Portable Fluorescence Sensor for Lyme Disease Antibody Detection

Sponsor:

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Motivation

- Create Demonstration of Everix thin filter technology
- Create a portable fluorescence sensing device that can be used outside the laboratory environment
- Problems with traditional fluorescent sensing devices
  - Low Portability
  - High Cost

Goals & Objectives

- Create a device which accurately and precisely excites fluorophores of particular interest that are attached Lyme disease antibodies
  - Select an ailment and Fluorescent marker to detect
  - Determine the excitation and emission wavelength of the fluorescent marker
  - Find an illumination source with a peak emission wavelength equal to the excitation wavelength of the chosen fluorophore
- Create a device which accurately and precisely measures the concentration of fluorescent emission from the fluorophores
  - Photodetector chosen with a high sensitivity within the fluorescence emission wavelength range
  - Optical filter chosen to isolate the fluorescent emission wavelength, cutting off the excitation light
- Compact design with reduced weight and bulk compared to other fluorescent sensing devices
  - Compact optical design through use of angled illumination reflection and detection
  - Compact circuit design
- Portable design for use in the field outside of the lab
  - Portable power supply/long battery life
  - Re-chargeable battery
- Visual display of sample concentration of fluorophores representing a particular ailment detected through florescence
  - Display with a high enough pixel count to display the decimal quantities with units of molar concentration

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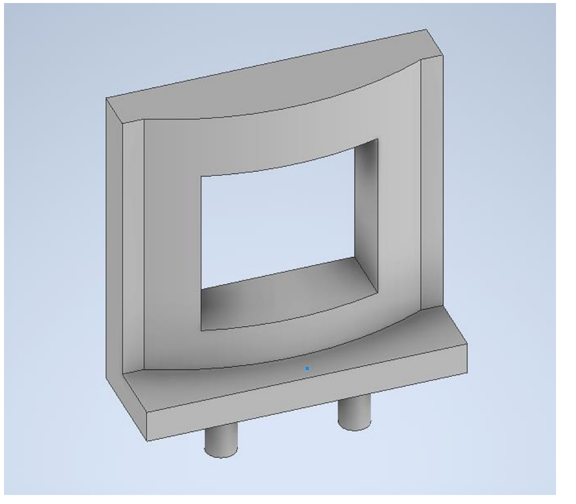
Specifications & Requirements

Description	Requirement / Specification
The LED shall emit a spectrum of light with the highest intensity peak centered within the excitation wavelength range for fluorescein	460 ± 10 nm
The LED, optical filters, and photodiode will be rotated and positioned with respect to the cuvette holding the fluorophore solution so that the ratio of fluorescent emission signal intensity to reflected LED light signal intensity (SLR) is high	SLR > 500 at concentration of 0.3 mM
The optical system will have a spacing between optics that allows for compact design	Fit within cube of 30 mm sides
The overall device limit of detection (LOD)	< 100 nanomolar (nM)

Device Enclosure



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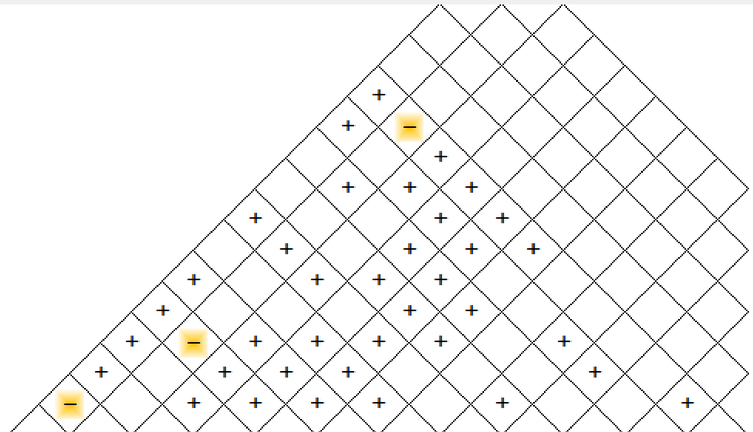
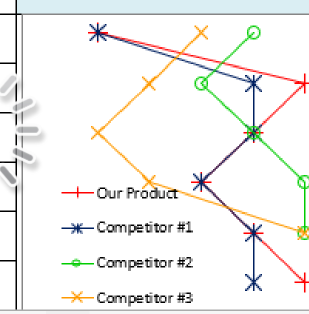
Correlations	
Positive	+
Negative	–
No Correlation	

Relationships	
Strong	●
Moderate	○
Weak	▽

Direction of Improvement	
Maximize	▲
Target	◇
Minimize	▼



Category	Weight	Customer Requirements (Explicit and Implicit) \ Engineering Requirements	Florescence detection accuracy	Optical System focal length	Fluorophore Excitation accuracy	Effective Optical Filter Switching	Accurate Display of Fluorescence Data	Effective user input to parameter adjustment	Simple GUI Operation	Accurate fluorescence calculation software	Accurate Fluorescence calibration software	Dimensions	Power use	Cost	Our Product	Portable Fluorescent Sensor for on-site detection of microalgae	Low-Cost, Portable Smart Instrument for Detecting Colorectal	Fluobeam	Customer Competitive Assesment (0 1 2 3 4 5)	Row #
		Column #	1	2	3	4	5	6	7	8	9	10	11	12						
		Direction of Improvement	▲	▼	▲	◇	▲	◇	◇	▲	▲	▼	▼	▼						
Safety	2	Blue LED light emission within safe intensity	○	▽	●		▽	○			▽		●	○	1	1	4	3		1
Everix	8	Compact size	○	▽	▽	●	▽	▽			▽	●	●	●	5	4	3	2		2
	4	Portability	▽	▽	▽	○	▽	○	○	▽	▽	●	●	○	4	4	4	1		3
	4	Low cost		○	○	●	○	○		▽		●	●	●	3	3	5	2		4
	8	Accurate fluorescence detection	●	▽	●	○	▽	▽				○	▽	▽	4	4	5	5		5
	4	Battery Life				●							●	●	5	4				6



