UV-VIS Spectrometer for Protein and DNA Quantification

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***Abstract* — This project aims to create a spectrophotometer specifically tailored for molecular biology labs that study proteins and genetic information. The device mainly will aim to be low-to-moderate in cost, capable of fast sample readings, and have a friendly user interface. The end result is to provide researchers a quick and easy solution for spectroscopic protocols without impacting lab efficiency and workflow.**

***Index Terms* — Biomedical engineering, biomedical equipment, CCD image sensors, geometrical optics, spectroscopy**

I. INTRODUCTION

Biomedical research is responsible for an increasingly significant portion of innovation in medical technology. Many modern drugs and procedures are made possible with the knowledge and manufacturing methods produced in these laboratories. One of the growing sectors of biomedical research is molecular biology, which studies the structures and behaviors of cellular structures using advanced machinery. Scientific endeavors such as the Human Genome Project rely on molecular biological research to understand the genetic information of lifeforms and how they correspond to traits and behaviors. These studies are made possible by increasingly sophisticated machinery to interact with the microscopic material, and one of the most important tools is the spectrophotometer.

Spectrometry is used in the lab to answer questions related to sample concentration, purity, and reactivity. This information is vital to understanding what is happening on the molecular level, and almost every protocol performed in a molecular lab uses a spectrometer of some kind to identify or verify experimental results. Many labs will contain several types of spectrometers, but the most common is a spectrophotometer capable of single-wavelength measurements with a minimum amount of sample usage. Often several of these devices will be available in a research laboratory due to their popularity in modern scientific protocols.

Overall this project hopes to produce an attractive product for newly-established laboratories aiming to work in molecular biology or older labs seeking to upgrade their equipment. It also serves as an excellent educational tool, providing an easy-to-use measurement device with quality results that reflect good technique in the lab.

II. BIOLOGY APPLICATION

Spectrophotometers are used in molecular biology research to quantify the molecules present in a liquid sample. It is impossible to visually understand the contents of a sample, which can contain a complex cocktail of DNA, protein fragments, various salts, pH buffer molecules, and other contaminants. Many protocols such as purification and cloning require a precise understanding of what is in the sample as well as how much. Fortunately, this is answered through Beer-Lambert Law:

$$A = \log\frac{I_0}{I} = \mathcal{J}\mu bc \qquad (1)$$

Where A is the absorbance (unitless), I_0 is the incident light through the sample (lux), I is the measured light by the sensor (lux), b is the path length of the sample measurement (cm), c is the concentration (mol/ml), and Îµ is the molar absorptivity coefficient (ml/cm*mol). This molar absorptivity coefficient is unique to each molecule and wavelength of light. Usually this coefficient will be known for the desired substance, or derived from known quantities. If the proportion of light absorbed by a sample is known for a given wavelength, the concentration can be derived. The spectrophotometer is a fixture in almost every biology lab, usually with several different devices in a single lab. Different models have important tradeoffs between accuracy, cost, speed, and specialization. A protocol done in a lab might require a very accurate measurement to work, but some may only need a quick comparison before proceeding. This project aims to introduce a very low-cost spectrometer with good enough accuracy for these less precise measurements, while introducing improved usability over existing models in the low price bracket.

Interviews with molecular biology researchers show that a spectral range of 200-800nm is required for commonly used protocols in this application range. Some improvements over existing models include reduced post-analysis formatting, full standalone functionality, and a simple but flexible user interfaces. This project achieves these goals through a simple LCD and keypad interface, as well as USB flash drive compatibility for easy measurement extraction.

III. SENSOR PROCESSING

The linear CCD used in the project (the TCD1304) is a row of pixels formed by MOS capacitors and a shift register. When the internal circuitry is activated, the pixels are exposed to photons that create hole-electron pairs in the surface silicon. The electrons from these pairs are pulled to a gate by a positive charge behind the pixel. When readout is requested, these charges are placed in a shift register and read out to the output buffer. Each charge directly relates to the intensity of the light striking the pixel, which has been split by the diffraction grating to produce a spectrum.

The CCD requires precise timing pulses to begin readout. These pulses are shown in Fig. 1. The OS line represents the output of the CCD. The master clock is provided by the microcontroller and controls the data rate of the sensor. Every 4 cycles, a new pixel is read. The icg line shows the integration clear gate, which keeps the pixels blank until readout is called. The sh line is the shift-hold register control, and signals the sensor to place pixel values into the shift register for readout.

