SASSPR

Semi-Automated Sensing for Surface Plasmon Resonance

Group 2
Robin Howell - Photonics Engineering
Robert Ballentine - Photonics Engineering
James Henderson - Computer Engineering

Motivation

- Viruses such as COVID-19 show the importance of rapid and accurate drug development.
- Observing molecular binding between an analyte and ligand is a crucial step in many biological research projects, including drug development.
- SPR sensors monitor the binding kinetics of the interaction between biomolecules without the need for labels, while also providing information on the on and off rates.
- However, commercial SPR sensors are very expensive, average cost $40,000.
- Affordable SPR sensors would provide biochemistry labs across the world with more accurate data on biomolecular interactions.

Goals and Objectives

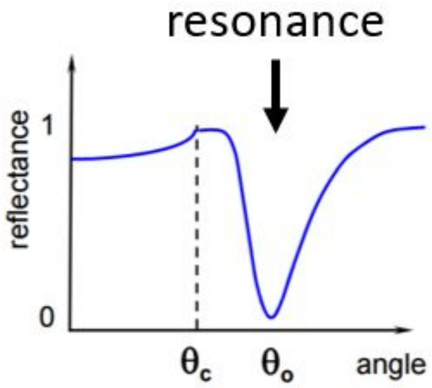
- Design a more affordable SPR sensor compared to commercial SPR sensors on the market.
- SPR sensor will be fully automated to detect the surface plasmon resonance angle and display the information to the consumer in a graphical representation.
- Sensor location will be easy to reuse, lowers the cost of consumables.
- Provides accurate data on the angle where excitation of surface plasmon polaritons (SPP) occurs.
- Experiments will be easily repeatable.

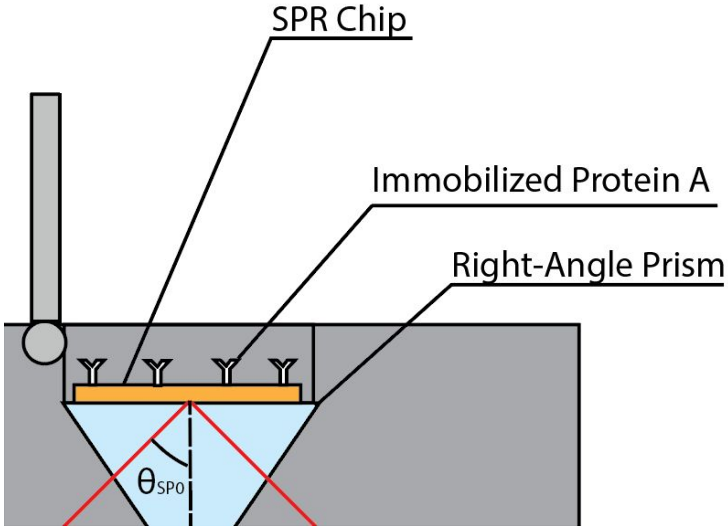
Biological Samples to Test

Category	Item	Manufacturer	Price	Quantity	Additional Information
Ligand	Protein A	Sofchip	$285	3	• Immobilized to SPR chip. • Binds to mammalian IgGs through Fc region.
Analyte	Immunoglobulin G (IgG) antibodies	Jackson ImmunoResearch Laboratories, Inc.	$99	2.0 mg	• Target: Goat • Host: Rabbit • Specificity: IgG (H+L)
Buffer	Phosphate-buffer saline (PBS)	ThermoFisher Scientific	$49.82	500 mL	• pH: 7.4 • Concentration: 10X

How Do SPR Sensors Work?

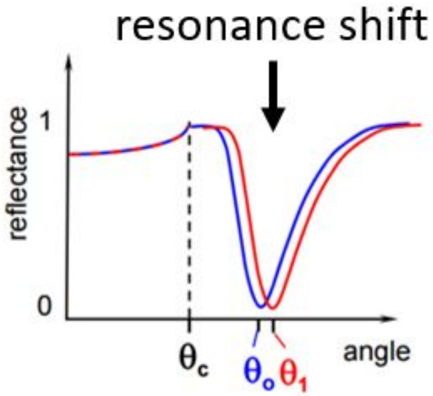
- SPR chip contains immobilized Protein A to the gold surface.
- Before solution containing IgG is added, the software detects a drop in intensity from the reflected beam.
- The reflected angle where the drop in intensity occurs is our initial SP angle, θ_{SP0}.

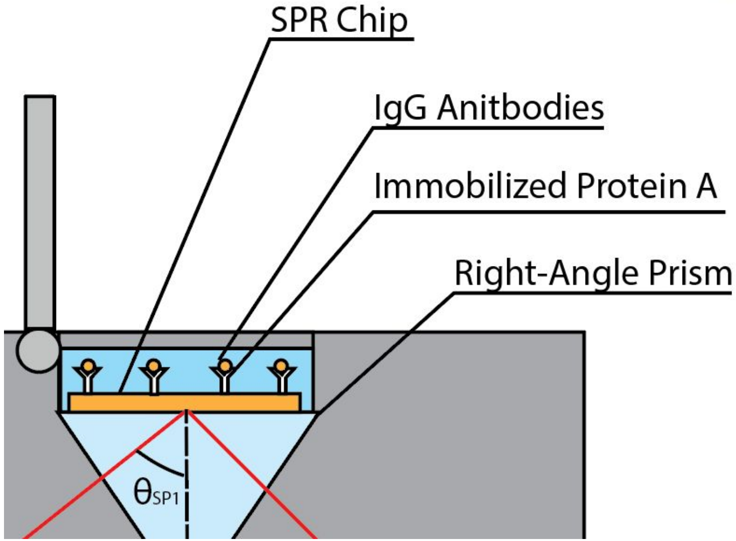




How Do SPR Sensors Work?

- When the IgG solution is added, the antibodies begin to bind to the Protein A.
- The binding between the ligand and analyte causes the refractive index of the gold surface to change.
- The change in refractive index leads to a change in the SP angle, labeled θ_{SP1}, which the software detects as an increase in intensity.