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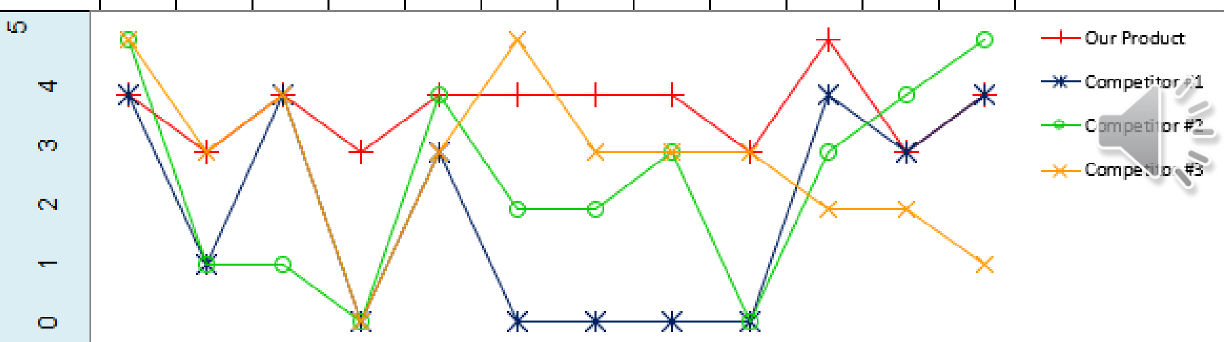
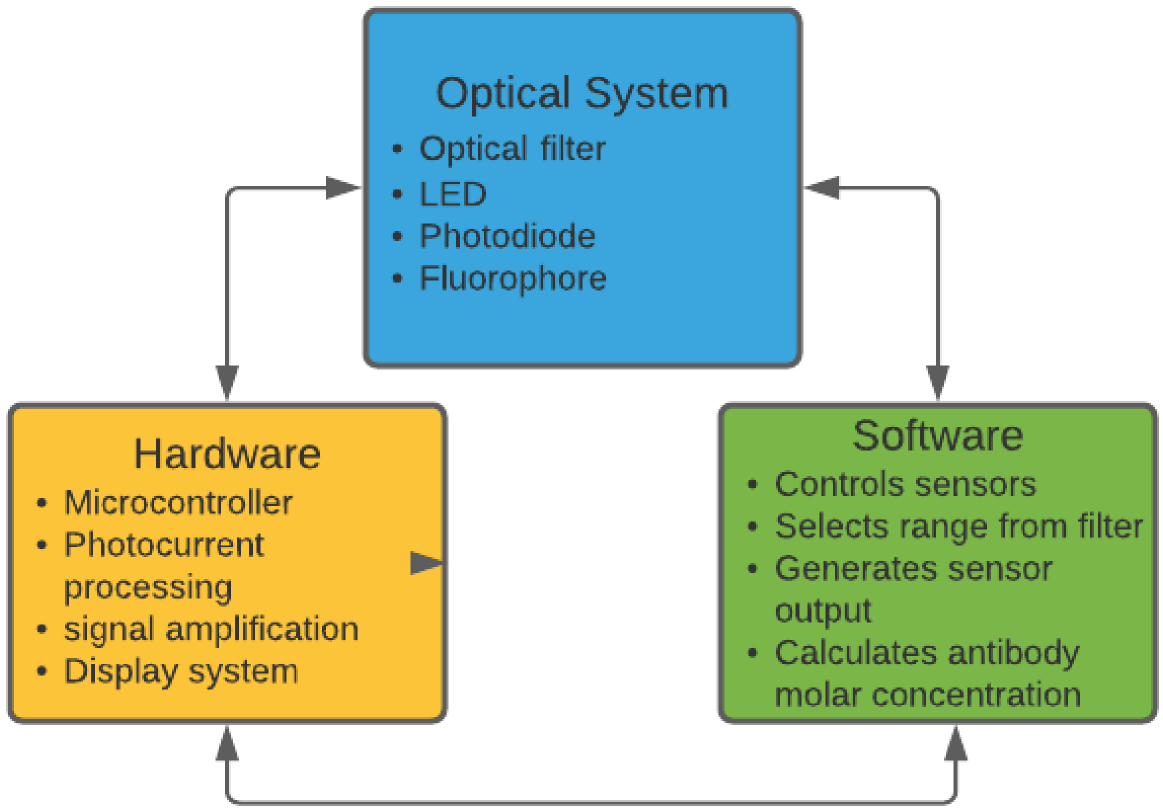
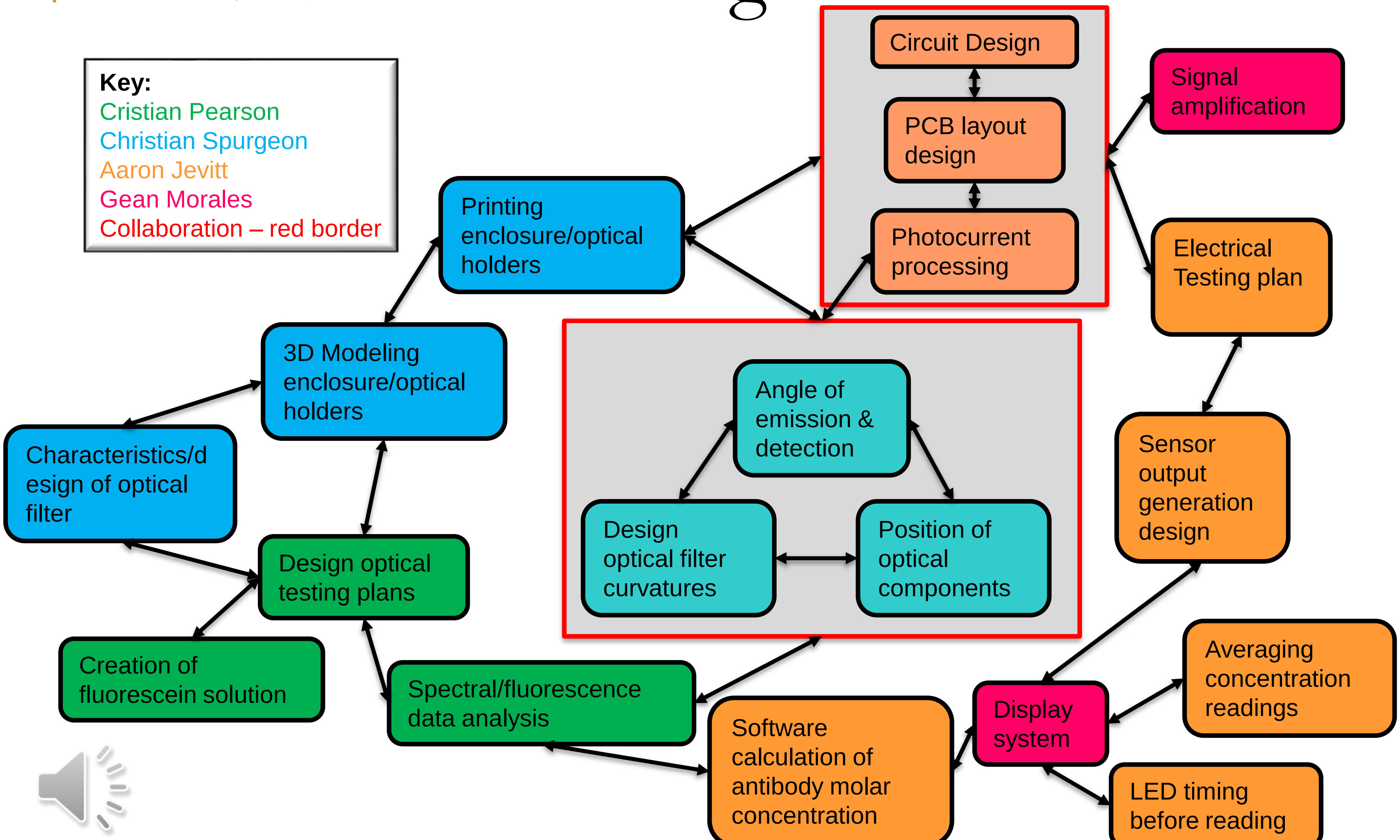
Target	Florescence detection accuracy	Optical System focal length	Fluorophore Excitation accuracy	Effective Optical Filter Switching	Accurate Display of Fluorescence Data	Effective user input to parameter adjustment	Simple GUI Operation	Accurate fluorescence calculation software	Accurate Fluorescence calibration software	Dimensions	Power use	Cost
Max Relationship	9	3	9	9	3	3	3	1	1	9	9	9
Technical Importance Rating	10600	3400	11400	18000	3400	4600	1200	800	1400	16800	20600	17000
Relative Weight	10%	3%	10%	16%	3%	4%	1%	1%	1%	15%	19%	16%
Weight Chart												
Our Product	4	3	4	3	4	4	4	4	3	5	3	4
Portable Fluorescent Sensor for on-site detection of microalgae	4	1	4	0	3	0	0	0	0	4	3	4
Low-Cost, Portable Smart Instrument for Detecting Colorectal Cancer Cells	5	1	1	0	4	2	2	3	0	3	4	5
Fluobeam	5	3	4	0	3	5	3	3	3	2	2	1



