Low-Shift Raman Microscope

Group # 17

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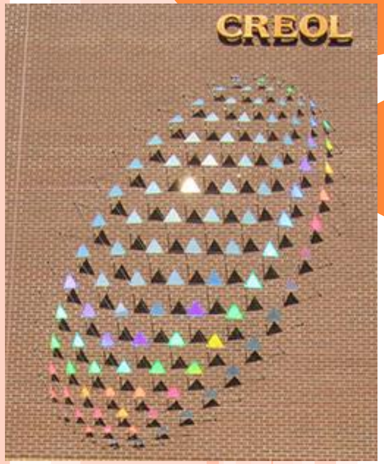
Brandon Seesahai - PSE

Matt Aviles – EE/CpE

Chris Beck - PSE



UCF

College of Engineering and Computer Science

Motivation

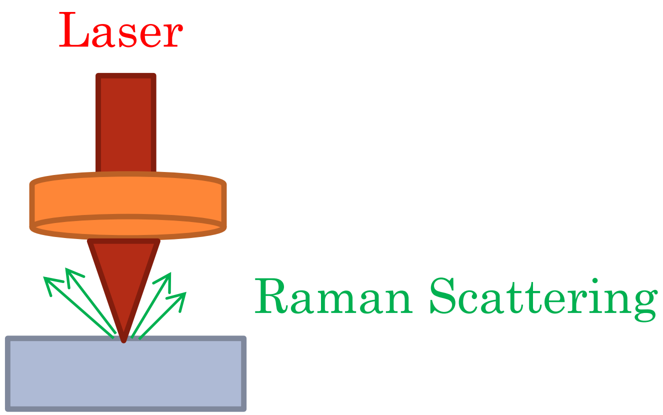
- Sponsor: Professor of Chemistry and Forensic Science wants a Raman spectroscopy system in his lab that detects low-shift signals.
- Raman spectroscopy has applications in forensic science for analyzing drugs, explosive substances, and other materials for forensic applications.
- Raman spectroscopy and microscope integration: Microscope allows for easy sampling, sample visualization with high magnification, and can focus light to a small point to easily create a Raman Signal.
- Low-shift signals provides a more detailed “fingerprint” of a sample.

GOALS

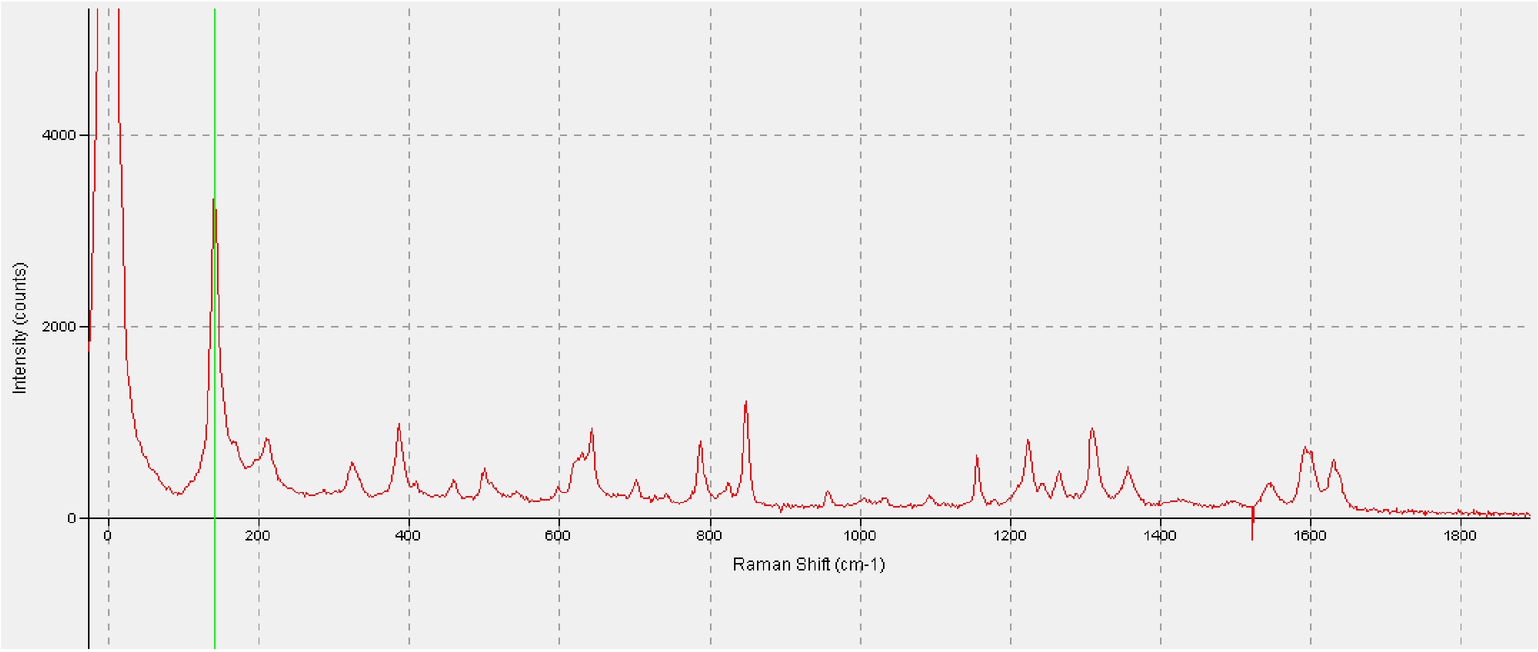
- Integrate Raman spectroscopy with a microscope.
- Create a Raman spectroscopy system that can detect low-shift Raman signals.
- "Cheaper", safe, and easy to use system for non-optics majors.

What is Raman Spectroscopy?

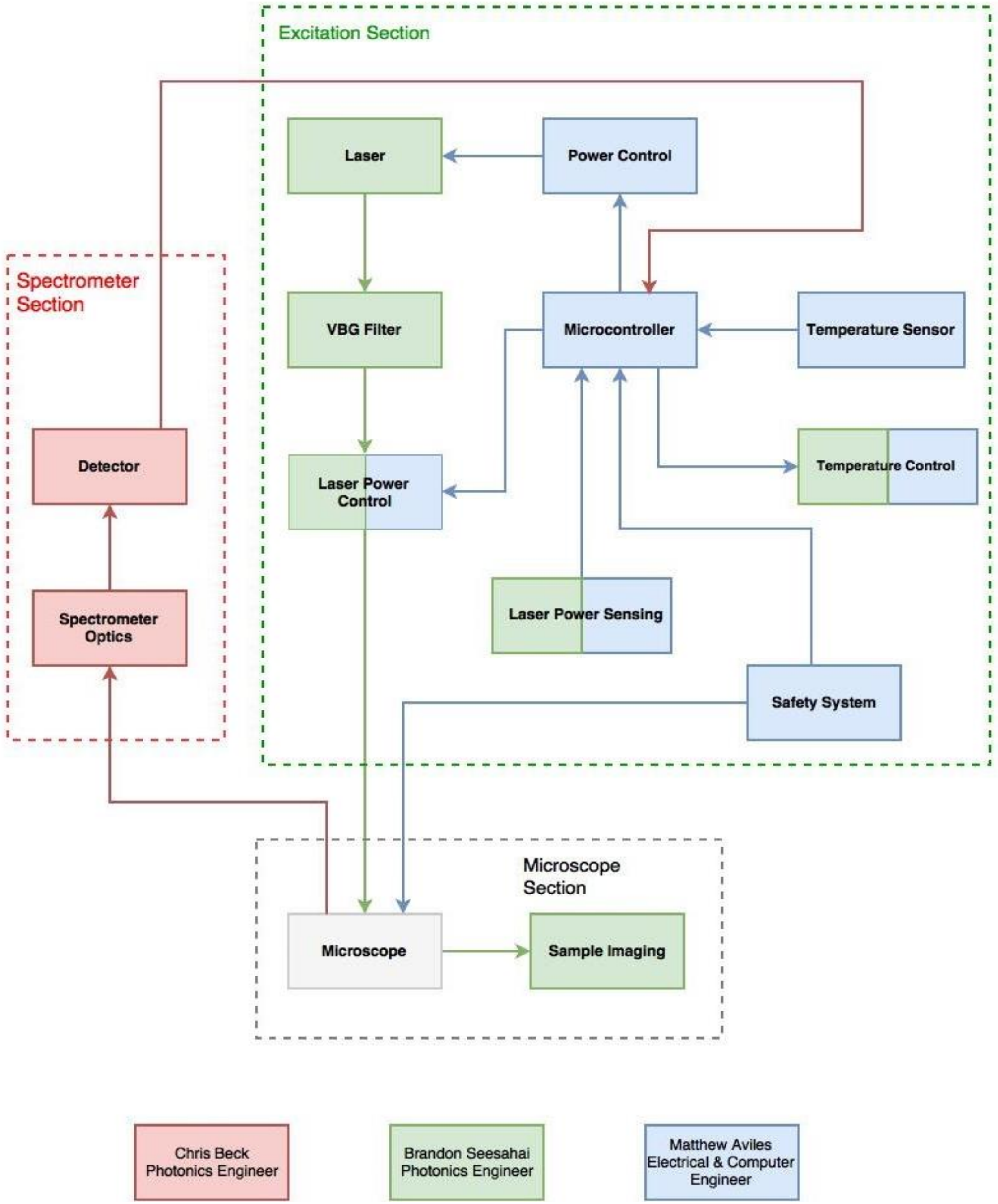
- Focus Laser light to a sample to create Raman scattering.
- Raman scattering can provide a spectrum that provides the molecular signature of a material.
- Raman scattering: Rayleigh scattering and inelastic scattering (stokes and anti-stokes).



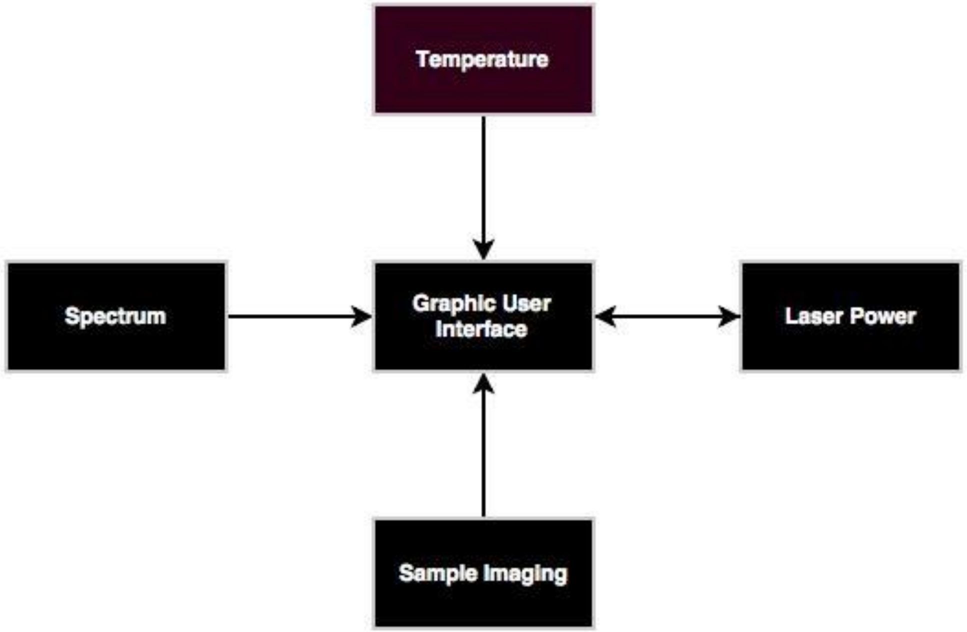
Stokes Signals of Excedrin Tablet

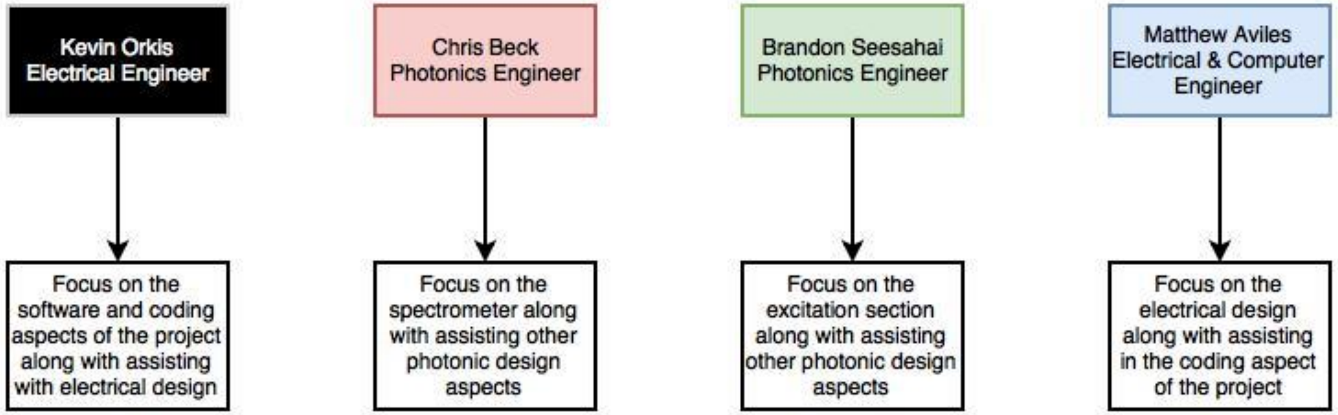


Hardware Diagram



Software Diagram





Work Distribution

	Main	Secondary
Spectrometer	Chris Beck	Brandon Seesahai
Excitation	Brandon Seesahai	Chris Beck
Hardware	Matt Aviles	Kevin Orkis
Software	Kevin Orkis	Matt Aviles

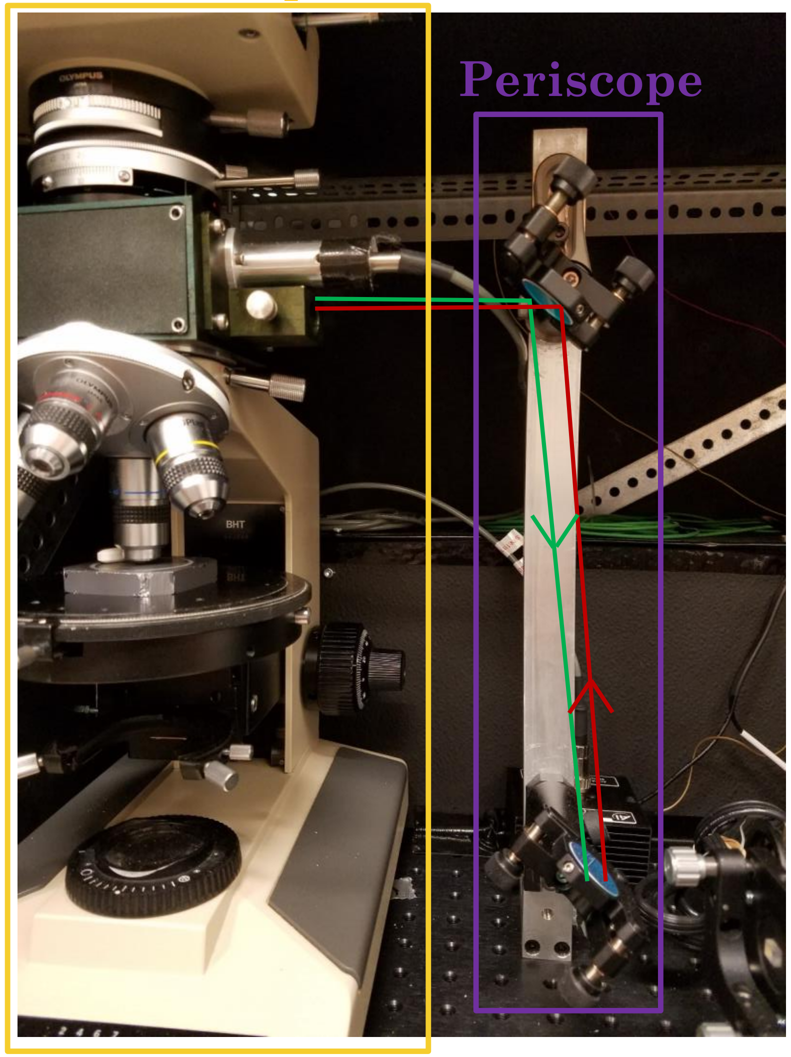
Overall Requirement Specification

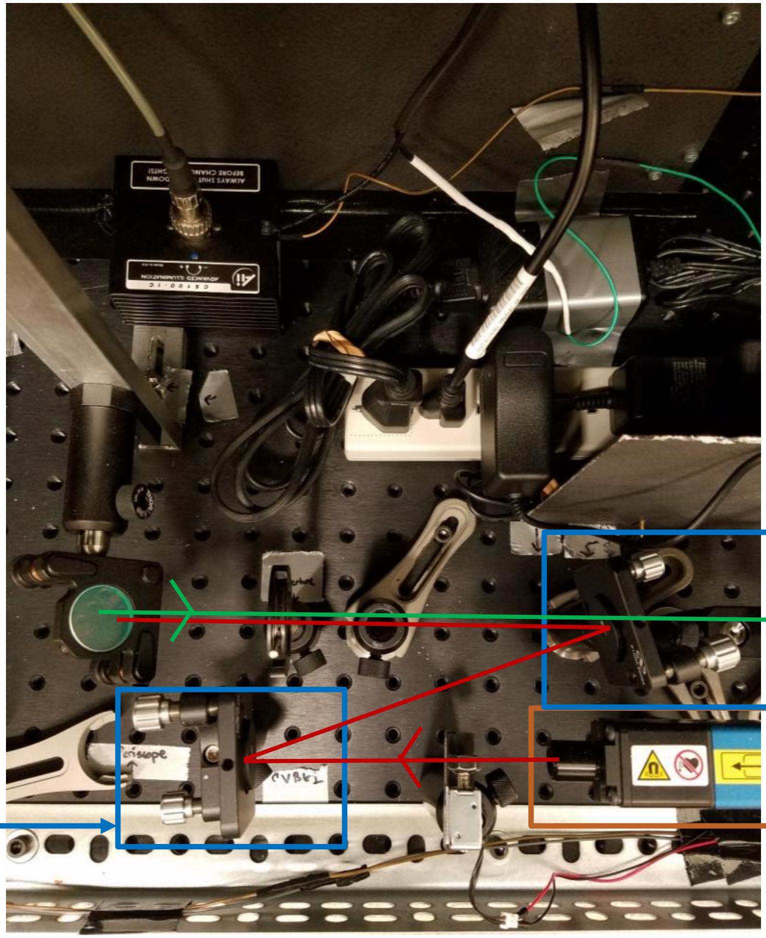
- Laser Wavelength = 785 nm
- Resolution ≤ 5 cm^{-1}
- Detect Peaks ± 200 cm^{-1} (770.87 nm to 799.13 nm).
- Class 1 Laser System
- Fit on a Chemistry Lab Table