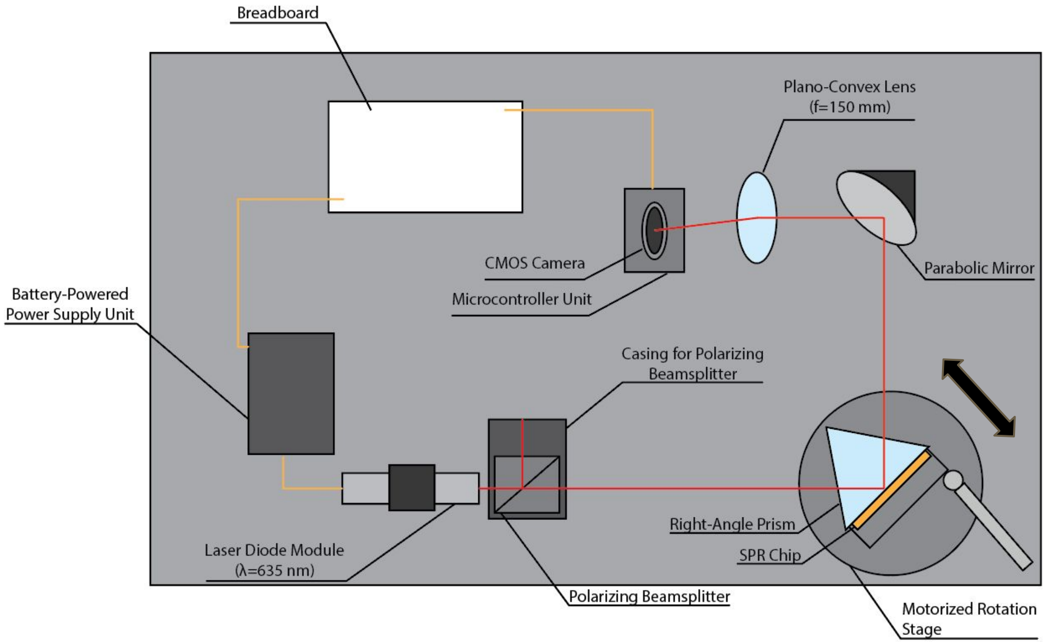




Design Description

Step 1: Determine Initial SPR Angle

- SPR chip is placed onto base of prism by user, affixed to surface using index matching gel.
- System turned on using software application. Laser, motorized rotation stage, camera, and MCU fully controlled using software app.
- Motorized rotation stage rotates automatically from range 42-50° in 0.1° increments.
- Camera captures 1 image every 0.5 s at every 0.1° increment of the rotation stage. Camera stops image capture after 50°.
- Application measures the intensity profile of the captured images taken during the measurements. From data, the angle where max drop in intensity occurred can be determined.
- Rotation stage is rotated to angle were max drop in intensity was observed.



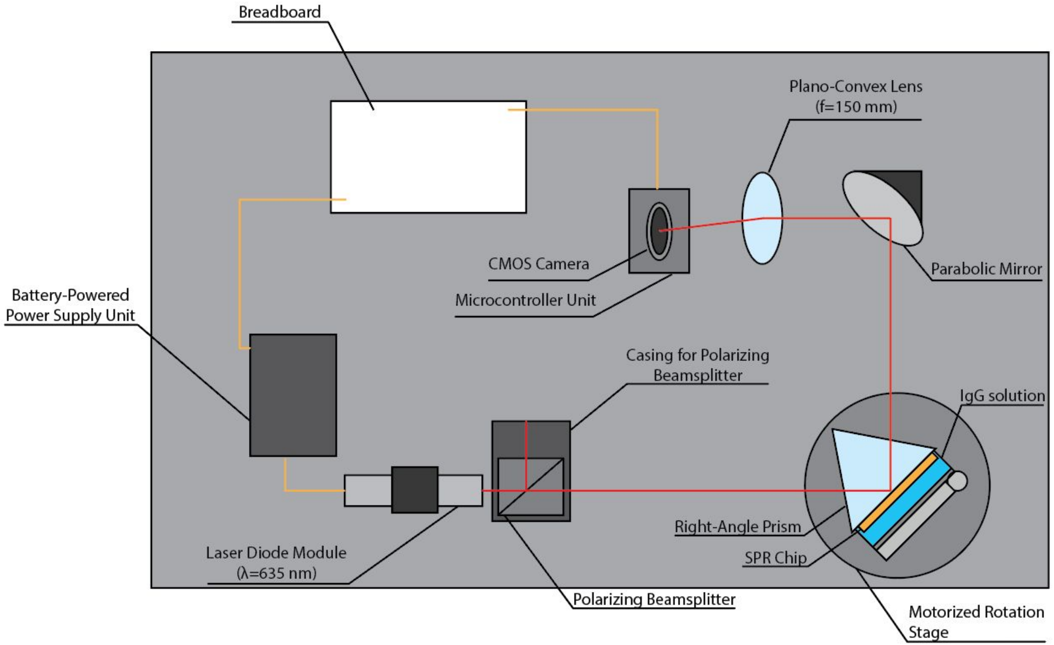
Design Description

Step 2: Observe Molecular Binding

- Analyte solution to be tested is added by user to sensing chamber. The rotation stage remains fixed at angle of lowest intensity.
- Camera begins capture of 1 image every 0.5 s until an increase in measured intensity is observed from intensity profile of processed images.

Final Results

- Software displays two graphs to user:
  - Intensity vs. incident angle of beam.
  - Intensity vs. time.
- Data from previous experiments will be saved by the software for future reference.

