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Miniature Fluorescent Microscope

Final Report

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1. Abstract

Microscopes are essential for understanding the structure of cells, microorganisms, and other molecular structures. Many educational institutions and scientists rely on these devices for important research, yet modern microscopes, while available to well-financed labs, are often not an option for a classroom setting. Therefore, many students are unable to use these devices to practice processes that they are expected to understand later. A typical epifluorescent microscope can cost over $100,000, which far exceeds a typical course budget. The client, Professor Matthew Merrins, teaches a human biochemistry lab at the University of Wisconsin-Madison. His lab currently uses Laconic, a Fluorescence Resonance Energy Transfer (FRET)-based biosensor to detect the presence of Lactate in cells. Ideally, this lab will allow students to learn about microscopy through experimentation, but with the cost constraint of the course a “typical” microscope is out of the question. The goal of this design is to build an affordable, FRET-capable microscope that can be repeatedly manufactured for his students. The current proposed design involves a simplified microscope with a sample stand, LED light source, objective platform with filter-switching interface, tube lens, and camera. The data collected from the camera will be submitted to a proper software service for data analysis and extraction. Current design plans include assembly and testing of the excitation source.

2. Introduction

2.1. Problem Statement

The client, Professor Matthew Merrins, teaches human biochemistry lab at the University of Wisconsin-Madison. The course focuses on the enzyme lactate dehydrogenase, which produces lactate from pyruvate. Currently, his lab utilizes Laconic, a Förster Resonance Energy Transfer (FRET)-based biosensor. This biosensor detects the presence of lactate in healthy, living cells, but the fluorescence must be monitored over a period of time using an expensive microscope. This microscope excites the lactate biosensor using a complicated system of LEDs and filters. The fluorescence emission between the two different wavelengths is recorded. Since the current microscope in his lab in extremely expensive, the goal is to simplify the microscope and build a low-cost alternative specific to the Laconic biosensor.

2.2. Project Motivation

Current microscopes are extremely expensive due to their broad functionality. Even though this can be beneficial in a research lab, the client does not require as much flexibility for his simplified microscopes. The client would like to measure FRET, but with a specific focus on a single metabolic enzyme, lactate dehydrogenase. Ideally he will have multiple devices for his class to maximize his students’ educational experience. The design should be reproducible so that in the future he will have six to eight microscopes for his class.

2.3. Background

2.3.1 FRET

Fluorescence Resonance Energy Transfer (FRET) is the transfer of energy between two light-sensitive molecules. These molecules are known as chromophores, and they are referred to as the donor and the acceptor. FRET is a measurement of the different intensities of emission in order to determine the proximity of the two chromophores [1]. This is done by using a light source (usually an LED or laser) that will excite the donor chromophore. As the donor chromophore gets excited, it emits photons and transfers energy to excite the nearby acceptor chromophore. Usually the intensity of these sources is mapped using an absorption/emission spectrum, and a ratio of acceptor to donor emission intensity is obtained [2]. Many dynamic processes, such as protein-protein interactions, can be identified with various FRET biosensors. FRET is a popular method because of its ability to measure low concentration of molecules, and it has the capability to determine molecular dynamics of a given complex over time [3].

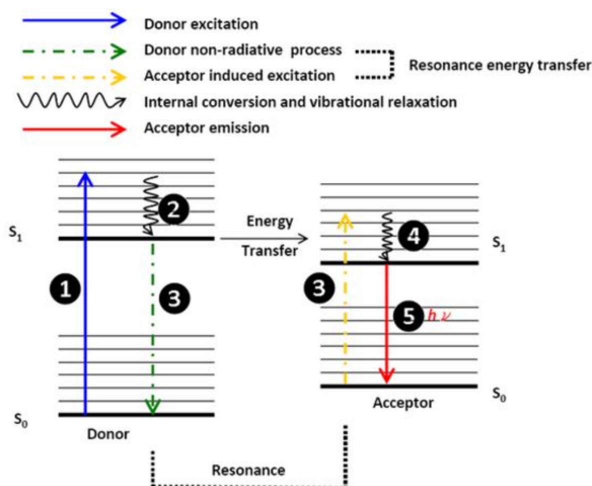


Figure 1. Schematic of Fluorescence Resonance Energy Transfer [2]. This image was obtained from the University of California, Davis, and shows of visual representation of FRET. S_0 represents the ground state, whereas S_1 represents the lowest excited state after donor group is excited by a photon and then relaxed. After the electron reaches the ground state it will then excite a nearby chromophore, which also will emit a photon and later return to the ground state.

2.3.2 Laconic: Lactate Biosensor

Lactate is produced from pyruvate by the enzyme lactate dehydrogenase (LDH) in mammalian cells [1]. LDH is found in almost all body tissues, and is vital in cellular respiration, signaling, and metabolic processes in healthy tissues [4]. In addition, if lactate is not regulated properly, this can lead to various risks to a person's health. Frequently, tumor cells have high rates of lactate production when oxygen is present [5]. As a result, many studies have been trying to further understand this process in living cells.

