Portable Wide - Illumination Microscope

Group 2 - Andres Parra, David Ponce, Jacob Roth, Xuan Luo

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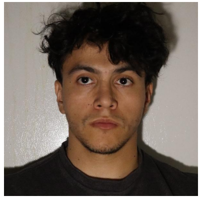
Overview

- Our portable microscope is a battery powered microscope which employs a mobile app to control the stage, the magnification, and the wavelength of light to observe various specimens.

Motivation and Background



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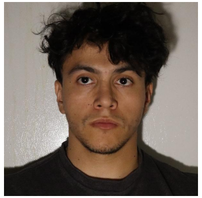
- Collaboration between students specializing in photonics, electrical engineering, and software engineering
- Project objective: Streamlining and enhancing microscopy through automation
- Designing and constructing a portable microscope with a digital interface
- Enabling easy control of the microscope and specimen observation on a display
- Incorporating a wide spectrum illumination range from ultraviolet to infrared for versatile observation
- Designing and constructing a portable microscope with a digital interface Enabling easy control of the microscope and specimen observation on a display Incorporating a wide spectrum illumination range from ultraviolet to infrared for versatile observation Demonstrating the project's impact on improving the field of microscopy and its applications.

Basic goals



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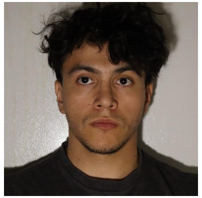
- Display a clear magnified image compared to commercial microscopes in the market.
- Our microscope will be able to change between two magnification levels with motor controls.
- The sample will be illuminated by a designed LED light source with three spectra.
- Our microscope should be able to resolve biological specimens around 100 μm.
- Able to focus the image appropriately for each level of magnification through vertical axis control.
- The microscope will be portable, lightweight, and easily carried having dimensions that do not exceed.
- The system will be powered via rechargeable batteries.
- A camera module controlled by a mobile app will be in place to capture the image displayed by the microscope.
- Images will be transmitted and displayed on a mobile app through the Raspberry pi.
- Axis controls with motors will be achieved through a mobile app.
- Store images using an image processing software app.

Advanced goals & Stretch Goals



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Advance Goals

- Images can be stored in a database with labels in real time
- Magnification and image resolution will be improved and balanced
- LED light source will be improved to view specimens in better lighting for all wavelengths (390 – 940 nm)
- Microscope will be improved to resolve biological samples around 80 μm.
- Motor system will be improved to make smoother and more accurate movements

Stretch Goals

- The microscope will have an “Educational mode” which will utilize machine learning to differentiate between plant and animal cells, and identify the different structures that make up a cell.
- Microscope will be able to resolve biological samples around 20 μm.

Objectives

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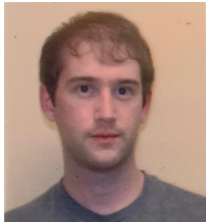
Objectives

- UV, white, and IR LEDs will be used for LED light source design on PCB (390 – 980 nm spectrum)
- Small lenses will be used (small, portable, and lightweight)
- Two objective lenses will be used (magnifications of 40x and 100x)
- Image sensor will have high resolution and small pixel size (diffraction – limited)
- Doublet lenses will be used (achromatic)
- Wavelength control will be achieved by LED control

Engineering Specifications

Table 1 - Engineering Specifications			
Component(s)	Parameter	Specification	Unit(s)
Camera Module	Image resolution	1920 x 1080	Pixels
Image Sensor	Pixel size	1.25 x 1.25	μm
Vertical Focusing motors	Latency	<100	ms
Objective lens motor	Latency	< 2	Seconds
Turntable for objective lens	Turn radius	360	Degrees
Objective Lens 1	Magnification	40x	Unitless
Objective Lens 1	Focal length	4	mm
Objective Lens 2	Magnification	100x	Unitless
Objective Lens 2	Focal length	1.6	mm
Tubular Lens	Focal length	75	mm
White LED	Wavelength	450 - 700	nm
850nm IR LED	Wavelength	850	nm
940nm IR LED	Wavelength	940	nm
UV LED	Wavelength	390 - 395	nm
Batteries	Discharge Time	≥1	Hour



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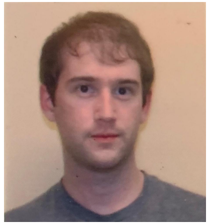
Hardware Design - Block Diagram



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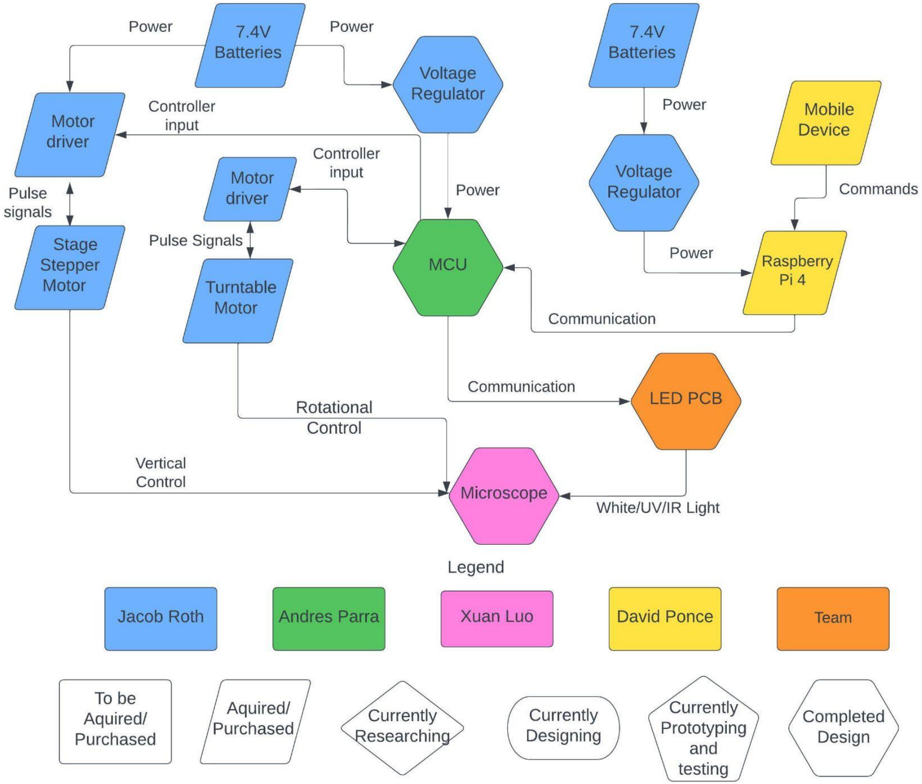
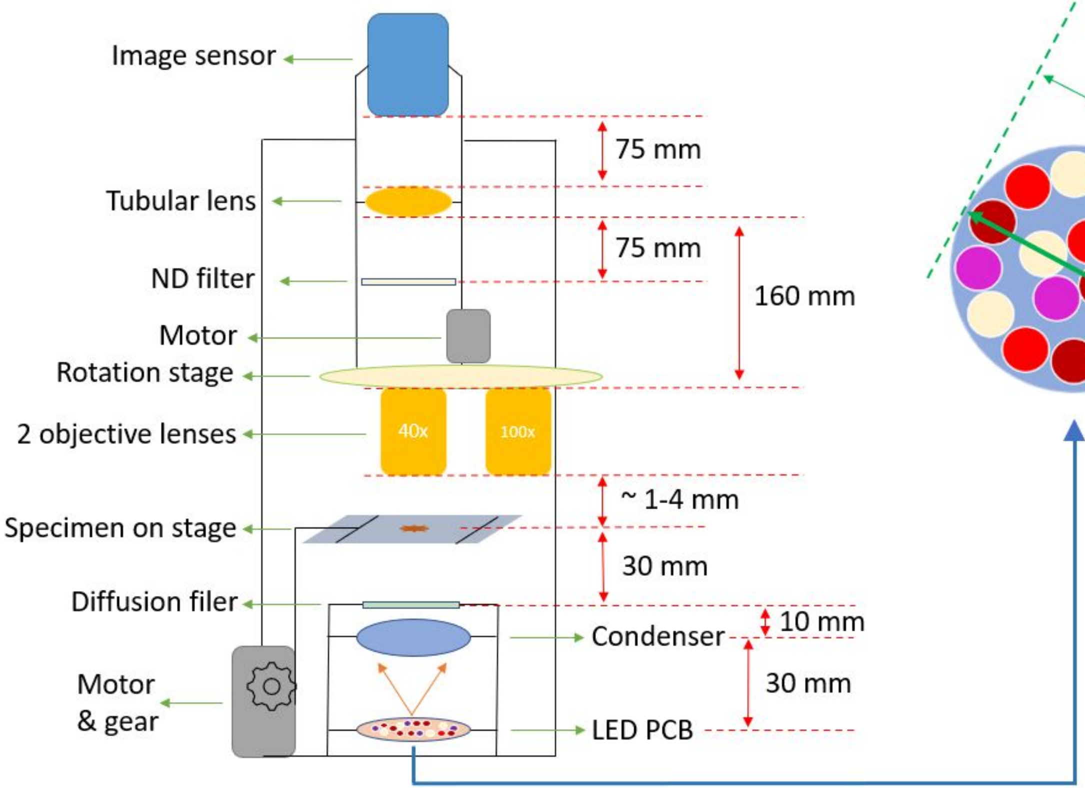
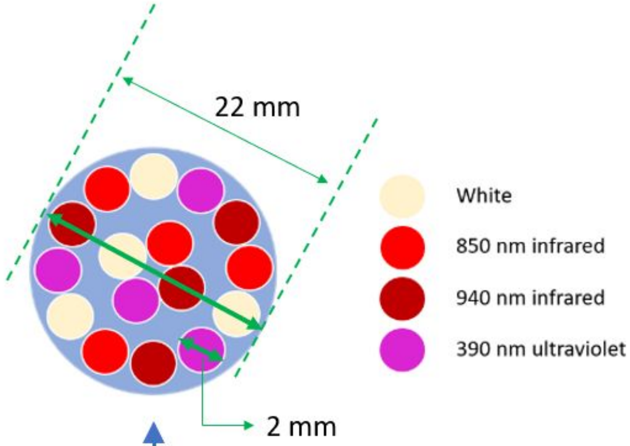