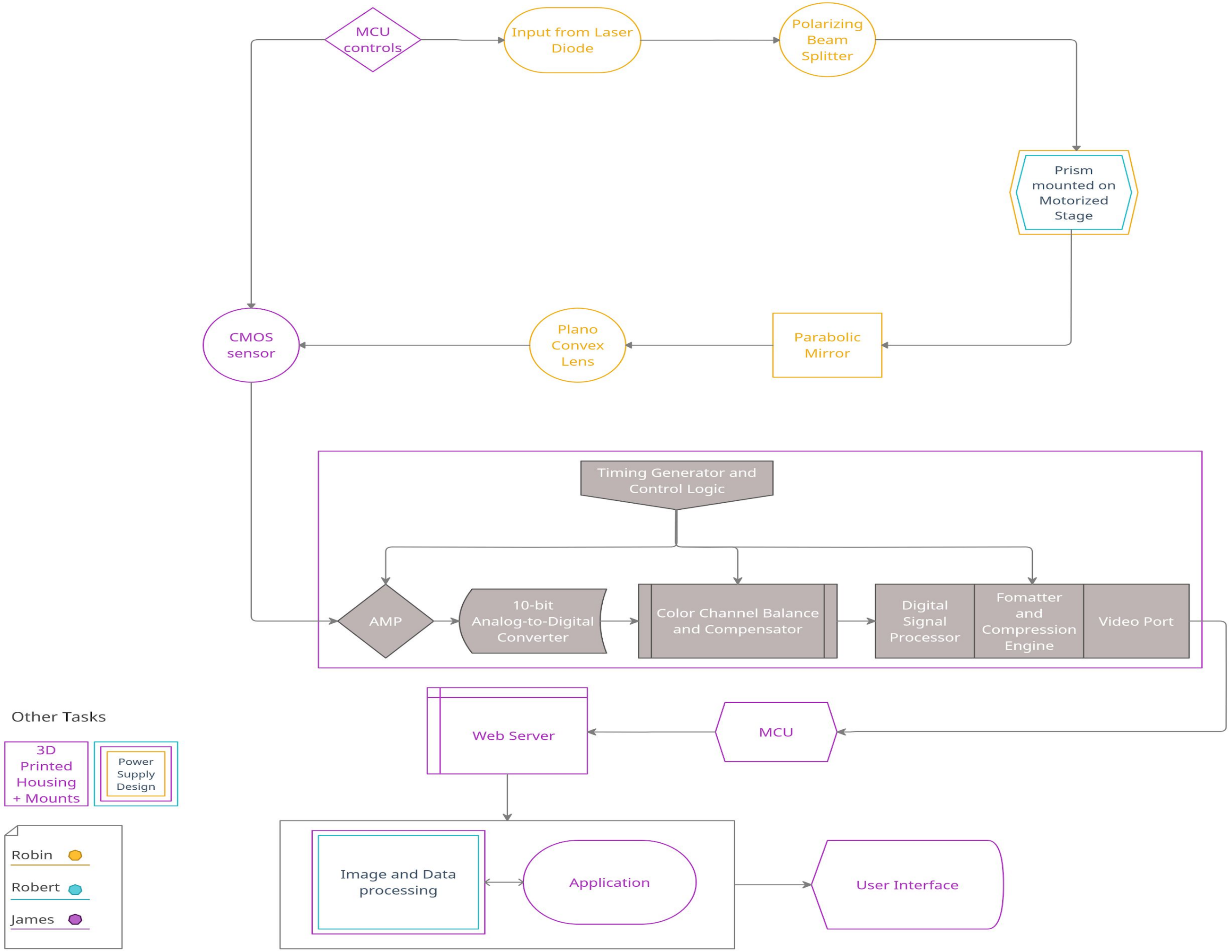


Engineering Specifications

Requirements	Units
The wavelength of laser ensures minimum broadening of SPR angular dependence.	635 nm
The CMOS camera will have a fast response time to collect necessary data.	1 image per 0.5 seconds
The motorized rotation stage provides high precision to create excitation of SPP.	25 arcsec achievable incremental motion 0.1% percentage accuracy
The software application will collect the intensity profile of images captured and process the data into graphs showing the SPR angle when molecular binding occurs within the specified time.	< 500 ms
The SPR sensor will detect concentrations levels of IgG combined with PBS solution.	7.11 µg/1 mL of IgG/PBS solution
The system shall be as portable as possible within the specified weight and dimensions.	≤ 4 Pounds ≤ 8″ x 10″ x 5″
The power draw of system will be minimal to optimize lifetime.	<10 W

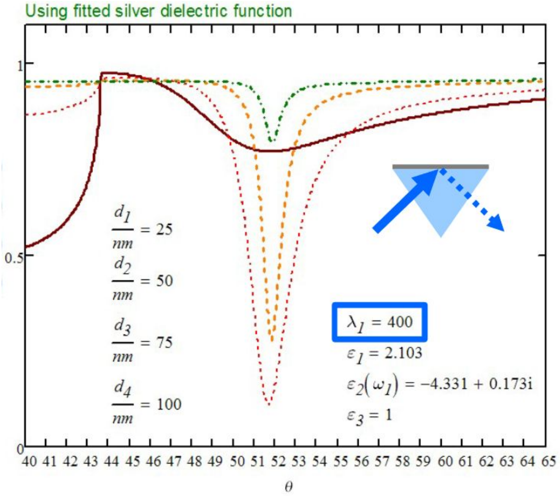
Hardware Design

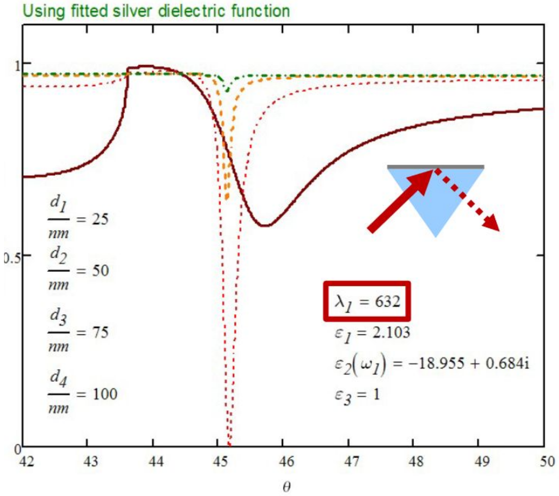
Hardware Block Diagram



Laser Diode Module

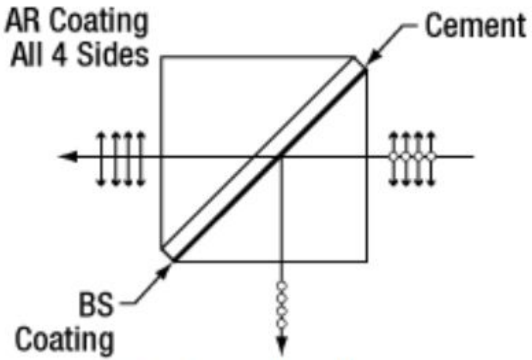
Wavelength	Manufacturer	Price	Beam divergence	Optical Output Power (CW)	Operating Voltage
532 nm	Thorlabs	$172.06	0.5 mrad	0.9 mW	4.9-5.2 V
635 nm	Thorlabs	$97.39	0.6 mrad	1.2 mW	4.9-5.2 V