Excitation Section Objectives

- Inject a narrow line width laser into a microscope
- Focus as much laser power as possible to a sample
- Generate Raman scattering that can be detected by a spectrometer.
- Camera imaging of sample

EXCITATION OPTICS

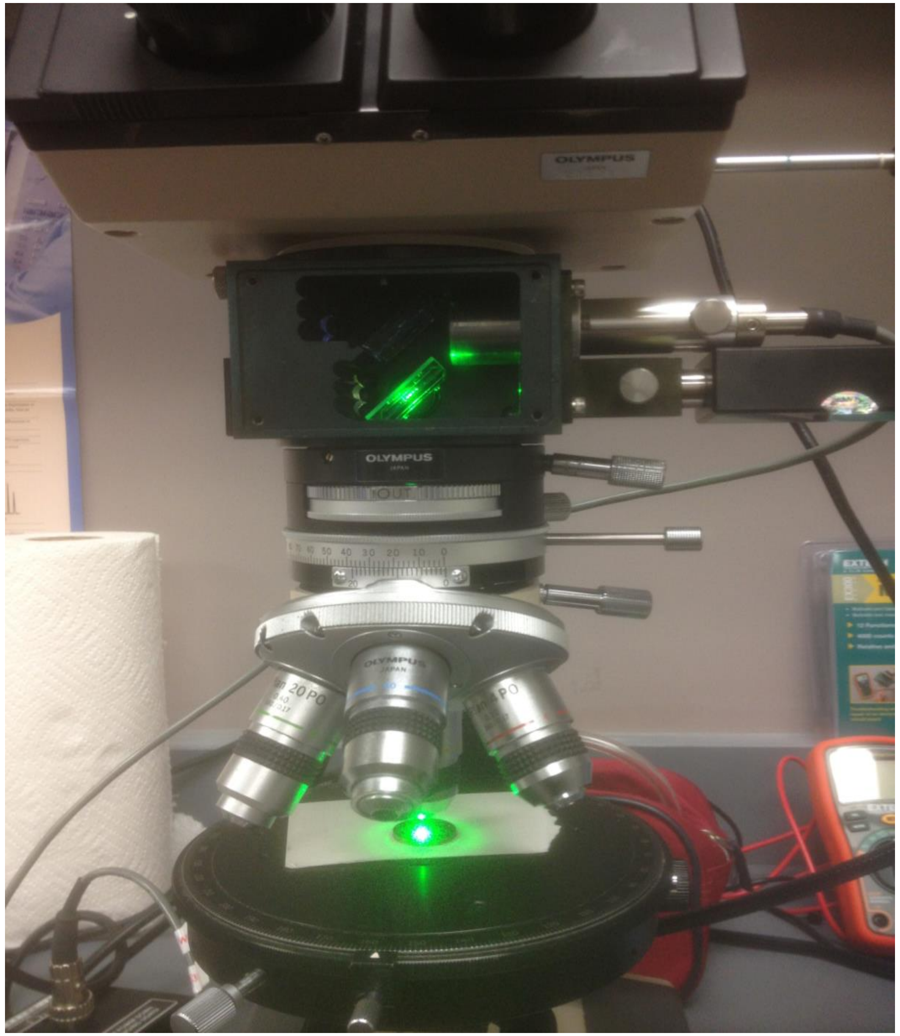




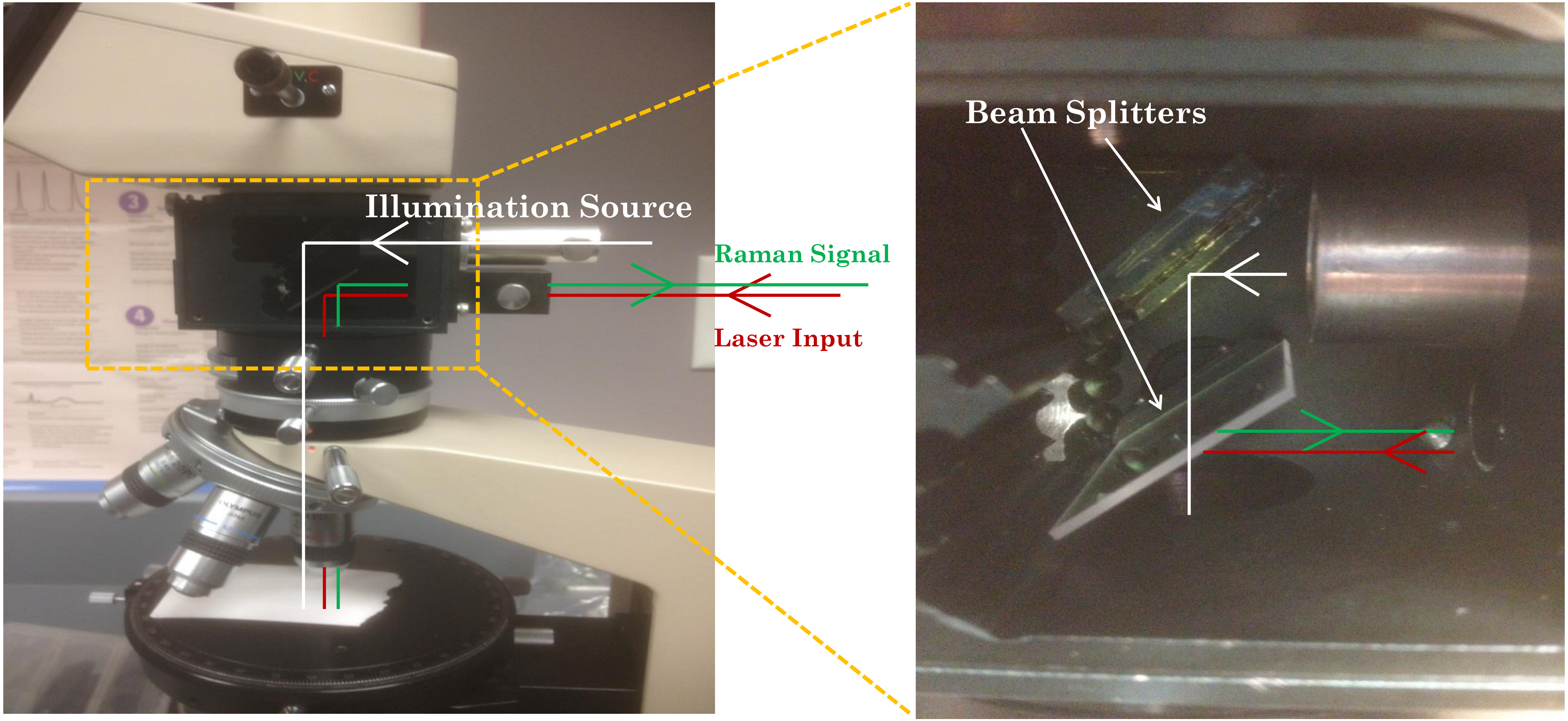
Microscope

- Olympus BH2 Microscope

Magnification	4 X	10 X	20 X	40 X
N.A.	0.10	0.25	0.40	0.65
Focal Length (mm)	34.23	17.69	8.99	4.61

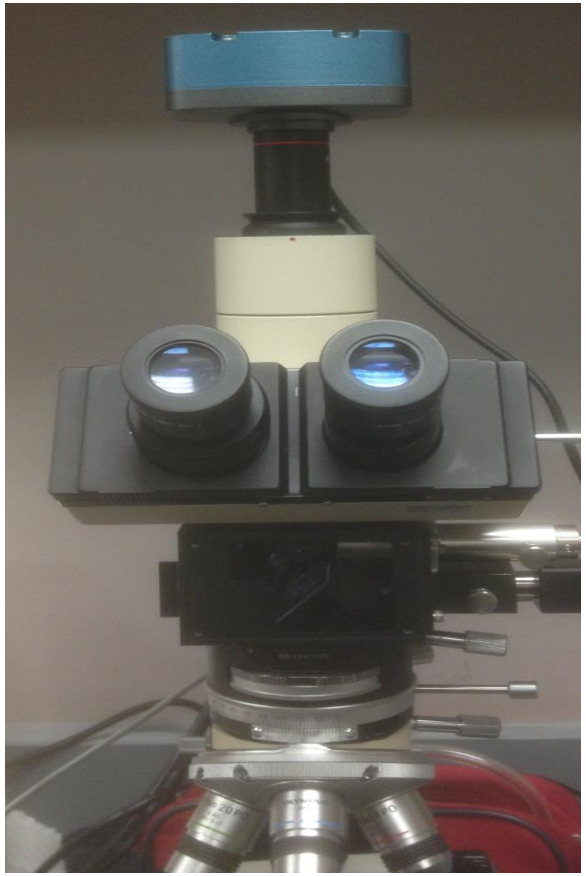


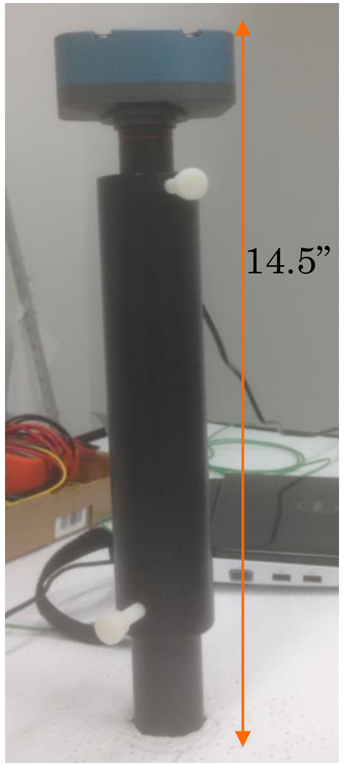
Inside the Microscope



Camera

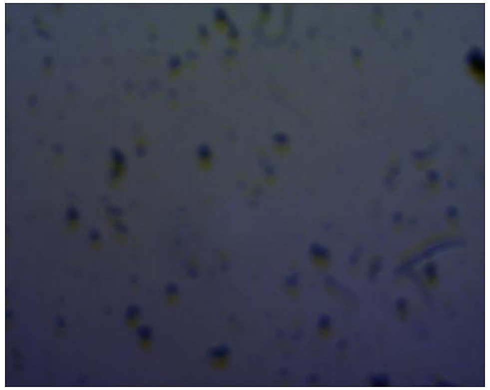
- Camera is on top of the microscope.
- Camera has the same field of view as the objective
- Camera imaging of sample
- Matching focal planes



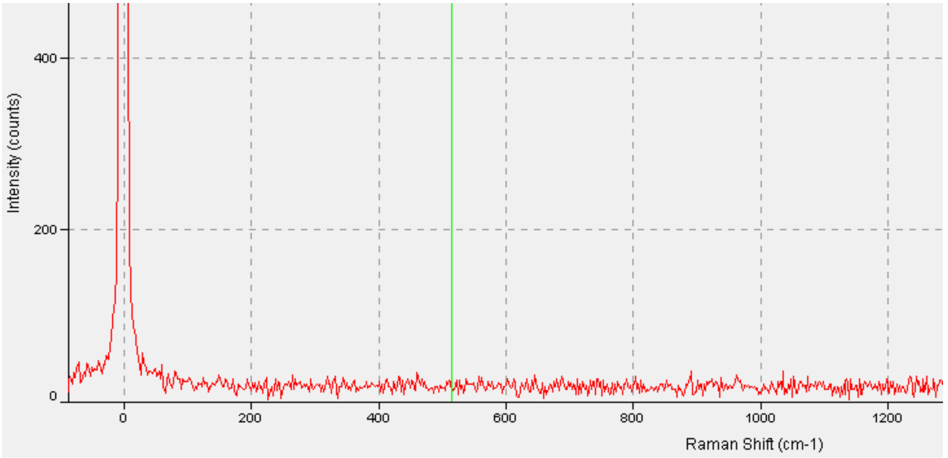


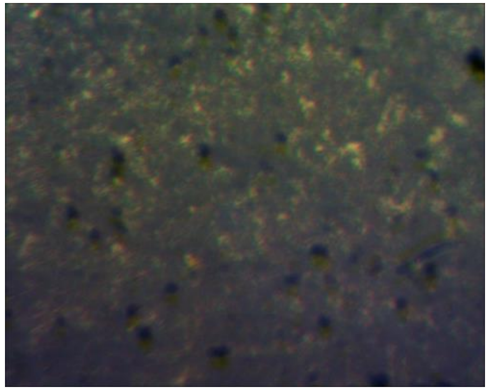
Camera

- Matching focal planes

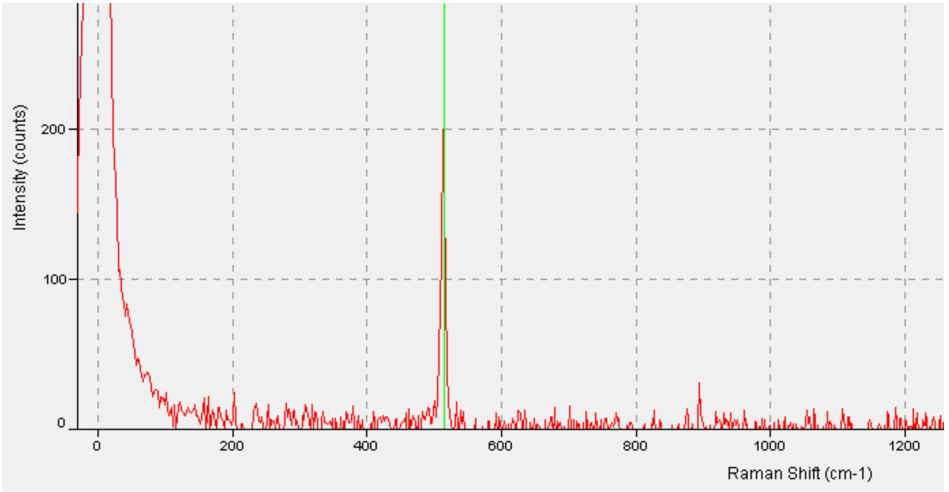


Defocused





Focused



LASER

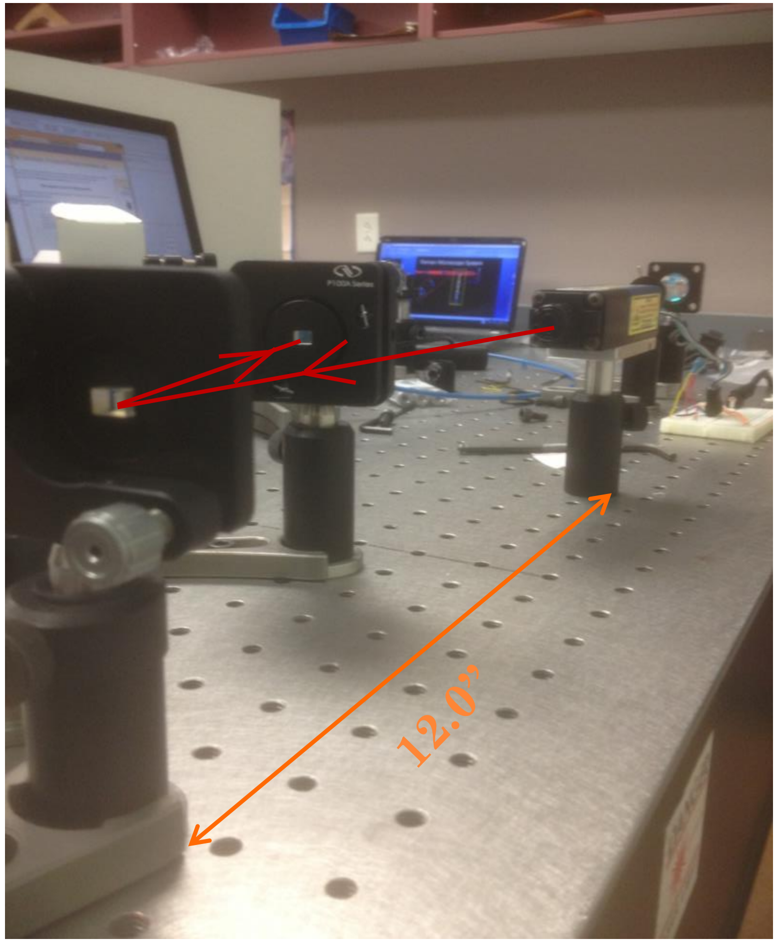
- Laser Wavelength = 785 nm from Innovative Photonic Solutions (IPS)
- Single Mode
- Collimated Output Beam with FWHM 0.018 nm.
- Maximum output ~100 mW
- Optical Isolator