Microscope Prototype Design





LED layout

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Microscope Prototype

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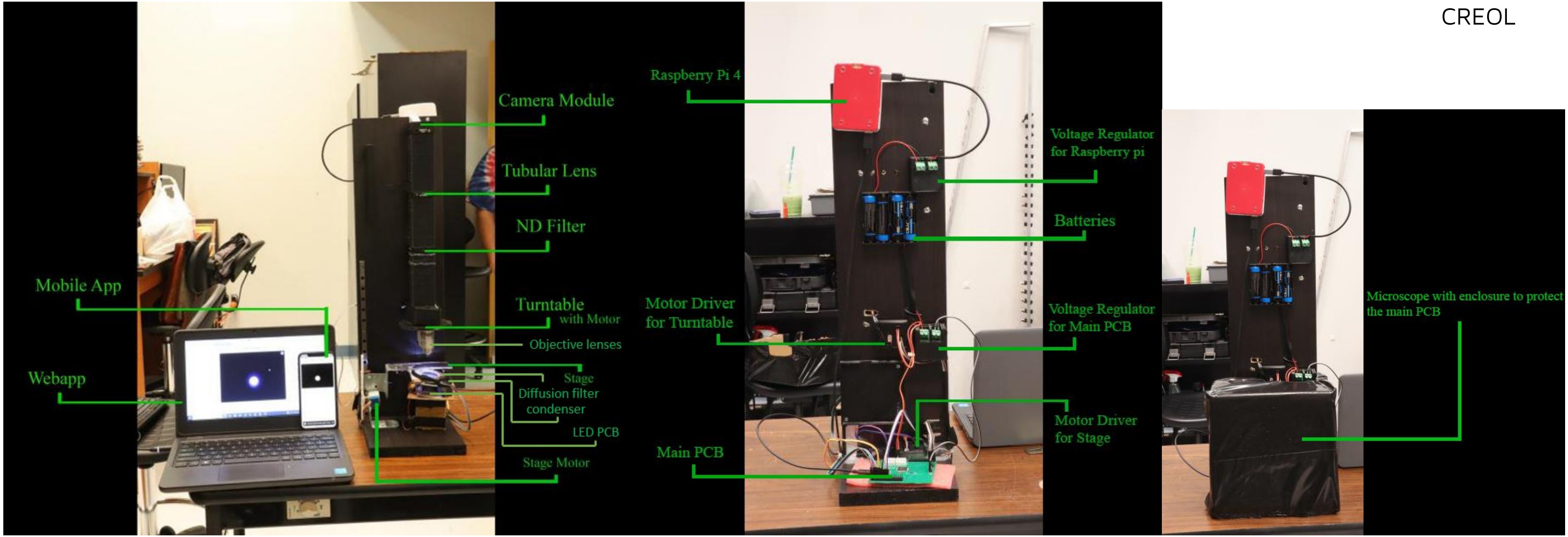

Front

Back

PCB protector

Part Selection

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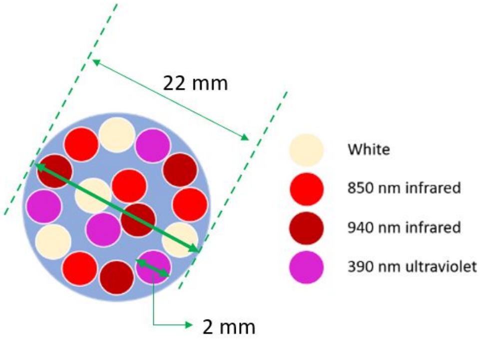
Achromatic objective lens selection:

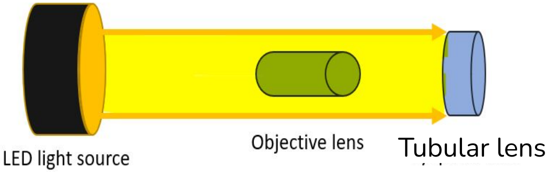
- Dark-mode: the objective lens only receives light scattered by the sample, not from the light source
- $\tan\theta$ = 11 mm/15 mm = 0.73, θ = 36°
- 90-θ = 90 - 36 = 54°
- NA = sin(54°) = 0.8
- The closest NA of objective lenses on market is 0.65
- Focal length of 40x obj is 4 mm and focal length of 100x obj is 1.6 mm, with a tube length of 160 mm

Tubular lens selection:

- $\Delta r = \lambda/2NA$ =390 nm/(2x0.65)=300 nm
- d = 1.12 μm (see image sensor section)
- optimal sampling: Δr>5 pixels
- Δr/5=300 nm/5=60 nm
- magnification = 1.12 μm/60 nm=18.67x
- focal length of the tubular lens = 18.67x4 mm=74.68 mm ~75 mm

LED selection:





- d = 2 mm, separation = (22 mm-4x2 mm)/3 =~ 5 mm
- actual illumination area: π(11mm)^2 = 121π
- Tubular lens area: π(12.7mm)^2 =~ 161π
- Illuminating ratio = 121π/161π = 75%

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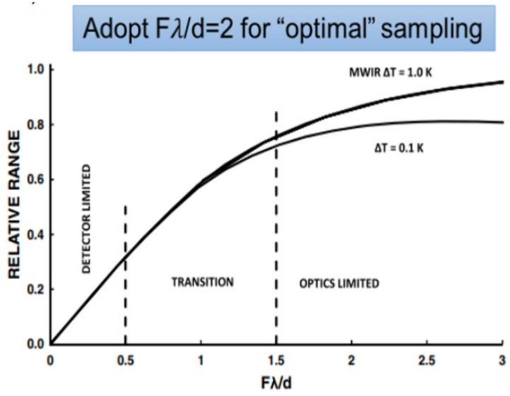
Part Selection (cont.)

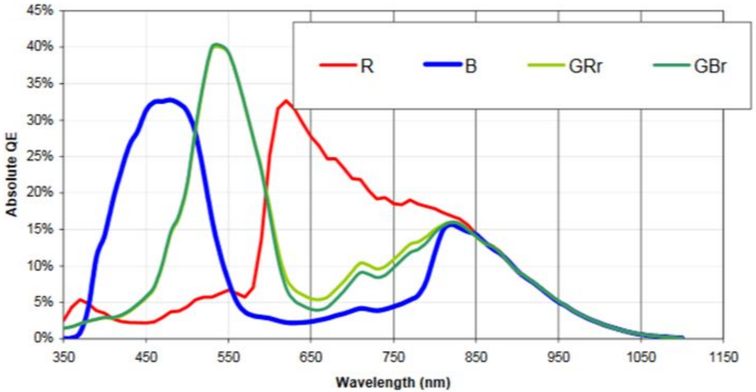
Image sensor selection:



"Imaging system performance based upon Fλ/d," G. Holst, Opt. Eng. 46(10), 103204 (2007)
"Design considerations for advanced MWIR target acquisition systems," G. Holst, R. Driggers and O. Furxhi, Appl. Opt. 59(14), 4339 (2020)

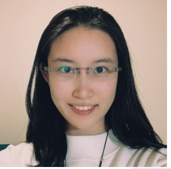
- diffraction blur is 2.44Fλ, Fλ= 2d for optimal sampling
- 2.44x2d= 4.88d
- stretch goal is to resolute around 20 μm
- 4.88d= 20 μm, d= 4.1 μm.
- When pixel size is smaller than 4.1 μm, the image sensor can achieve a diffraction-limited performance in imaging system.

Raspberry Pi Camera Module v2:



Working wavelength spectrum

- pixel size is 1.12 μm
- working wavelength is from 350 nm to 1100 nm



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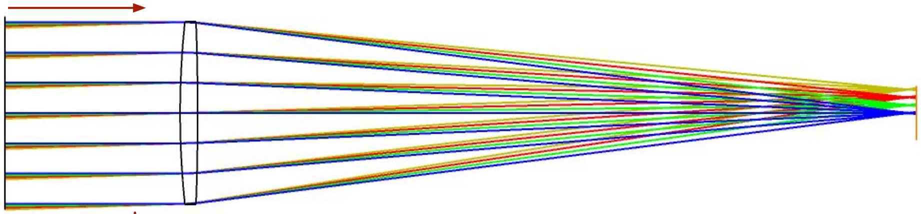
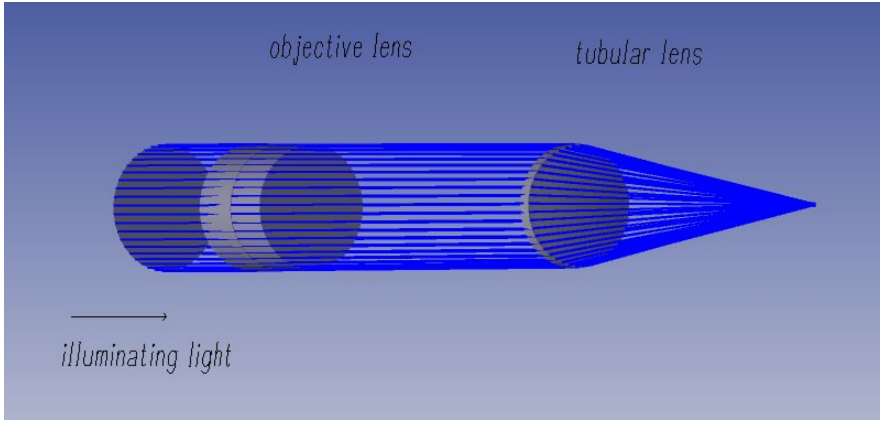
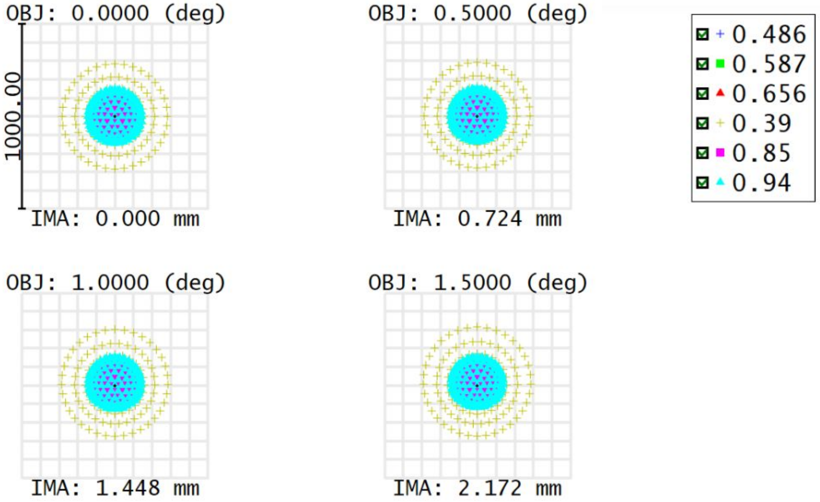
Microscope Design: Two-Lens Imaging System