Polarizing Beam Splitter

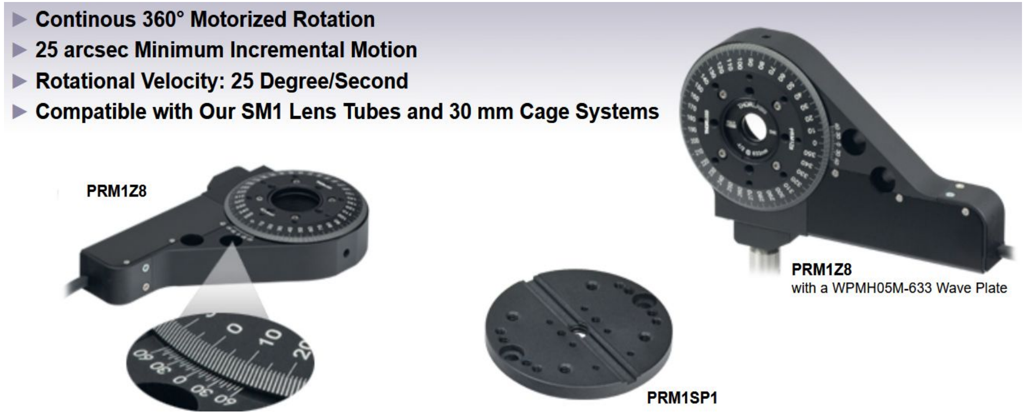
Manufacturer	Thorlabs
Price	$282.44
Dimensions	16x16 mm
Extinction Ratio	T_p:T_s >1000:1
Surface Flatness	<λ/4 @ 633 nm



Cube Beamsplitter Diagram
(Coating and Cement Layer Not to Scale)

Motorized Precision Rotation Stage

Manufacturer	Thorlabs
Price	$1,483.58
Dimensions	131x23x83 mm
Achievable Incremental Motion (min)	25 arcsec
Percentage Accuracy	0.1%
Backlash	±0.3°
Bidirectional Repeatability	±0.1°

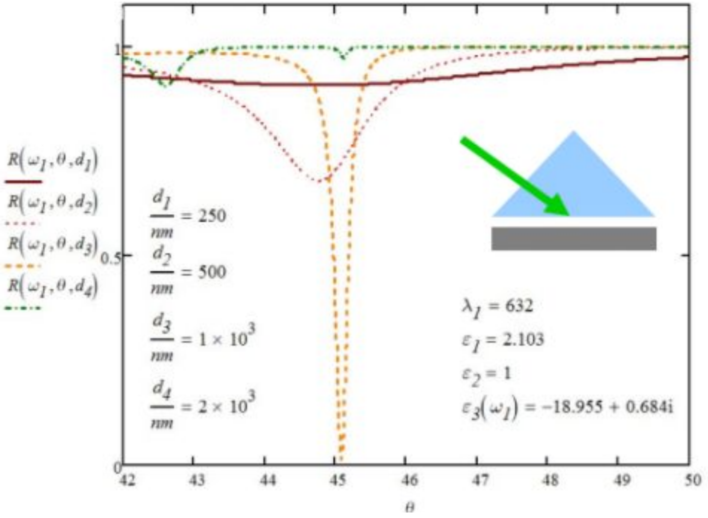


Surface Plasmon Sensor Material

Material	Cost	Upkeep	Characteristics
Fiber Optic Cable	\$8.50/meter	• Needs replacement after each use. • Metal film must be coated evenly on fiber optic core surface.	• Single mode • Operating wavelengths: 488-633 nm • Cladding diameter: 125μm • Coating diameter: 245μm
BK7 Right Angle Prism	\$51.68	• Easy to clean with acetone or isopropanol. • Manufactured SPR chip can be attached to surface.	• Dimensions: 20x20x20 mm • Refractive index: 1.52 • Transmission: 93% at λ=635 nm

Adhesive for SPR Chip

- Gap size between prism and gold metal surface determines if incident energy from light source transfers to surface plasmon polariton (SPP) mode.
- Small air gap: leaky plasmon, incident angle not critical.
- Large air gap: narrow resonance, weak coupling of SPP.
- Intermediate air gap: almost 100% of incident energy transferred to SPP mode.
- **Index matching gel (n=1.5) will be injected between prism and gold metal SPR chip. Ensures gold and prism remain in close contact for incident light to travel from prism to gold and transfer energy to SPP mode.**

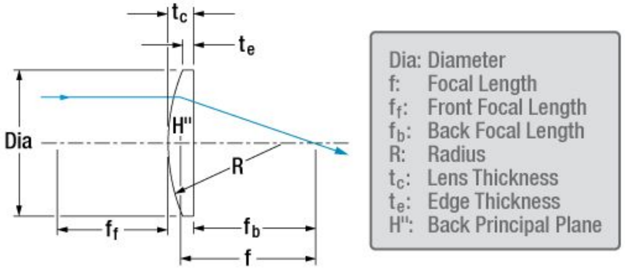


Manufacturer	Thorlabs
Price	$44.10
Quantity	3 cc Syringe
Refractive Index	1.46 @ 589.3 nm

90°

Parabolic Mirror and Plano-Convex Lens

Manufacturer	Thorlabs
Price	$265.12
Diameter	50.8mm
Reflected Focal Length	152.4mm
Parent Focal Length	76.2mm
Reflected Wavefront Error	<λ/4 at 633 nm
Off axis angle	90°



Manufacturer	Thorlabs
Price	$102.54
Diameter	75mm
Focal Length	149.5mm
Back Focal Length	141.1
Radius of Curvature	77.3
AR coating	350-700
Center Thickness	12.7
Edge Thickness	3.0

Microcontroller Unit

- Modified from original ESP32 to support Pseudostatic RAM and a parallel camera interface, enhancing IoT functionality
- Low-Energy Bluetooth
- Dual Wi-Fi
- Small, cheap and abundant for testing

The processing power of the ESP32 was potentially too slow for our engineering specifications but with the aforementioned qualities, we decided to work with what the device offered.