Professor Merrins' Lab specifically focuses on the nutrient metabolism in pancreatic islet

beta cells. His goal is to further understand the cause of insulin release and how to cause cell proliferation as soon as insulin is needed. By using rodents that are obese or have diabetes, Professor Merrins is able to use FRET in order to monitor metabolite production in different cells types, such as in yeast and cancer cells.

The biosensor that Professor Merrins hopes to use in his lab course is a protein that consists of two connected fluorophores and a lactate binding site [1]. The donor fluorophore is teal fluorescent protein (mTFP), which is excited by 430 nm light and fluoresces at 470 nm (Figure 2). The acceptor fluorophore is Venus, which is excited by light around 470 nm and fluoresces at 535 nm (Figure 3).

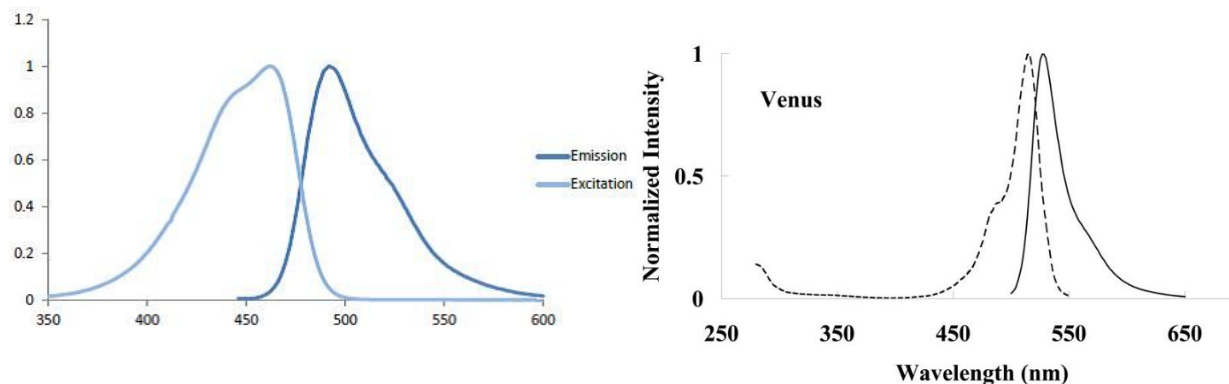


Figure 2-3. (2.) Excitation and emission curves for mTFP with normalized intensity on the y-axis and wavelength (nm) on the x-axis [6]. (3.) Excitation (dashed line) and emission (full line) curves for Venus [7].

This biosensor can be used to quantify lactate levels between 1 uM an 10 mM based on the FRET efficiency. Without lactate, the proteins are positioned well to allow for energy transfer between mTFP and Venus. Upon lactate binding, the FRET efficiency of the sensor decreases due to a conformational change induced by binding. Therefore, the change in FRET efficiency can be correlated with lactate concentration.

2.3.3 Client Background

Professor Matthew Merrins is an assistant professor in the Biomolecular Chemistry Department with a laboratory under the Department of Medicine at the University of Wisconsin School of Medicine and Public Health. His research is focused on nutrient metabolism in pancreatic islet beta cells using biochemistry, patch clamp electrophysiology, and quantitative imaging. Professor Merrins received his B.A in Chemistry and Biology at Oberlin College and his PhD in Physiology from the University of Michigan. He teaches Human Biochemistry Lab (BMC504) at the University of Wisconsin-Madison, where they use an epifluorescent microscope to image cells.

2.3.4 Competing Designs

This project will specifically target the research done in Professor Merrins' human biochemistry lab. As a result, there is no current device on the market that caters to a low cost device that perfectly meets his lab's requirements. However, there are many similar devices on the market that could be modified for his needs.

The Dino-Lite is a small fluorescence microscope that is able to filter a specific wavelength of light [8]. In addition, it can be designed for the different fluorophores used. A Dino-Lite fluorescence microscope can cost between $948-$968 which is dependent upon the type of LEDs needed and emission filters. In addition, software is included and comes with a stand with additional features for adjustments. Even though this device is low-cost, which is what the client requires, this device is not ideal for FRET since FRET requires the use and detection of two fluorophores and their emission wavelengths. As a result, the device would need to be modified to compensate for this.

Figure 4. Dino-Lite Fluorescent Microscope [8]. A small hand held microscope that connects to your computer.

The Lumascope 620 uses FRET to image living cells. This microscope is one of the most powerful devices on the market, and has a 5-filter set with 3 exciting filters, 1 triple-band emitting filter, and 1 beam-splitting filter. Utilizing confocal microscopy, one can obtain nanoscale resolution of specimens. In addition, the cells remain alive because this microscope minimizes photobleaching. The microscope also features different configurations of the objective lens, multiple laser options, filters, and detectors [9]. Even though the client would be able to use this microscope for his research, it is too expensive to obtain for a classroom setting because of the microscope's broad capabilities. The team requested a quote of the microscope, and the CEO of Etaluma Inc., Chris Shumate, said it is depended upon the chassis, filter and lens, illumination source, electronics, and type of camera. Many of the current designs with this type of equipment cost over $100,000, based on the variable cost within the quality of the parts requested for the microscope.