Tubular lens design



Two-lens imaging system illustration



Spot diagram for four fields at different wavelengths

Microscope Design: Resolution

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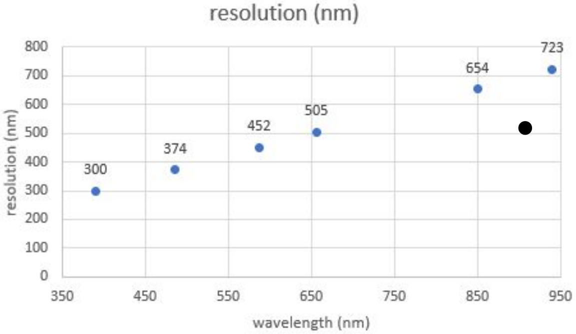
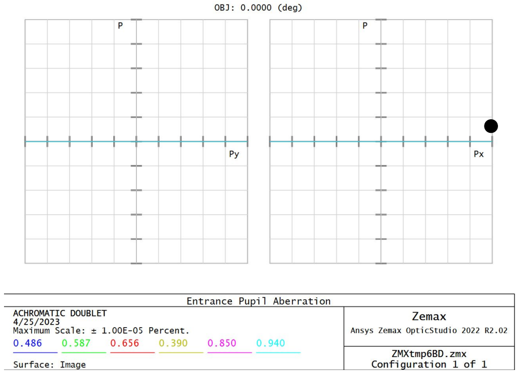


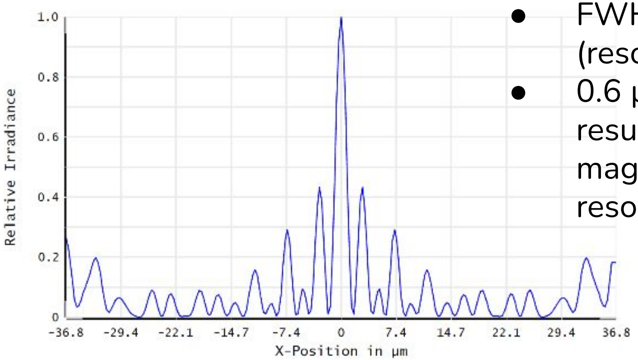
Resolution-wavelength plot

- Formula $\Delta r = \lambda/2NA$ is used to obtain resolution-wavelength plot



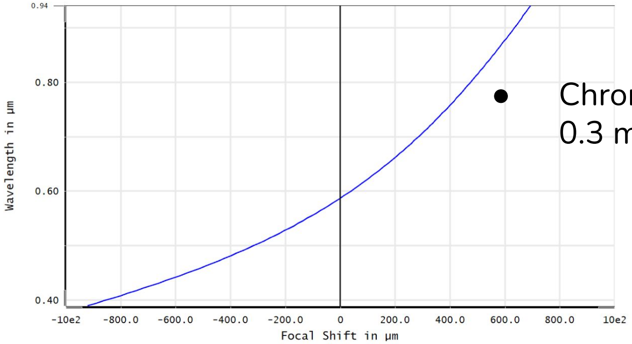
Pupil aberration under all wavelengths

- Pupil aberration is 0 along x-axis and y-axis



PSF function

- FWHM is ~0.6μm (resolution ~0.6μm)
- 0.6 μm resolution is the result of balancing the magnification and resolution



Chromatic focal shift

- Chromatic shift is around 0.3 mm/100 nm

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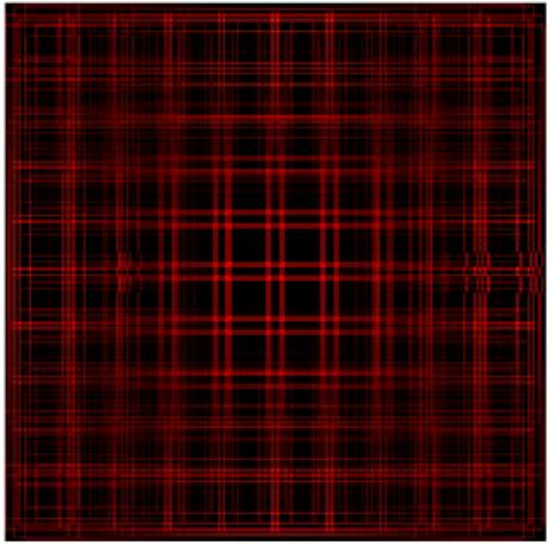
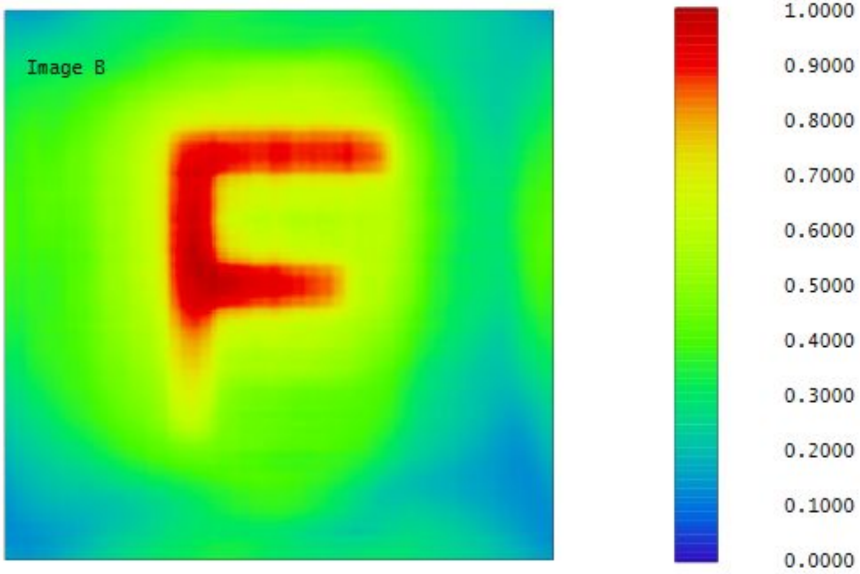
Microscope Design: Resolution (cont.)

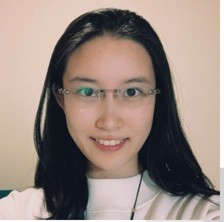
Image simulation:

Image A





- Image A: simulation of a test image with a grid of lines that is 0.001 mm
- Image B: diffraction image obtained by simulating the performance of our imaging system with a letter ‘F’
- The central gives a satisfactory imaging result



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Microscope Design: Magnification & FOV

Magnification:

for 40x objective:

- objective focal length is 160 mm/40=4 mm
- tubular lens focal length is 75 mm
- overall magnification of the entire imaging system is 75 mm/4 mm=18.5x

for 100x objective:

- objective focal length is 160 mm/100=1.6 mm
- overall magnification is 75 mm/1.6 mm=46.875x ~ 47x

FOV:

- pixel size of the image sensor we are using is 1.12 μm x 1.12 μm. The sensor has 3280(H) × 2464(V) pixels.
- for 40x objective lens, in the sample plane, one pixel is 1.12 μm/18.5=60.5 nm. Hence, the horizontal field of view is 3280x60.5 nm=198 μm in the sample plane; the vertical field of view is 2464x60.5 nm=149 μm in the sample plane.
- for 100x objective lens, one pixel is 1.12 μm/47=24 nm. Thus, the horizontal field of view is 3280x24 nm=79 μm in the sample plane; the vertical field of view is 2464x24 nm=59 μm in the sample plane.

Microscope Design: LED Light Source

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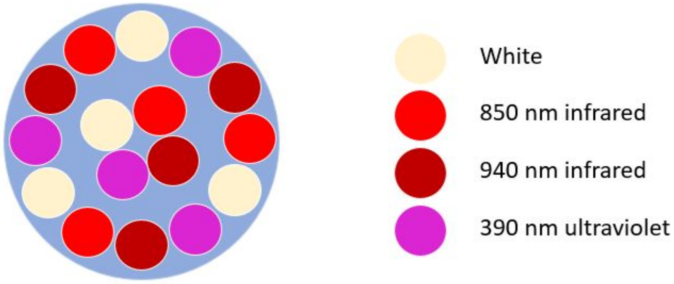
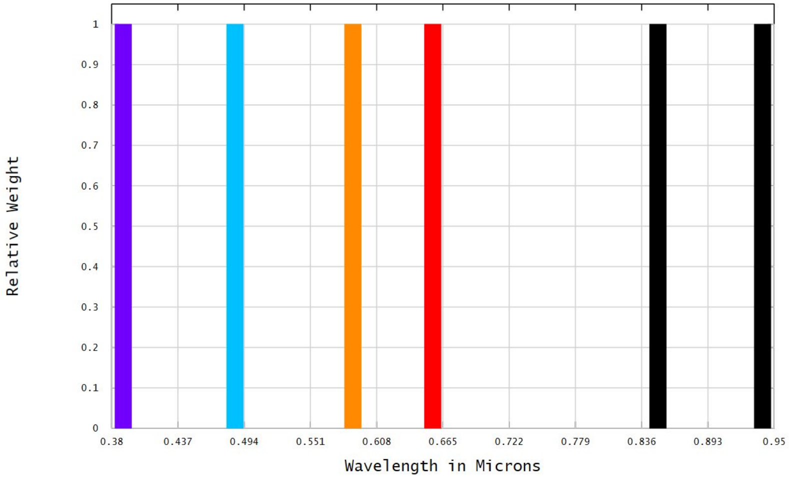
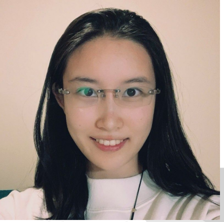


LED layout design



Illuminating spectrum (illustration)

- UV gives a higher resolution
- White light can provide enough brightness
- IR can image the structure of the sample



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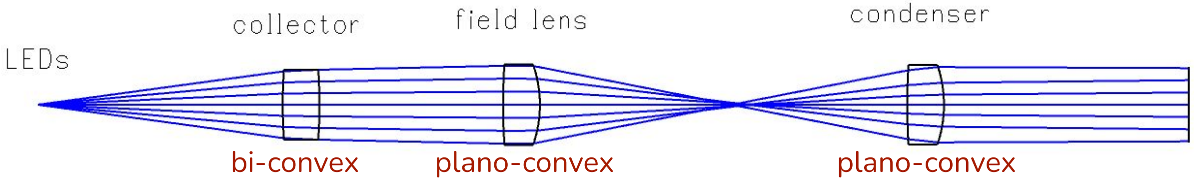
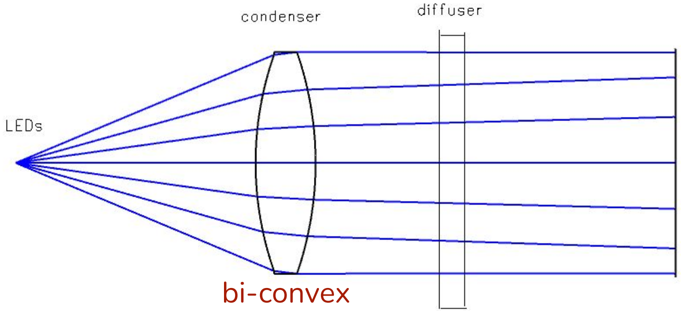
Microscope Design: Illumination System