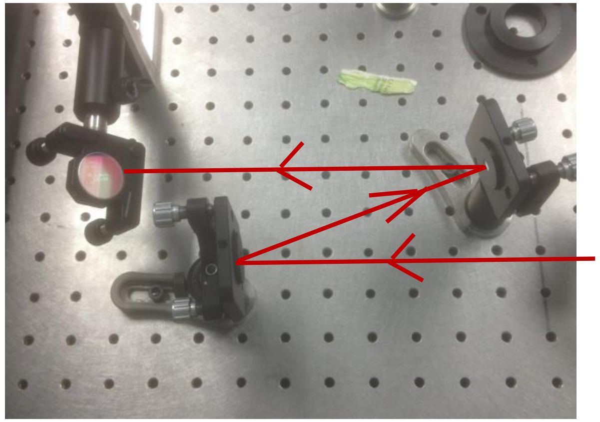
	532 nm	785 nm	1064 nm
Excitation Efficiency	high	medium	low
Fluorescence	high	medium	low
Heat Absorption	low	medium	High

$$P_{scattered} \propto \frac{I_o}{\lambda^4}$$

Volume Bragg Gratings (VBG)

- VBG is a dispersive element for a single wavelength at a single angle.
- Reflects 785 nm and transmits other wavelengths.
- Narrow spectral profile of laser down to less than 5 cm^{-1} or 0.31 nm.
- Cleans intensity profile.





Periscope Mirrors

- Broadband Dielectric Mirror
- 0.5” or 1” mirrors? Raman signal will have a diameter of 0.49” if 10 X is used. 1” Mirrors cost ~$24 more than 0.5”

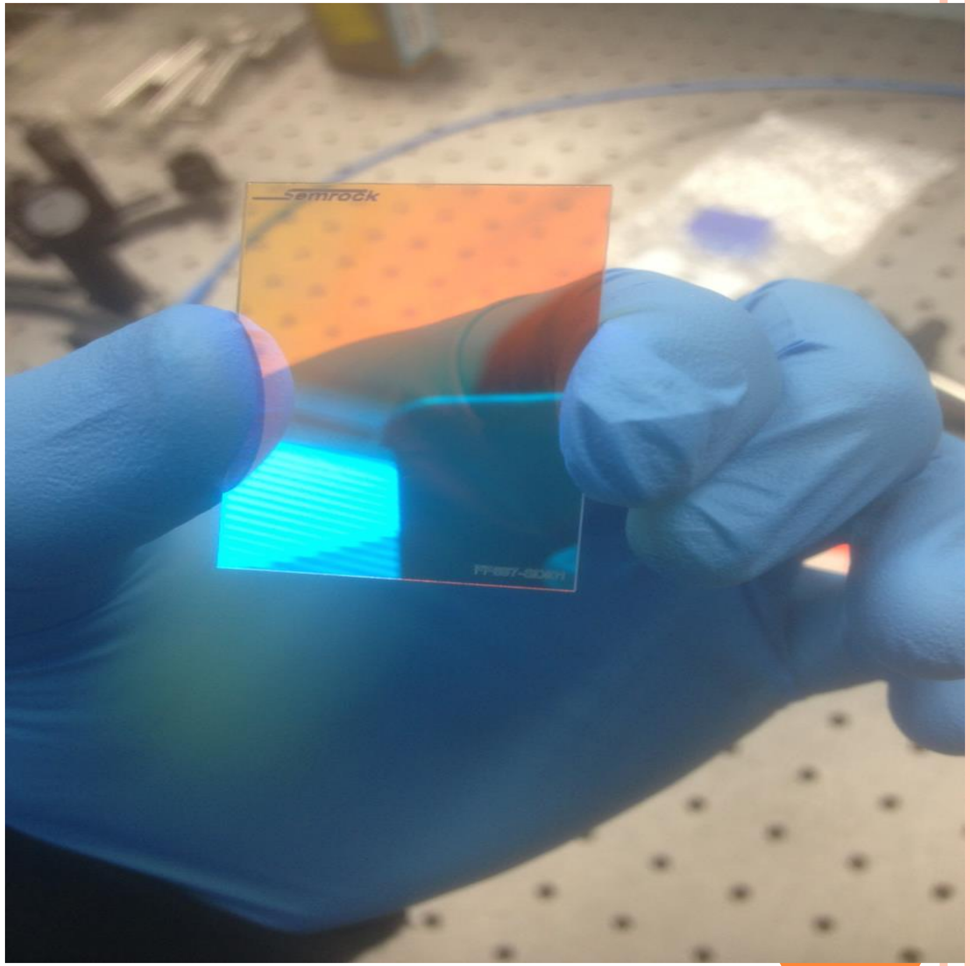
Part	BB1 – E03
Wavelength Range (nm)	(99 %) 750 – 1100
Cost	$75.10

Magnification	4 X	10 X	20 X	40 X
Pupil Diameter (in)	0.27	0.35	0.28	0.24
Raman signal diameter on Mirror (in)	0.38	0.49	0.40	0.34

Semrock Filter

- Single-Edge Short Pass Dichroic Beamsplitter
- Efficient at 45 degrees

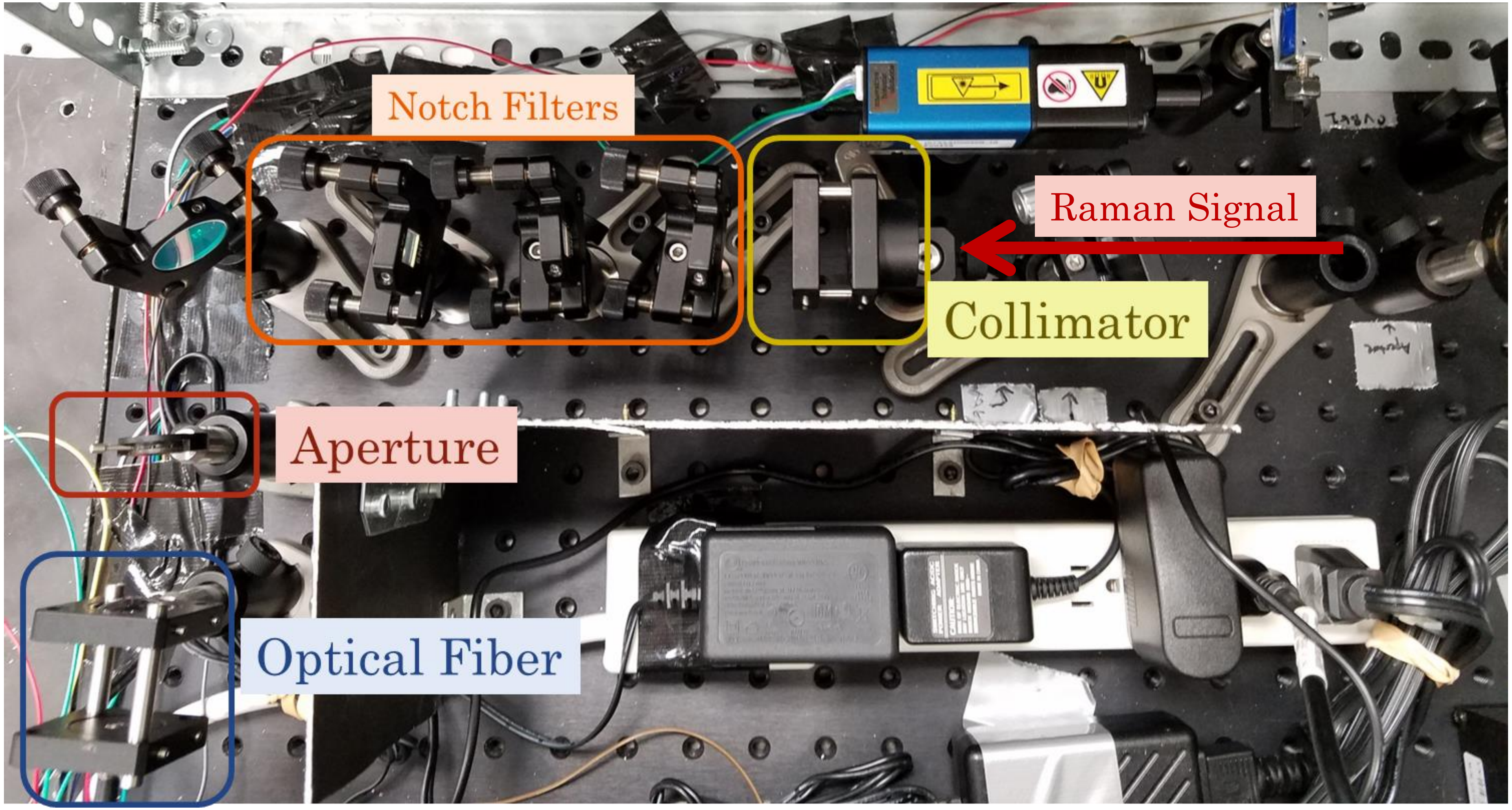
Cost	$ 225	$335	$335
Reflection Band (nm)	(97 %) 705 – 900 nm	(90 %) 750 – 1140 nm	(96 %) 770 – 1100 nm
Tranmission Band (nm)	(93 %) 532 – 690 nm	(90 %) 430 – 700 nm	(93 %) 400 – 730 nm



Filtering

- Correct collimation (Two-lens collimator)
- Reduce laser line (Notch Filters)
- Remove scattering (Iris aperture)
- Pinhole (Optical Fiber)

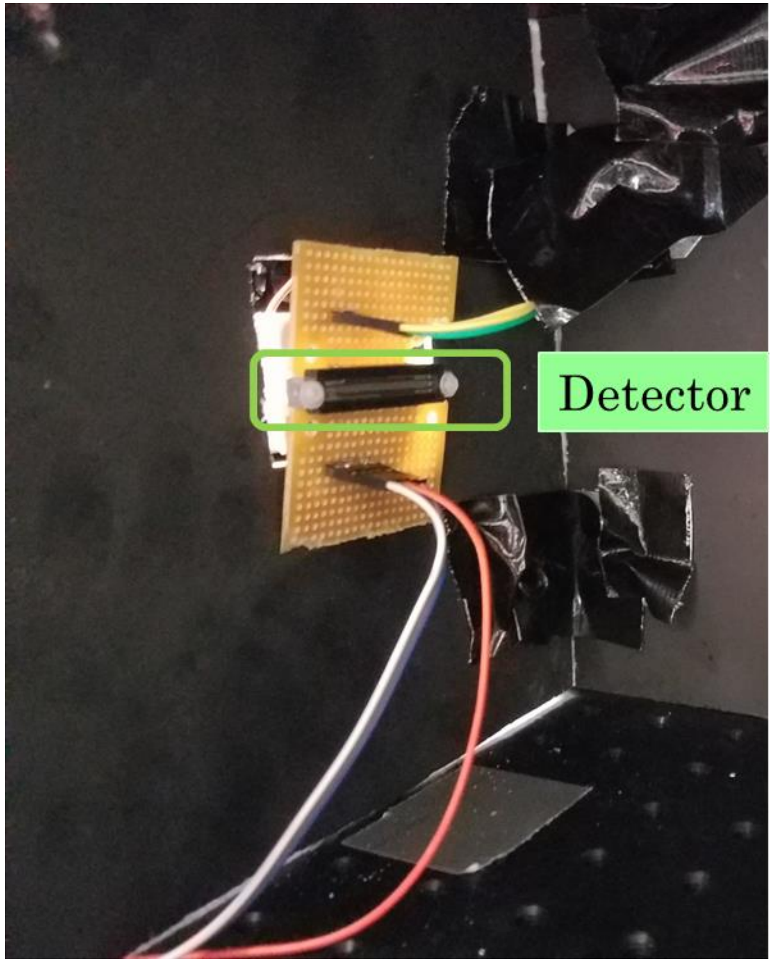
Filtering

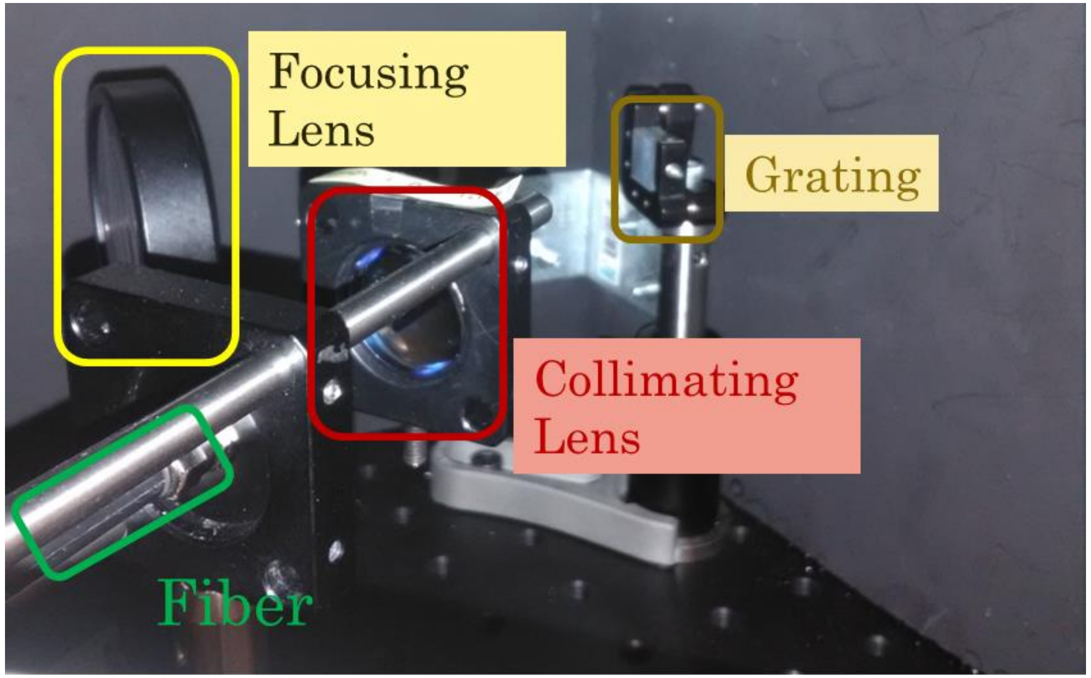

Notch Filters
Raman Signal
Collimator
Aperture
Optical Fiber

Spectrometer

- Design based on Czerny-Turner spectrometer
- Lens (f = 50 mm) collimates light from fiber
- Grating (1200 lines/mm, 12.5x12.5 mm) for dispersion
- Lens (f = 400 mm) focuses light onto detector

