Portable Fluorescence Sensor for Lyme Disease Antibody Detection

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***Abstract* — An original Optics and Photonics Engineering, Computer Engineering, and Electrical Engineering project, the Portable Fluorescence Sensor for Lyme Disease Antibody Detection. Is a practical demonstration of what is possible with Everix thin-film optical filters. The project is a self-contained system for Illuminating, Measuring, and analyzing fluorescent emissions down to the nanomolar range in a very small form factor. With tight integration of the optical and electrical hardware systems, our device successfully demonstrates precise detection of fluorescein concentration from 3 µM – 50 nM, handheld dimensions, and portable rechargeable battery power.**

***Index Terms* — Optical filters, Fluorescence, Optical signal detection, photodiode, PWM.**

I. Introduction

One of our project goals is to create a device which accurately excites a fluorophore (Fluorescein sodium salt) that attaches to antibodies (Lyme's disease antibodies IgG and IgM a type of antibody). Our device will precisely measure the concentration of fluorophore solutions from the corresponding fluorescence emission readings. Compact design with reduced weight and bulk enabling a portable design for use in the field outside of the lab. The compact size and portability of our device give it an advantage over most fluorescence sensing devices which are predominantly made for benchtop use. The optical system will have a spacing between optics that allows for a compact design, keeping the optical system within a cube with 30mm sides. We will be utilizing our sponsor Everix's thin optical filters which have a thickness of tens to hundreds of microns. The small thickness of the Everix optical filter enables our optical system to have a reduced size compared to using other optical filter providers with a larger thickness. Everix's optical filters can be curved to accommodate the emitted excitation light without the use of a collimating lens which will enable our optical system to have a further decreased size. The short pass (SP) optical filter shall be curved such that, in combination with the long pass (LP) optical filter, the fluorescence signal to reflected excitation light ratio (SLR) will be at or above 500:1. The LED, optical filters, and photodetector will be rotated at an angle with respect to the normal of the container holding the fluorophore solution so that the ratio of fluorescent light emission signal intensity to reflected LED light signal intensity is greater than 500:1 at a 0.3 milli-molar (mM) concentration. The optical system shall have a limit of detection (LOD) <100 nanomolar (nM) concentration for fluorescence emission detection. With a financial sponsorship budget from Everix of $1,500, we planned to have the cost of a singular prototype of our device be less than a value of $750. We had 8 months from the end of August 2021 to design, test, and redesign our device before demonstrating the completed product at the end of the Senior Design 2 course in April 2022.

II. Fluorescence Spectroscopy Standards

Since our device will be used for detection of Lyme disease antibodies using fluorescence detection, our device is subject to the standards designated for fluorescence spectroscopy as prescribed by the National Institute of Standards and Technology in conjunction with the Technology Administration and the U.S. Department of Commerce. When a fluorophore solution is excited by light of a particular wavelength then the fluorophore will emit light of a longer wavelength, called fluorescence emission [1]. There are two general modes of measuring fluorescence from a sample: qualitative and quantitative [1]. Our device will provide qualitative as well as quantitative measurements. Qualitative fluorescence measurements detect the presence of particular antibodies, yielding a positive or negative answer [1]. Quantitative fluorescence measurements determine the concentrations of antibodies within an unknown sample [1]. A linear proportionality between the fluorescence emission signal and fluorophore concentration when the absorbance of the fluorophore is less than 0.05 at a path length of 1 cm [1].

Fluorescein isothiocyanate (FITC) is a fluorophore which will attach to Lyme's disease antibodies IgG and IgM [2]-[3]. FITC has a fluorescence emission peak at a wavelength of 519 nm. FITC costs $250 for a 1 mg size [2]-[3]. We needed to have a large quantity of fluorescent marker for prototype testing and determination of our device's limit of detection. We chose to use fluorescein sodium salt because it has a similar fluorescence emission peak compared to FITC and costs $30.5 for a size of 100 g which better fit our budget.