Method 1: Köhler illumination system



Method 2: Lambertian diffuser illumination system



- LED light is Gaussian-distributed, which results in the central part of the specimen brighter than the edge.
- Illumination system can make the LED light homogenous, so the specimen gets uniform illuminating light.
- We chose method 2 for the entire system to be more compact
- Focal length of condenser is 30 mm

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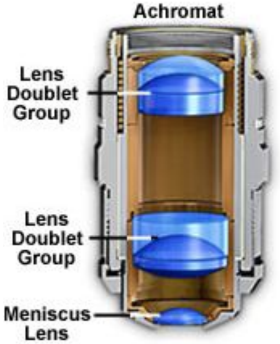
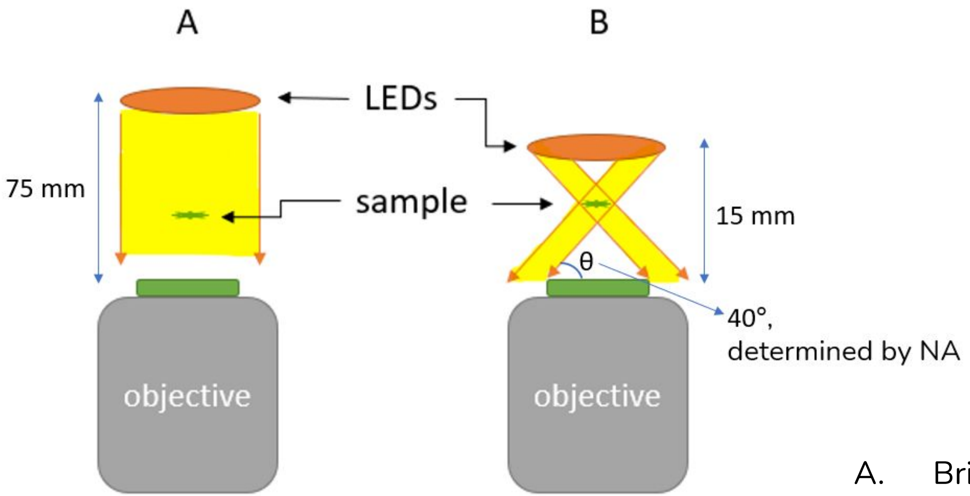
Microscope Design: 2 in 1 Feature



achr. obj



bright-field

dark-field

A. Bright Mode:
- central and outer circles of LEDs: on
- objective lens captures the LED light

B. Dark Mode:
- central circle of LEDs: off
- outer circle of LEDs: on
- objective lens only captures light scattered by the sample

Comparison and Selection of Hardware - Image sensor vs. Camera module

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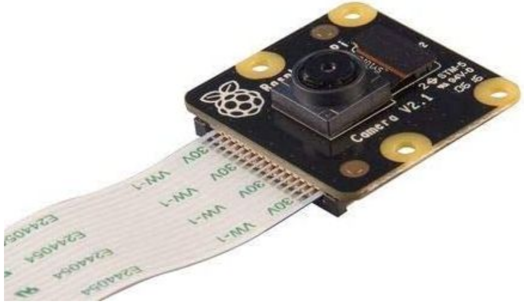
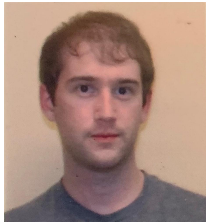
Table 7 - Image Sensor vs Camera Module Technology Comparison		
-	Image Sensor	Camera Module
Applications	Security cameras, drones, robotics	Security cameras, drones, robotics
Functionality	No on-board pre-processing	On-board pre-processing
Cost	Cheaper	More expensive
Ease of use	Harder to use	Easier to use
Size	Lighter weight	Heavier weight



- On-board pre-processing
- Ease of use

Motor Technology Comparison

Table 9 - Motor Technology Comparison		
-	Servo Motors	Stepper Motors
Applications	Used for robotics, electronic manufacturing, CNC machines	3D-Printers, milling machines, automated machines
Capabilities/Features	Runs smoother than stepper motors	Can use microstepping to overcome misstepping issue
Noise	Quiet at high speeds	Lots of noise at high speed operation
Speed	Can provide 2-3 times the speed of stepper motors while maintaining the same torque	Inversely related to torque. Good for low speed and high torque applications
Torque	Constant torque at low and high speeds	Inversely related to speed. At low speeds, stepper motors provide higher torque compared to servo motors.
Feedback	Speed and positional feedback to monitor position. Misstep correction	No feedback to monitor position. Could result in missteps
Accuracy	High Accuracy. Continuously corrects positional errors using its encoder and use of feedback	Low accuracy. No feedback to monitor positional errors.
Efficiency	High efficiency	Low efficiency



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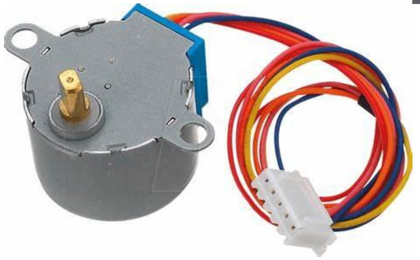

- Stepper motor is more precise
- Good for low speed designs/projects

- Low noise
- Good for high speed designs/projects

Stepper Motor

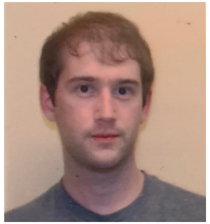

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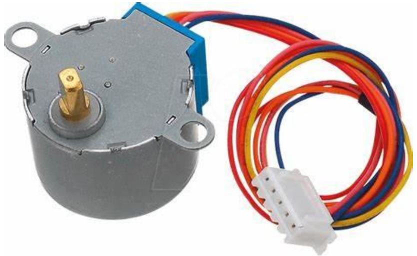
Table 10 - Stepper Motor Part Comparison			
-	28BYJ-48	42-23 Nema 17	28-28 Nema 11
Size	42 x 30 x 29 mm	42 x 42 x 23mm	28 x 28 x 28 mm
Weight	40 grams	132 grams	80 grams
Rated Voltage	5V	4.1V	3.8V
Step Angle	5.625°	1.8°	1.8°
Torque	Self-positioning torque: 0.0343 N x m	Holding torque: 0.13N x m	Holding torque: 0.065N x m
Cost	$9.99	$8.99	$17.99



Voltage Regulator

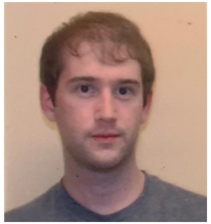


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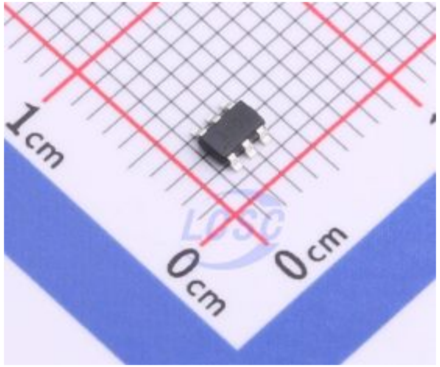
Table 15 - Voltage Regulator Part Comparison				
-	LM2598	TPS54202	TPS563300	TPS564201DDCR
Output voltage Range	3.3V, 5V, 12V - fixed 1.2V to 37V - adjustable	0.6V to 26V	0.8V to 22V	0.76 V to 7 V
Input Voltage Range	Up to 40V	4.5 to 28V	3.8V to 28V	4.5 V to 17 V
Output Current	1A	2A	3A	4A
Switching Frequency	150kHz	500kHz	500kHz	560kHz
Cost	$3.33	$1.08	$0.31	$0.35



Stepper Motor Driver Comparison

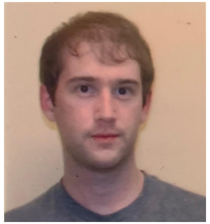


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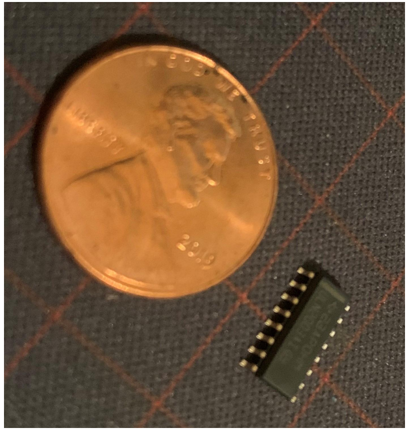
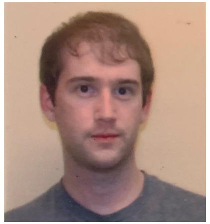
Table 11 - Stepper Motor Driver Part Comparison			
-	ULN2003	A4988	DRV8825
Features	4 phases, 4 step state LEDs	5 step revolutions up to 1/16-step	6 step resolutions up to 1/32-step
Rated Voltage or Max voltage	5V to 12V	Maximum voltage 35V	Maximum voltage 45V
Rated Current or Max Current	500mA	2A	2.5A
Advantages	Simple and ships with the 28BYJ-48	5 selectable step modes	6 selectable step modes
Disadvantages	Only compatible with the 28BYJ-48. Max current rating and voltage rating are low	Higher frequency noise. Less step modes than the DRV8825	Susceptible to voltage spikes due to its use of low-ESR ceramic capacitors
Cost	$1.32	$1.99	$3.99





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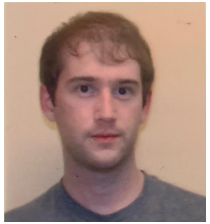
Voltage Regulator Comparison

Table 14 - Voltage Regulator Comparison

-	Linear Regulator	Switching Regulator
Efficiency	Low efficiency due to waste heat. More sensitive to input/output voltage difference	Very high efficiency, and no heat waste. Deliver more on the desired current and voltage
Noise	Low noise	High noise
Dropout voltage	Higher dropout voltages	Lower dropout voltages
Ripple voltage	Lower ripple voltage	Higher ripple voltage
Cost	Cheap compared to standard switching regulators	More components leads to a higher cost
Simplicity	Linear regulators can be made with few parts. Less complex	More components than linear regulators. More complex

Battery Technology Comparison

Table 12 - Battery Technology Comparison		
-	NiMH	Li-ion
Self-discharge rate	higher self-discharge rate	Lower self-discharge rate
Nominal Voltage	1.2 V. Lower than Li-ion	Varies (3.6V to 3.85V). Higher than NiMH
Cycle Durability	Estimated 180 to 2000	Estimated 400 to 1200



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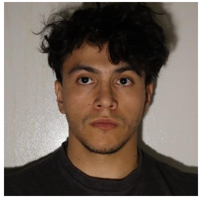
Comparison and Selection of Software



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Database

Sql vs Nosql

- SQL and NoSQL are two types of database management systems with different data storage models, query languages, and scalability.

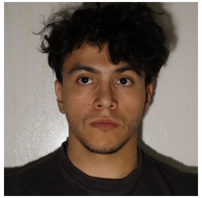
Query Language

- SQL databases use SQL as the standardized query language, while NoSQL databases use different query languages based on their data models.