Spectrometer





Detector

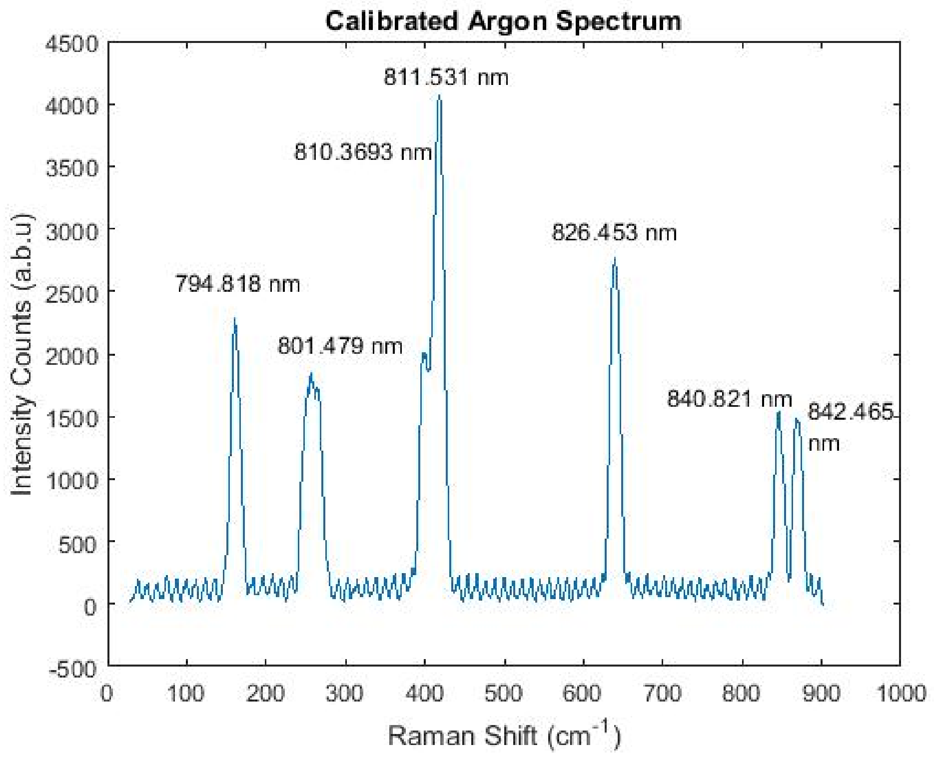
- Collects spectrum
- Each pixel represents a single wavelength

- TCD1304AP
  - Highly sensitive, low dark current linear image sensor
  - 3648 Pixels
  - 8 um x 200um Pixel Size
- Commonly used, cheap, easy to use

CALIBRATION

- Calibration assigns pixel # to a wavelength or wavenumber.
- Argon lamp used for calibration

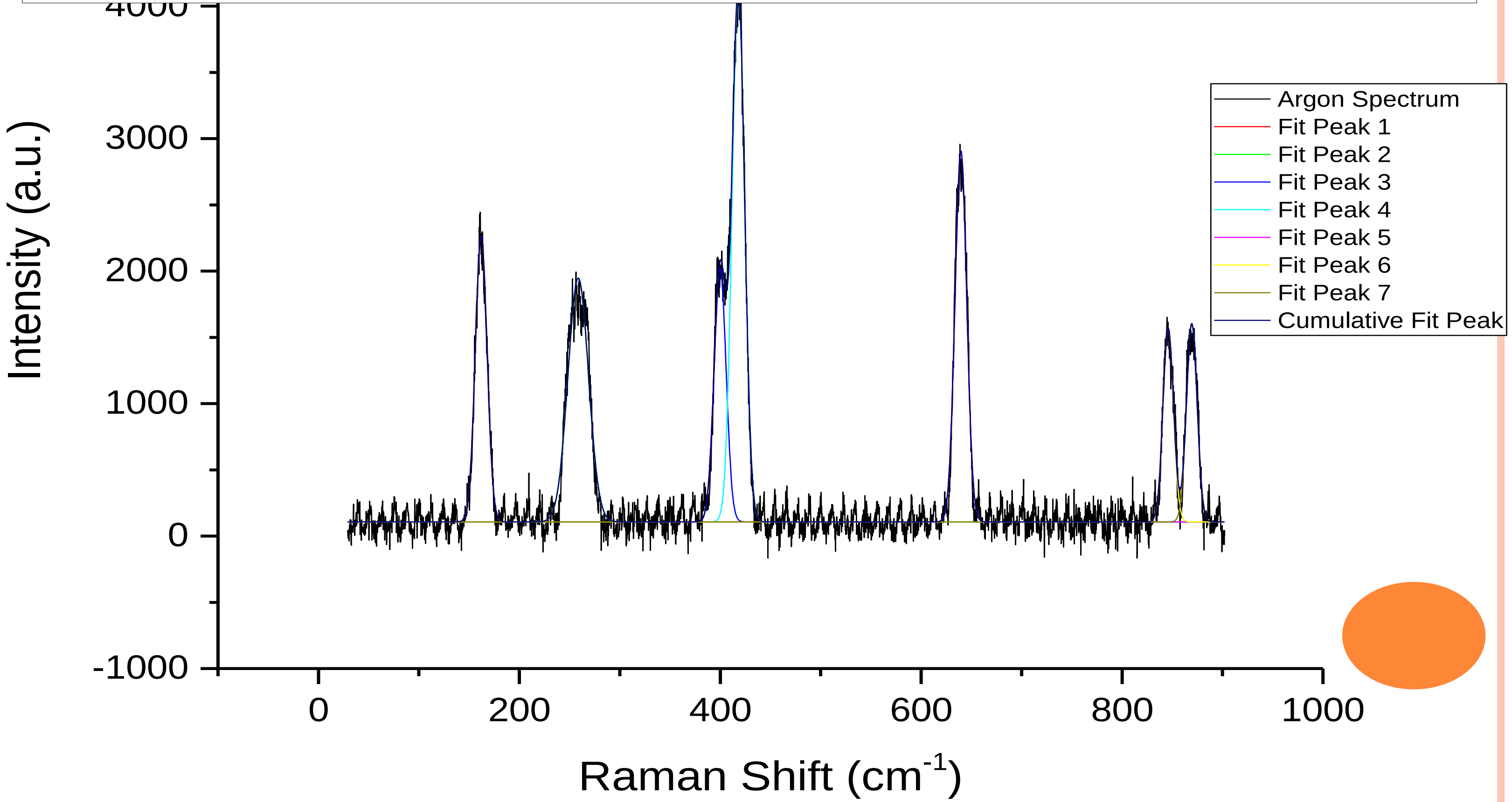
Pixel Number	Wavelength (nm)
154	842.465
256	840.821
1166	826.453
2108	811.531
2187	810.369
2777	801.479
3164	794.818



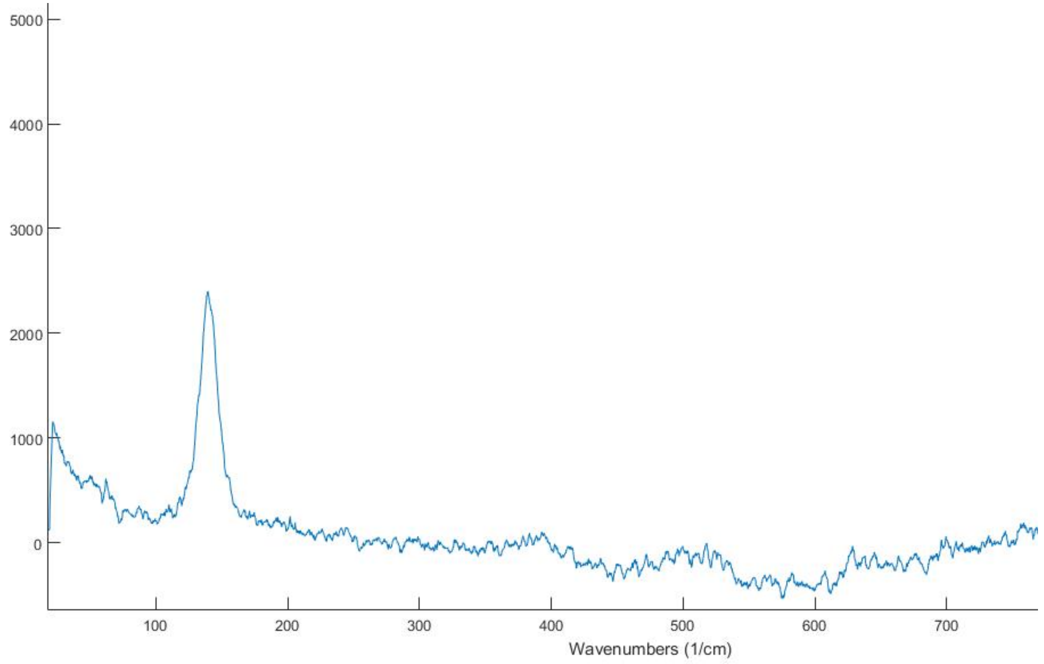
Resolution Calculation

- ~ 10.3 cm-1

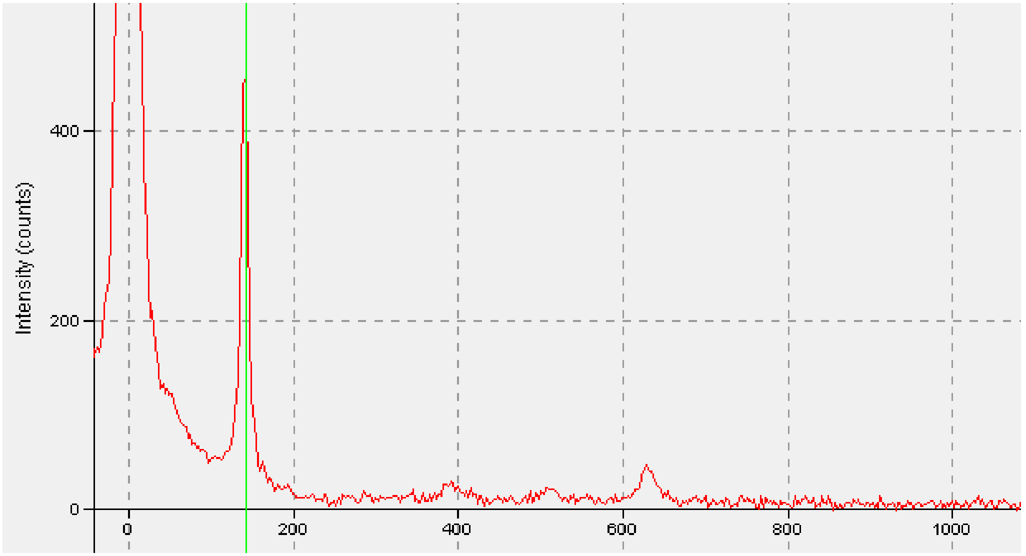
Model	Gauss						
Equation	y=y0 + (A/(w*sqrt(PI/2)))*exp(-2*((x-xc)/w)^2)						
Plot	Peak1(B)	Peak2(B)	Peak3(B)	Peak4(B)	Peak5(B)	Peak6(B)	Peak7(B)
y0	107.35017 ± 1.89806	107.35017 ± 1.89806	107.35017 ± 1.89806	107.35017 ± 1.89806	107.35017 ± 1.89806	107.35017 ± 1.89806	107.35017 ± 1.8980
xc	162.0012 ± 0.0591	258.61762 ± 0.09109	399.84318 ± 0.10894	418.01533 ± 0.05325	639.54873 ± 0.04392	846.00722 ± 0.07867	869.42043 ± 0.0773
w	11.6083 ± 0.11908	20.29634 ± 0.18457	11.84137 ± 0.20711	12.49648 ± 0.10336	11.65547 ± 0.08854	10.35455 ± 0.16135	10.52721 ± 0.1587
A	31447.96048 ± 283.44	46811.7107 ± 378.02	28381.90823 ± 485.96	62995.01762 ± 492.96	40961.97879 ± 273.73	19007.59945 ± 256.50	19778.3108 ± 258.2
Reduced Chi-S	10017.45253						
R-Square(COD	0.97839						
Adj. R-Square	0.97827						



RESULTS – EXCEDRIN TABLET

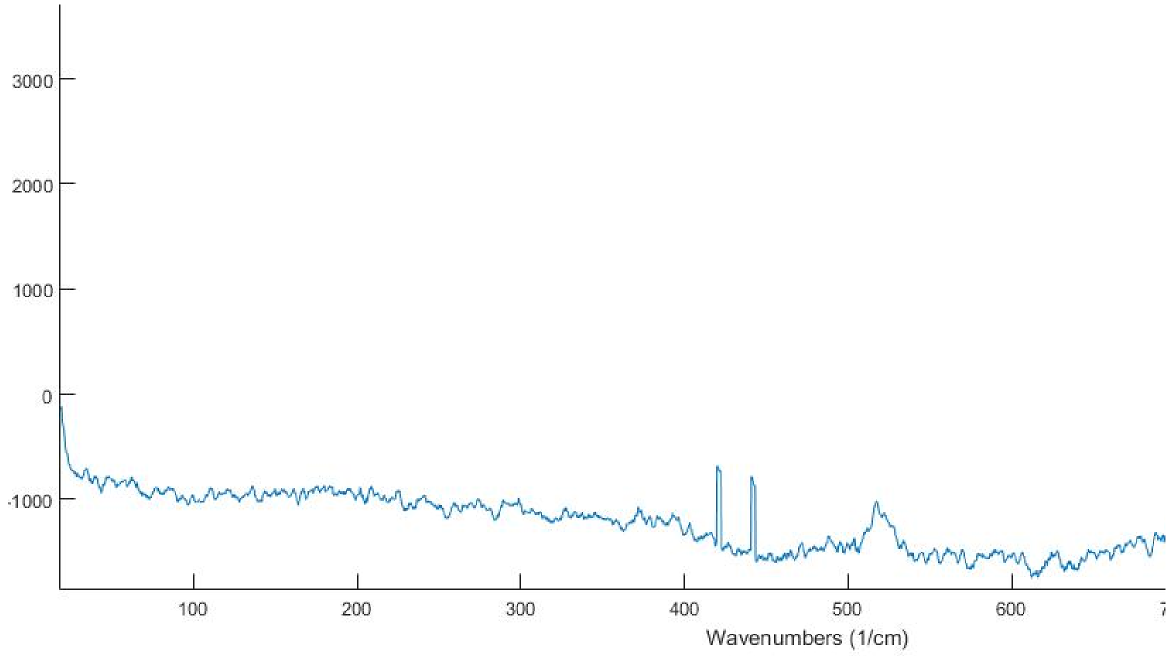


Our Spectrometer

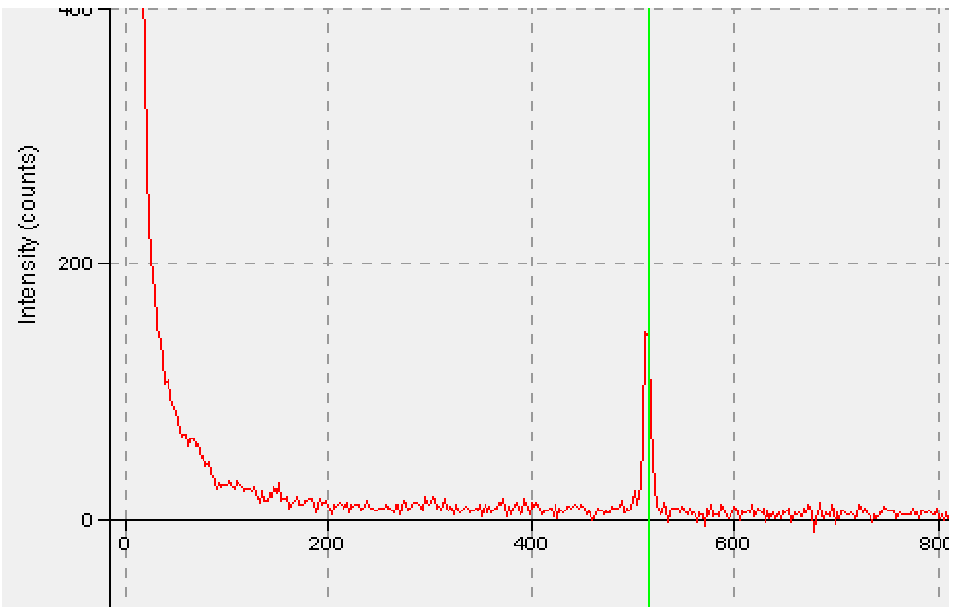


Ocean Optics Spectrometer

RESULTS – SILICON



Our Spectrometer



Ocean Optics Spectrometer

Electrical Design Specifications

- Temperature Monitoring
- Laser blocking
- Backlight Control
- Laser Power Control
- Obtain spectrum with CCD