Figure 5. The Lumascope 620 [9] is an expensive option that does more than FRET analysis.

The Nightsea converts a stereo microscope into a simple fluorescence system. The product accomplishes this by using an attached filter and an external excitation source. The light source and the filter are assembled to be used with specific fluorophores. The Nightsea comes with one-color complete setup, modular excitation/emission sets, and a modular white head for a total cost between $1,880-$2,180. Although this device is relatively cheap, this device is not ideal for FRET. This is because FRET uses two fluorophores and would thus require swapping two filters relatively quickly. There is no current data acquisition system as well, which would need to be integrated in the design in order to extract relevant data for determining the FRET ratio [10].

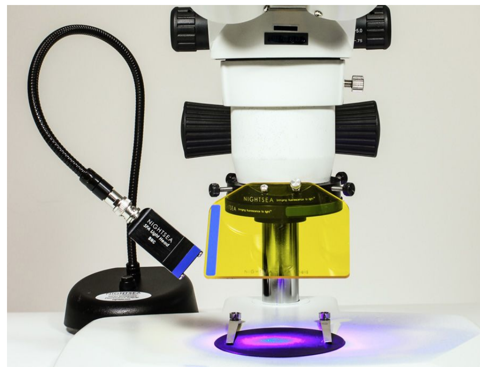


Figure 6. The NightSea Model SFA[10] consists of an excitation source and a filter that attaches to stereoscope.

2.4. Product Design Specifications

The final product will be a simplified single prototype microscope that will allow the client's students to measure FRET in a classroom setting. This device will be similar to his lab's microscope, as it will contain an excitation source at 430 nm, two different filters for the FRET response (one at 470 nm for the donor emission and the other at 535 nm for the acceptor emission), and a camera. The camera will capture the images of the specimen in the solution chamber and upload them to a compatible computer for image analysis. The goal of the device is to extract accurate acceptor-donor FRET ratios from the images collected. This accuracy does not have to be research-grade, but the microscope should be accurate enough that students can detect a change in lactate expression.

Along with this, the device must be intuitive to use, and the students should have to put in minimal work to obtain the image outputs. The students are not expected to have an extensive microscopy background; therefore, they should have to do little to no image processing. The product must be under $2,000 so that the lab would be able to purchase at least one device annually with its current budget. To accomplish this goal, most unnecessary/excessive parts of a microscope, such as eyepieces and other components, will be eliminated in this prototype. An estimate of the size of the microscope is a 20 cm by 30 cm base with a height less than 45 cm. If additional software for image analysis is needed, the software used must be free and capable to pair with the microscope to reduce cost. The client requires that the microscope be inverted and that a degree of versatility be present in the design for future applications. A full list of specifications can be found in the PDS in Appendix A.

3. Designs

3.1. Design Possibilities

The team decided on three potential designs for this simplified epi-fluorescent microscope for FRET imaging with various pros and cons. All will achieve the same goal of imaging cells expressing Professor Merrins' biosensor as well as outputting a FRET ratio. The three designs are presented in the following section.

3.2. Design One: Single-Shoot

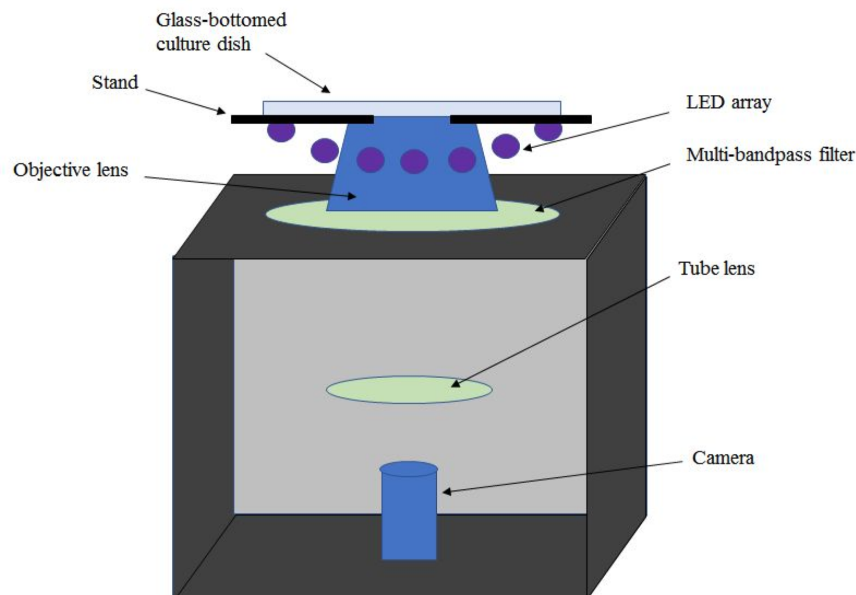


Figure 7. Single-Shot Design Schematic. This is the first design idea proposed and it consists of no moving parts.