CMOS Sensor

- Omnivsion image sensor that supports up to 1600 x 1200 resolution, 15 captures per second
- Popular amongst tech giants, widely available documentation
- Onboard JPEG compression for low latency upload, removing load from MCU
- Compatible with low-end ARM microprocessors

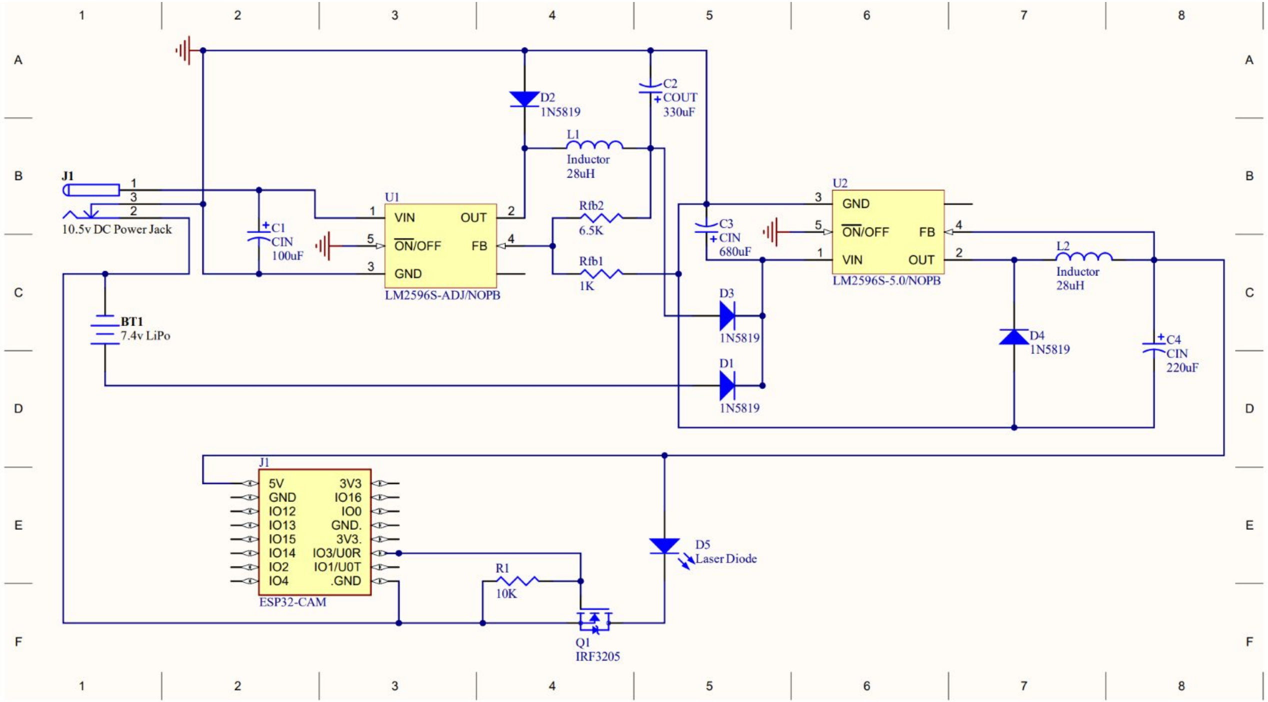
The OV2640 was a no-brainer for its similarities with smartphone cameras which is something used often for portable SPR systems.

MCU and CMOS Sensor	
Manufacturer	Xiuxin
Price	$17.99
Wireless Type	Bluetooth, 802.11bgn

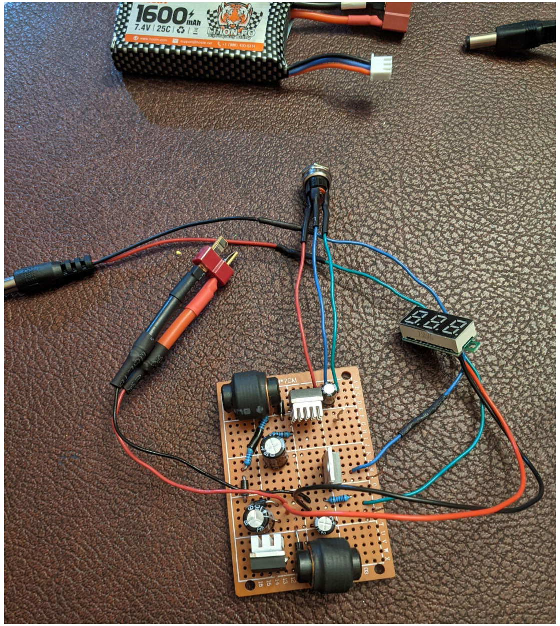
ESP32-CAM Capture vs Clock Frequency

Clock Rate	Pixel Resolution	Captures per Second	Average Processing Time	Maximum/ Minimum Processing Time
240 MHz	1600 x 1200	12.2	79 ms	209 ms/ 73 ms
240 MHz	800 x 600	25.6	40 ms	61 ms/ 38 ms
160 MHz	1600 x 1200	11.9	83 ms	162 ms/ 74 ms
160 MHz	800 x 600	27.1	39 ms	98 ms/ 42 ms
80 MHz	1600 x 1200	8.3	112 ms	185 ms/ 67 ms
80 MHz	800 x 600	25.6	39 ms	72 ms/ 34 ms

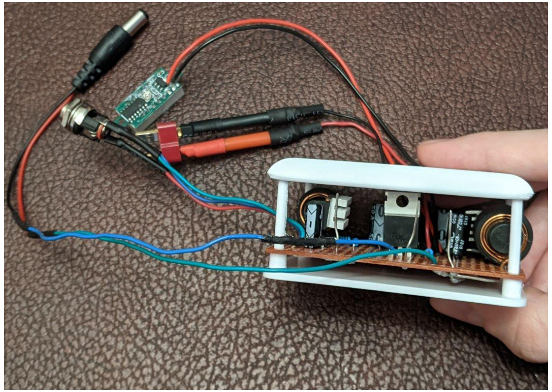
Original Power Design (Before Rotation Platform)



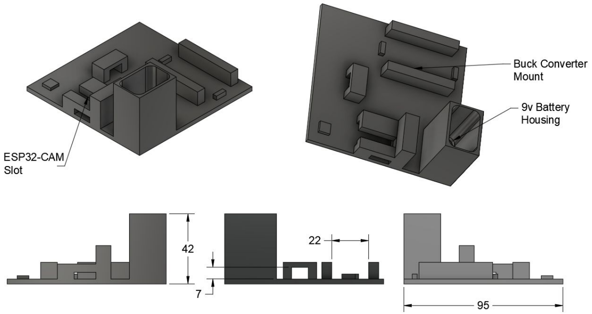
Perfboard for AC Adaptor and Battery Power Regulation