Fluorescein sodium salt typically has an excitation wavelength peak of 460 nm and a fluorescence emission peak at a wavelength of 515 nm [4]. We found through testing that the fluorescein we used has a fluorescence emission peak at a wavelength of 518 nm. Tests to determine the limit of detection of our device will be conducted through, multiple measurements of each sample out of a range of samples solutions, with varying concentrations from 3 mM to 5 nanomolar (nM) of fluorescein. Limit of detection is typically defined as the sample concentration at which the signal is equal to three times the noise level [4].

III. Power

A. Battery

A 3.7 V, standard 18650 cell powers our device. Since the device requires very little power, the 2.6 Amp hours provided by the battery will power our device for an approximate 4 hours. The battery is rechargeable and will recharge within about 3 hours, this is done while the device is plugged in with the USB C, the user is able to still make use of the device while charging.

B. Protection Circuit

Since we made use of a battery it was necessary to have a battery protection circuit to prevent overcharging to the battery which would create a safety hazard, it will also include an over discharge protection. The TP4056 is being used to charge the battery. The TP4056 is then connected to the DW01A IC with MOSFETS which is designed to protect the lithium-ion battery from damage or degrading due to overcharge, discharge and over current.

The battery protection circuit is connected to the USB C which is the emerging standard for charging and transfer of data, although we will only be using it as a charge port to the charging circuit. To accomplish this we used resistors to create the required circuit for USB standard to produce output voltage. The USB 5V Bus voltage is connected to the input of the input of the TP4056 IC, as well as the power regulator.

C. Switching Regulator

The chosen battery produces around 4.2V, and the USB input is a 5V supply. For that reason, a 5V regulator and a 3.3V Regulator will be needed to power the various ICs and components. A switching regulator like a DC-DC converter has the advantage of increased efficiency due to the nature of a switching regulator, but a higher quiescent current than a linear regulator. since our device will be powered off while not in use, the team has chosen to use a DC-DC converter for the 5V Regulator. The LTC3118 voltage regulator was used in our circuit design, it allows an input voltage range of 1.5V-10V, which allows our input voltage of 3.7-4.2V from the battery.

IV. Optical Design

A. Optical Filter

There are three main types of optical filters used in fluorescence sensing and, in our case, fluorescein detection which are bandpass filters (BP), long pass (LP), and short pass filters (SP). Bandpass optical filters only allow transmission of light within a wavelength range dictated by maximum and minimum wavelength cut off values. Long pass optical filters only allow transmission of light with a wavelength above a certain threshold wavelength value. Short pass optical filters only allow transmission of light with a wavelength below a threshold wavelength value. The key characteristics of an optical filter include thickness, optical density, flexibility, and transmission spectrum.

Edmund optics filters are all very thick, with thickness (1 - 5 mm), which would not allow our optical system to be scaled down enough to meet our design constraints. With this issue in mind, we decided to use ultra-thin optical filters, with thickness (200 – 500 μm), designed by our sponsor company Everix. In our optical system we chose a short pass optical filter with a cut off wavelength of 500 nm directly in front of the LED so that the excitation light from the LED will not overlap with the peak fluorescence emission of fluorescein. The excitation light intensity from the LED is much greater than the fluorescence emission intensity making the fluorescence signal indistinguishable from the LED light when their spectrums overlap. We also used a long pass optical filter, with a cut off wavelength of 500 nm, directly in front of our photodiode to cut off the reflected LED light from reaching the detector. It is important that we decrease the amount of LED light which reaches the photodiode because the photodiode does not discriminate between different wavelengths of light and will give us a false reading of the quantity of antibodies in a solution being higher than the true antibody concentration. In the application of fluorescence detection, because the emission and excitation wavelength are so close in proximity, and we are sensing at a very tight scale, we will need an optical density (OD) of 6. Since we are making use of both short pass and long pass optical filters with an OD of 3 each than our combined OD will be higher than the individual filter OD but less equivalent to the use of one filter with an OD of 6.