Design one, Single-Shot, uses ten LEDs to emit light with a wavelength of 430 nm. These LEDs will excite the mTFP donor molecule, which emits photons with a wavelength of 470nm. The 470 nm photons excite the Venus fluorophore, which has a 535 nm emission wavelength. A

40x objective collects and collimates the light from the fluorophores.

The light then passes through a multi bandpass filter which blocks all the light except for light with wavelengths of 470 nm (± 20 nm) and 535 nm (± 20 nm). See Appendix B for the transmission curve of the multi bandpass filter. The multi bandpass filter ensures only the light of interest is passed to the rest of the system. The light then travels to a tube lens. The tube lens focuses the light onto the detector. The detector in this design is a color camera which will tell the difference between 470 nm light and 535 nm light. The color camera sends the data to a computer. An image processing software, most likely FIJI, will be used to analyze the images.

3.3. Design Two: Filter-Swap

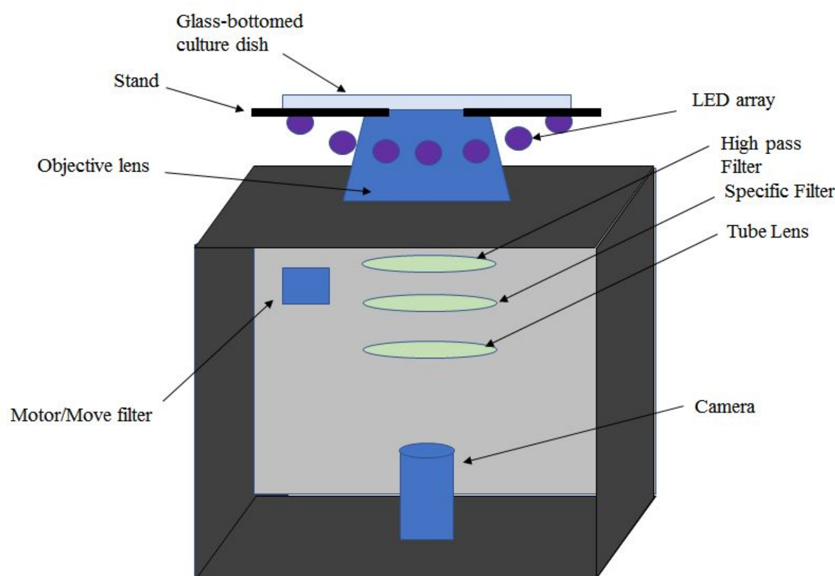


Figure 8. Filter-Swap Design Schematic. This is the second design idea proposed and it consists of a motor that swaps out the filters.

The Filter-Swap design alternative is both similar to and distinct from the Single-Shoot preliminary design. The specimen is placed on an open platform above the rest of the interface, and a hole drilled through the platform allows an LED source and detector access to the sample for an excitation and emission spectrum for FRET Imaging. The LED source is composed of a ring of 430 nm LEDs for excitation of the sample. A 40x objective centered through the LED excitation ring is brought up to the sample dish for image collection. A box or structure would be inserted between the LED source and the camera detector to limit interference from the 430 nm light. A high pass filter is likewise put in the beam path to limit interference that may pass through the objective lens.

Following this, the incident light travels through two rotating filters. The 470 nm and 535 nm filter are placed in a sliding mechanism and electronically controlled to be switched into the beam path for data collection. This sliding mechanism can be accomplished by either a linear solenoid, linear actuator, 2-bar-linkage rotor, or other integrated mechatronic circuit. The goal of the filter swap is to allow the camera to detect good, in-focus images of both emission sources while maintaining a practical shifting rate to capture both types of images. This filter collection

pattern would be coordinated with the LED light source and camera detector to shine and collect, respectively, at the best time for image analysis. A tube lens is placed in the beam path to properly collimate the sources on the camera. A monochrome camera is used to send these image captures to a software package for an analysis protocol that can determine the FRET ratio for the client's lactate.