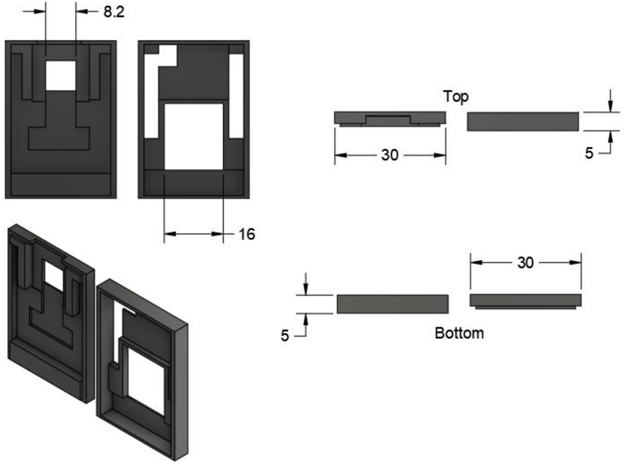


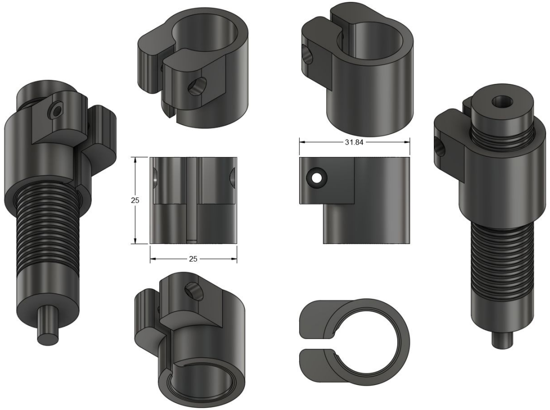
- Uses AC from a wall adaptor and DC from a LiPo battery, prioritizing AC when both are attached.
- Delivers power to microcontroller, laser diode, and rotation platform
- Uses 2 switching regulators
- Voltmeter to read battery charge level
- Houses transistor for binary control of power delivery to laser diode.