Optical filters exhibit different properties depending on the incident light onto the filter. Snell's law shows that as the incident light shines into the filter the angle changes

after the light enters the filter. Most optical filters are made with a thick glass material and the deviation after the filter would need to be accounted for. Everix filters are so thin, that the deviation in angle out of the filter will be negligible with the long pass filter placed 2 mm from the photodiode. As an interference-based filter, such as Everix's ultra-thin optical filter, is tilted away from normal, the transmission spectrum is "blue shifted," which means the spectral cut-off of the optical filter is shifted to shorter wavelengths [5]. This angular shift can be calculated for relatively small angles of incidence using the following formula:

$$\lambda_\theta = \lambda_0 \sqrt{1 - (\frac{n_0}{n_{eff}} \sin\theta)^2} \quad (1)$$

This angular shift becomes more pronounced with increasing incidence angles [5]. The "blue shift" in optical filters is typically mitigated using a collimating lens to collimate the light before it reaches the optical filter, reducing the angle of incidence on the optical filter. Using a collimating lens would increase the size of our optical system, therefore we decided to pursue the option of curving Everix's ultra-thin optical filters in a way to mitigate the 'blue shift" of our optical filters by reducing the angle of incidence on the filter. Everix's ultra-thin optical filter's flexibility enables a smaller optical design in our device.

B. Photodiode

We are using a PS11.9-5-TO5 photodiode as the photodetector in our device. The photodiode's 4.5 mm length and 8 mm diameter mean that it can help meet the small size requirements for our device [6]. The photodiode has a spectral range of detection that includes the 518 nm fluorescence emission that we expect from the fluorescein. The linear response of the photodiode enables a more precise linear equation to be developed between the photocurrent produced by the photodiode and the concentration of fluorophores in the fluorescent solution. Identifying the concentration of fluorophores within a solution enables the determination of the concentration of antibodies in a solution. The PS11.9-5-TO5 active area size is 3.45x3.45 mm which is very large compared to most photodiodes [6]. We chose a photodiode with a large active area so that it could capture as much of the fluorescence emission as possible. This is important because the fluorescence emission intensity is much lower than the LED light intensity, especially at low concentrations of fluorescent solution. The responsivity of our photodiode is approximately 0.25 A/W at the fluorescence emission peak of 518 nm.

$$I = 2.303(K')\varepsilon b c P_0 \quad (2)$$

Fluorescence emission intensity (I) can be calculated using equation 2 with respect to excitation light optical power (P_0) as well as other factors. Geometry based factors and quantum yield are represented in the constant K'. The molar absorptivity is represented by ε in equation 2. Fluorophore concentration is represented by "c". The optical path length that the fluorescent light emission travels is represented as "b" in equation 2. An approximate optical path length for the fluorescent light emission from the sample fluorophore concentration in our device is 1.2 cm. The molar absorptivity of fluorescein is 70,000 $cm^{-1}M^{-1}$. The Thorlabs LED465E that we have chosen as our excitation source has a 20-mW optical power. The lowest concentration of fluorophore solutions that we tested is 5 nM while the highest concentration of fluorophore solution that we tested is 3 mM. The calculated fluorescence emission intensity for fluorescein sample concentrations without accounting for changes in factors relating to K', ranges from 19.345 µW with a concentration of 5 nM to 11.607 W with a concentration of 3 mM.

$$I_{ph} = PR \quad (3)$$

Equation 3 is a formula for solving for photocurrent (I_{ph}) given responsivity (R) and incident power (P). Using the calculated fluorescence emission intensity 11.607 W at a concentration of 3 mM and intensity of 19.345 µW at a concentration of 5 nM, along with the photodiode responsivity of 0.25 A/W, the photocurrent was calculated to range from 2.902 A at high concentration to 4.836 µA at our lowest test concentration. A fluorescent solution with a concentration that gives our device a 3- or 9.54-dB signal to noise ratio (SNR) is the limit of detection for our device. The voltage noise level of our device is 0.01 V. This means that the signal at our limit of detection ideally would be 0.03 V.

For the photodiode amplifier we made use of the LTC1050 operational amplifier in our design. A circuit using the LTC1050 optimized the precision of the photodiode sensor by amplifying the current which depended on the light seen by the photodiode. Furthermore, the LTC1050 converted the current generated from the photodiode to an output voltage.