3.4. Design Three: Beam-Splitter

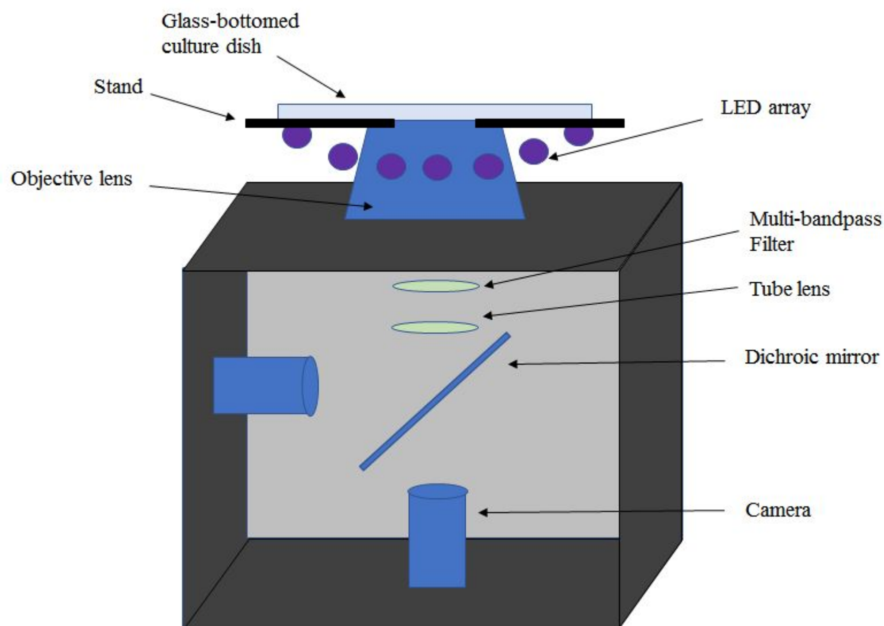


Figure 9. Beam-Splitter Design Schematic. This is the third proposed design and it consists of mirror that splits the two wavelengths of interest.

Design three, Beam-Splitter, uses an array of ten LEDs that emit light with a wavelength of 430 nm. A 40x objective will collect and collimate the emitted light. The light passes through a multi-bandpass filter allowing only the light of interest to pass through to the rest of the system. See Appendix B for the transmission curve of the filter. The light travels to a tube lens which will focus the light onto the dichroic mirror. The dichroic mirror passes longer wavelengths and reflects shorter wavelengths. In this design, the dichroic mirror would pass 535 nm and reflect the 470 nm light. The two wavelengths of light would be detected simultaneously by two monochrome cameras. The cameras would pass the information to a computer where image analysis would occur.

3.5. Design Matrix

After thoroughly researching these four designs, the team created a design matrix to rank them against one another in order to determine which should be pursued. The team considered six different categories in order to determine the best option: cost, client input, image quality, ergonomics, dependability, and manufacturability. Considering the advantages and disadvantages of each option, the team collaborated to give each design idea a ranking out of 5

for each component of the design matrix. Design scores highlighted in blue won their category (or tied for the top) and the total highlighted in green is the score for the design idea the team chose.

Design Criteria	Single-Shoot	Filter-Swap	Beam-Splitter
Cost (25)	**3/5: 15**	**3/5 : 15**	2/5: 10
Client Input (20)	3/5 : 12	**5/5 : 20**	1/5 : 4
Image Quality (15)	3/5 : 9	4/5 : 12	**5/5 : 15**
Ergonomics (15)	3/5 : 9	3/5 : 9	**5/5 : 15**
Dependability (15)	**4/5 : 12**	3/5 : 9	**4/5 : 12**
Manufacturability (10)	**5/5: 10**	3/5 : 6	3/5 : 6
Total:	67	**71**	62

Figure 10. Design Matrix. This figure represents the design matrix for the three different design ideas. The highest scoring design(s) for each respective criterion is highlighted in blue, and the highest scoring design total is highlighted in green.

3.6 Design Criteria

Cost was chosen as the most important design criterion since the client required that there be a strict budget of $2,000. Every semester the client teaches human biochemistry lab, he is provided with a $5,000 budget for the course. Ideally, he wants to obtain 5-8 microscopes for the course, resulting in a total cost of up to $16,000. Therefore, his plan is to purchase one to two microscopes each year given that each is under the $2,000 budget. With this in mind, the team hopes to make the microscope as cheap as possible while still maintaining image quality. To determine which microscope is the most cost effective option, the team researched cost for all of the components and compiled total prices. Lists of the items required for each microscope can be seen in Appendix C. Based on these cost spreadsheets, Single-Shoot and Filter-Swap were nearly identically priced, so they were both given a three out of five. However, Beam-Splitter was almost $500 more due to the added dichroic mirror and extra camera. Therefore, cost was ranked the lowest in design 3.

Client Input was also chosen as one of the highest weighted categories because Professor Merrins has relevant experience working with an epi-fluorescent microscope and FRET in his lab. He also works closely with students each semester to teach them about fluorescent microscopy. Therefore, he has a great understanding of his precise design specifications. Additionally, once the client saw the predicted prices for each of the microscopes, he was excited that there were two options under $1,500. As a result, he was intrigued about the possibility of adding in the ability to change filters to do FRET with a different biosensor. Based on this, Professor Merrins thought that the Filter-Swap design was best since it did not require the extensive image processing like that of Single-Shoot, and the filters could easily be exchanged.

The client thought that the two cameras of the third design was unnecessary. Therefore, Filter-Swap was given the highest score of five out of five, Single-Shoot received a three out of five, and Beam-Splitter received a one out of five.

