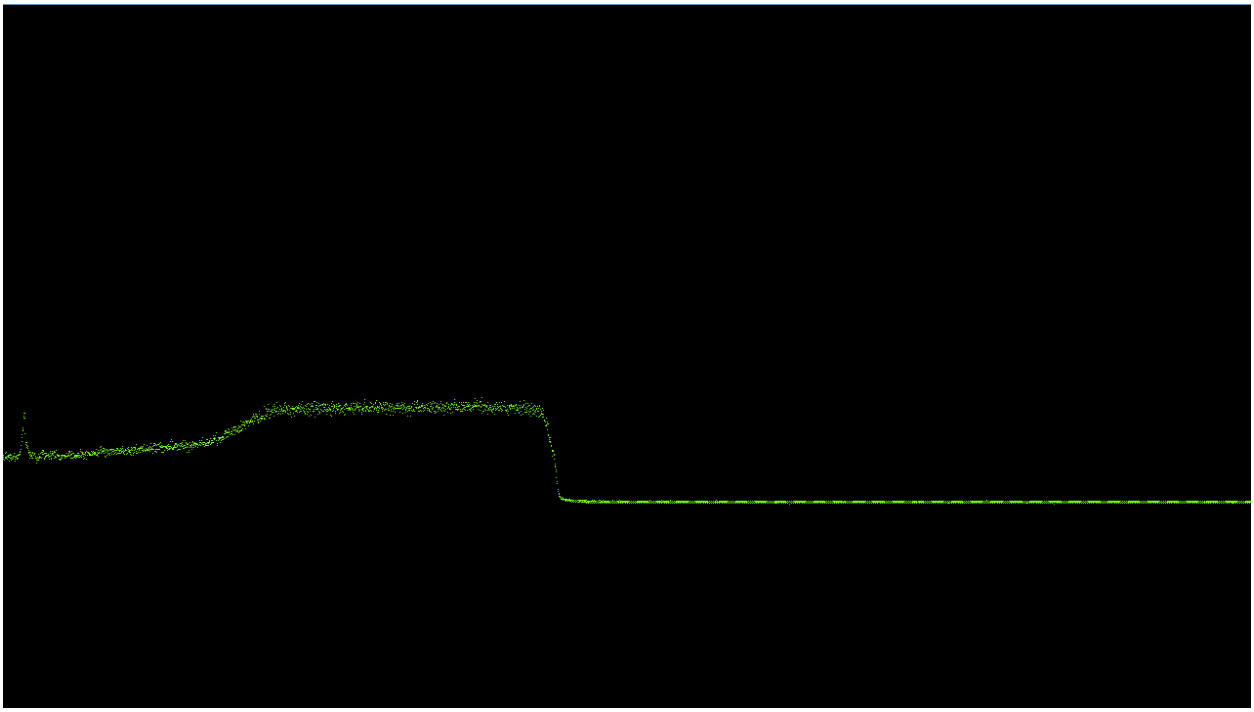

Figure 5: Baseline data for the Raman spectrometer. X axis is wavelength with the left side being 532, right side being 675. The Y axis is intensity.

Though this is baseline data, it is clear that data is being received even when no sample is present. If there was no Raman or Fluorescence signal, the graph would be completely flat in the entire

wavelength range. However, there are two distinct signatures: a sharp peak on the leftmost side of the graph, and another very broad peak closer to the middle. It appears that the broad peak is from fluorescence. Fluorescence peaks are typically Gaussian shaped, however this peak appears to have been so strong that it saturated the CCD. The sharp peak is characteristic of a Raman spectra.

After some analysis, it was determined that the Raman and fluorescence data were most likely coming from the 3D printed plastic that holds the filtering optics. The holder was printed out of Polylactic Acid (PLA). Because lactic acid, which is a biomolecule, is present, it is no surprise that a Raman peak is also present.

After the baseline data was established, and the noise examined, it became possible to analyze samples. Figure 6 shows the spectra obtained from a leaf sample.

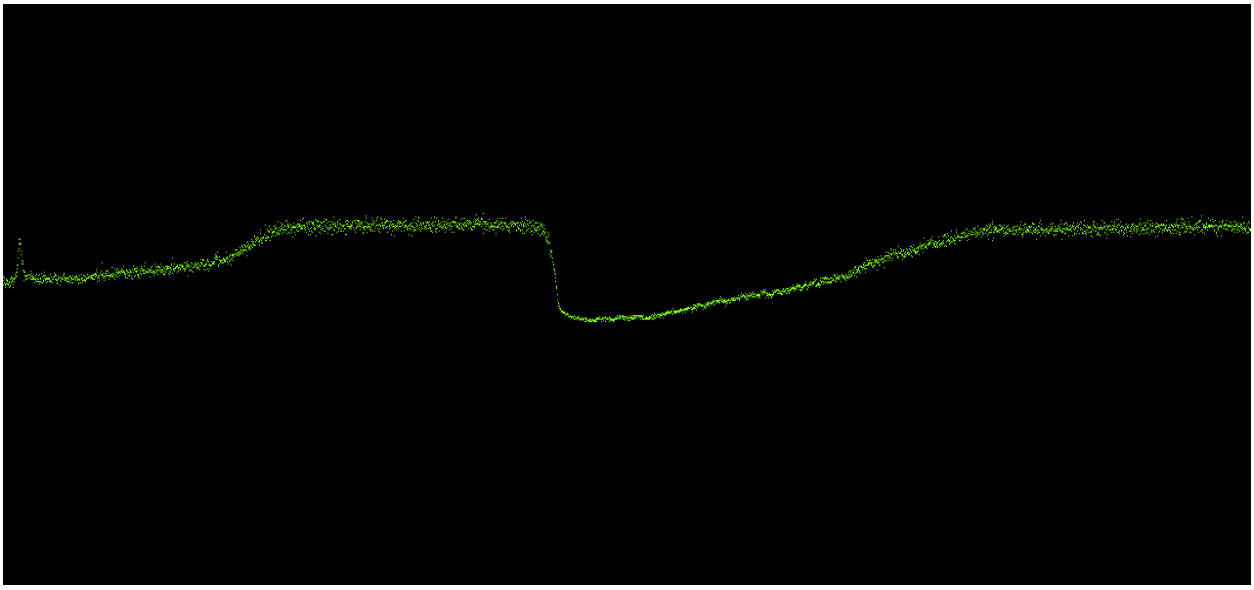

Figure 6: Spectra obtained from a leaf sample

The results from figure 6 show the same baseline data from Figure 5 in addition to what appears to be another Fluorescence spectra that has caused the CCD to saturate.

CONCLUSIONS

Results show that it is possible to build a functional Raman-Fluorescence spectrometer for under 2,500 USD. The spectrometer, designed completely by undergraduates, was able to detect both Raman and fluorescence peaks. Though the CCD camera is not fully functional, the data is clear enough to determine that spectra were being observed.

In the future the CCD will be optimized to allow for full detection of fluorescence spectra that would otherwise saturate the camera. This will require optimization of code to reduce sensitivity as well as a high frequency amplifier. Additionally, a pulsed laser and a chopper wheel may be introduced in order to eliminate fluorescence signals that may overpower any Raman spectra. These components are also expensive and will have to be designed by the Missouri University of Science and Technology Mars Rover Design Team.

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