C. Excitation Source

The excitation source we have chosen is a Thorlabs 465E LED which has a central wavelength of 465 nm [7]. Comparing the theoretical normalized intensity distribution to the lab tested distribution we can see a shift in the lab test by about 9 nm. This shift in the spectrum will not affect the excitation of the fluorescein as there is a broad range for the excitation to occur. The LED465E has an intensity distribution full width at half the maximum (FWHM) intensity of about 25 nm [7]. When accounting for the wavelength shift to a 474 nm wavelength peak when testing the LED and adding half of the FWHM value 12.5 nm. The LED465E based on specifications and preliminary testing should only reach as high as a 486.5 nm wavelength. When

conducting further testing it was found the FWHM does not accurately represent the spectral spread of an LED with a high intensity such as LED465E when compared to the lower fluorescence emission intensity which will be less than 50% the intensity of the LED. The higher intensity of the LED leads to a spectral overlap which was found to completely smother the fluorescence emission signal at certain angles. This larger spectral overlap provided the motivation to utilize a short pass optical filter to cut off the LED light above the 500 nm wavelength.

When discussing our optical system, one of the most important parameters of the LED is the beam profile and angle of the LED. The beam angle will decide how close the LED needs to be to the sample to allow both the correct amount of fluorescent marker excitation and the proper detection of fluorescent emission. The radial intensity of the LED is about 30°. The half viewing angle of LED465E as specified in the specs sheet is ±8° but upon further testing the half viewing angle was found to be ±16°, with a full angle of 32° [7]. The full angle measured matches the radial intensity of 30° previously approximated. The Thorlabs LED465E emits an isotropic pattern characteristic of an LED with a dome shaped surface. The LED used here is a parabolic shape meaning that the light intensity of the LED decreases as the angle increases beyond the normal light axis of the LED.

Blue light, in the range of 380-550nm wavelengths, can induce photochemical damage to the retina [8]. This blue-light retinal injury can result from viewing extremely bright light for a short duration of time or less bright light for a longer duration [8]. Since our project emits blue light within this wavelength range, there are certain safety standards that we must follow. These blue light LED safety standards come from the Lawrence Berkeley National Laboratory Environment, Health & Safety (LBL EHS) standards. The following are the blue light limit threshold values: time-integrated radiance, weighted by the blue-light hazard function, should not exceed 100 J/(cm2-sr) over a total viewing time of 167 minutes in a day [8]. If the viewing duration is longer than 167 minutes, the radiance weighted by the blue-light hazard function should not exceed 10 mW/(cm2-sr). If these limits are exceeded by our device, then we must provide safety control measures [8]. Our LED will not be continuously on and will only be on for a number of seconds per reading taken by our device. The optical enclosure and the sample cuvette will both be covered which will keep a majority of the LED465E light emission within our device and will not require additional blue light safety control measures.

The LED also needed a driver circuit in order to be powered by the MCU with the correct light amount. The MCU sends a PWM signal to the LED driver and power is applied to the LED. The LED will then emit the specified amount of light to the sample which is then detected by the photodiode.