Electrical Design Specifications

Electrical System 1

- Temperature Monitoring
- Laser blocking
- Backlight Control
- Laser Power Control

Electrical System 2

- Obtain spectrum with CCD
- CCD Cooling to reduce noise

Microcontrollers

Electrical System 1 - Atmega328P

- 5V System Voltage
- 16 MHz
- 23 Programmable I/O
- 6 Pulse Width Modulation

- Easier to Solder (28 pin DIP)
- Cheap - $2.21
- Easy to use

Electrical System 2 – Atmega1284P

- 5V System Voltage
- 16 MHz
- 32 Programmable I/O
- 6 Pulse Width Modulation

- Easier to Solder (44 pin DIP)
- Cheap - $5.50
- Needs Arduino Flash

Other Considerations – Atmega2560

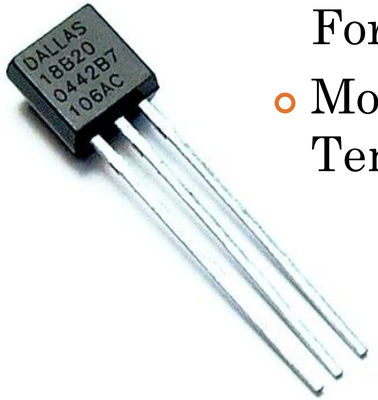
- 5V System Voltage
- 16 MHz
- 32 Programmable I/O
- 6 Pulse Width Modulation

- Hard to Solder (100 pin TQFP)
- Cheap - $2.31
- Easy to use

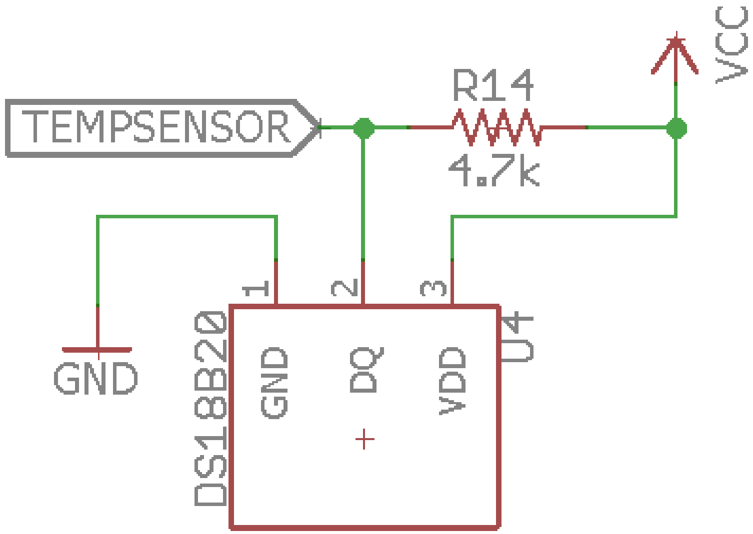
Temperature Monitoring

- Constantly Monitor temperature with Automatic Cooling
- Parts Used:
  - Temperature Sensor – Digital
  - Fans

- DS18B20 - Digital Temperature Sensor
  - Uses “One – Wire” Communication For multiple sensors on a single bus
  - More Accurate than Analog Temperature Sensors



- Circuit Diagram

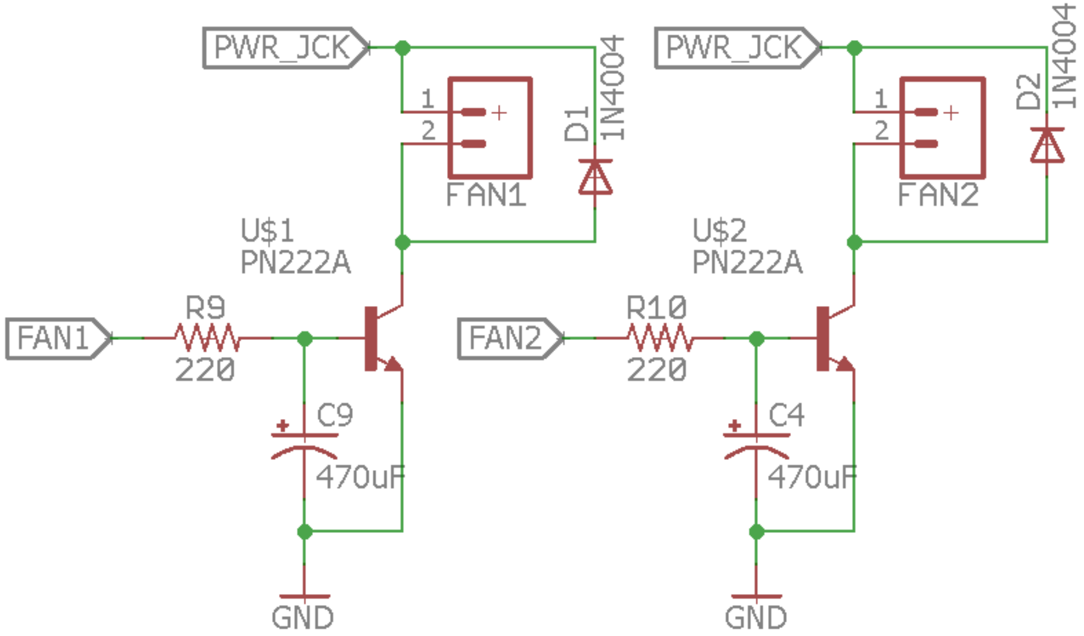


Temperature Monitoring

- Provides air flow to the system
- 1 Intake fan and 1 Outtake fan
- Multicomp MC36031
  - 5V
  - 115mA
  - 600 mW
  - Pushes 3cu.ft/min

- Pulse Width Modulation for variable fan speed (0V = 0 PWM and 5V = 255 PWM)

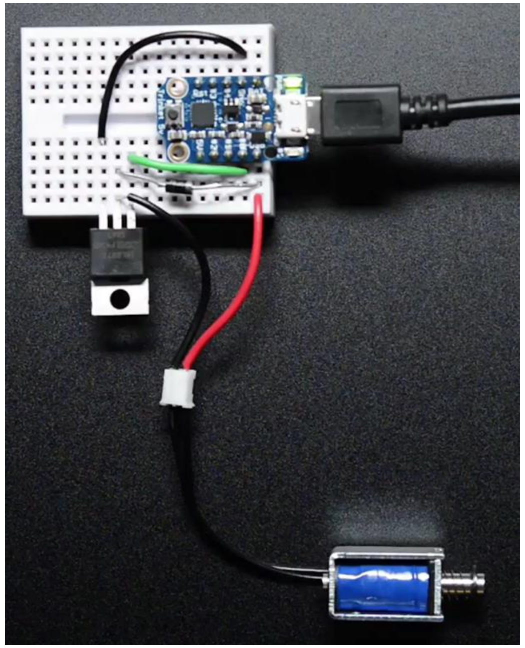
Duty Cycle	Voltage	Temperature Range	PWM Value	Fan Speed
0	23mV	Less than 70 F °	0	0
30%	.778 V	70 F °	72	30%
40%	1.123 V	74 F °	102	40%
60%	2.553 V	78 F °	153	60%
80%	3.753V	82 F °	204	80%
100%	4.42 V	86 F °	255	100%

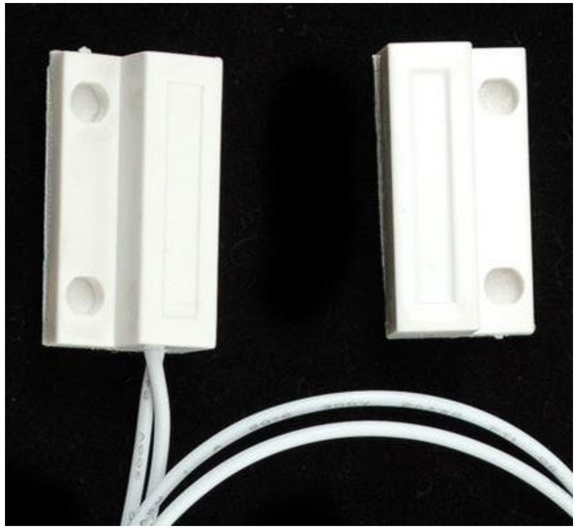


Laser Blocking

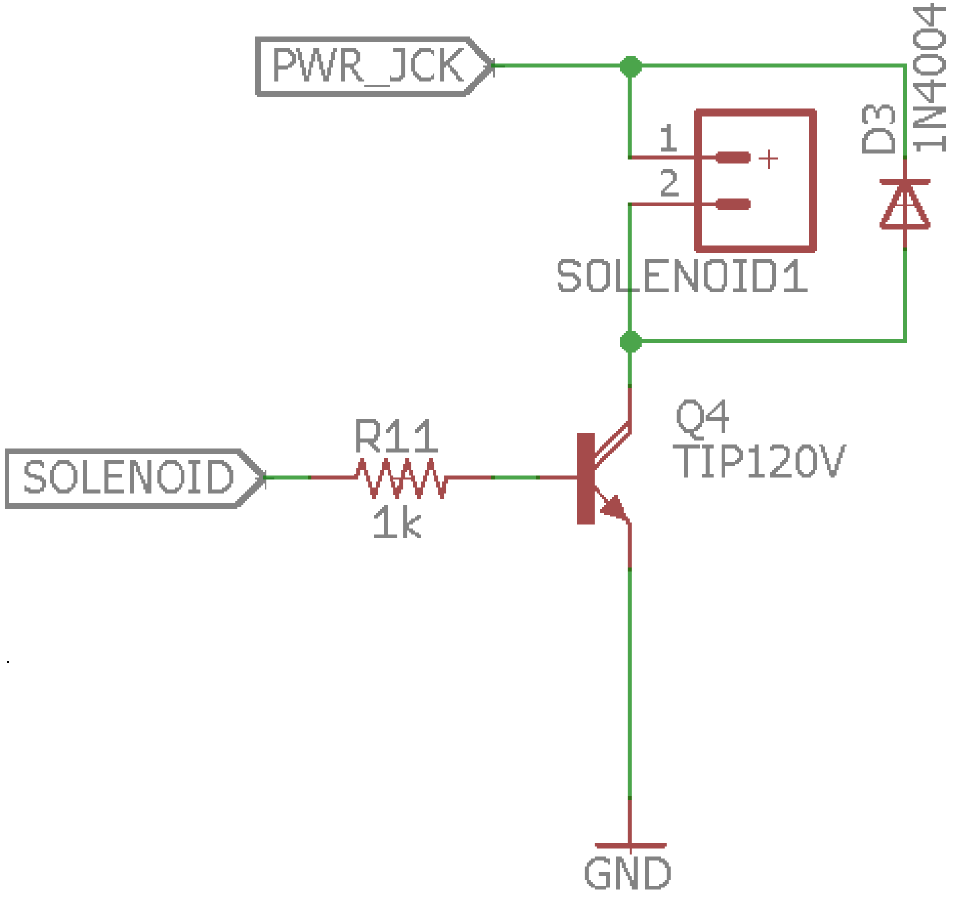
- Needed to block the laser while a sample was being loaded on to the sampling stage
- Must be quick

- Mini Push-Pull 5V Solenoid
  - Faster than a motor for our application
  - Small and cheap - $4.95





- Circuit Diagram

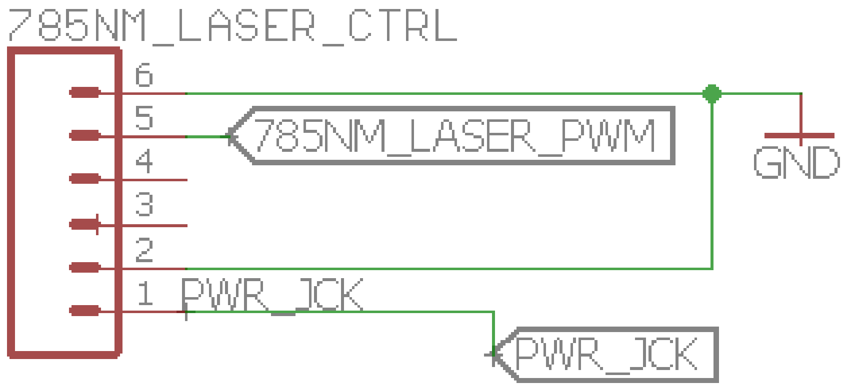


Laser Power Control

- Display Laser Power (Software) & Control Laser Power.
- Laser uses 100mW and runs on a 5V source.
- Pulse Width Modulation to modulate the power.

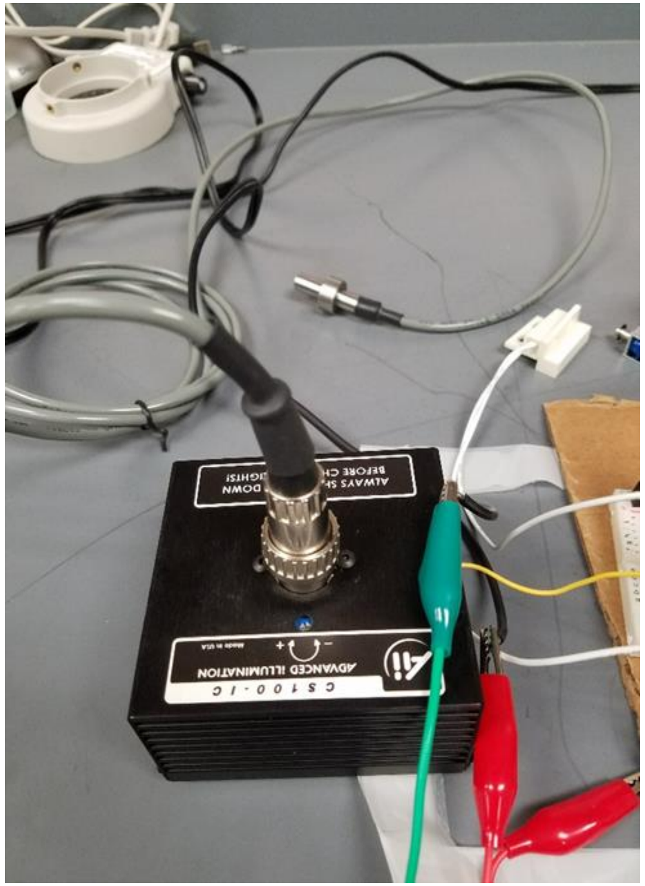
Duty Cycle	Voltage	Power Prior to Calibration	PWM Value
10%	.5	10 mW	26
20%	1	20 mW	51
30%	1.5	30 mW	77
40%	2	40 mW	102
50	2.5	50 mW	128
60	3	60 mW	153
70	3.5	70 mW	179
80	4	80 mW	204
90	4.5	90 mW	230
100%	5 V	100 mW	255