Image Quality is making sure that the camera receives enough signal from the fluorophores to create a useful image. This means that the intensity of the donor and acceptor wavelengths should be detectable and small changes will need to be discerned as well. The team determined that the final design, Beam-Splitter, should win this category with a five out of five because there would be two cameras, which would be able to each detect an individual image of the two different wavelengths. Therefore, all of the pixels are dedicated to detecting only one wavelength, so the image quality would be better. This meant that the second design was the second best option since it would have the same quality of image, but the images will be not taken at exactly the same time. With a small time offset this should not affect the results much. Since Single-Shoot detected both images simultaneously with one camera, it will have the worst image quality. Therefore, it received a three out of five.

Ergonomics is meant to quantify user-friendliness of each design. Therefore, the team considered how much image processing would need to be done for each design and whether or not it would be easy for a student to use. Since Single-Shoot will require some image processing and Filter-Swap may require the student to push a button to swap filters, they both received a three out of five. Beam-Splitter will require only minimal image processing and does not require the student to swap filters, so it received a five out of five.

Dependability is one of the design criteria because the design should be able to withstand student use for as long as possible. For a cost effective microscope, ideally the client should not need to purchase new parts or new devices for as long as possible. Thus, the team decided that any designs with moving parts that could fail with repeated use may not last as long as designs with parts that do not move. This meant that Filter-Swap received the lowest score in this category with a three out of five. The other two designs both received fours out of five since there are still problems with the cameras or circuitry that could arise.

Manufacturability is how easy it is to build and assemble each of the designs. This includes aligning all of the components so that the image is focused onto the detector of the camera as well as the manufacture of any circuitry used to power the design. If there is anything unusual about the stand set-up, it is also included in this category. Therefore, Filter-Swap and Beam-Splitter each received a three out of five since single Filter-Swap requires the design of a system to swap the filters and Beam-Splitter will require a stand to hold more components. These components will also need to be meticulously aligned, which will be more difficult with two cameras pointing in different directions. Single-Shoot won this category with a five out of five because everything is aligned in one path and there are no moving parts.

3.7 Power and Control of Excitation Source

The team chose to use Thorlabs' 430 nm LEDs to excite the sample[11]. These LEDs were chosen because their spectrum peak was at 430 nm, which is what the design required (see Appendix D). The LEDs were also brighter compared to other 430 nm LEDs on the market and appeared to have a narrower spectrum than others that were being considered. The team wanted to use an Arduino microcontroller to control the LEDs. A constant current sink 8-bit shift register was used to control the LEDs through the Arduino. The Texas Instruments TLC5917 8-bit shift

register was used. See Appendix E for a pinout of the shift register. The shift register was chosen because the SparkFun community had created a software library, which has the ability to easily manipulate the LEDs [12]. The amount of current delivered to the LEDs was determined by a programmable resistor on the shift register. A potentiometer was used as the programmable resistor since it allowed the team to easily change the current of the LEDs. The output pins of the 8-shift register were controlled by the clock pin, data input pin, and latch pin. The clock pin on the shift register received pulsed voltages at a frequency of 16MHz [12]. If the clock pin and data pin were both set a high volt at the same time, then a high voltage was sent to the register. When the latch pin received a high voltage then a low voltage, the values in the register were sent to the output pins. The program controlling the shift register was set up such that the the entire register contained a high voltage and the latch pin signal was pulsed such that all the output pins on the shifter register were always on. The TLC5917 also has a disable pin. When the disable pin is set to high all the output pins are turned off. A push button-switch was wired to the Arduino. When the button was pushed the LEDs turned off by setting the disable pin high. A schematic of the circuit can be seen in Figure 11 and the program used to control the LEDs can be seen in Appendix F.