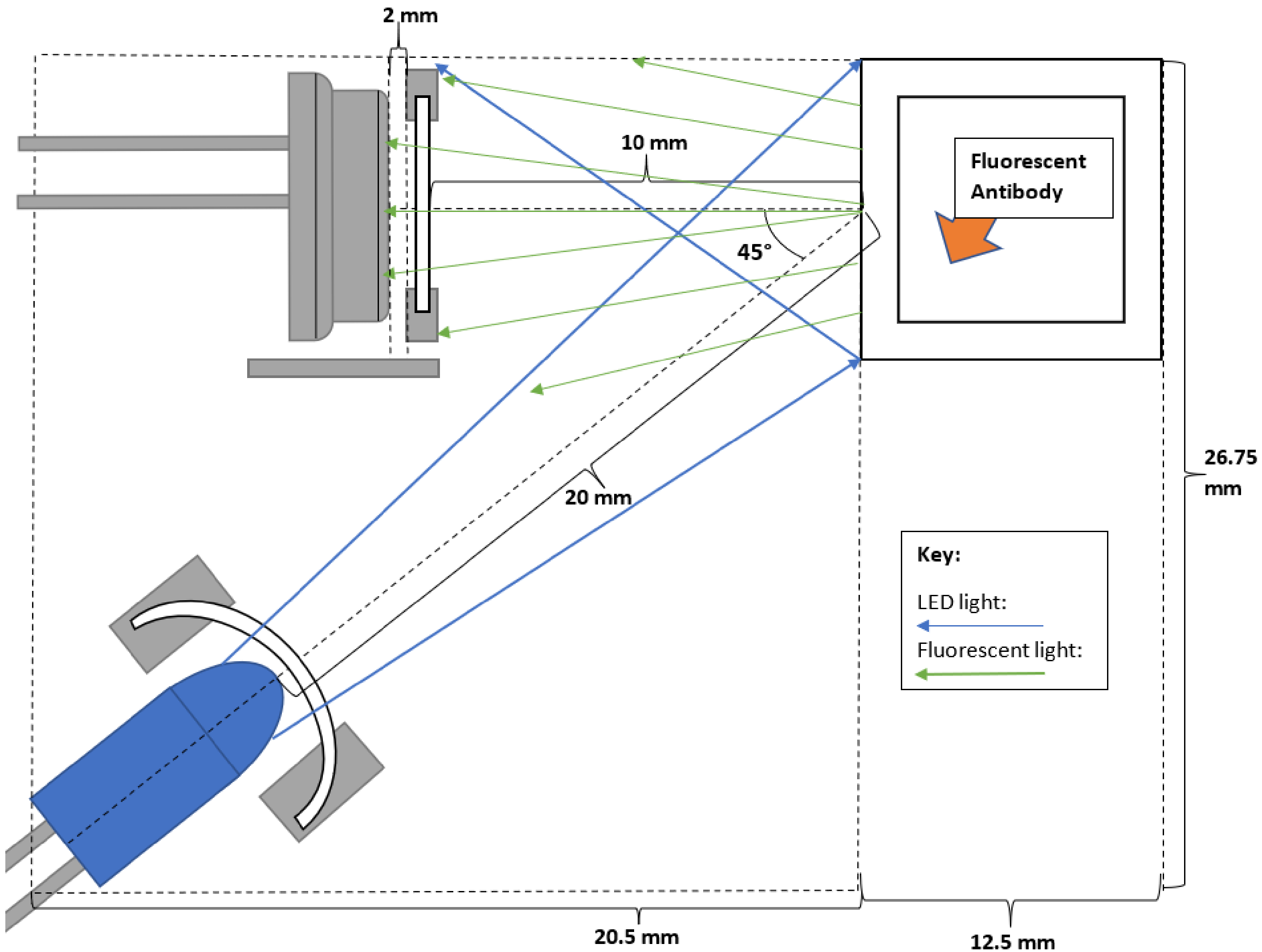
Overall Block Diagram



Individual Role Diagram



Optical System Design Diagram



Explain Design Approach

- Reflection based fluorescence detection
  - Higher signal detection from fluorescent light emission
  - Compact optical system due to angled illumination
  - Reduced intensity of LED light reflected off cuvette compared to direct LED light intensity from transmission

Optical Filter considerations

- Optical Filter
  - Long pass filter (LP)
    - Cut-off light wavelengths below 500 nm
  - Short pass filter (SP)
    - Cut-off light wavelengths above 500 nm
  - Unwanted LED illumination leakage at non-normal angles
  - Everix thin filter decreasing size of optic system
  - SP Filter curved for normal incidence at all angles of LED light emission incident on filter
    - 15 mm radius of curvature

Overcoming Design Challenges

- Challenges
  - LED Spectral bandwidth overlap with fluorescence signal
  - Intensity of LED light in direct reflection path too high for effective light filtration
- Use of SP optical filter in front of LED to stop spectral overlap with fluorescence signal
- Angling of photodiode and LP filter outside of the direct LED reflection path generated a signal to LED light ratio (SLR) of 1919:1

Overall Optical Component Decision

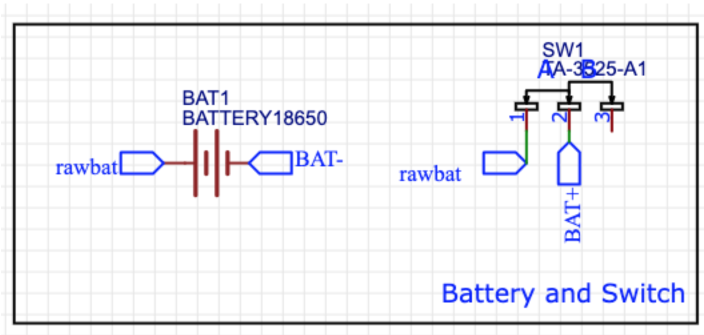
- LED excitation source
  - Peak LED emission wavelength 474 nm within fluorescein excitation wavelength range
- Photodiode detector
  - Linear signal response
  - High responsivity
  - Large active area
- Optical filter
  - 500nm wavelength cut off chosen for LP and SP filter, to isolate fluorescent light signal
  - Curved to stop overlap of fluorescence and reflected LED signals by decreasing spectral spread of LED light