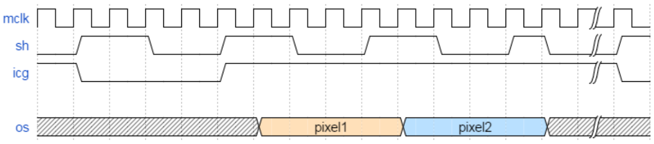

Fig. 1 Timing pulses of the CCD

The signal from the CCD needs to be buffered and gained to match the range of the ADC module that will interpret it. Fig. 2 shows the simple two-stage op-amp circuit used for this purpose. The first stage buffers the signal and isolates the sensor from the processing circuitry. The second stage inverts, gains, and offsets the signal to fit into the 3V range of the onboard ADC. The resistors presented in this schematic are examples, and must be tuned for each individual sensor that is used.

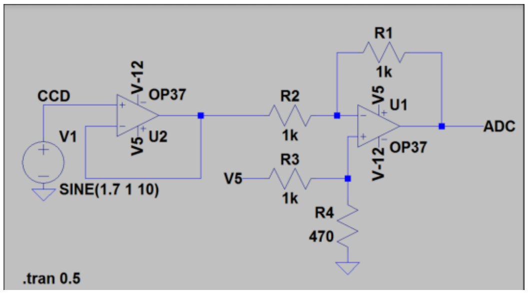

Fig. 2 Two-stage op-amp circuit for range matching

Once the signal has been captured, the software must interpret the values in a meaningful way. Fig. 3 shows the calibration pathway for each spectral measurement. The collected pixels are first binned down to match the number of desired values. This reduces the impact of shot noise on the pixel values for each wavelength. Then a dark frame is subtracted from the measurement, representing the voltage present in each pixel when no light is present. These values are then compared to a calibrated full frame, which is collected before any sample is present to compare to the measured values. The proportion of light on each pixel after this operation represents the transmittance at that wavelength.

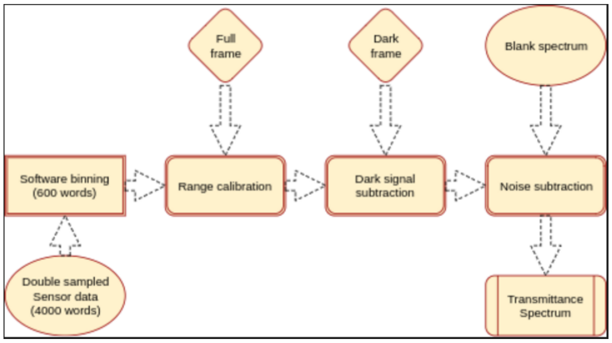

Fig. 3 Calibration pathway for each spectral measurement

IV. SPECTROMETER

A. Part Selection

The mains parts that make the spectrometer of a spectrophotometer are the light source, the focusing elements, the dispersing element, and the detector. For this device to be used in the ultraviolet and visible spectra, the light source would have to span the wavelength corresponding to these spectra. A xenon lamp is a suitable choice for this case. The lamp used for the spectrometer is a BulbAmerica H7 xenon bulb, rated at 12 V and 55 W.

The focusing elements and the dispersing element together create the spectrograph. Either convex lenses or concave mirrors can be used as focusing elements. For this device, concave mirrors are used because they are budget-friendly, easy to handle, and do not exhibit chromatic aberration. The first mirror of the system, called the collimating mirror. As the name entails, it collimates the divergent light from the entrance slit onto the grating. The mirror that proceeds the grating is called focusing mirror. This mirror will focus the dispersed light onto the detector. The mirrors used for this device are two ThorLabs CM254-075-F01 concave mirrors with UV-Enhanced Aluminum. The focal length of these mirrors is 75 mm, which is an adequate focal length for this application. The UV-Enhanced Aluminum coating increases the reflectance of UV wavelengths to over 85%. This is important because the response of the optical system to UV light is very poor due to several factors. The coating enables the use of as much UV light as possible.

The dispersing element is the key component involved in any sort of wavelength selective or spectra-based instrumentation. A dispersing element of a spectrograph or spectrometer determines the wavelength range of the system and partially determines the optical resolution. The ability to select a certain wavelength or range of wavelengths from a broadband source is important to different fields in optics and photonics, such as spectroscopy. The diffraction grating is the dispersing element of choice for this device. A diffraction grating is a periodic series of grooves that act as many miniature mirrors. Ruled gratings are the classic example of a diffraction grating. The ruled grating is created by physically cutting grooves into the reflective layer of the grating substrate. The diffraction grating we chose for our spectrophotometer is the ThorLabs GR25-0305 Ruled Reflective Diffraction Grating. This grating has a blaze wavelength of 500 nm and a groove density of 300 lines/mm. The blaze wavelength of 500 nm is roughly in the center of the UV-VIS range. At the blaze wavelength, the diffraction efficiency of the grating is at its highest. The strength of the diffraction decreases at wavelengths away from the blaze wavelength. In general, the maximum diffraction efficiency is cut in half at 2/3 of the blaze wavelength and at 1.8 times the blaze wavelength. The groove frequency is directly proportional to the dispersion of the grating, directly proportional to optical resolution, and inversely proportional to the wavelength range. Since the wavelength range of this device is relatively large (about 500 nm), a small groove density is much needed.