- Circuit Diagram

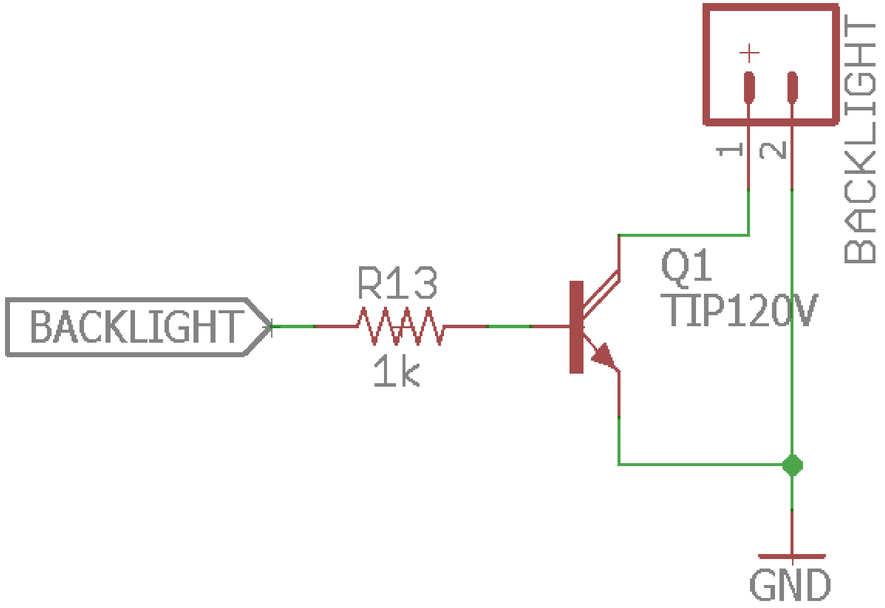


Backlight Control

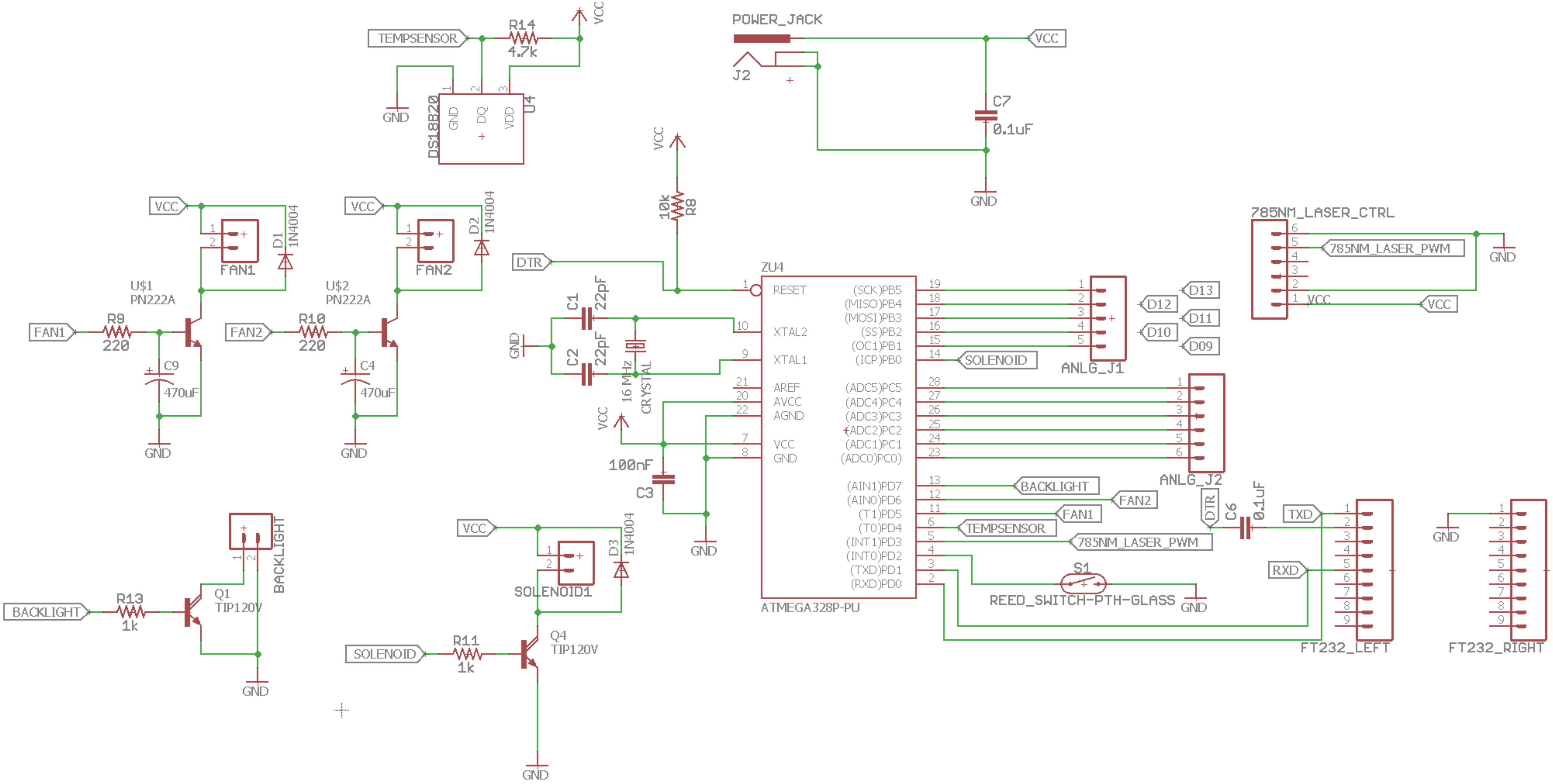
- Control Microscope backlight
  - Allows for user to toggle the backlight on/off



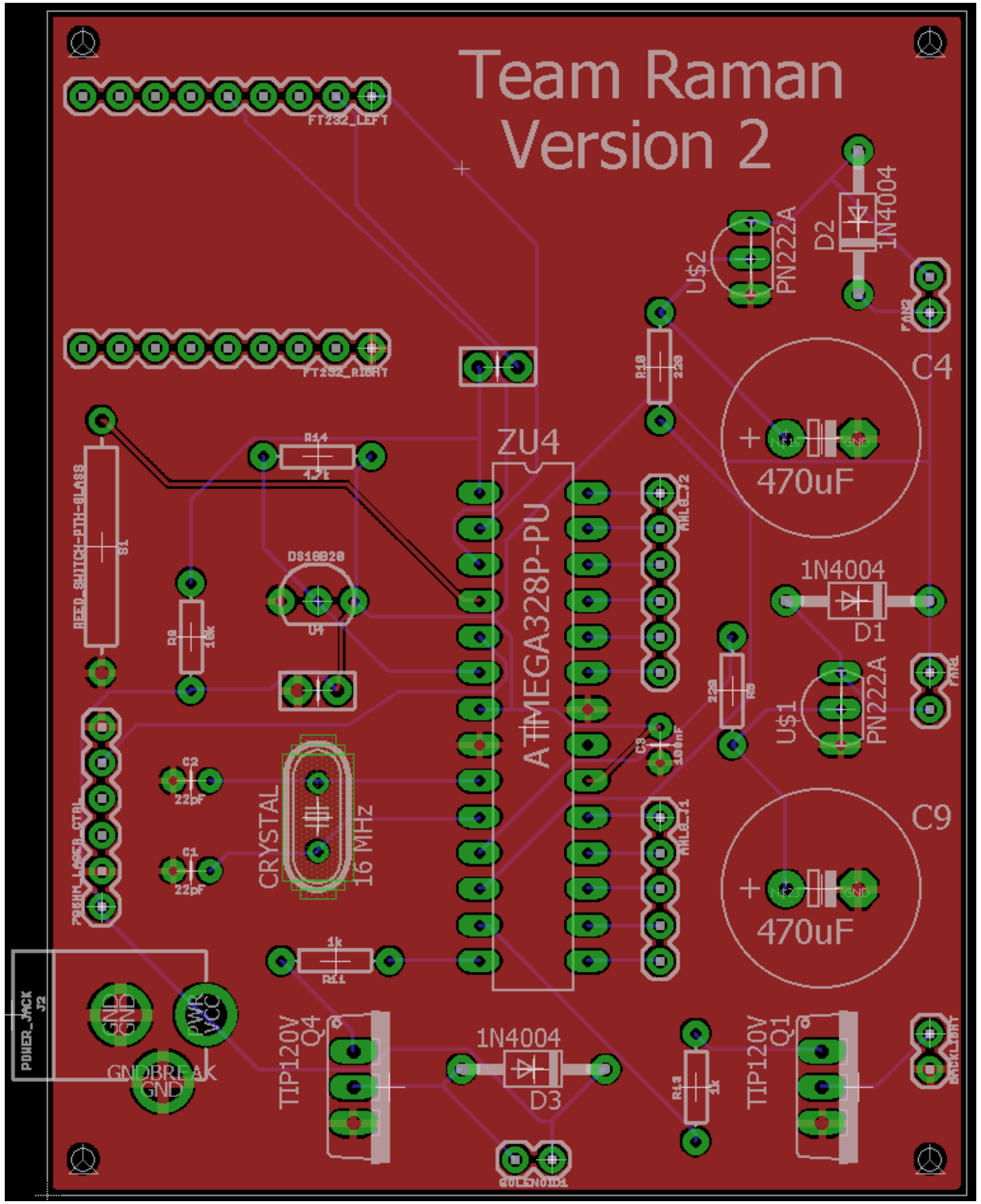
- Backlight Specifications:
  - 24V
  - 1.5A
  - Runs on separate power supply
  - Optional - Plan to integrate power supply into the system



Electronic System 1 - Circuit



Electronic System 1 - PCB



Spectrometer CCD Circuit

- Converts the intensity of light to an associated voltage
- This is done by "shifting" signals between stages

8 Bit CCD Circuit

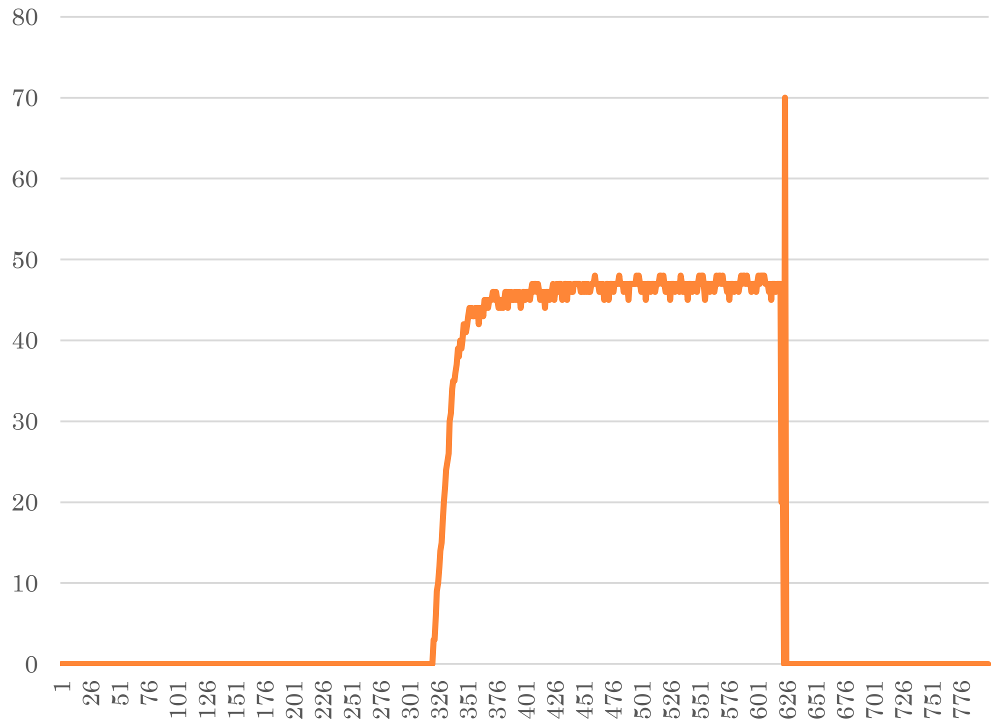
- Only allows for 256 different values.
- Reads 800 pixels continuously, not each single pixel.
- Doesn't capture milliVolt changes
- Easy Circuit
- For 1 Least significant bit = 4.8mV
- Did not work for our system

16-Bit CCD Circuit

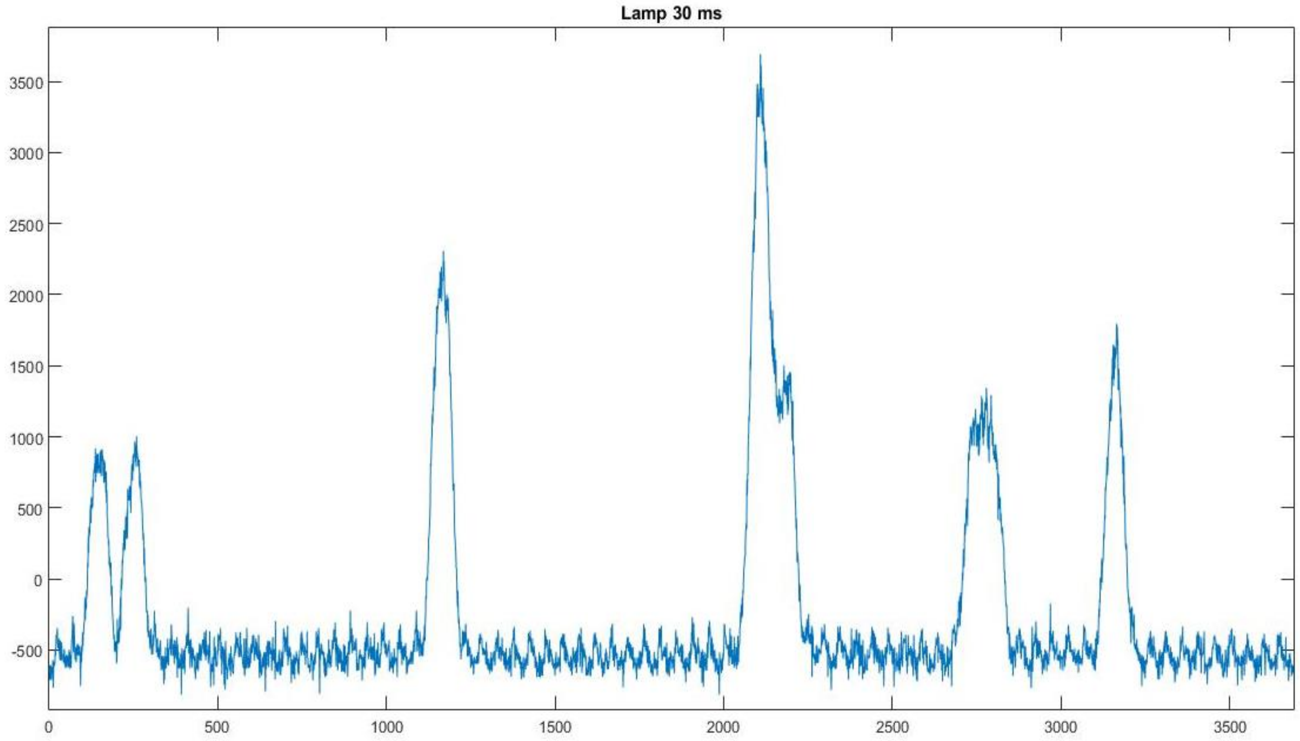
- Allows for 65,535 different values.
- Reads all 3648 pixels.
- Captures microVolt changes
- More difficult to implement
- For 1 least significant bit = 38uV
- Used in our final system