D. Reflection Mode of Fluorescence Detection

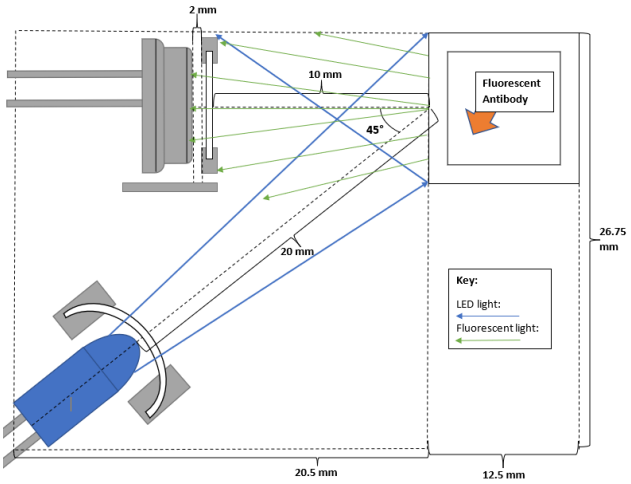

Fig. 1

The detection of fluorescence emission from a solution through a transmission based optical system leads to low brightness levels and a higher spectral noise level from a large concentration of scattered excitation light reaching the photodiode [9]. The scattered excitation LED light transmitted through the sample solution would produce a low fluorescence signal to LED light ratio (SLR). This is due to the intensity of excitation light being several orders of magnitude greater than fluorescence emission. The low brightness and low SLR led to our decision to use a reflection based optical system with the LED and photodiode facing the same face of the rectangular cuvette holding a solution of fluorescein. The LED light transmitted through the cuvette does not reach the photodiode and only the reflected LED light of reduced intensity will reach the photodiode. The light reaching the photodiode has a higher brightness in the reflection based optical system leading to a higher photocurrent output and higher SNR. Going forward SLR will be used to refer to the fluorescence signal to reflected LED light ratio. The SLR value is important to our design because the intensity of reflected LED light will create a spectral noise level at which the photodiode will falsely read a fluorescent signal when only LED light is detected. The reflected LED light intensity is greater than the noise level of our device and creates a threshold limit to our device's detection which is above what the limit would normally be if the fluorescent signal did not have to compete with the LED light.

For our sample holder cuvette, we chose to use Lifestyle Vision's Quartz Cuvette. Each cuvette can hold up to 3,500 microliters of solution. We chose a rectangular cuvette, as opposed to a cylindrical cuvette, to decrease the number of variables that we would have to factor into our optical

design. The quartz cuvette has two sides which are frosted (semi-opaque) and two sides transparent (transmissive). The cuvette has a size of 12.5 mm by 12.5 mm by 45 mm in width, length, and height respectively. The length and width of the quartz cuvette are reasonable dimensions to hold the needed solution concentrations and stay within our compact size constraints. The height however is larger than we would like to have for our device but is more than made up for by the transmissive properties of this cuvette. The cuvette has a 10 mm optical path length through the transparent sides of the sample container. These quartz cuvettes are advantageous in their property of above 83% transmittance of light through the transparent sides of the cuvette. This will enable more of the LED excitation light to be transmitted through the cuvette while simultaneously decreasing the intensity of the reflected LED light that the fluorescence signal will have to compete with. These quartz cuvettes cost $14.68 per cuvette.

Due to the 6 mm diameter of the LED and the 9 mm diameter of the photodiode, both components must be rotated at opposite angles with respect to the normal of the cuvette face in order to achieve a small distance between the components and the sample cuvette. Bringing the LED and photodiode close to the sample cuvette will increase the fluorescence signal seen by the photodiode and decrease the size of our optical system which are two main objectives of our optical design. At LED rotation angles larger than a 45° angle with respect to the normal of the cuvette, the angle between the LED and photodiode will become closer to 180° which will allow more direct LED light to leak into the long pass optical filter and photodiode. This led us to determine that a 45° angle for the LED and photodiode would be the largest angle that we could position our components at, to get close to the sample cuvette without direct LED light reaching the photodiode. Our optical design was initially planned to have the LED and photodiode rotated at equal and opposite angles with respect to the normal of the cuvette face. This would enable us to most effectively curve the LP optical filter in front of the photodiode due to the photodiode being angled into the known path of reflected LED light. When testing our optical design in our prototype we found that the ultra-thin LP filter could not block the high intensity light in the reflected LED light path leading to the fluorescence signal being overshadowed by the reflected LED light.

With this finding we decided to pursue decreasing the angle of the LP filter and photodiode while keeping the LED fixed at an angle of 45° with respect to the normal of the cuvette, so that the photodiode would be angled outside the reflected LED light path. With the half angle of the LED465E being 16° we determined through some basic math (45° - 16° = 29°) that we would need to angle the photodiode at a 29° angle with respect to the cuvette normal. We believed that an angle of 29° degrees would be optimal to position the LP filter outside of the reflected LED light path while having a large angle to bring our components close to the sample cuvette. When testing LP filter angles from 25°-0° degrees we found that as the angle decreased the SLR increased. We found the most significant increase at LP filter angle of 0° degrees. An angle of 0° degrees also enabled us to get closer to the cuvette without being directly in the LED reflection light path.