3D Printed Prototype Housing

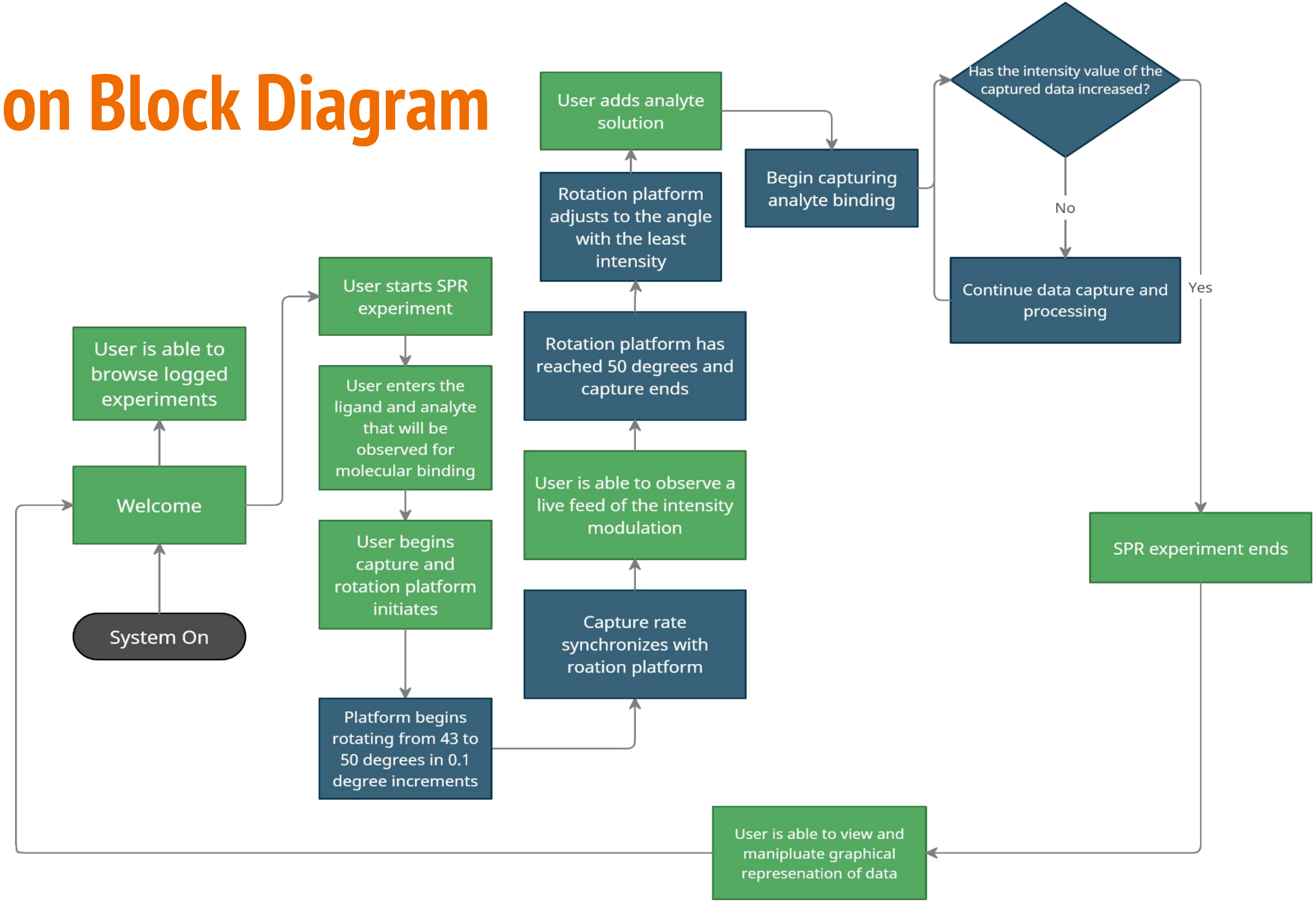






Software Design

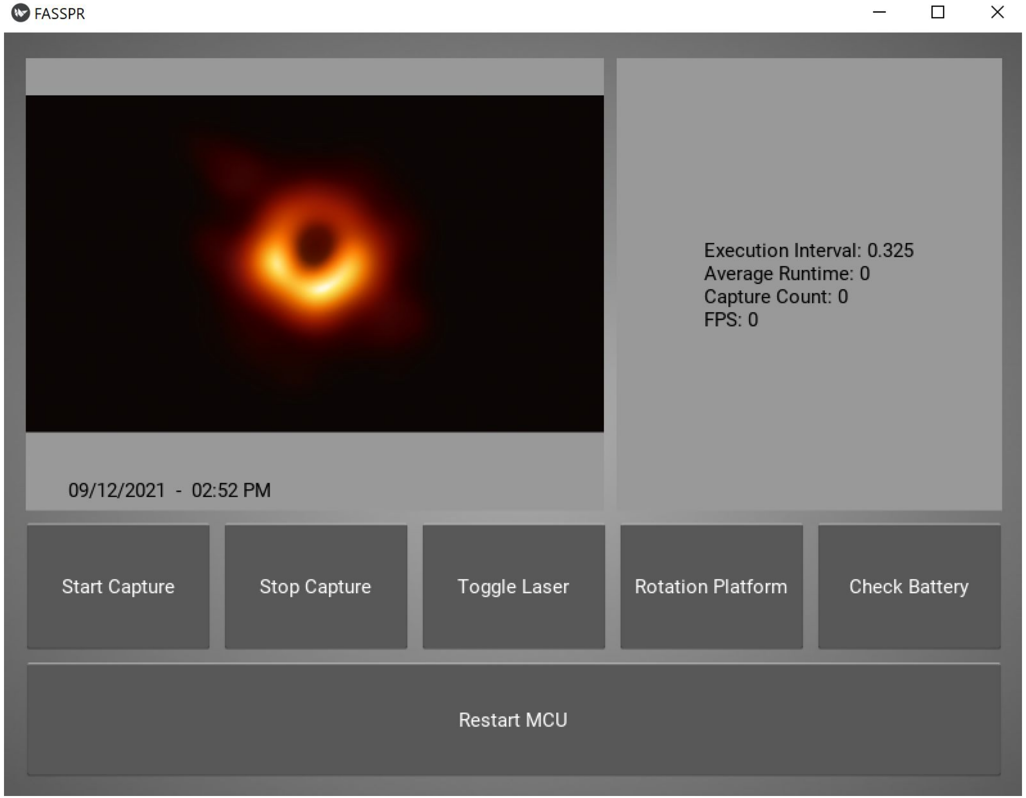
Application Block Diagram



Kivy Python Framework

- Written entirely in Python
- Fast response time to fulfill capture rate requirement
- Compatible with MacOS, iOS, Android, and Windows.
- Free and Open Source meaning wide availability of addons, libraries and documentation
- Enables rapid prototyping by building the application for all compatible systems

Prototype Software Solution for FASSPR



- Variable execution interval to maintain desired capture rate.
- Control over power delivery to laser diode.
- Control over motorized rotation platform
- Verify power source from wall or battery and check battery level
- Measure and record brightness of captured images and graph them over time
- Store and retrieve past experiments
- Compatible with Windows, Android, Mac OS and iOS

Administrative Content

Work Distribution

Member	Physical Optics	Biological Substances	Data Analysis	Hardware	Software Application
Robert	X		X		
Robin	X	X			
James				X	X

Project Budget and Financing

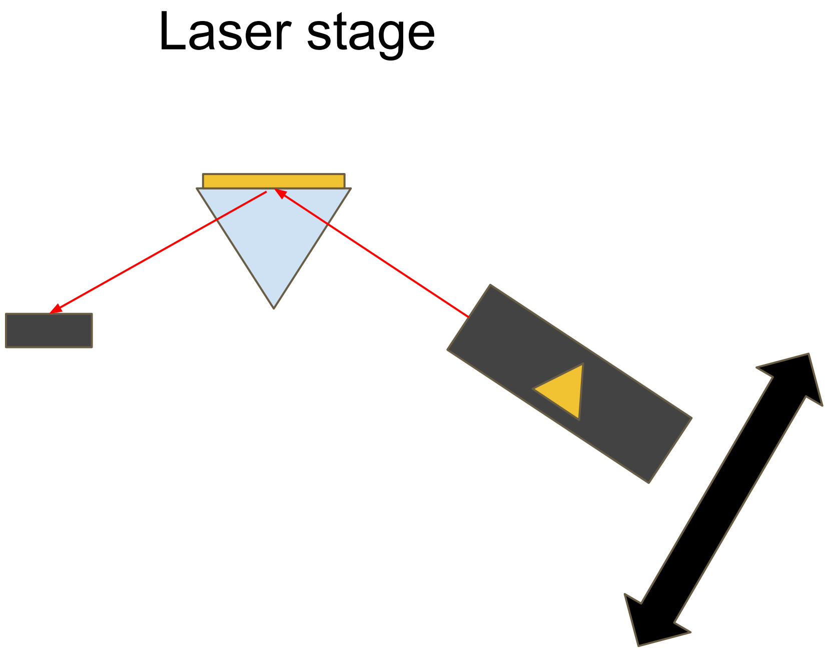
Surface Plasmon Resonance Sensor Equipment Cost				
Part #	Item	Quantity	Price	Out of Pocket Cost
CPS635R	Collimated Laser Diode Module, 635nm, 1.2mW, Round Beam	1	97.39	Covered by CREOL
CPS11K	Laser Diode Module Mounting Kit, 120V	1	217.51	Covered by CREOL
MPD269-601	2" Diameter Off-Axis Parabolic Mirror, Prot. Aluminum RFL=6"	1	265.12	Covered by CREOL
MP508P1	3" Outer Diameter Adapter for 2" Off-Axis Parabolic Mirror	1	33.28	Covered by CREOL
KS3	3" Precision Kinematic Mirror Mount, 2 Adjusters	1	204.52	Covered by CREOL
PRMTZ8	Motorized Precision Rotation Stage with 2.56" Platform (Imperial)	1	945.26	Covered by CREOL
KDC101	K-Cube Brushed DC Servo Motor Controller	1	677.41	Covered by CREOL
KPS101	15V 2.4A Power Supply Unit with 3.5mm Jack Connector for One K-Cube	1	35.36	Covered by CREOL
LA1002-A	Plano Convex Lens (f=150mm)	1	102.54	102.54
LMR75	75mm Diameter Lens Mount	1	56.81	56.81
M1	Mirror Mount 1.0" (compatible with 19.05mm mirror)	2	37	74
PS908	20mm Right-Angle Prism	1	51.68	51.68
1770126-3	Protein A immobilized SPR chip, thin (3 chip set)	1	285	285
CCM5-PBS201	16mm Cage-Cube-Mounted Polarizing Beamsplitter Cube, 420-680nm	1	282.44	282.44
AB_2339376	AffiniPure Rabbit Anti-Goat IgG (H+L)	2 mg	99	99
AM9624	Phosphate-Buffered Saline pH 7.4	500 mL	49.82	49.82
		Total Cost	3440.14	1001.29

Project Challenges

Challenge	Road Blocks	Solution(s)
Molecular Binding	Data can be affected when liquid analyte solution added to prism mounted on moving rotation stage.	• Prism remains fixed during molecular binding portion of the experiment.
Surface Plasmon Resonance Angle	Requires high precision to create excitation of SPP.	• Build a custom high precision instrument to rotate the incident angle of laser beam. • Galilean Laser Beam Expander used to expand reflected beam to aid in monitoring the shift in SP resonance angle.