An essential piece of equipment in a spectrophotometer is the photodetector of the system. The light may not be quantifiable to the human eye, so the detector enables a human to understand and analyze light. The charge coupled device (CCD) is the more popular choice of photodetector when it comes to spectrometers today. CCDs are usually created on a silicon substrate. The CCD array used for the spectrometer is the Toshiba TCD1304AP CCD linear image sensor. Reasons for the choice are related to relevant CCD technology and the price point.

B. Spectrometer Configuration

The most common spectrometer configurations are the Czerny-Turner configurations. The Czerny-Turner configurations consist of an entrance slit, two concave mirrors, a diffraction grating, and an array detector. All of these components were described previously. The Czerny-Turner models can be explained as follows. Light is incident on an entrance slit, which affects the photon flux and spectral resolution of the spectrometer. The light is directed to the first concave mirror, called the collimating mirror. This mirror collimates the white light and directs it toward the diffraction grating. The diffracted light is then incident on the second concave mirror, called the focusing mirror. The focusing mirror focuses the light to an exit slit to a detector array, or directly to a detector array. An exit slit is used if single wavelength measurements are wanted. In our case, we are imaging the diffracted light straight onto the array detector. An exit slit is not needed. The output display will show the full UV-VIS spectrum, along with the absorption peaks.

The Czerny-Turner configuration can be separated into two different types. These two types have their advantages and disadvantages. The first type is the crossed Czerny-Turner. It is a compact design, as shown above. By optimizing the geometry of the system, the spectral resolution of the system can be improved. Also due to its geometry, the image width of the slit is broadened. As a result, the stray light that hits the detector is relatively high. Therefore, this design is used for applications that only require low to medium resolution. The specific configuration used in this device is the unfolded Czerny-Turner configuration, shown in Fig. 4.

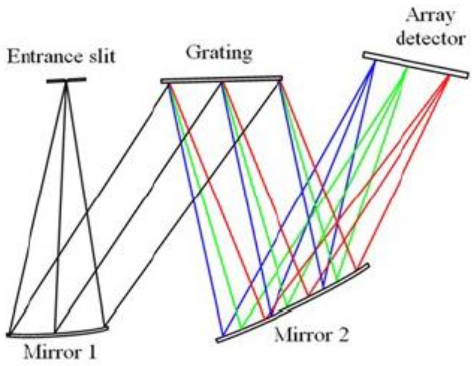

Fig. 4. Uncrossed Czerny-Turner Configuration

This configuration helps to alleviate stray light problems. Stray light is any unwanted light in the system that may or may not be detected by the photodetector. The reduction of stray light also reduces the optical noise of the system. Optical noise and stray light is a big problem for low light level UV applications, such as this spectrophotometer.

V. MCU AND LCD

The microcontroller unit is a key part of any electrical system. It allows the system to complete multiple tasks, and has many components to it. This device will be the center of the system, and will control the majority of other components in the system. It will do this by connecting to external hardware, and reading code loaded in either the device it's connecting to, or from code loaded onto it. For our particular system, we chose to use a MSP430F5528. The reason for choosing this controller is because all of us have experience with MSP430s from previous classes. This ensures that we can get right to work on it, and we don't need extra time to learn how it works. We are also provided resources for this component on campus, and through teachers who have helped us use this device. There are many choices of microcontrollers to choose from. Some of the more popular devices are Arduinos, and while there seems to be a good amount of support for these devices, it would be similar to using the MSP430. One of the main differences between these two devices is price. The Arduinos can cost anywhere from $20.00-$40.00, while the MSP430s cost from $1.00-$6.00. The catch here is that the MSP430s require a development board which can cost anywhere from $10.00 - $100.00. In order for us to save money here, we will be using the development boards from the TI lab on campus. Most microcontrollers have the same ports, but are usually structured differently or have a different number of ports. This means that most of the external devices needed for this project will be compatible with the MSP430, just as it is compatible with the Arduinos and other microcontrollers.