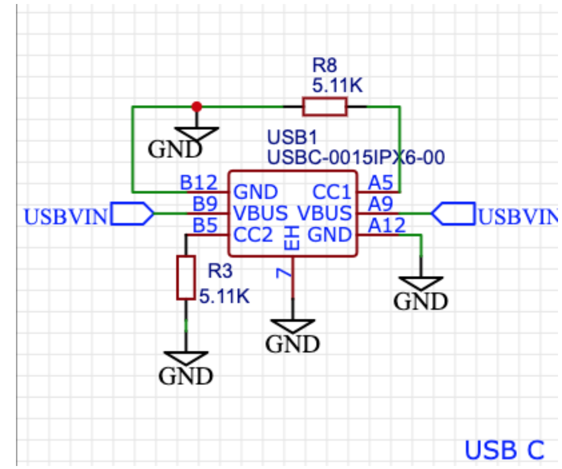
Electrical Design Overview

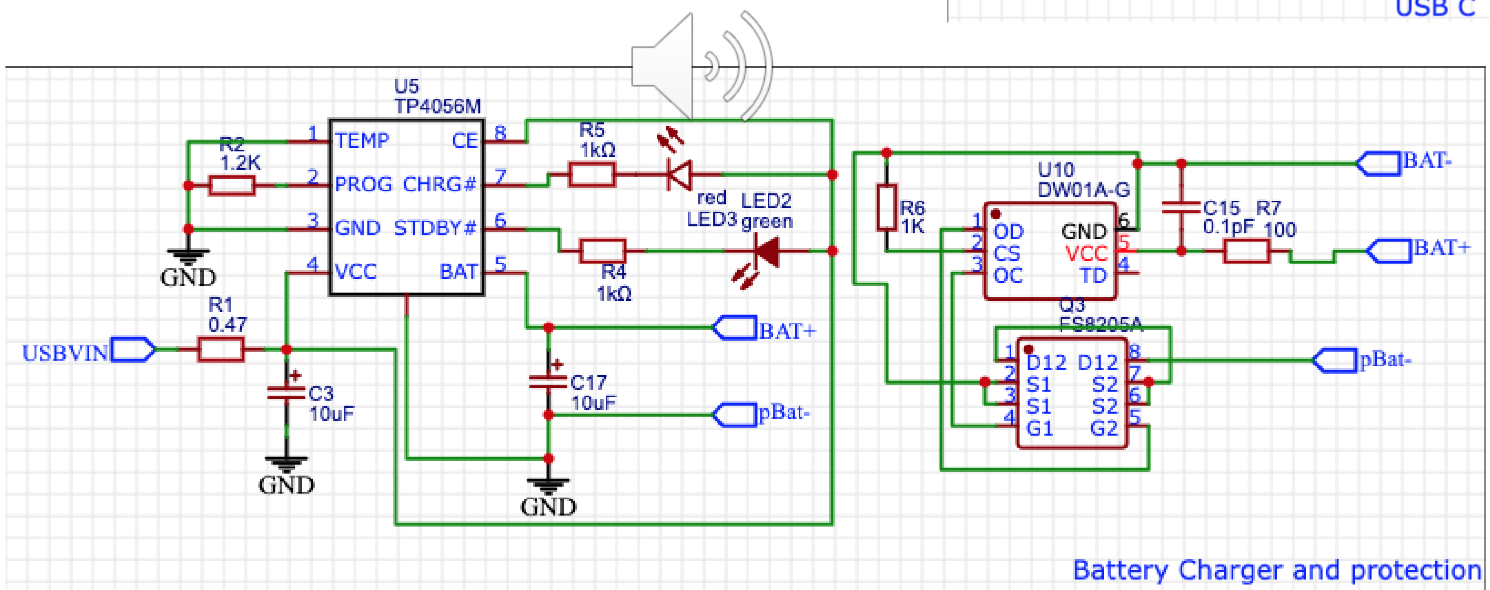
- The electrical design will be composed of a 3.3V and 5V Regulators, ESP32, Analog to Digital converter, Op-Amp for photodiode, rechargeable battery, battery charge charger IC and battery protection IC
- Powered by 5V USB Type C connector