We wanted to position the LED, optical filters, and photodiode at a close distance of 5 mm from the sample cuvette, but we knew that there would be a position at which the SLR would decrease at close distances. To test this hypothesis, we tested the SLR when moving the LED, with an SP filter, from 20 – 10 mm away from the cuvette while keeping the LP filter fixed at 20 mm away from the cuvette. During this testing we found that the SLR values decreased from 1919 – 725. This proved to us that we were already passed the point where the SLR would diminish with decreasing distance from the cuvette. We decided that the SLR value of 1919 was significant enough to warrant us increasing our optical size so that we could have a lower limit of detection for our optical system when scaling down to lower fluorescein concentrations. With this decision we chose to place the LED at a 20 mm distance from the cuvette.

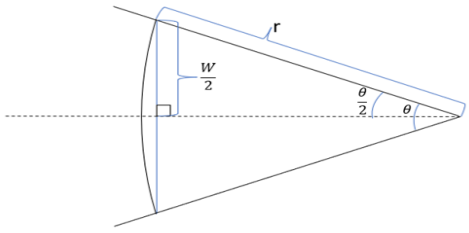

Fig. 2

$$r = \frac{W}{2\sin(\frac{\theta}{2})} \quad (4)$$

Equation 4 was used to determine the optimal radius of curvature for the SP filter in front of the LED465E. In equation 4, the LED diameter of 5 mm is substituted for W and the 32° full angle of the LED is substituted for θ. The radius of curvature of the SP filter was calculated to be 9.07 mm. When testing SP filter radius of curvatures from 10 – 20 mm at a 1 mm distance from the LED465E it was found that the reflected LED light intensity significantly decreased at a 15 mm radius of curvature. This decrease in intensity showed that at a 15 mm radius of curvature the SP filter most effectively cut off the LED light spectrum above 500 nm.

When testing LP filter distances to the cuvette of 30 – 20 mm, the SLR increased as distances from the cuvette decreased. We decreased the size of the LP filter to 8 mm to match the opening diameter of the photodiode and

minimize collision between components which had inhibited movement of our LP filter closer to the cuvette. Then we moved the LP filter and photodiode to 10 mm from the cuvette. We determined through calculation of the beam angles of the LED at a 20 mm distance from the cuvette that 10 mm was the minimum distance that the LP filter could be positioned at and be outside of the direct LED reflection path. At 10 mm from the sample cuvette with a 0.3 mM concentration, the fluorescence signal was observed to produce a 3.595 V photodiode output. This is significantly improved from our earlier signal output voltage of 0.21 V at 20 mm from the cuvette with the same concentration.

Our overall optical design manages to have dimensions of 33 mm by 26.75 mm by 15 mm. These optical system dimensions are very close to our compact size specification of less than a 30 mm cube.

V. Software Design

A simplified flowchart is shown in figure 3 to illustrate how the software operates. The software will be discussed in the same order that it is executed.

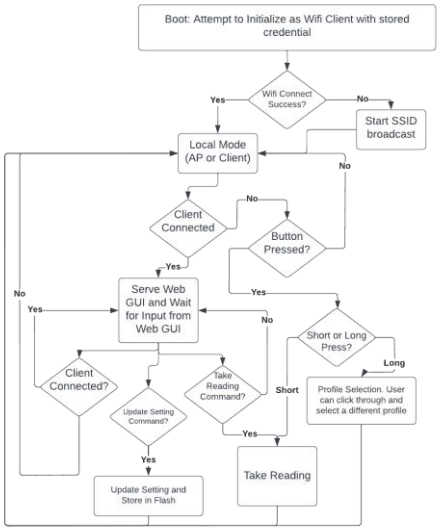

Fig. 3

A. Initialization

The system uses a basic state machine as the basis of the software. On Boot the device initializes its internal flash memory, and reads device configuration into memory. The device also initializes the display and ADC over I2C. The device then uses the WiFi SSID and Key that was stored in flash and attempts to connect the the network and get an IP address. The device waits for the WiFi driver to timeout if the connection is unsuccessful and tries 9 additional times to establish this connection. If a connection is established, the device moves into local mode with "wifi client" configuration. If no connection is established, the device initializes an internal WiFi access point, and begins broadcasting its own SSID with SSID and Password shown on the screen. Once the internal network is initialized, the device enters local mode with "AP Mode" configuration. The two different WiFi configurations enter the same logical state in the backend because client connections and HTTP requests are all handled by the backend libraries on the ESP32. Next the device initializes its web server, is able to respond to standard http requests. Next the Web Socket server is initialized and the device is able to receive Web Socket requests.