The ports needed for our microcontroller are the SPI, I2C, general pins, ADC, UART, and USB. These ports will be supporting the power to the light, the LCD, the buttons for the interface, and will receive information from the optics portion. One variable to consider for our microcontroller is the memory. There will be two types of memory used, nonvolatile, and the RAM. The non-volatile memory is saved on the controller, while the RAM is lost once the system is turned off. This means we need a microcontroller that has enough memory to support our external components and to run the code stored on it. This is why we chose a controller with a large amount of memory, this way we will have enough in case we need it. One factor in choosing the microcontroller was the ADC channels. For our system we need at least one ADC 12 bit channel. ADC stands for analog to digital converter, and it represents data given to it and converts it to bits. A 12 bit ADC will be able to convert data will be able to represent 2 to the 12th power numbers. This will be useful for connecting to our optics portion of the system and CCD in order to read in the data. The majority of MSP430s viewed had a slope ADC. This just means they have 2 to the 1 power of numbers to be represented. This limited our choices, but thankfully, since there are so many MSP430 microcontrollers, we were able to find one that fit with all the other specifications that we needed.

For our system, we will need a LCD in order to display the data on a graph to the user. The LCD we need must have a display resolution of at least 300x200, and be a large enough size for a graph to be easily seen from it. The best way to connect these LCDs will be through the SPI/I2C ports, meaning we will need to find a screen that supports these serial ports. Since there will be buttons on our system around the screen, we do not require the LCD to have touch screen technology, but since most modern screens have this feature, we will implement some functions for it if necessary. The screen must also come with an understandable datasheet and other resources to help us connect and program the LCD. One issue in finding LCDs is the size. Most screens we found are anywhere from two to four inches diagonally. For a reference, this would be the size of the screen of a smart phone, or a bit smaller. This is a problem since we want our screen to be big enough that the user can easily read a graph from. Although the resolution would be high enough in order to display a graph, we would like the user to not need to zoom into the screen in order to see the data clearly. This will create a demand for our LCD to have its own on board memory. When researching, we find that the bigger the screen is, the higher the resolution is. It is nearly impossible to find a screen that is the perfect size for our system, with a resolution of 320x240. Most screens past 5 inches have a resolution greater than or equal to 720x480.

The Kentec QVGA Display Booster Pack, one option for our LCD and seen in Figure 9 below, is fully compatible with the development board we have chosen. This pack is originally designed to connect to the development board, but since the microcontroller will be placed on a PCB board, we will have to manually connect the booster pack. This process will not be complicated since there are data sheets that will tell us where to plug in each pin. TI offers many resources, such as the data sheets for both the microcontroller and booster pack. These resources will be able to tell us where each connection will be made, why they are made, and what their function to the LCD is.

In order to design the graphical user interface for the system on the LCD, we used the C language on the IDE Code Composer Studio. Using the graphical library given to us by TI, we implemented functions to then draw onto the LCD. These functions include drawString, drawLine, drawRectangle, and then different parameters to set color, font-size, and other variables. We separated each function to draw a different screen, and in the main function implemented a loop to allow use for a keypad. The user will press the keypad, and whichever number they press will make the code call the corresponding function in order to jump to the next screen. In the latter screens, once the test has been conducted, the values obtained will be put in an array, which will then either be displayed on a graph, or will be searched through to find a specific value, depending on the test.

VI. POWER AND SPECTROPHOTOMETER ENCLOSURE

In the electrical industry nearly all power supplies are design to convert alternating current (AC) to direct current (DC). As for electric power distribution systems provide AC power, but nearly every device developed operates under DC power. Some examples of AC power would be power plants that provide homes, stores, business, the power used by a refrigerator, and washers. The devices which use DC power in order to function are cell phones, laptops, and flashlights.

Numerous circuits constructed are to convert AC to DC power, some are easy to design as for other circuits are multifaceted and safer for projects. For example, the half wave circuit which is developed of one transformer, one diode in series with a capacitor, permits for one-half wave of DC to flow through the load. The circuit is not very suitable for many applications, due to the harmonic content of the circuit's waveform is enormously large and difficult to filter with an op-amp. Furthermore, the design only delivers power to the load every half wave during every full cycle.

The most common mistake typically made when building the half wave rectifier is the diodes repetitive peak reverse voltage rating is overlooked. The voltage rating of the diode must be bigger than the peak voltage of the output voltage produced by the transformer. If the diode's peak inverse voltage (PIV) rating is inadequate, the diode will not be able to endure the loads of the circuit. When calculating the PIV rating of the diode, make sure to analyze the peak output voltage from the transformer's secondary windings. Each half-cycle of the AC waveform should be measured, taking into account the voltage loss across each device. After completion of the full cycle analysis, the required diode result should be attained.