Storage model

- SQL databases organize data in tables with a fixed schema, suitable for structured data like lists or tables.
- NoSQL databases offer more flexibility in data storage, making them ideal for handling unstructured data like social media posts.

Comparison and Selection of Software



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Stacks

MERN

- MERN is a JavaScript-based stack (MongoDB, Express.js, React, and Node.js) that simplifies full-stack web development, leveraging MongoDB for scalability with unstructured data, React for dynamic UI components, and Express.js for built-in middleware support.

LAMP

- LAMP (Linux, Apache, MySQL, and PHP) is an open-source stack widely used in web development, offering stability, scalability, and strong community support. While Linux provides security, Apache may have performance issues, and managing large codebases or handling substantial data in MySQL can pose challenges.

Django

- Python with Django (Model-View-Template) is a secure and feature-rich framework suitable for large-scale, high-traffic websites. It provides built-in security, an admin panel, and authentication system. While highly scalable and flexible, it may have limited flexibility in some cases and a steep learning curve for new developers. Additionally, Django has limited support for NoSQL databases.

Development Board Comparison

Table 16 - Development Board Comparison			
-	Arduino Uno	MSP4306968	Raspberry Pi 4 B
Microcontroller	ATMega328P 8-bit AVR RISC architecture (single core)	16 bit RISC architecture (single core)	Broadcom BCM2711, quad core Cortex-A72 64 bit, ARM v8 architecture (quad-core)
Clock speed	Up to 16-MHz	Up to 16-MHz	1.8-GHz
Memory	2KB SRAM 32KB FRAM	2KB SRAM 128KB FRAM	Up to 8GB SDRAM
Power consumption	Active mode: 6.9mA/MHz Sleep mode: 50µA/MHz	Active mode: 100µA/MHz Standby: 0.4µA/MHz	1280mA at 400% CPU load
Wifi Chip	802.11b/g/n Up to 150Mb/s 2.4GHz (Module sold separately)	802.11b/g/n Up to 150Mb/s 2.4GHz (Module sold separately)	802.11b/g/n Up to 150Mb/s 2.4GHz
Manufacturer	Adafruit Industries	Texas Instruments	Broadcom
Size	68.6x53.4mm	16x16mm	56.5x85.6mm
Price	$27.60 USD	$20.00 USD	$35.00 USD

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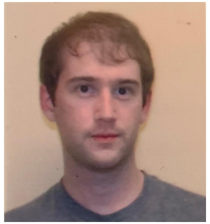
Schematics - Voltage Regulator



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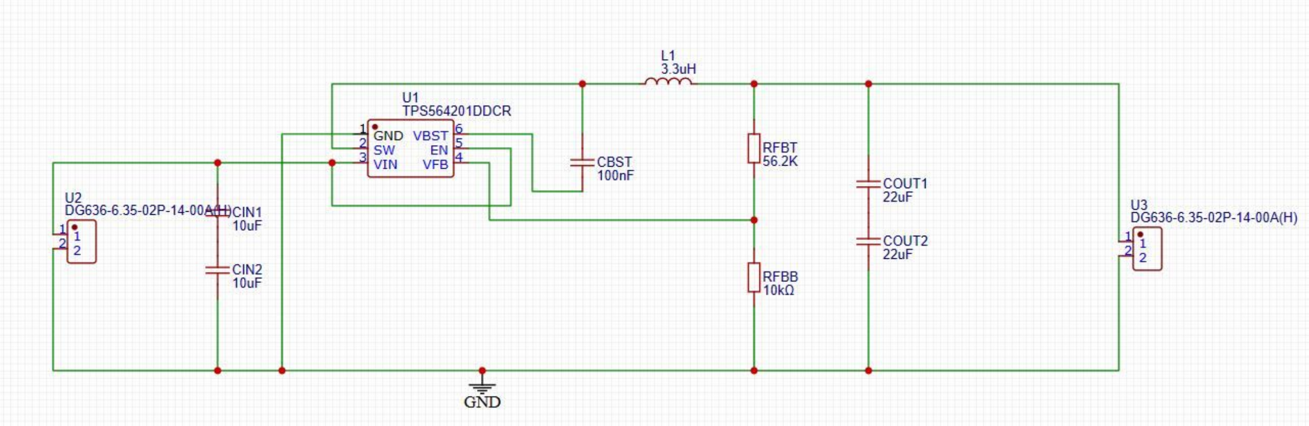
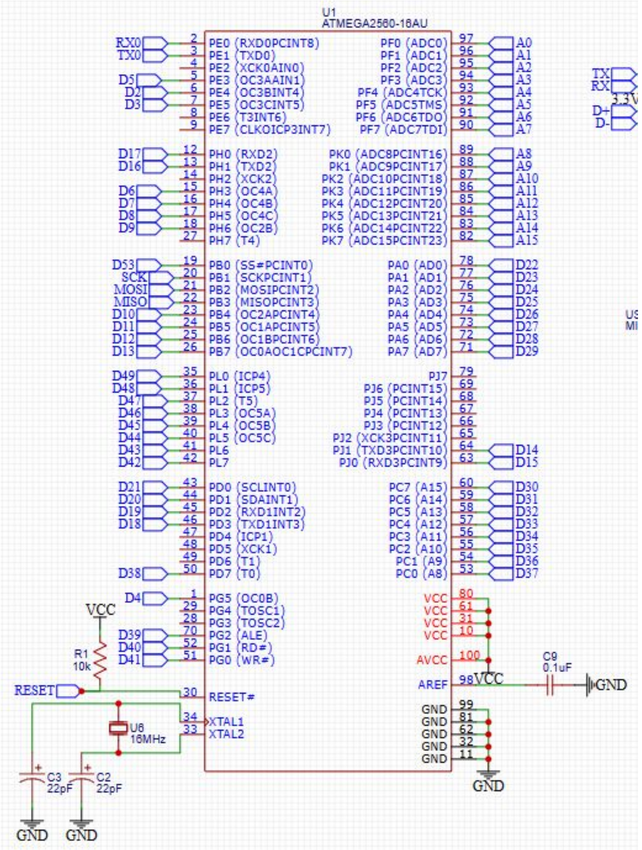
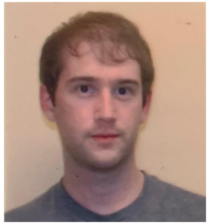


ATMEGA2560 - Power





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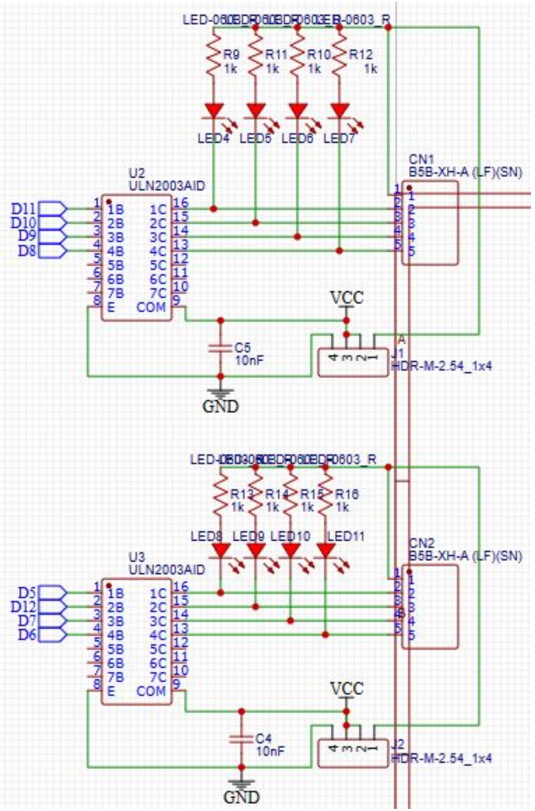
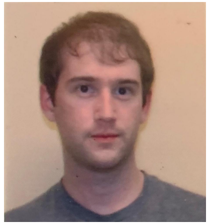
ULN2003 Stepper Motor Drivers





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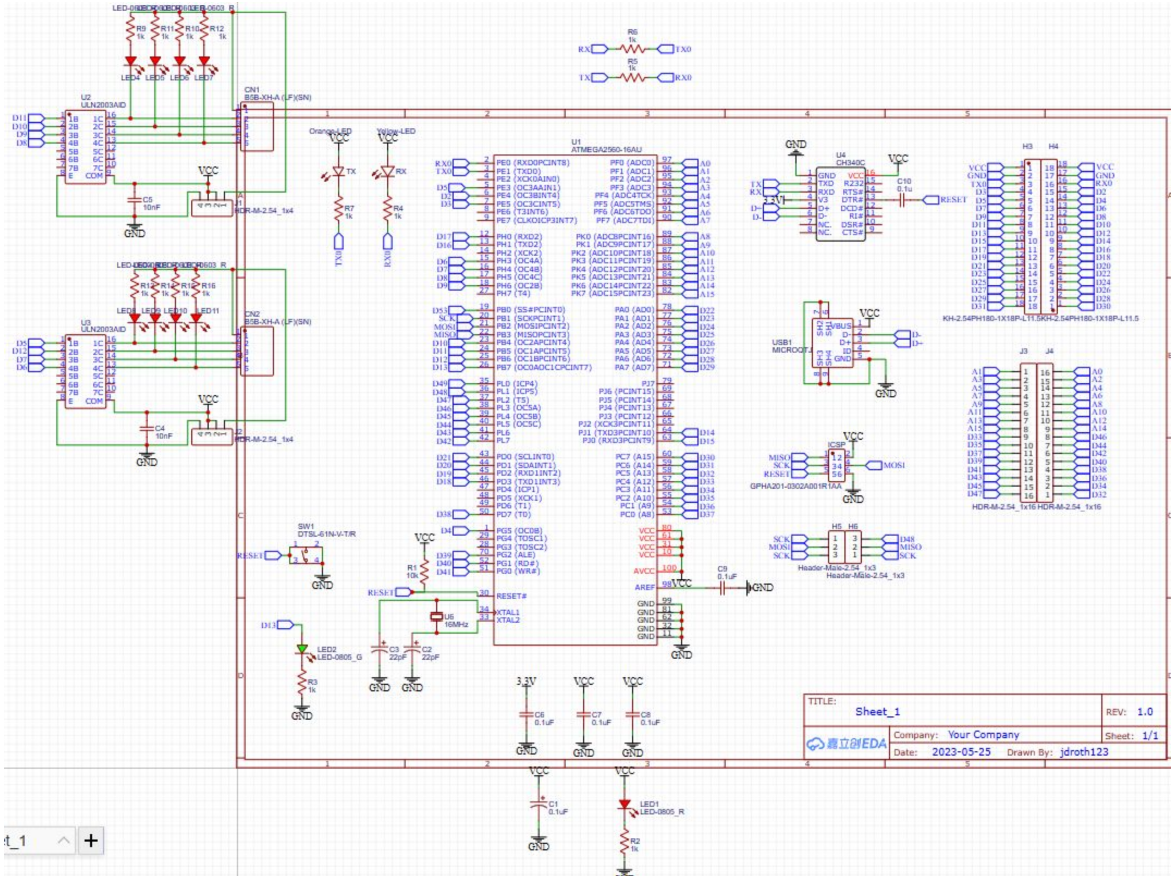
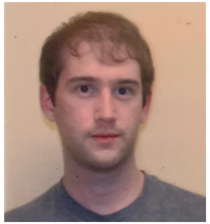
Hardware Design - Overall Schematics