B. Local Mode

In Local Mode the device displays basic information like the SSID and IP Address to connect to the device. The device is waiting for Web Clients to connect. In this state, it also makes available to the user a local interface with the display and a button for control. A user can initiate a reading by short pressing the control button, or enter a menu to switch through the available profiles by long pressing the control button. The process of taking a reading will be discussed below. From the Profile selection menu, a short press on the profile button will advance the device to the next profile, and a long press will return the the main screen. When the user switches the profile, the index of the current profile is stored in the devices flash memory so it can be recalled after a reboot and the device will stay on the desired profile. If a web client initiates a Web Socket connection to the device, it will enter web mode.

C. Web Mode

In Web Mode, the device is actively being controlled by an HTML based web app that allows advanced control of the device. In web mode, the user is able to select the SSID and password that the device will attempt to connect to. When a new SSID and password are entered, the device essentially reboots and attempts to connect to the newly configured network. These credentials are stored in the devices flash memory so they can be stored persistently between reboots. The user is also able to view and edit the 16 sampling profiles that are stored on the device. The interface allows configuration of the profile name, number of samples per reading, delay between samples, conversion factor, and offset values. These can be modified from the web UI and are stored in the flash memory for persistent recall. The Web UI also allows the user to take a reading and calibrate the device.

D. *Taking a Reading*

When a reading is triggered the device enters the same function with either Web UI or local trigger. It illuminates the LED on the sample, Samples the voltage from the photodiode, turns off the LED, and then waits the amount of time configured in the profile for delay. This process is repeated based on the number of samples configured in the profile. Once all samples have been taken, the device averages the samples, and multiplies and offsets the value based on the values from the profile, and publishes the reading to the web UI if there are any connected, or the local display if no web UI is connected.

VI. Design

We have gone through two main overall device prototype designs. All the protypes were modeled in Autodesk and printed on an ender 3s1. The first overall device enclosure was made from silk white filament which allowed more light to be reflected on the walls inside the enclosure and allow more light into the design. The first overall device prototype had a working display, button, circuit board, power supply, and optical enclosure.

The first optical enclosure prototype was successful in having the LP filter and LED465E at equal and opposite 45° angles with respect to cylindrical vial that was used. There were many challenges discovered with this prototype including: filter holders could not properly curve the LP filter, pegs on LP filter holder did not work, unreliable use of tape for positioning, and significant background reflections off the enclosure walls. The second optical enclosure prototype had reliable modular positioning using pegs and holes. The inside walls of the enclosure were painted with black 3.0 paint which significantly reduced the background reflections coming off the wall. We implemented the use of our previously mentioned quartz cuvettes to reduce LED light reflection and reduce the number variables needed to account for when determining the optimal optical filter curvature.

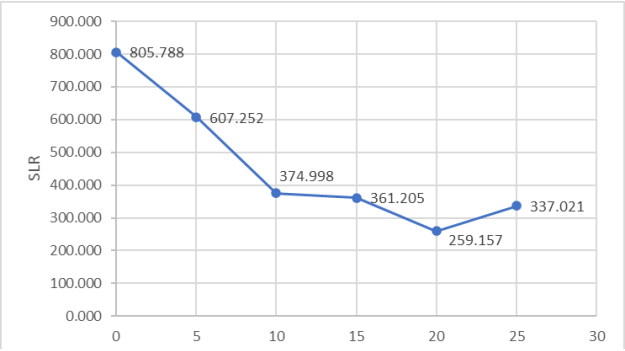

Fig. 4

The third optical enclosure prototype learned from the successes of the second optical enclosure prototype and additionally made it possible to test the LP filter at different angles from 25° - 0° with respect to the sample cuvette. The concentration in the sample cuvette was 0.3 mM and stayed fixed throughout testing. The third optical enclosure prototype kept the LED465E fixed at an angle of 45°.