Spectrum Comparison

8 Bit Spectrum



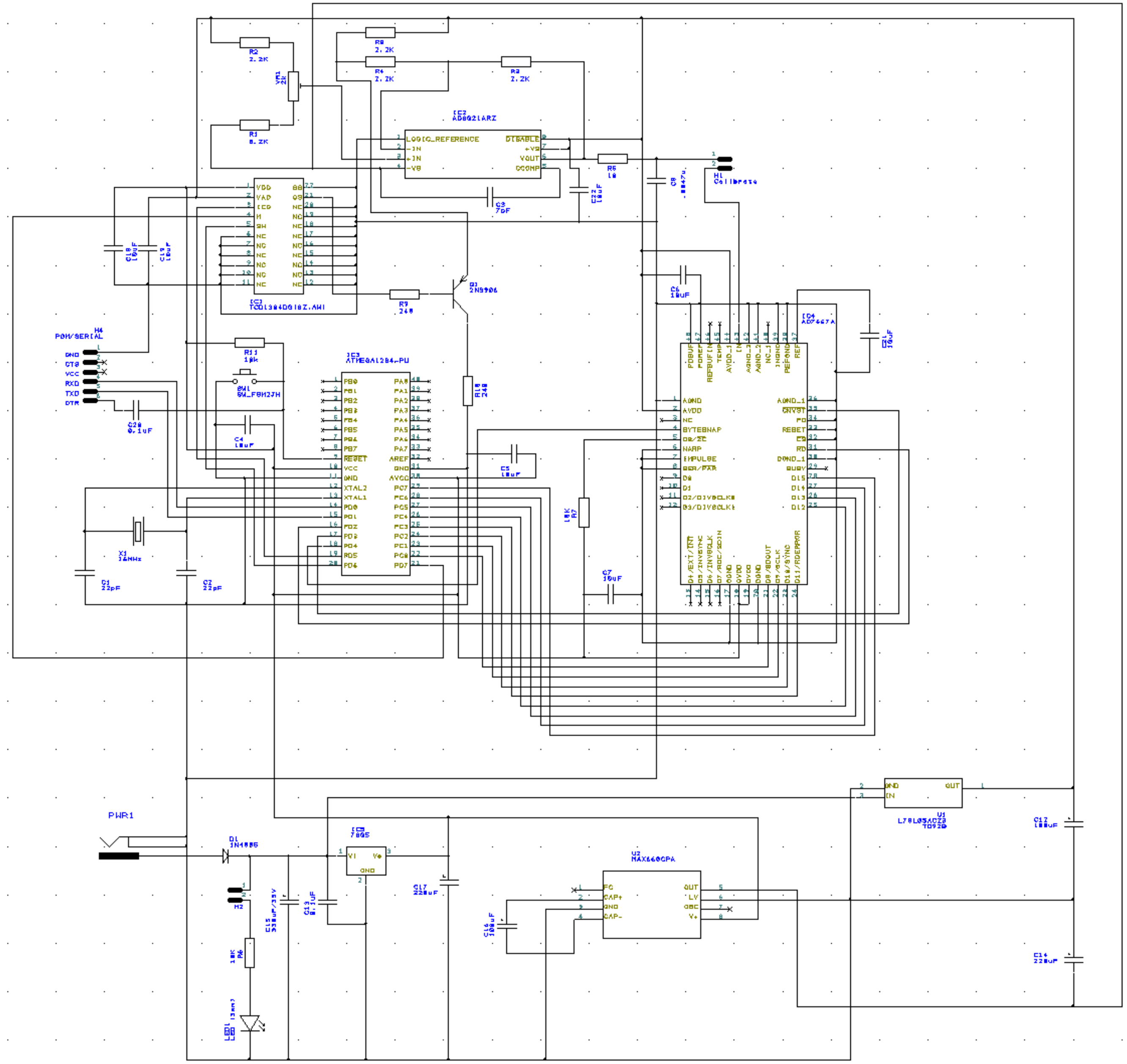
16-Bit Spectrum



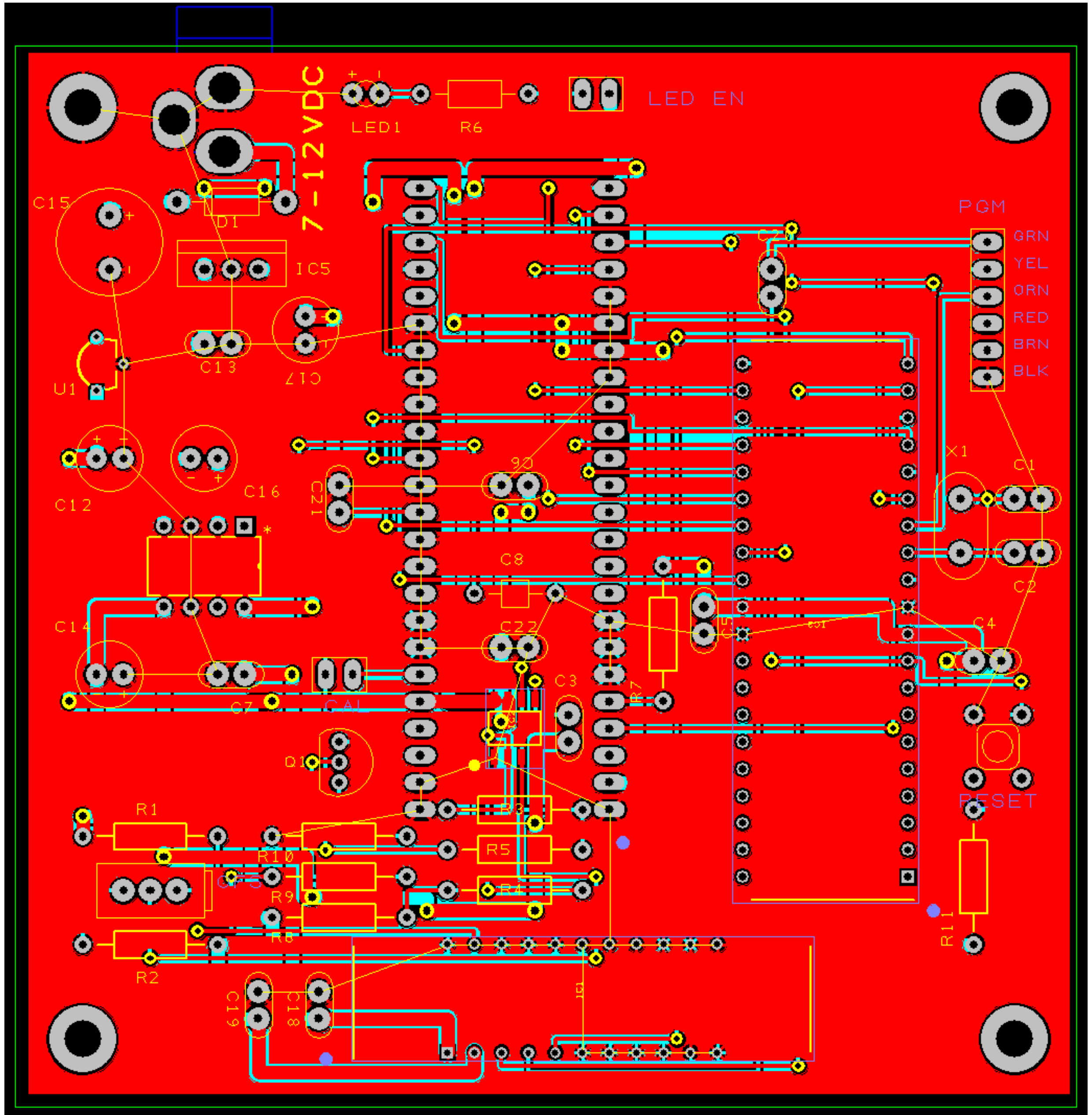
Spectrometer CCD Circuit

- The Atmega1284 has to generate clocks to drive the CCD and ADC.
  - The Master Clock, the Shift Gate, and the Integration Clear Gate.
  - The CNVST, RD, and the BYTESWAP control lines on the ADC.
- To generate the pulses, the ATmega1284 timers were used.
- An AD7667 16-bit 1 MSPS converter was used which can digitize a frame in 16ms
- The sensitivity of the 16 bit converter is 2.5V / 65536 = 38µV

Electronic System 2 - Schematic

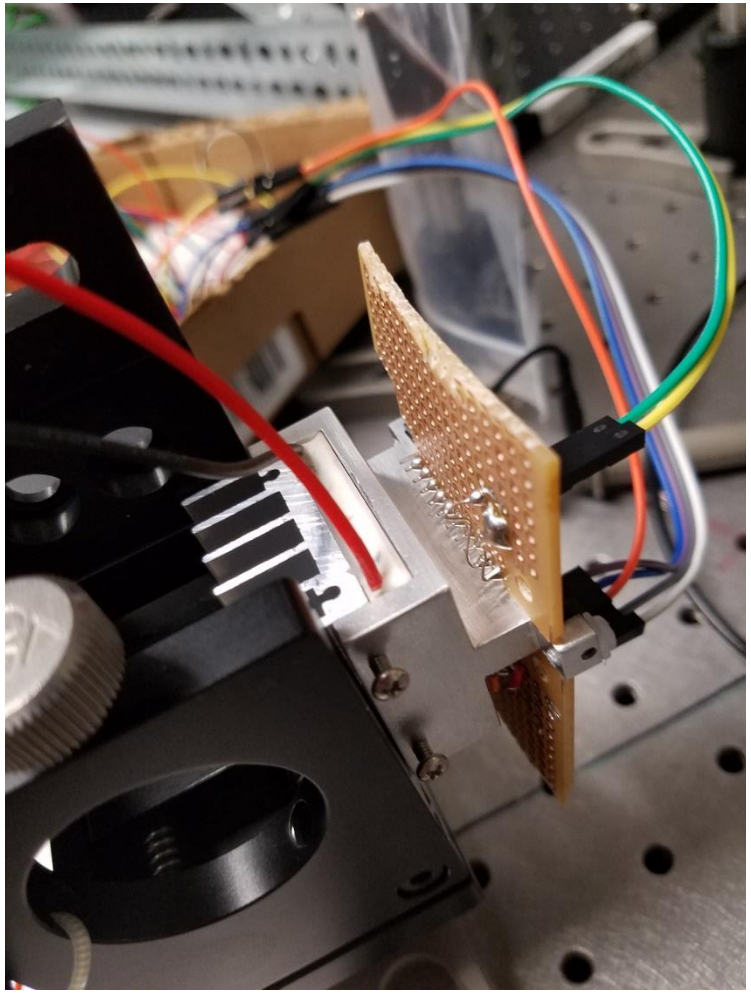


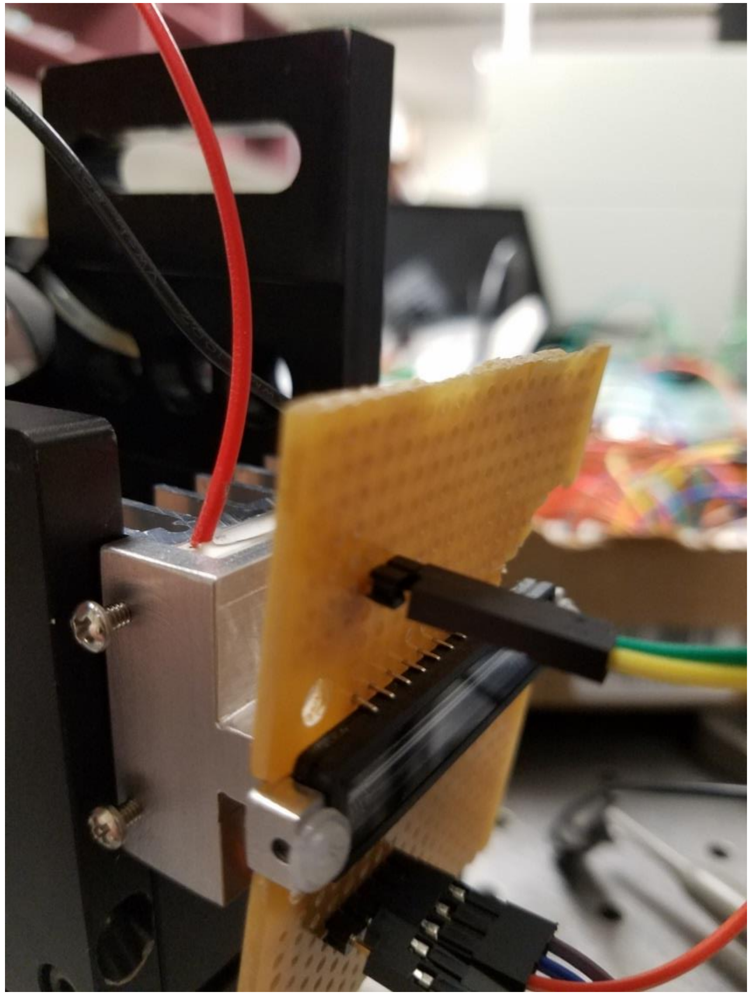
Electronic System 2 - PCB



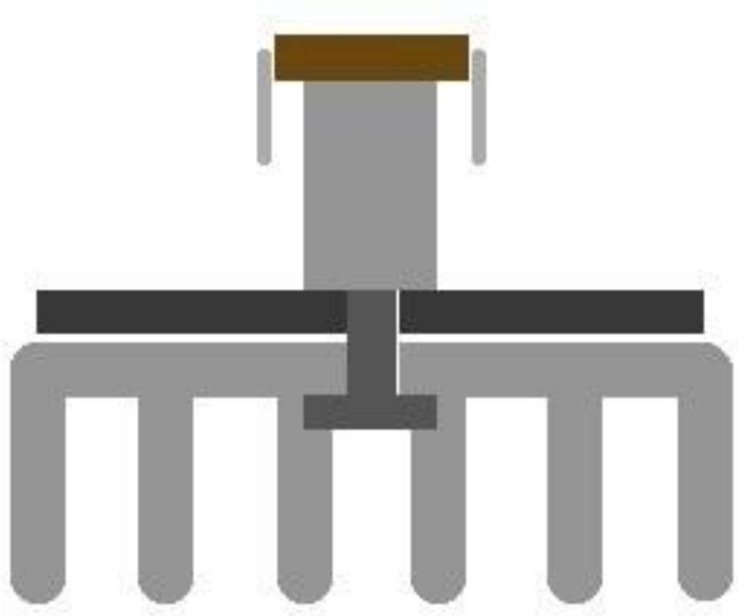
CCD Cooling

- A TEC was built because thermal noise was too much when looking at low voltage signals.
- 5V, 1.5A TEC plate was used while a fan and a heatsink blow out heat.