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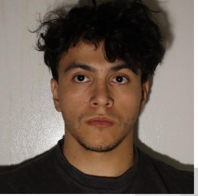

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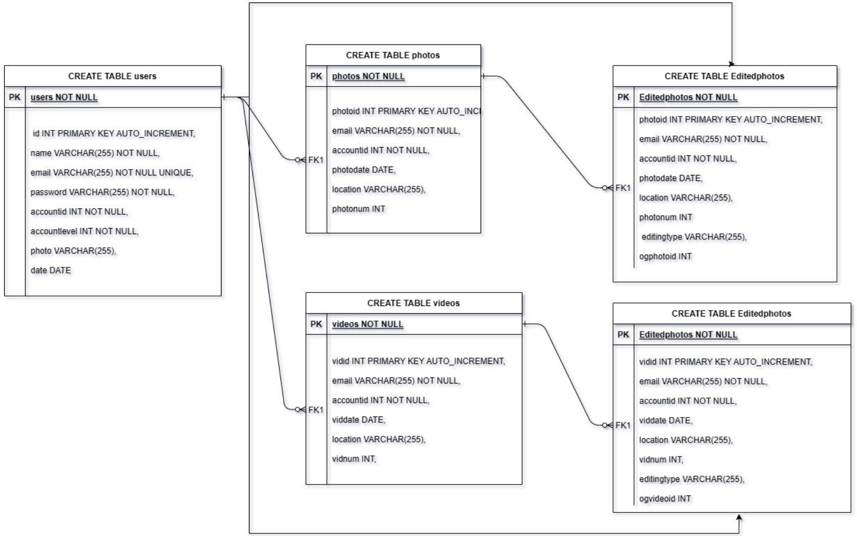
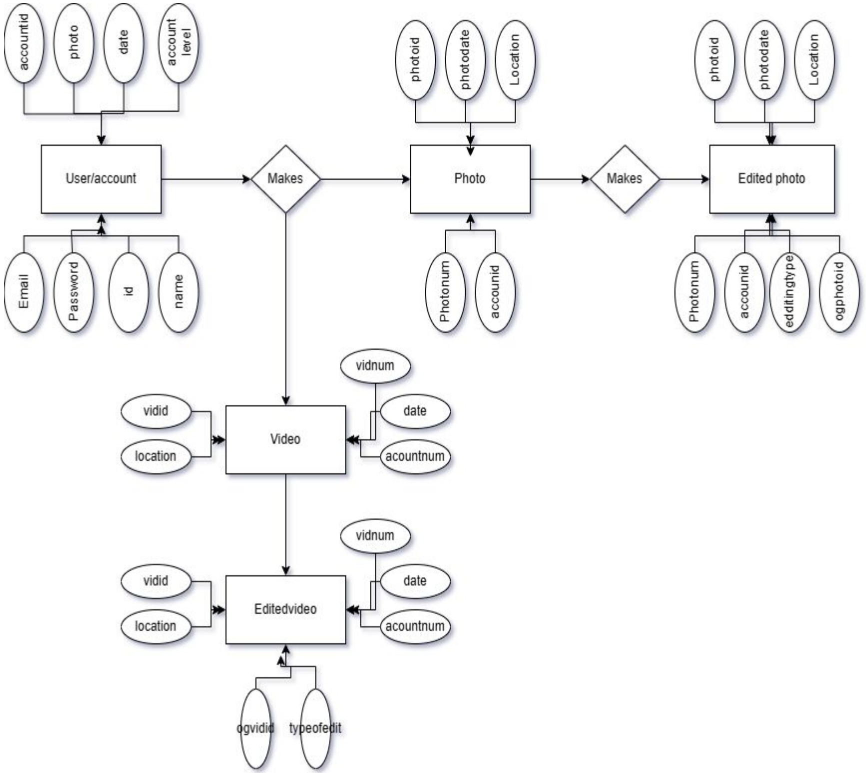
Software Design Database

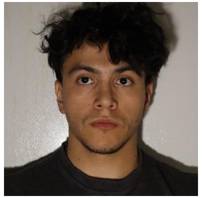
Database Design

Entity Relationship Diagrams





Software Design Ecosystem



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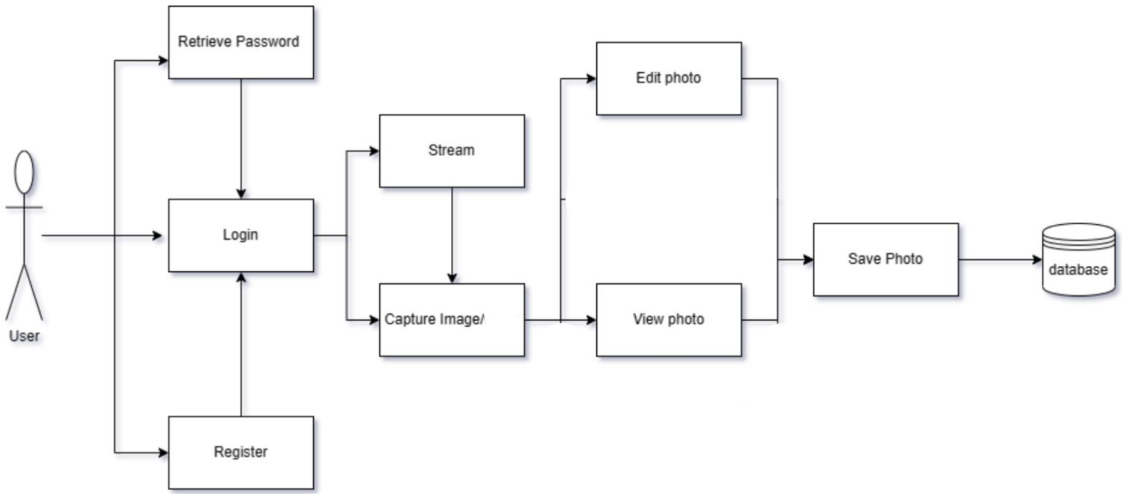
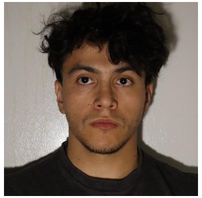
Overall App Design

Use case



UI Design



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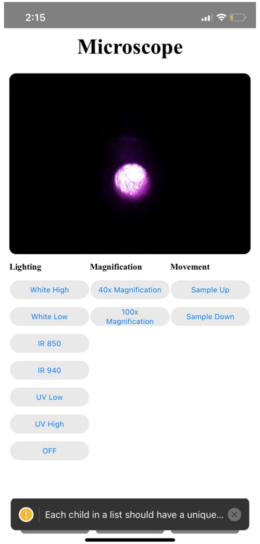
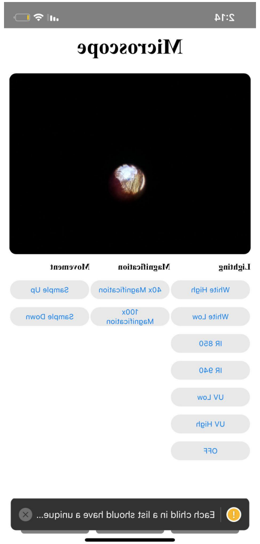
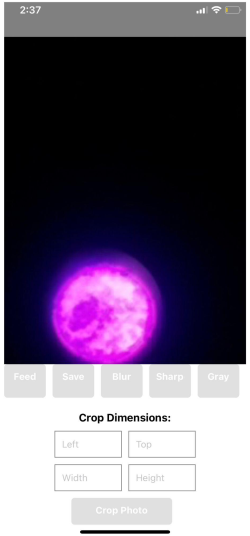
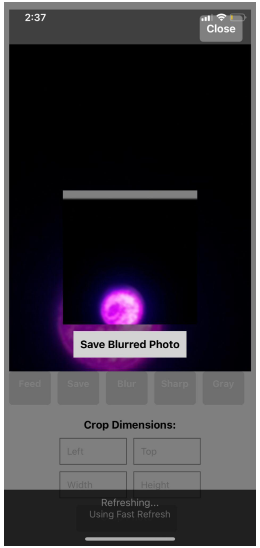
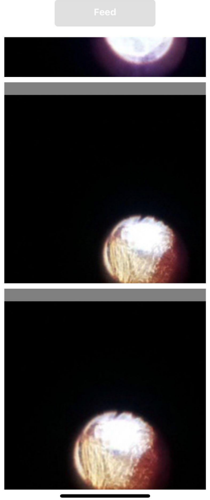