Researching began on the type of power supply that would be needed for the project, being one of the most important parts of the project. The configuration of the power supply that was needed to be designed was AC to DC power. In order to be able to convert AC to DC power, a transform is used with a 1:10 ratio. The ratio is the amount of winding from the primary side compared to that of the secondary side. Following the transformer is a bridge rectifier used to give a full wave function during every full cycle, providing the circuit with efficient power and also making it easier to filter. After the bridge rectifier was implemented the rails were developed. In order to know what rails will be needed you'll have to see what needs to be powered. For this project the rails needed were a 3.3 V rail is used for the micro controller, 5 V rail is used for the LCD and sensor, and a 12 V rail is used for the keypad and light source. Followed by two capacitors in parallel, the first being a 1 μF and the second being a 100nF for both capacitors. As trial and error continued, the designed changed and began to improve.

The final schematic for the power supply is in Fig. 5. The power supply below begins with an on and off switch, the transformer was removed because it didn't provide us with the amount of power needed for the project. The device that needed the most power in the project is the light bulb, consuming 55 watts of power. The transformer added to the circuit is a Quans transformer that came with a 12 VDC output, having only to add a 3.3 V and 5 V regulators. Since the regulators used are linear regulators instead of switching regulators, the 5 V regulator was assisted with a heat sink to keep it from burning up. Also, the light source needed a switching MOSFET turn the light on and off. The switching MOSFET used for the project is a 3205. After all changes were done to the power supply, it reduced the final price by nine dollars.

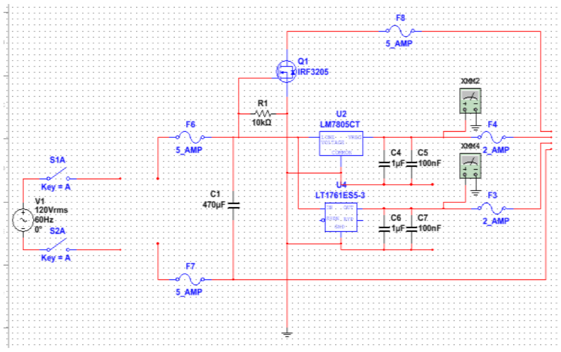

Fig. 5 Power supply schematic

Every project needs and enclosure, as it helps with protecting components from weather or animals. The project enclosure was initially designed using a software called solid works. The enclosure was to be 12in in length, 8in in width, and 6in in height, and be 3D printed in the TI lab. The issue encountered was that the lab was backed up with files and running out of material to print. So we decided to go with a toolbox, which isn't the right dimensions needed but they were close enough. The dimensions of the toolbox were 15.56in in length, 8.5in in width, and 6.4in in height. The enclosure will hold the power supply, micro controller, PCB, light source, switching MOSFET, LCD, keypad, the sample, and the optics (light source, mirrors, and grating).

VII. CONCLUSION

This project was chosen with a handful of goals. It was chosen as a showcase of our ability to collaborate as professional engineers and break down a complicated product into distinct parts. It was chosen to highlight each of our unique backgrounds, spanning the fields of biology, photonics, computer architecture, power systems and electronic design. It was chosen to improve upon an existing technology using modern components and research, targeting a growing audience for a unique yet widespread application. And importantly, it was chosen to demonstrate that each of us are competent engineers capable of realizing a practical solution to a problem.

We were warned when selecting this project that it would be very difficult. There is a great deal of careful consideration in the circuitry, and small details can cause big problems in the final system. While this quickly turned out to be true, we felt as engineers that the only thing separating us from previously successful designers was our dedication and commitment. This document contains our best efforts at understanding the devices necessary to build this product. While a larger team of more seasoned engineers might be able to produce a document twice the size with similar considerations, we felt this was a good starting point. We are eager to put these works into practice.

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BIOGRAPHY

Evan Zaldivar is currently a senior at the University of Central Florida and will receive his Bachelor of Science in Computer Engineering degree in May 2017. He has attended the University of Central Florida for four years, and plans on pursuing a Master's degree here.

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Sean Pope is a senior engineering student studying Electrical and Bioengineering at UCF. He is graduating with honors in May 2017 and plans to work in systems engineering for a few years before pursuing graduate studies in biomedical engineering. He is a member of Eta Kappa Nu honors society and the National Society of Collegiate Scholars.

Jimmy Vallejo, a senior student of the electrical engineering department at University of Central Florida. He interned with Peace River Electric Cooperation during the summers of 2015 and 2016. He hopes to pursue a working career in power engineering and continue studies for a master's degree in business administration and a math minor.