- Considerations:
  - Size
  - Battery Life
  - Photodiode Measurement Precision

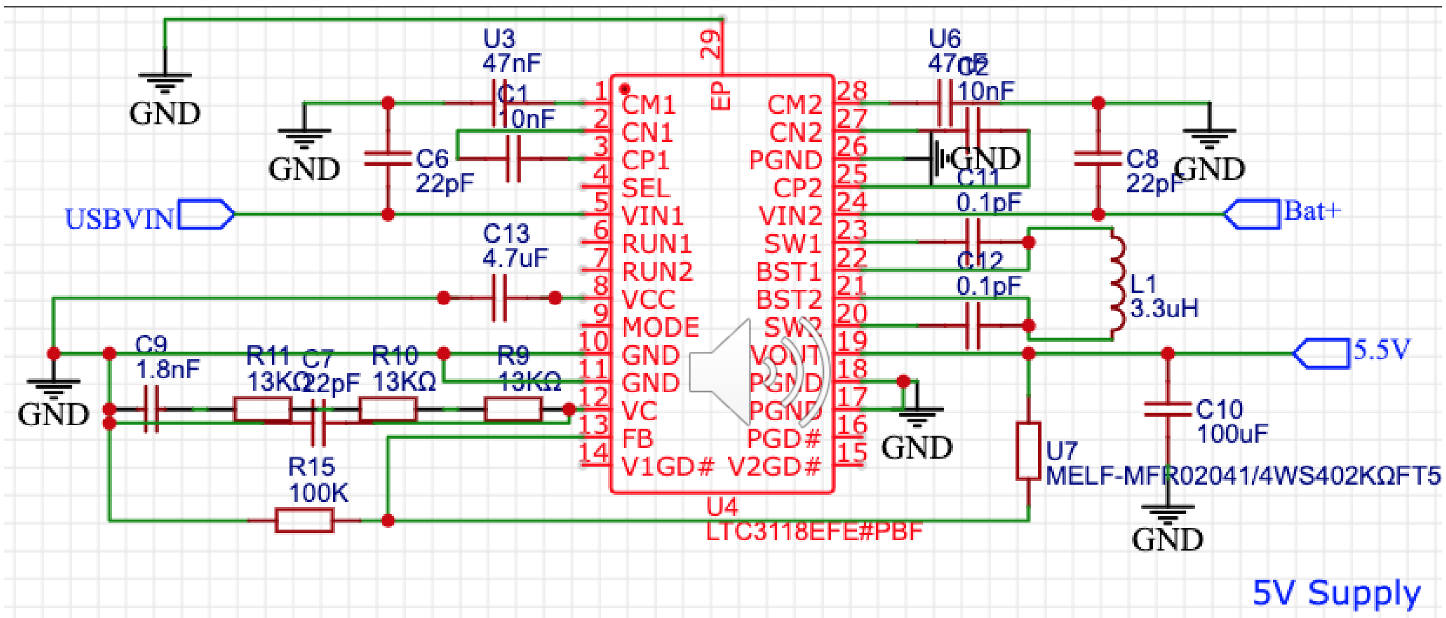
Battery, Switch and Battery Circuit



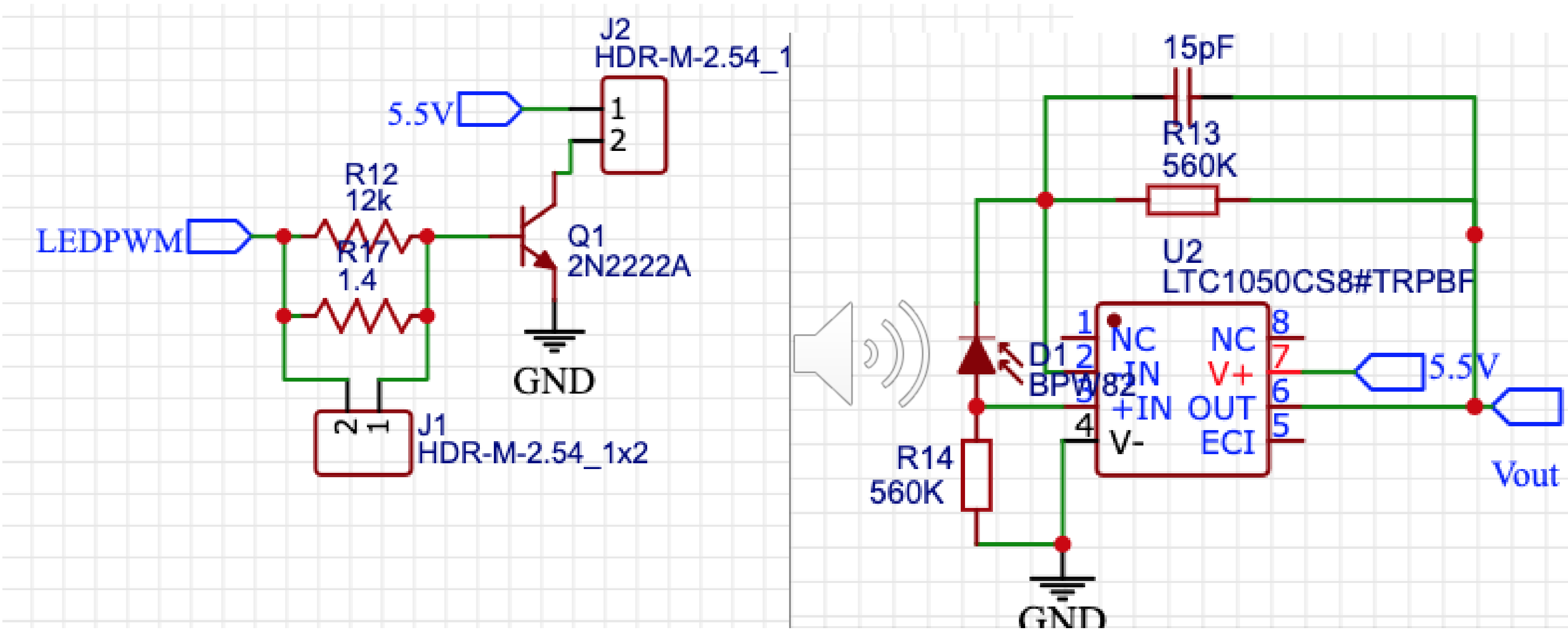




DC-DC Converter



LED Driver and Photodiode



Microcontroller

- Considerations
  - WiFi Communication
  - I2C Bus
  - SPI Bus
  - Non-Volatile Flash Memory
- We chose the ESP32 because it has a Wifi Transceiver, the required communication buses, and a built in Flash Memory Chip.