My Photos



Display with controls

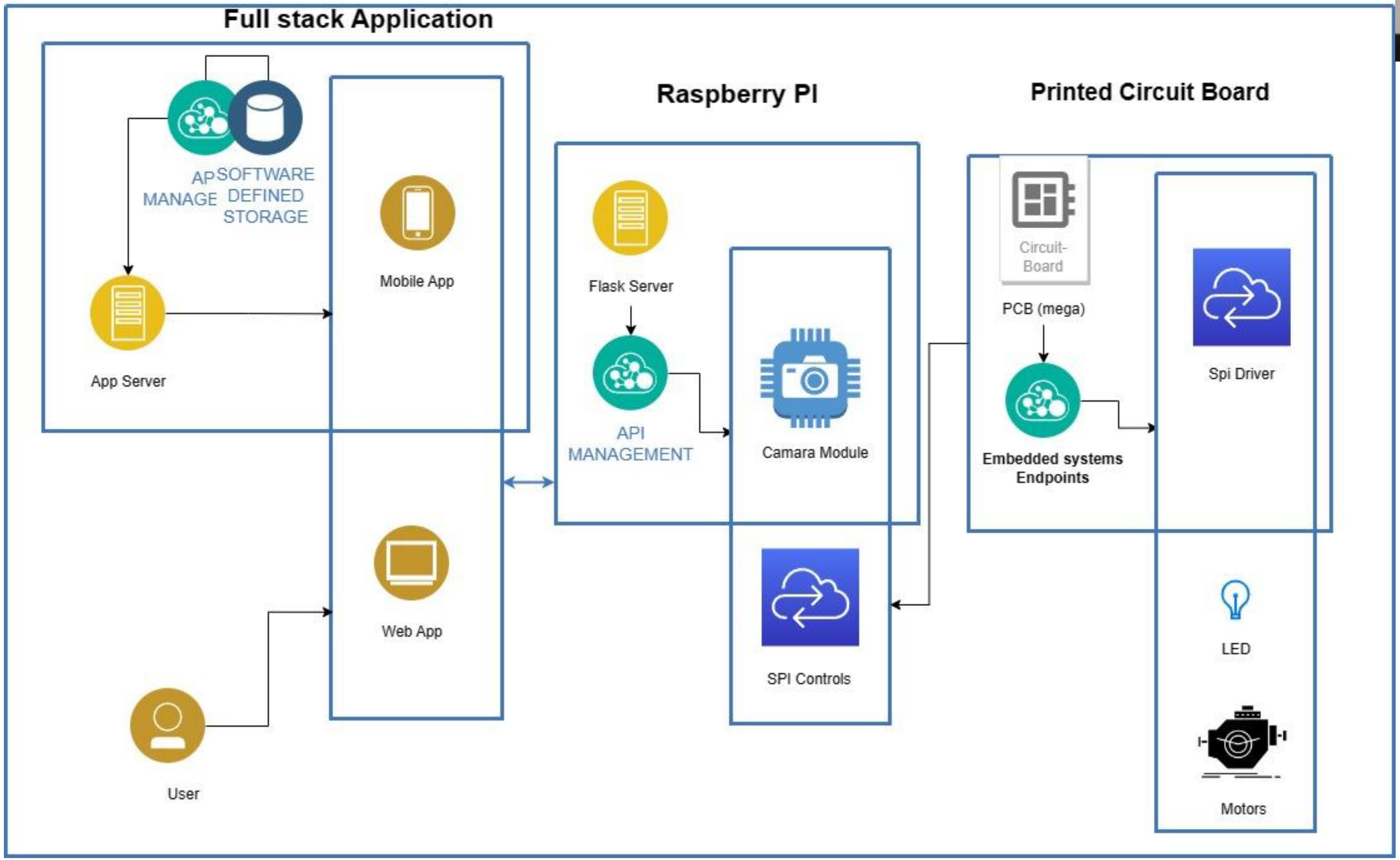
Editing

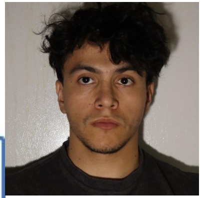
Login

Edited Photo

Gallery

Overall Software Design





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Significant PCB Design

Design Flowchart

Raspberry Pi works alongside the main PCB

Peripheral Control handled by main PCB

Image Processing and network communication handled by Raspberry Pi

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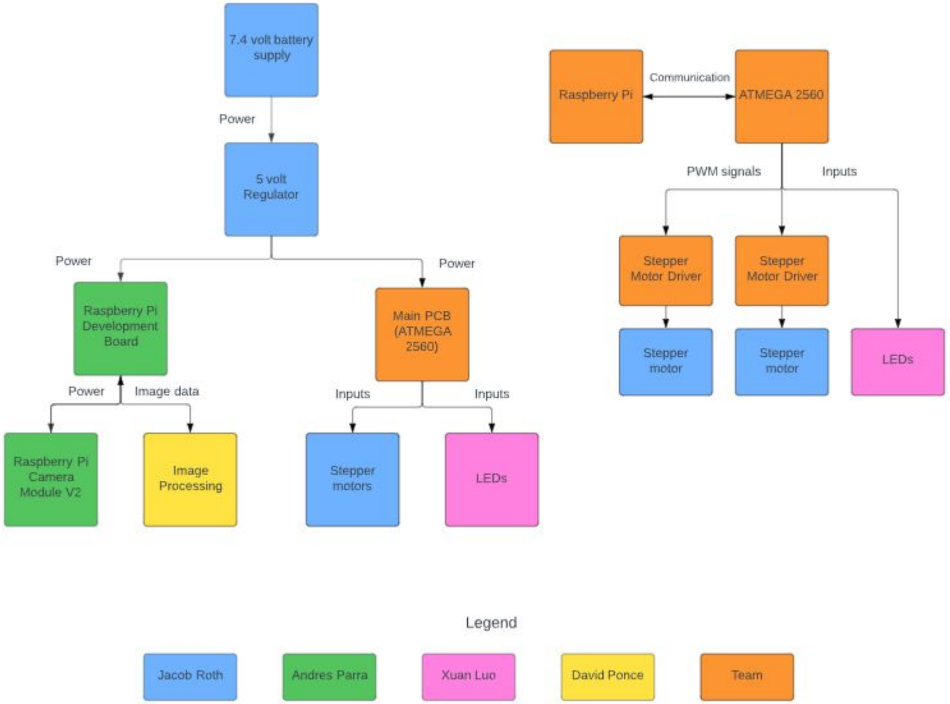

Significant PCB design

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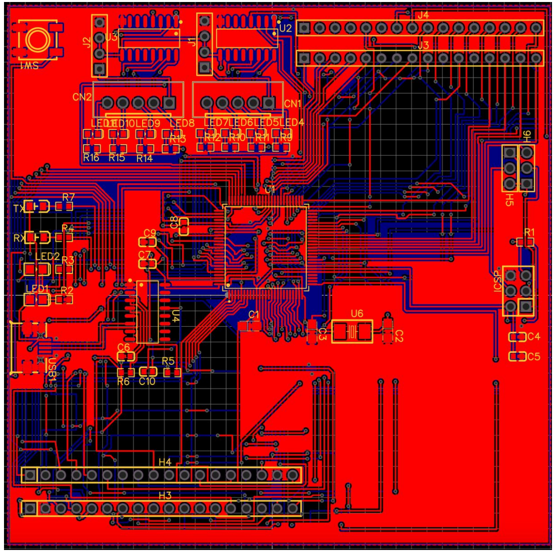
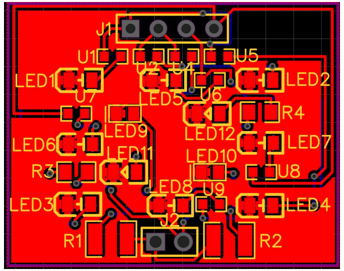
Main PCB

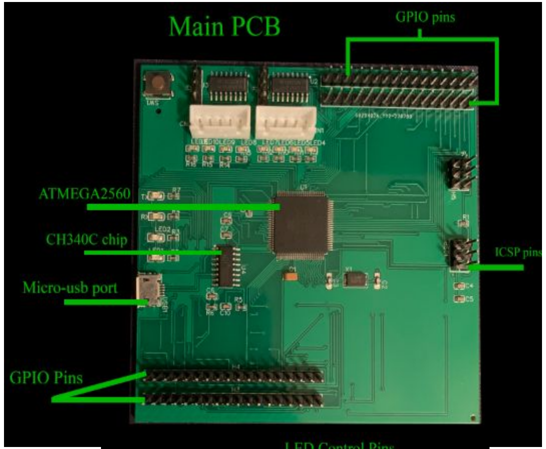
- AtMega2560
- Minimalist design
- I/O pins

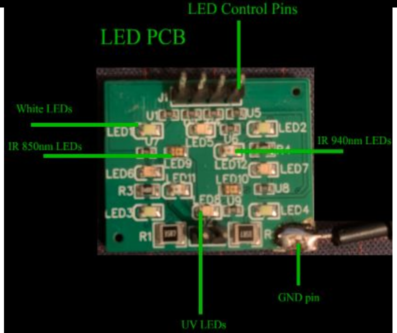
LED PCB

- Uniform Lighting
- Various Lighting Modes







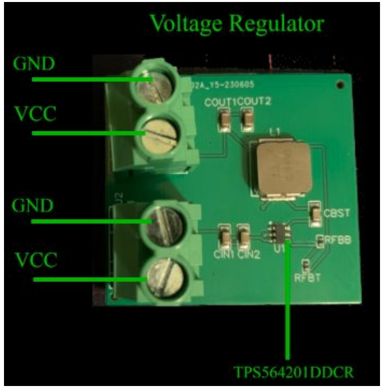


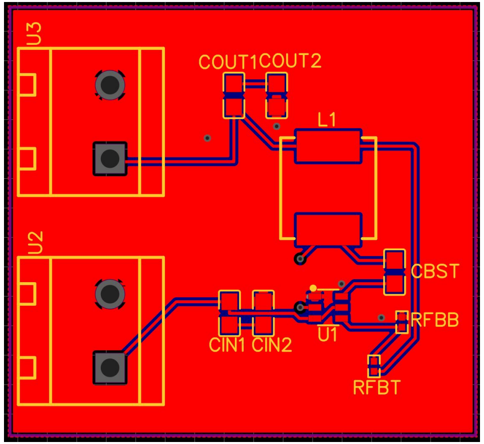
Voltage Regulator

Designed using EasyEDA

Input Voltage range: 7.4V to 13V

Output Voltage: 5.03V





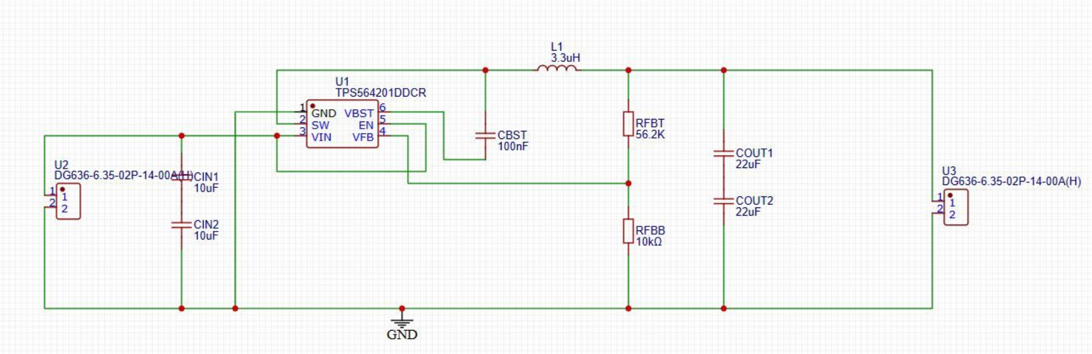


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Image Results


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CREOL

Image Results:

1. Microscope overall performance:
   - Multiple specimens under three spectra

2. UV vs. white vs. IR
   - Same specimen under three spectra

3. 40x vs. 100x
   - Same specimen under two magnifications

4. Bright-mode vs. Dark-mode
   - Same specimen under two modes

5. Actual resolution
   - Calculated by measuring a width that is resolvable

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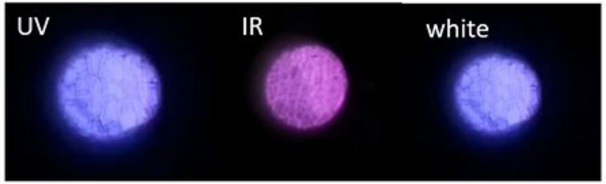
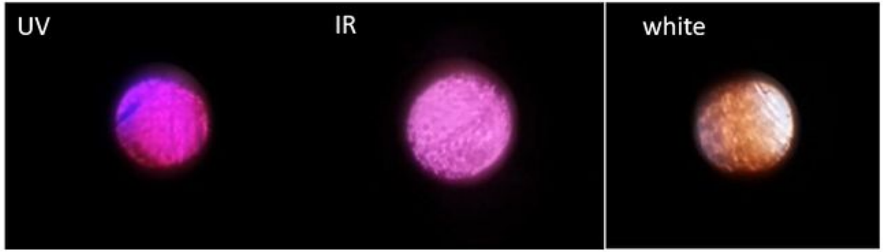
Image Results

1.