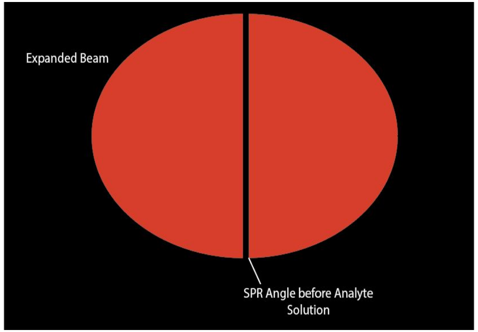
Potential Redesign 1: Moveable Laser Stage

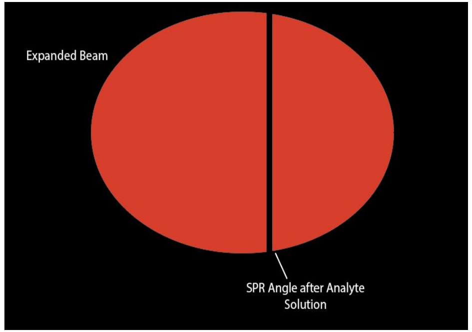
- Mount the laser onto a mechanism that changes the incident angle of laser.
- Used to detect changes in angle of SPP as analyte solution added to sensing location.
- Added cost: ≥ $1,000



Potential Redesign 2: Galilean Beam Expander

- Place an achromatic AR-coated Galilean beam expander after the polarizing beam splitter.
- Expands the diameter of the beam, allows us to monitor the change in surface plasmon resonance angle as analyte solution introduced.
- Added cost: $400-$500





Current Progress and Future Plans

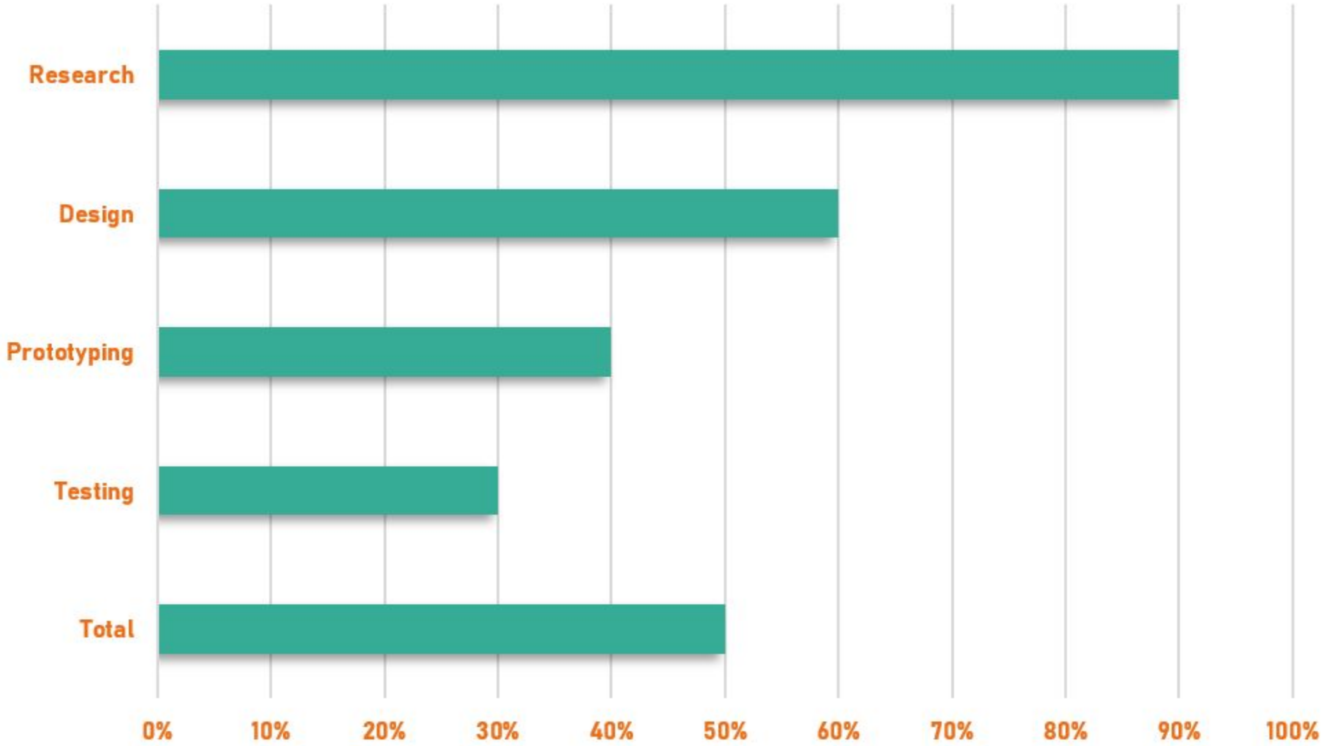
Current Progress

- Camera and microcontroller is working, camera can capture 1 image every 0.5 seconds.
- Power supply has been designed, however redesign is required to incorporate the motorized rotation stage.
- Software application in development, prototype designed and tested. Collects and processes the data in less than 500 ms.
- 3D printed prototype of casing for laser diode module created, a second prototype will be designed to properly attach to new laser diode dimensions.
- Optical equipment has been ordered and delivered as of September 16, building of optical setup for testing can begin.

Future Plans

- Research Galilean beam expander, determine if appropriate for current system.
- Redesign power supply to include motorized rotation stage.
- Redesign software application for communication with motorized rotation stage.
- Create surface plasmon excitation with current optical setup.
- Build prototypes of housing unit and casing for each optical and electrical component.
- Purchase biological samples and begin testing for molecular binding.

Progress



Research
Design
Prototyping
Testing
Total
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Questions