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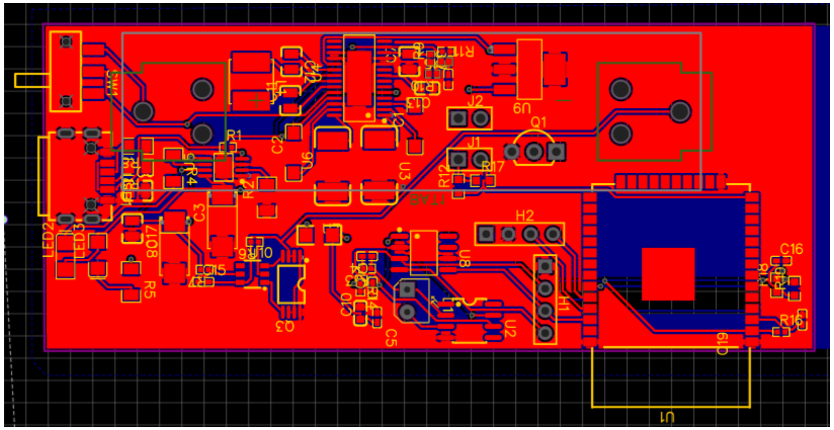
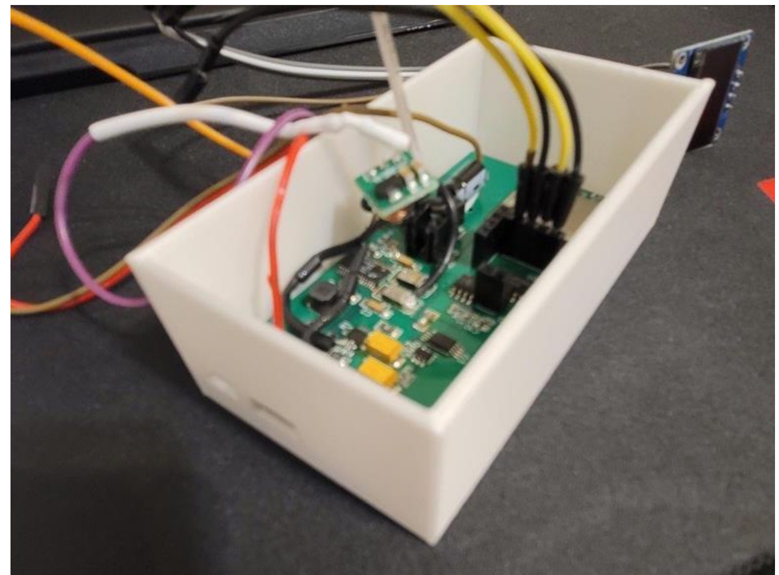
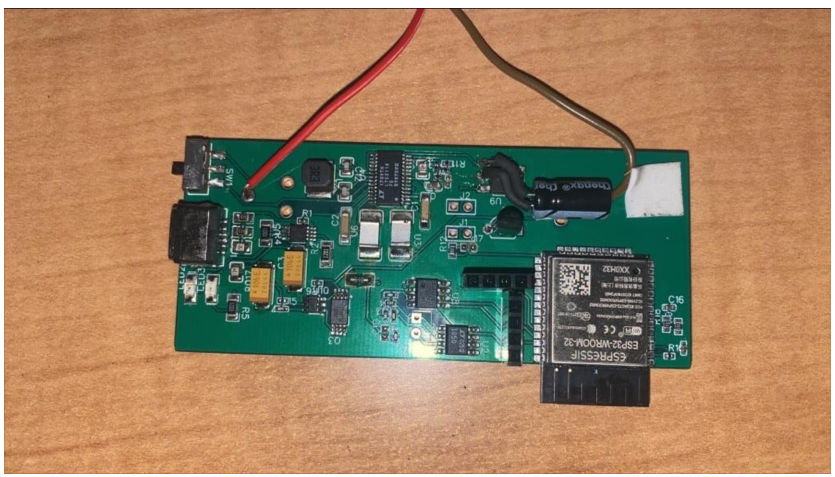
Photodiode Amplifier

- Considerations
  - Small photocurrent
  - Minimize dark current to reduce error
  - Create voltage signal that can be fed to analog to digital converter
- We chose the LTC1050 op-amp configured as a transimpedance amplifier because of its low drift, offset voltage of <5 µV, and availability.

Electrical System PCB







Dimensions: 37mm x 88 mm

Electrical Design Standards

- This project uses the following standards:
  - 802.11 b/g/n (WiFi)
  - I2C
  - SPI