The following procedure was conducted for all of our SLR values obtained through our optical testing. The spectrums of fluorescence and LED465E reflection were obtained with the aid of a fiber optic cable attached to a blue wave spectrometer. The noise was subtracted from the fluorescence and reflected LED signals within these spectrums. The intensity of the fluorescence signal and LED reflection were then compared to obtain a SLR value for each test in our optical enclosure. As can be seen in figure 2 when we moved the LP filter from 25° – 0°, the SLR drastically increased from 375 to 607 at an angle of 5° and increased from 607 to 806 at an angle of 0°. The result of this prototype testing determined that the LP filter should be at a 0° angle with respect to the cuvette for our device to have a high SLR value.

The fourth optical enclosure prototype was made similarly to the second optical enclosure prototype with the LP filter instead set at an angle of 0° with respect to the cuvette. The fourth optical enclosure was built with holes to position the LP filter at 30 – 10 mm and LED465E at 20 – 10 mm from the quartz cuvette. The LP filter positions from 30 – 10 mm were tested at each LED465E position from 20 - 10 mm from the cuvette. While testing the LED465E positions we found that the SLR decreased as the distance from LED to cuvette decreased. We determined that the LED465E position of 20 mm from the cuvette produced the largest SLR value of 1919. When testing positions of the LP filter from 30 - 20 mm the SLR increased up to 1919. This led us to decide that we needed to bring the LP filter as close to the cuvette as possible which due to the size of the LP filter was limited to 20 mm from the cuvette. To get passed the limitation of the LP filter size we cut the LP filter to an 8 mm by 8 mm size, and this allowed us to move the LP filter to a minimum distance of 10 mm away from the cuvette. We could not bring the LP filter closer to the cuvette without the LP filter and photodiode getting in the direct LED reflection path.

The SP filter was tested at radii of curvature from 20 – 10 mm and it was found that at 15 mm radius of the SP filter, the reflected LED light intensity was at a minimum. When testing radii of curvature of LP filter from 20 – 10 mm, voltage read out by the photodiode circuit changed by 0.01 V which was very close to our noise value of 0.01 V leading

us to decide that the curvature of the LP filter has little impact on our device's operation. With this result in mind, we decided to use a flat LP filter to simplify our modeling and make our optical system smaller.

To determine our overall device's limit of detection we used cuvettes with concentrations varying from 3.331 mM to 12.708 nM. We found that our device has a limit of 50 nM with a 12.708 nM concentration being below the reflected LED light voltage value, showing that at 12.708 nM we are only detecting reflected LED light. We found that the voltage output by our photodiode circuit has a linear relation for 3 µM – 50 nM fluorescein concentrations and an exponential relationship between 3 mM – 3 µM. This linear relation at micro-molar to nano-molar concentrations enables us to precisely map our photodiode output to the true molar concentration.

VII. Conclusion

This 8-month project has been a very valuable experience. Through this project, we have learned how to work as a team to solve critical challenges, how to schedule and conduct professional weekly meetings, how to deal with conflicts within our team, how to design a device from a customer's specifications, and how to test and re-design prototypes. We have effectively applied engineering concepts from our individual disciplines to solve critical challenges which would otherwise stunt our device's success.

We are very proud of our device's ability to detect fluorescence concentrations at the nano-molar level while having a compact size which can easily be held in one hand. Our device can be comfortably carried around while running off its own re-chargeable battery. Our device has a low cost of $377.66 which is significantly lower than our planned cost of $750. We have successfully developed a compact optical system which demonstrates our sponsor Everix's thin optical filter technology.

BIOGRAPHY

Cristian Pearson, a senior engineering student of the CREOL, college of optics and photonics., at the University of Central Florida, will be working as a fiber laser engineer for Leidos. He is also interested in pursuing a higher education after gaining experience in the engineering profession.

Christian Spurgeon, a senior design engineering student of CREOL, college of optics and photonics at the University of Central Florida. Currently an optical and quality engineering intern at Everix, optical filters. Looking to go to graduate school for a Masters.

Gean Morales, a 23-year-old senior Electrical Engineering student at UCF. Plans on working with the power distribution branch of electrical engineering.

Aaron Jevitt, a Computer Engineering student from UCF. Currently an working on Artemis for Jacobs Engineering under TOSC contract, with plans to continue after graduation.

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