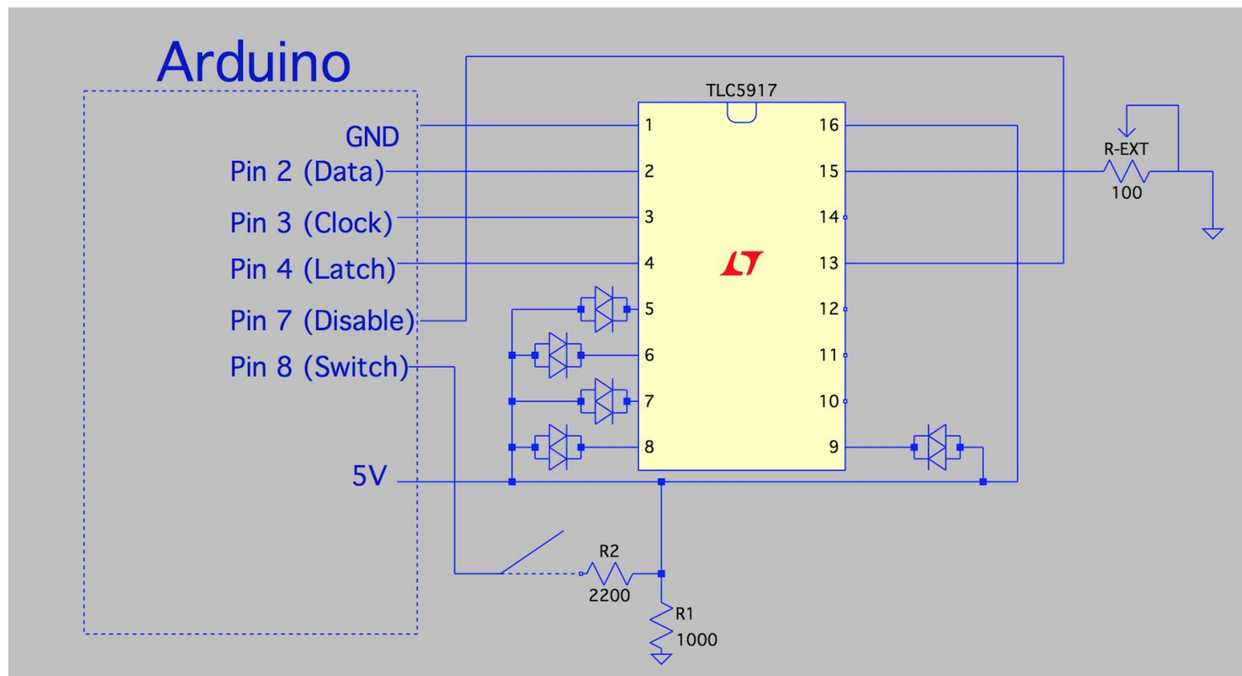


Figure 11. Schematic of Circuit

3.8 Preliminary LED Holder Designs

In order to test the excitation source, the team wanted to design an aperture for holding the LEDs in place. The first prototype made can be seen in Figure 12. The team attempted to use this prototype for testing, although the prototype had many issues with the LEDs. One of the issues that occurred was that the wires were unable to fit through the small hole made. In addition, the LEDs were unable to be angled up to concentrate the light. This is because the center platform was flat instead of on an angle. To improve this prototype, the team decided to

add more holes in order for wires to fit through. The piece was also angled instead of flat so that the LEDs would be able to concentrate the light. The final design can be seen in Figure 13. The team plans to use this design for future experiments and the final miniature microscope.

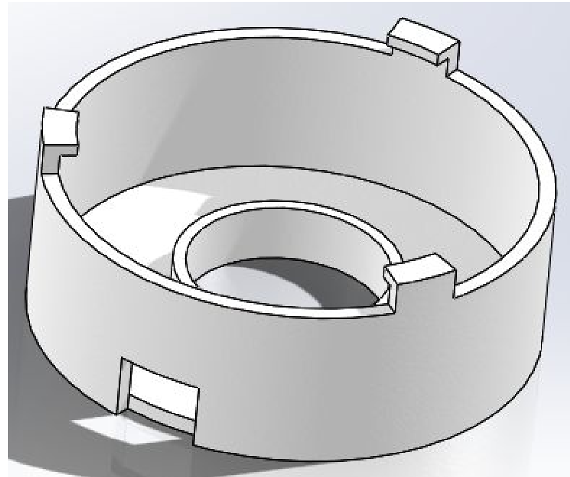


Figure 12. Preliminary Sample Holder Design. This image represents a 3D printed SolidWorks design of the first sample holder. This prototype was designed in order to hold the LEDs in place and organize the wires from the LEDs through a small hole.

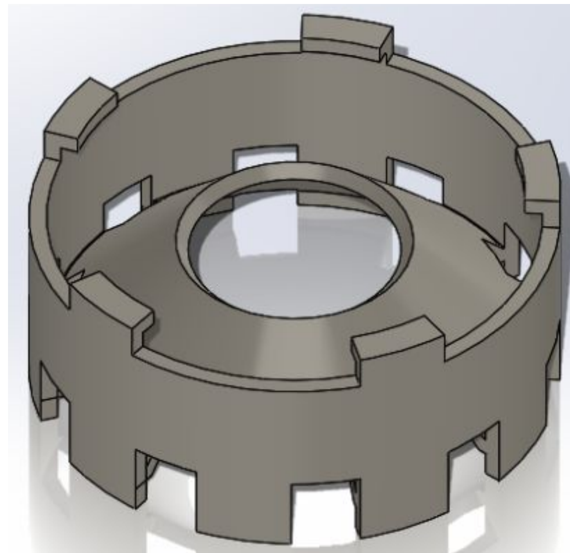


Figure 13. Final Sample Holder Design. This holder has a conical center for the LEDs. The design is no longer flat so that the LEDs are able to be angled to concentrate the light.

3.8 Final Proposed Design

Upon evaluating results from iterative designs during the semester, the team has decided to propose and pursue a final design for the semester that will be evaluated during subsequent design periods. A conceptual mock-up of the design can be viewed in Figure 14. The design

consists of a skeleton frame holding each of the required components for FRET microscopy. Only three components will be custom designed for this device: the sample platform/excitation source, filter holder, and filter post/objective holder. These custom components will be 3D-printed to reduce complexity of fabrication; all other components are completely off-the-shelf or are with minimal modification.