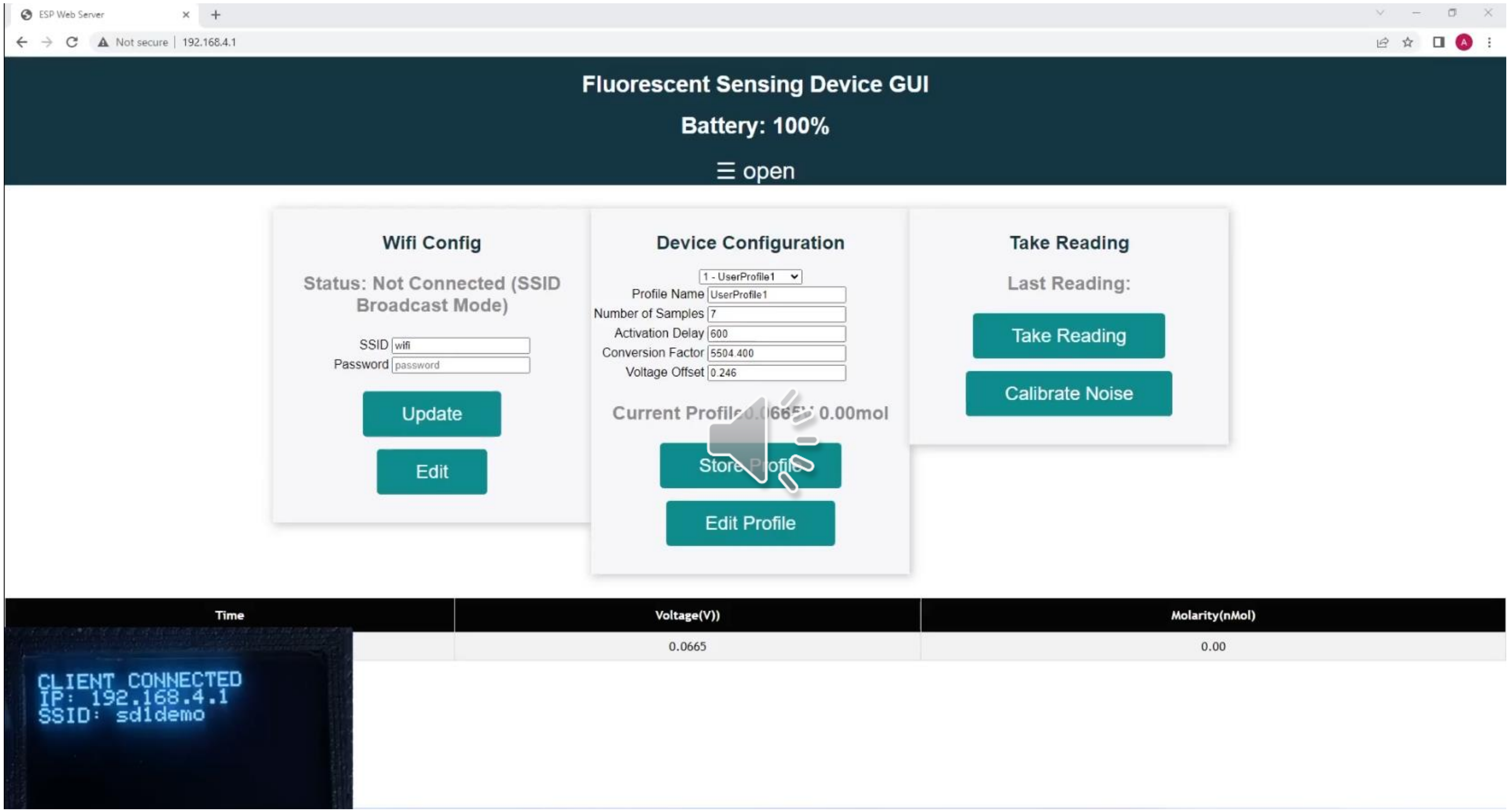
Device Software Design Overview

- Necessary Functionality
  - WiFi client and access point
  - Web Server
  - ADC interfacing via I2C
  - Display interfacing via SPI
- The ESP32 was programmed using Arduino IDE due to library availability for major components like WiFi, Display, I2C, and SPI

Web GUI Design Overview

- Functionality
  - Home Page for initiating sampling, and viewing most recent sample
  - WiFi Configuration
  - Profile Configuration
- The Web GUI was programmed in HTML using JavaScript to facilitate the transfer of data to and from the device

Web GUI


ESP Web Server
Not secure | 192.168.4.1
Fluorescent Sensing Device GUI
Battery: 100%
☰ open
Wifi Config
Status: Not Connected (SSID Broadcast Mode)
SSID wifi
Password password
Update
Edit
Device Configuration
1 - UserProfile1
Profile Name UserProfile1
Number of Samples 7
Activation Delay 600
Conversion Factor 5504.400
Voltage Offset 0.246
Edit Profile
Take Reading
Last Reading:
Take Reading
Calibrate Noise
Time
Voltage(V))
Molarity(nMol)
0.0665
0.00
CLIENT CONNECTED
IP: 192.168.4.1
SSID: sd1demo

Local GUI Design Overview

- Functionality
  - Select Profile
  - Take Reading

- The Local GUI is displayed on the local device display, and shows some information about the device and wifi configuration

Design Constraints

- Budget
- Safety
- Fluorophore (fluorescent marker)
- Size
- Battery-Powered

Standards related to our project

- Safety
  - Low blue light radiance value
  - Safe battery storage
- Fluorescence spectroscopy standards
  - Qualitative measurement
  - Quantitative measurement
- Electrical housing standards
  - Protect electrical hardware from outer environment

Successes & Difficulties

- Difficulties
  - Complex ray trace due to angled detection & illumination system
  - Optical, electrical, and software integration issues
  - Complex design of curved optical filter
- Success
  - Handheld dimensions (L: 95 mm, W: 50 mm, H: 78.5 mm)
  - Battery power for portability
  - Precise detection of fluorescein concentration from 3 µM – 50 nM

Budget & Financing – Deliverable

Costs for Fluorescence Sensor					
	Item	Quantity	Price/Unit	Projected Cost	Actual Cost
1	LED	1	$4.992	$4.992	$4.992
2	**Photodiode**	**1**	**$48.71**	**$48.71**	**$48.71**
3	Optical Filter	1	$115	$230	FREE
4	Fluorescein	100g	$30.5	$30.5	$30.5
5	Microcontroller	1	$ 4	$ 4	FREE
6	PCB	1	$2.04	$2.04	$2.04
7	**Display**	**1**	**$3.00**	**$3.00**	**$3.00**
8	**Custom Enclosure**	**1**	**$5.00**	**$5.00**	**$5.00**
9	Circuit Components	1	$ 34.84	$ 34.84	$25
10	Quartz Cuvette	**1**	**$14.58**	**$14.58**	**$14.58**
				$377.662	**$133.822**
Team Budget		$200.00			
Sponsorship		$1,000.00			

References

- https://www.everixopticalfilters.com/