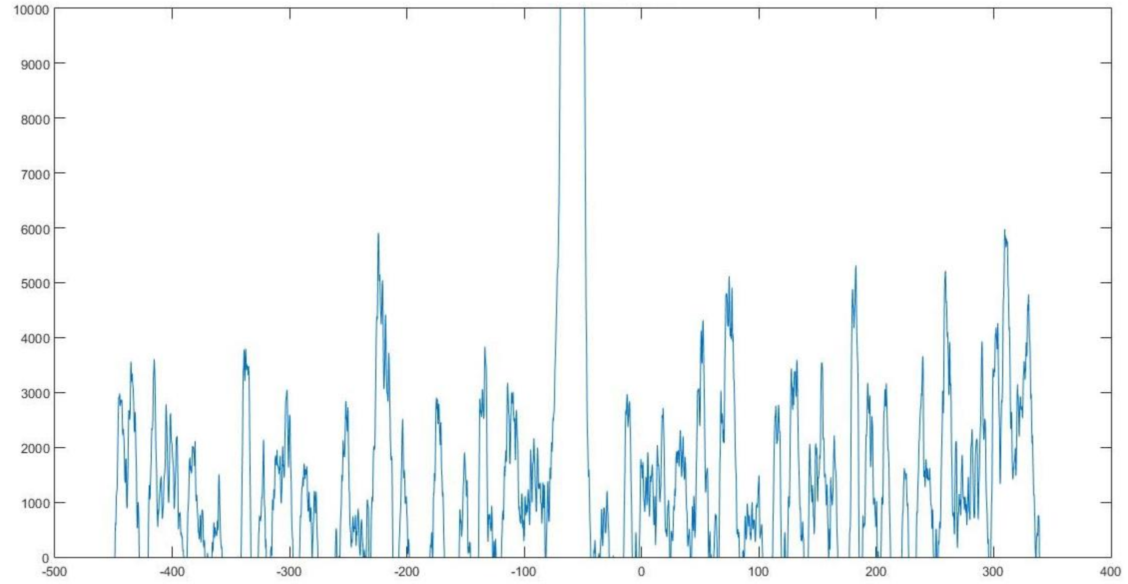




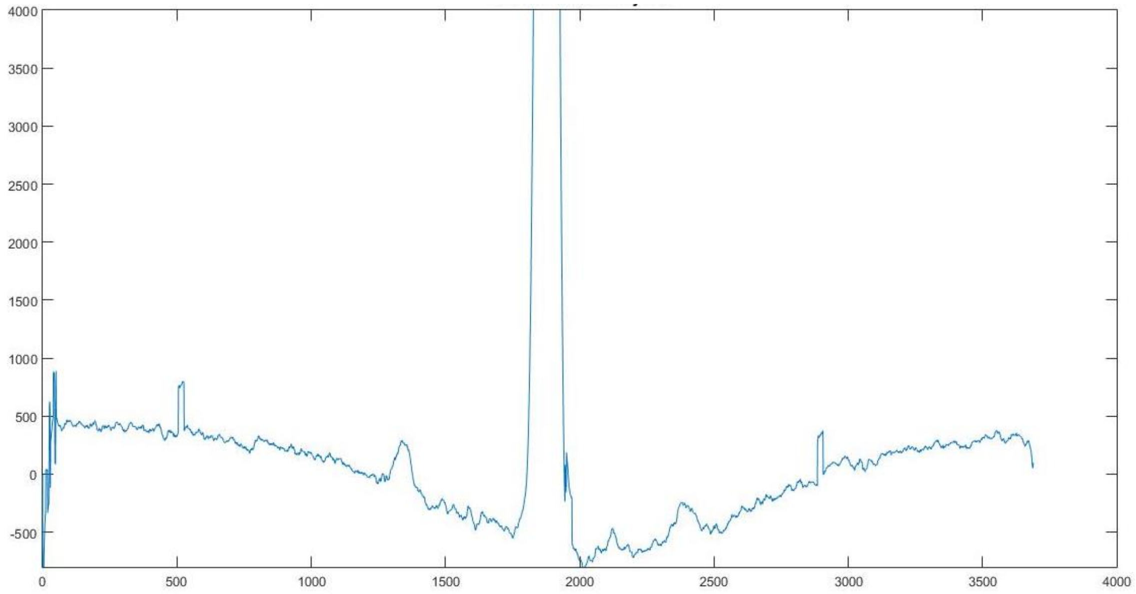
- CCD Cooling design



Spectrum Comparison using the TEC

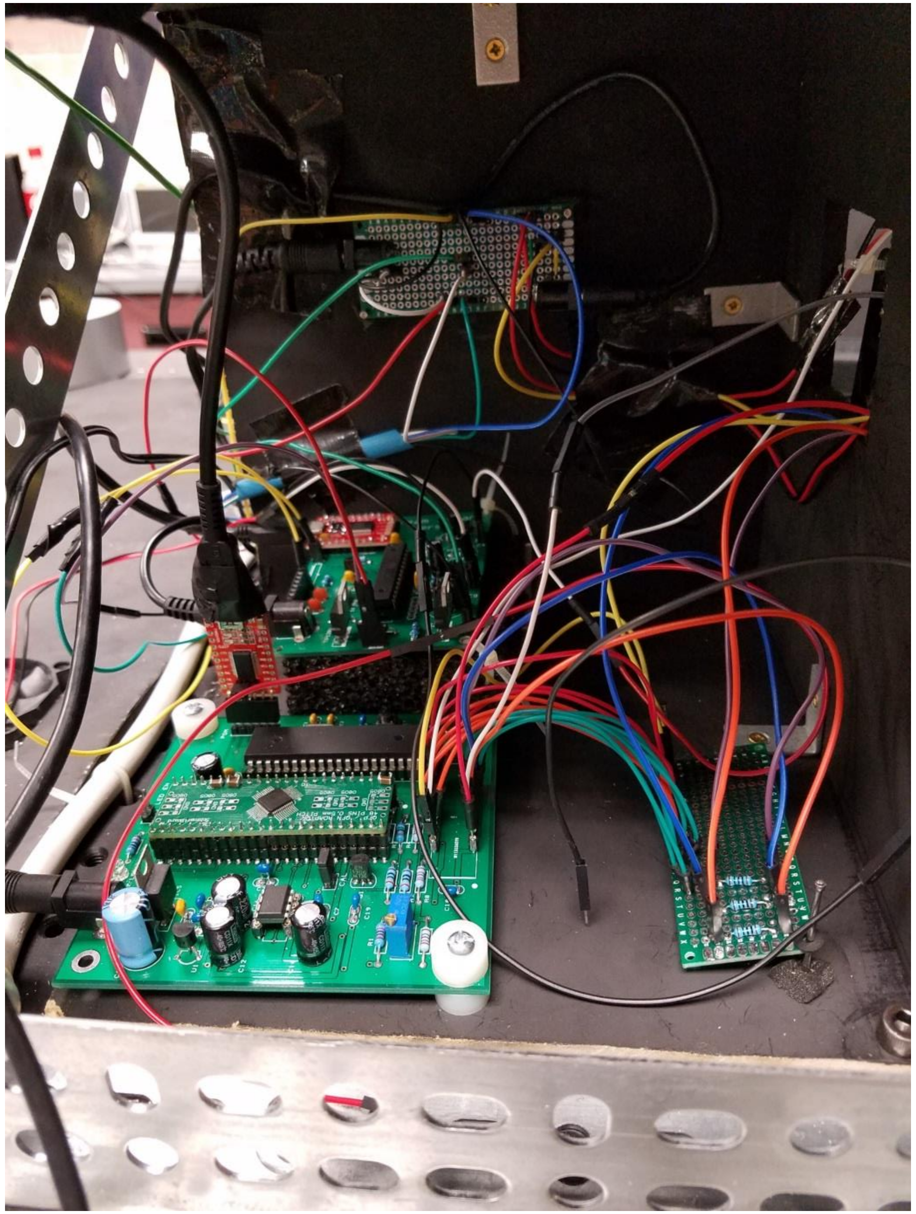


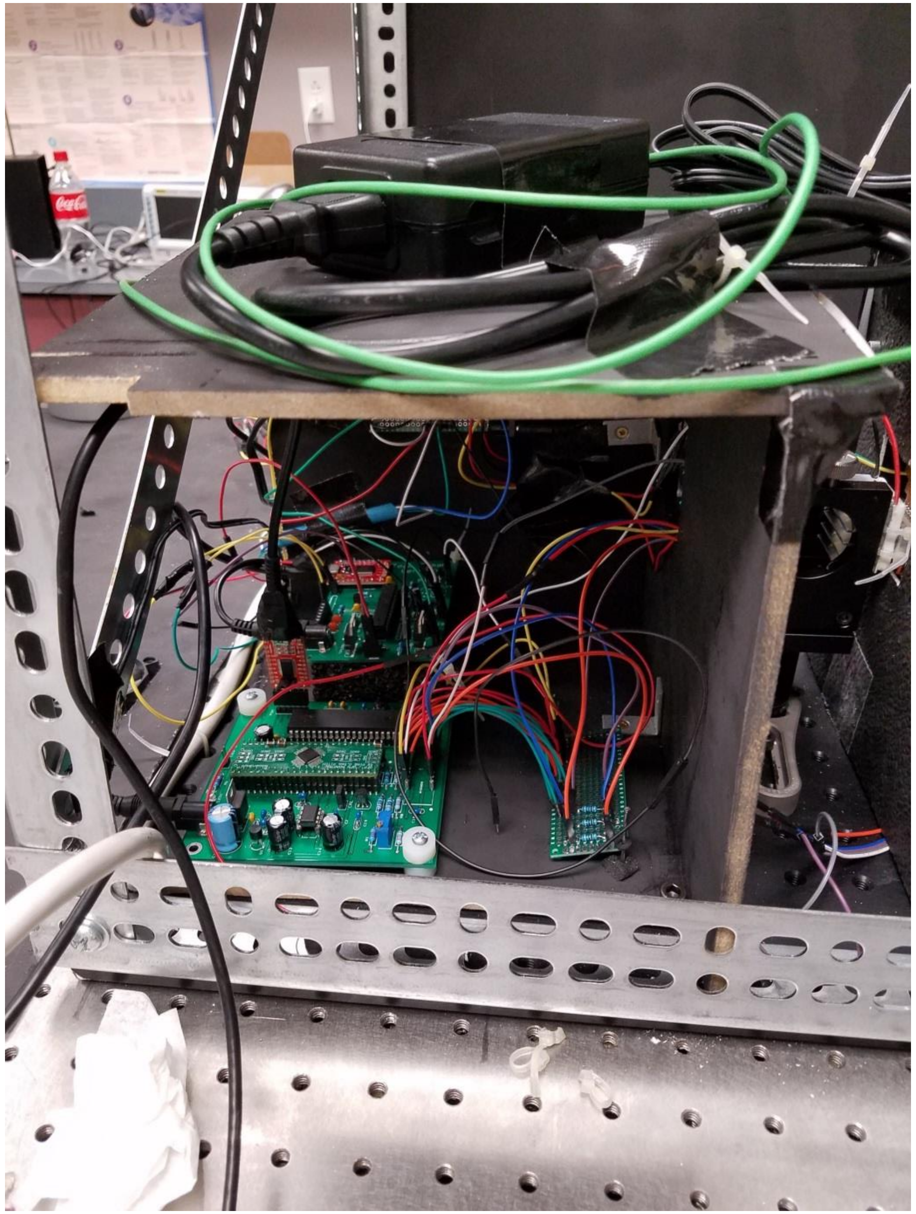
- Spectrum before cooling.
- Noise at its highest is at 6000 analog to digital unit.



- Spectrum after cooling.
- Noise at its highest is at 800 analog to digital unit

Electrical Systems enclosed





Graphical User Interface

- MATLAB
- Used to Control
  - Laser Power
  - Fan Speeds
  - Temperature
  - Door Sensor
  - Graph Wavenumber vs Intensity
  - Camera

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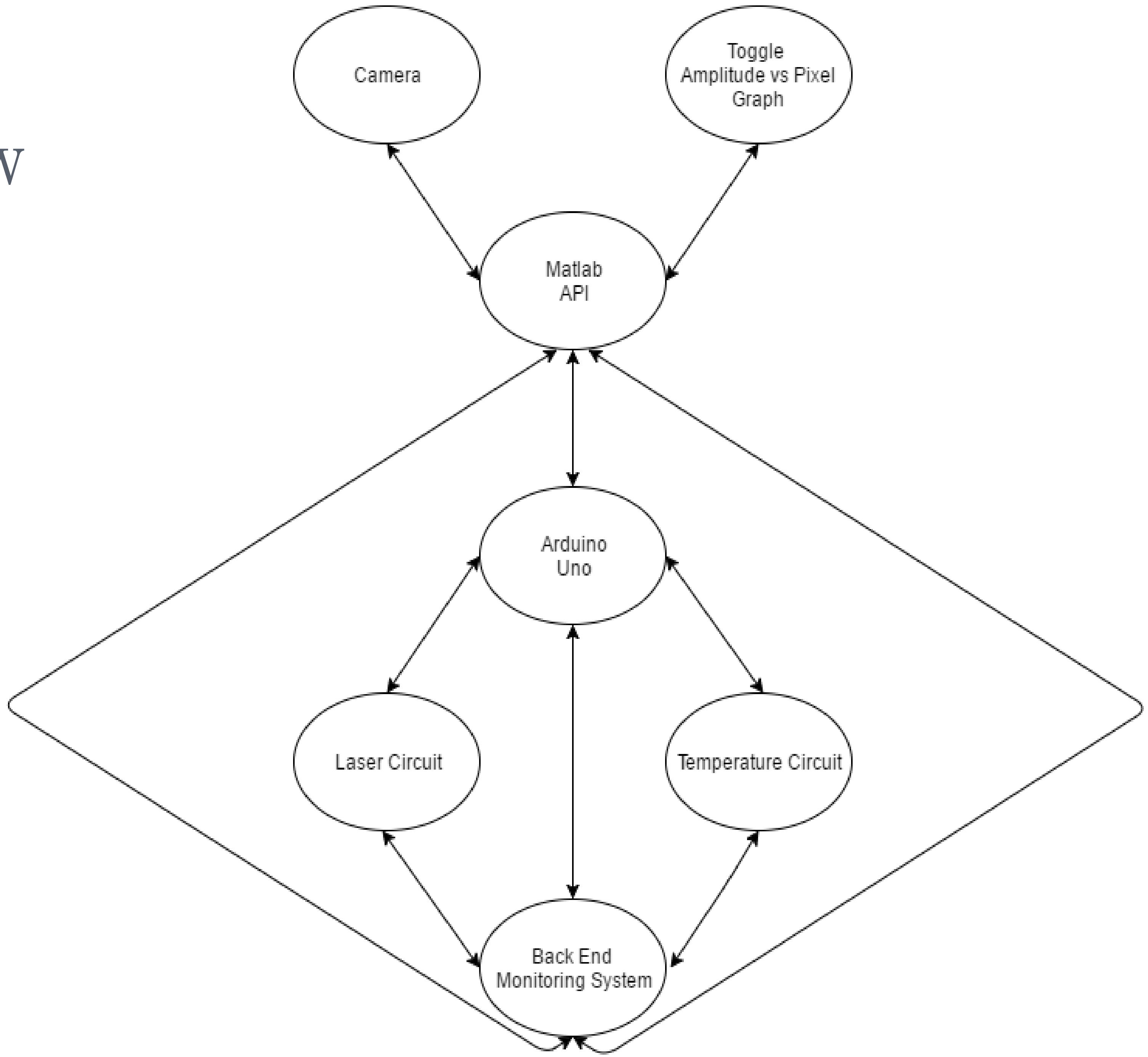
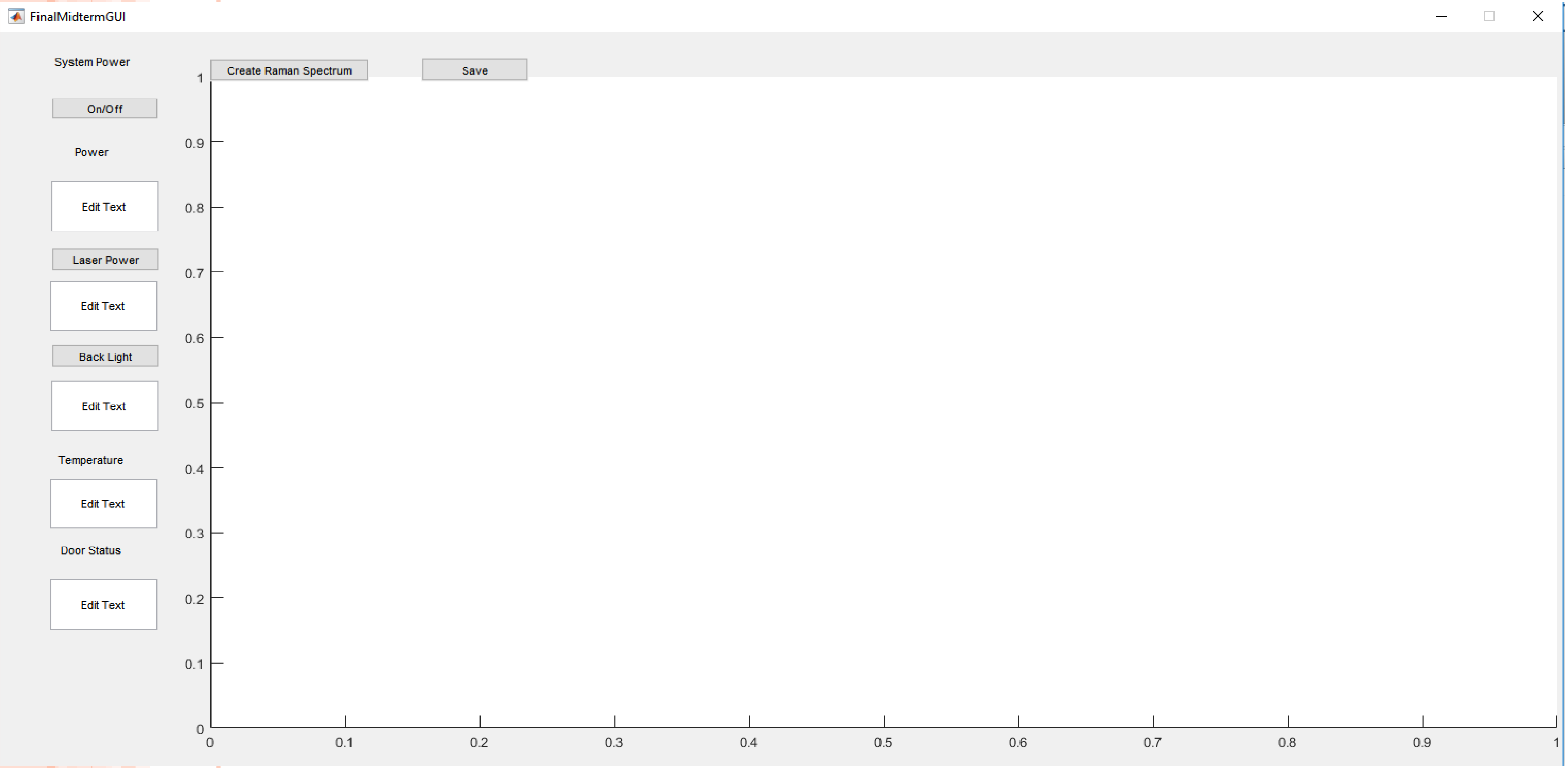
GUI Flow


Camera
Toggle Amplitude vs Pixel Graph
Matlab API
Arduino Uno
Laser Circuit
Temperature Circuit
Back End Monitoring System

Gui Picture



FinalMidtermGUI
System Power
On/Off
Power
Edit Text
Laser Power
Edit Text
Back Light
Edit Text
Temperature
Edit Text
Door Status
Edit Text
Create Raman Spectrum
Save
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

Issues

- Optical Issues:
  - Resolution specification not met
  - Detector is noisy
  - Ambient light causing issues

- Electrical Issues
  - Sometimes the Electrical system 1 – Arduino runs “hot” and may “lose connection to the host PC”
  - Solenoid gets very warm when blocking laser
  - CCD cooling system generates dew on the CCD.

- Software
  - High serial throughput for a single MATlab interface.
  - The camera the professor wants to use does not connect with MATlab. Therefore we used the a separate software for the camera connection.

Budget

Electronics Parts	Price
Arduino Uno	24.95
5V 2A Power Supply	7.95
Magnetic Contact Switch	3.95
DS18B20	3.95
Solenoid	4.95
TO-220 Heatsink	0.75
Diode Kit	5.99
Transistor Kit	20
Capacitor kit	20
Resistor Kit	10.99
5V DC Fan	7.99
TCD1304	3.5
LM324 Op Amp	0.58
break-away pin stip male	4.95
FT232RL	14.95
PCB-Team Raman	$33
PCB -Electrical System 2	$33
Atmega1284	$6
Max660 Charge Pump	$10
Schmartboard	$6
ad7667	$22
LM7805	$1
L78L05 Linear Regulator	$1
AD8021	$3
Total	$250.48

Optics Parts	Price
Grating	64.40
Grating Mount	65.90
Focusing Lens	40.50
Focusing Lens Mount	25.25
1st Lens	41.21
1st Lens Mount	16.00
2nd Lens	33.10
2nd Lens Mount	34.70
Cage 4pack	26.37
2" 5-pack Post Holders	38.50
2" 5-pack Posts	23.36
Notch Filter Mounts	38.70
Mount for Detector	59.20
Kinetic Mirror Mount with a 1" BB1-E03 Mirror	103.50
Semrock Beamsplitter	255.00
Total	$ 865.69

Questions?