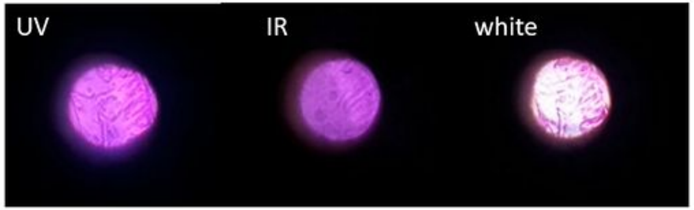
Capsocum:



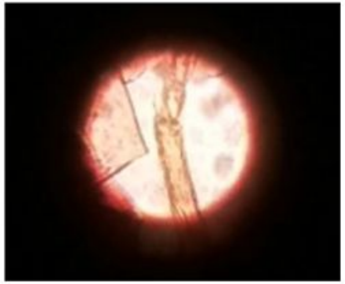
Honeybee foreleg:



Female worm:



Ant under white LED:



Housefly leg under IR LED:

Image Results

2.

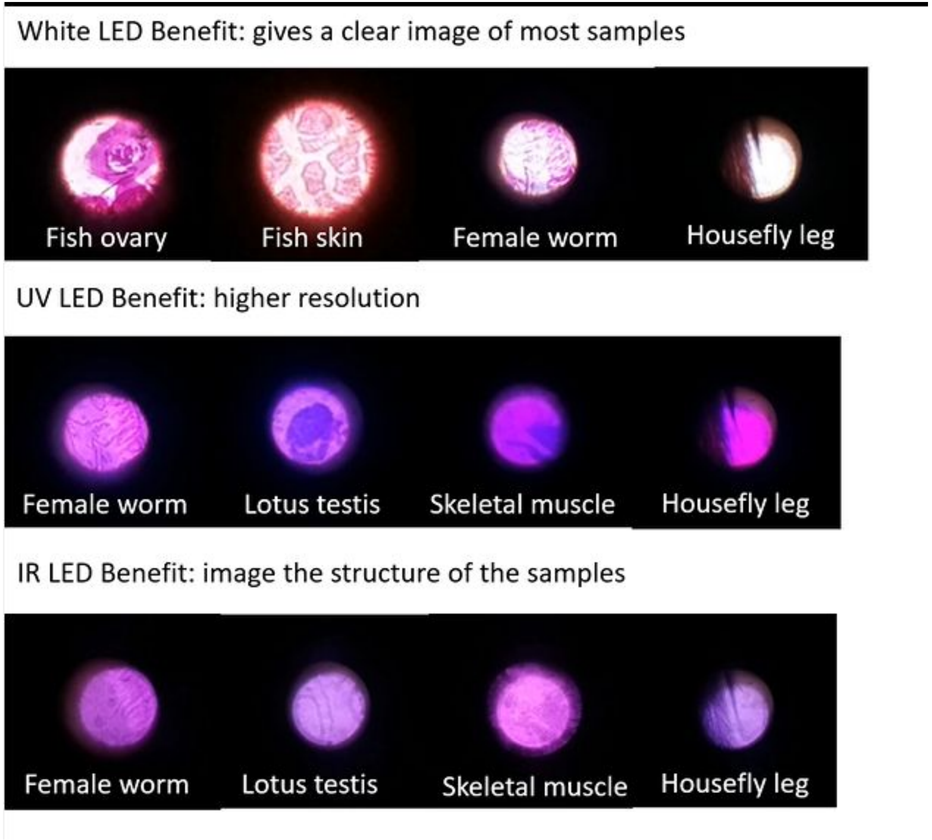


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Image Results

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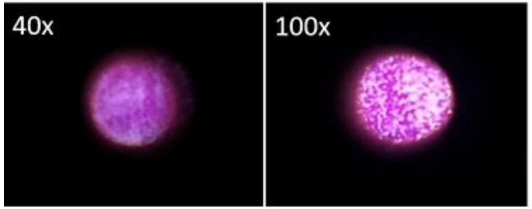
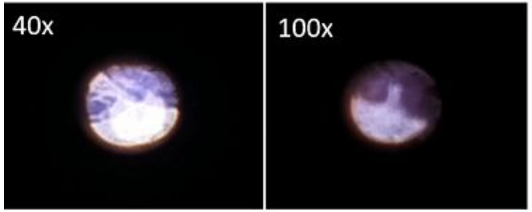
3, 4, 5.

40x vs. 100x:

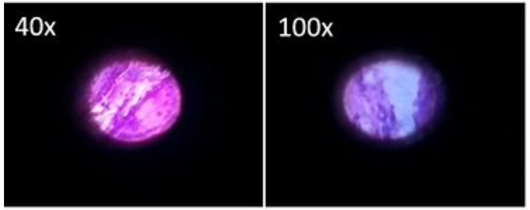
Human blood smear:



Skeletal muscle:

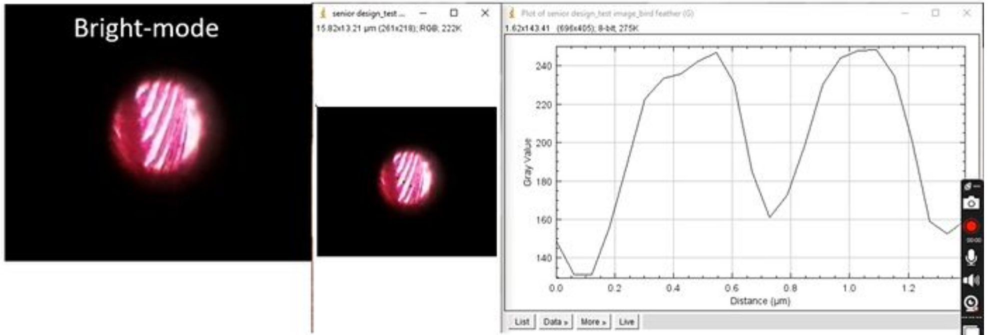


Small intestine:

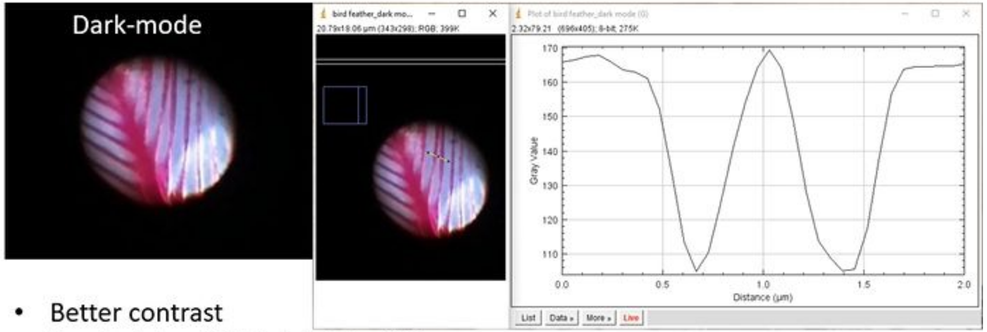


Bright-mode vs. dark-mode:

Bird feather:



- Resolvable width is 1.03-0.485 = 0.545 μm



- Better contrast
- Resolvable width is 1.4-0.8 = 0.6 μm

Administrative

done: In progress:

Table 24 - Project Budget/Financing Early Estimate				
Item	Quantity	Per Unit Cost ($)	Total ($)	Extra
Objective Lens	2	34	34	2 lenses
Eyepiece Lens	1	70	70	
Flange Nut	1	0.99	0.99	
Silicone Sealant	1	6.28	6.28	(optional) For setting the lenses
Printer Filament	1	19.59	19.59	For use in the 3D printer
ATMEGA2560	1	21	21	
Stepper Motor	2	7.99 - 20.99	7.99 - 20.99	2-piece set
Stepper Motor Driver	1	10.79	10.79	2-Piece set
Batteries for motors	1	20.00	20.00	Set of four
Battery holders	1	10.00	10.00	Set of ten
Camera Module	1	20.00-25.00	20.00-25.00	
Estimated Total	$220.64 - 238.64			

Table 27 - Hardware SENIOR DESIGN 1 Work Delegation		
Hardware		
Microscope		
	Design	Xuan
	Testing	Xuan
Power		
	Design	Jacob
	Testing	Jacob
LED		
	Design	Jacob/Xuan
	Testing	Jacob/Xuan
Breadboard		
	Design	Jacob
	Testing	Jacob
Motors		
	Design	Jacob
	Testing	Jacob
PCB		
	Design	Jacob/David
3d printed designs		
	Design	Andres

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Administrative (cont.)

done: In progress:

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Table 28 - Software SENIOR DESIGN 1 Work Delegation		
Software		
Database		
	Design	David
	Testing	David
Phone app Front end		
	Design	David
	Testing	David
Web app Front end		
	Design	David
	Testing	David
OpenCv		
	Design	David
	Testing	David
Motor controls		
	Design	Jacob
LED controls		
	Design	Jacob
Mega 2560 connections to PI4		
	Design	Andres

Table 29 - Hardware SENIOR DESIGN 2 Work Delegation		
Hardware		
Microscope Assembly with 3d parts		
	Design	Xuan/Andres
	Testing	Xuan/Andres
Power		
	Design	Jacob
	Testing	Jacob
LED/ Implementation with microscope		
	Design	Jacob/Xuan
	Testing	Jacob/Xuan
Motors//implementation with microscope		
	Design	Jacob/Xuan
	Testing	Jacob/Xuan
PCB/ integrations with pi		
	Design	Jacob/David/Andres
Camera module/ implementation with app		
	Testing	Andres/David

Administrative (cont.)

Table 30 - Software SENIOR DESIGN 2 Work Delegation		
Software		
Database		
	Design	David
	Testing	David
Phone app Front end		
	Design	David/Andres
	Testing	David/Andres
Web app Front end		
	Design	David
	Testing	David
OpenCv/ Implementation with camera module		
	Design	David/Andres
	Testing	David/Andres
Motor controls/With Application		
	Design	Jacob/David
LED controls//With Application		
	Design	Jacob/Xuan/David
Mega 2560 connections to PI4 to Application		
	Design	Andres/David/Jacob

done:

In progress:

Xuan Luo
CREOL