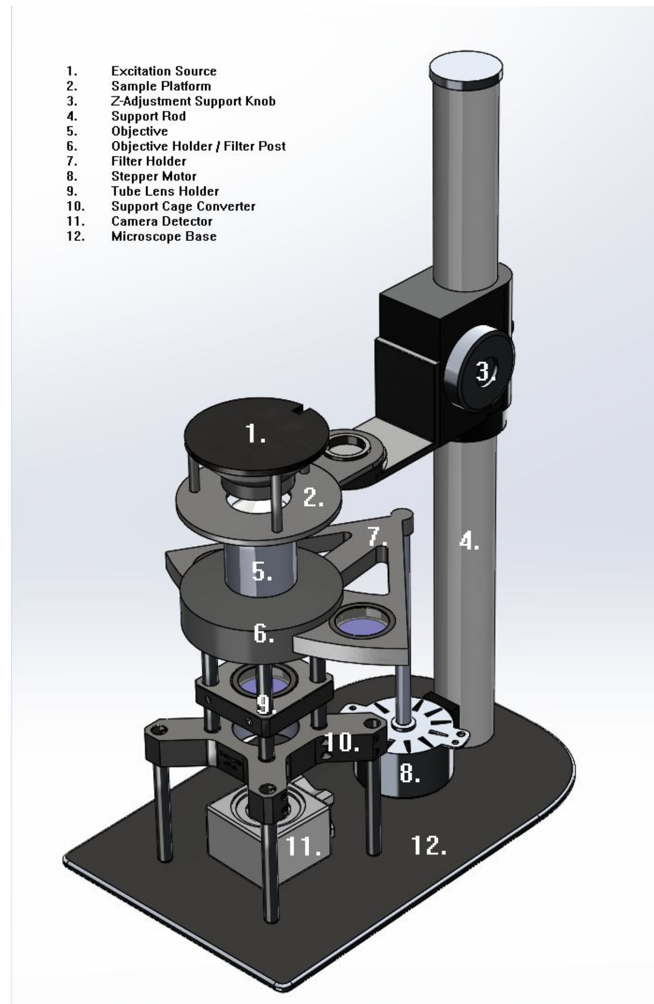


Figure 14. Overview of proposed microscope structure. A legend is included to identify all important components of the design.

After evaluating the excitation source of LEDs, an excitation filter is implemented in the final design to provide excitation only between 400 and 440 nm. In order to account for this extra filter, the excitation source will be situated above, rather than below, the sample platform (Figure 15). The sample platform is likewise attached to a z-adjustment support knob to move the sample in focus for experimentation. This adjustment is the only current manually adjustable source for student use, as every other component is planned to be either fixed or automated. The emission filter swapping mechanism is adjustable but electronically controlled, and all other components in the beam path, including the objective, tube lens, and camera, are assumed to be rigid with minimal adjustment during calibration.

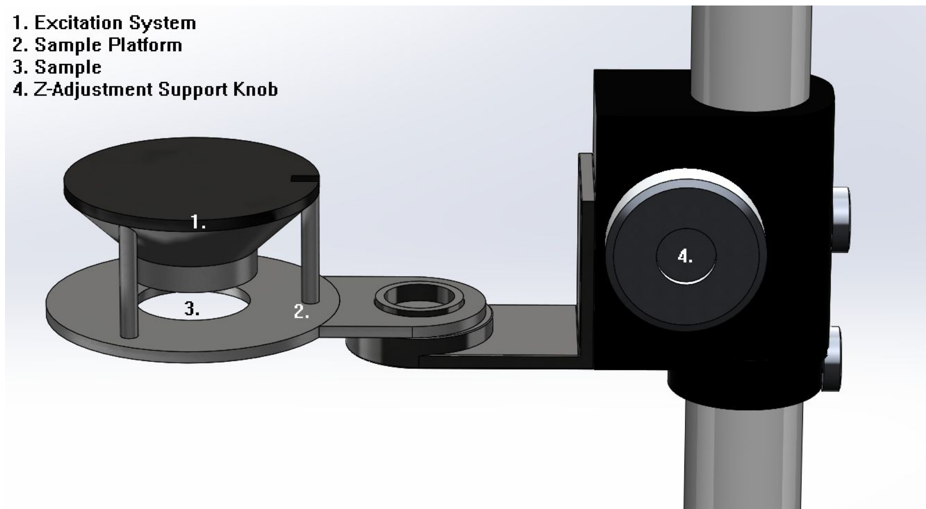


Figure 15. An exploded view of the excitation source and sample platform. This section is the only free-standing component of the design that will be held by the support rod. The system is adjusted by a support knob attached to the rod.

As stated above, a motor-driven filter swapping mechanism will be implemented in the final design to control which emitted light will be collected by the camera detector. The current state of this design involves a stepper motor, custom semi-circular filter holder, and a filter post in the beam path of the microscope (Figure 16). A stepper motor with sufficient speed is used to switch between the 470 nm and 535 nm emission filters with needed accuracy and can be remotely controlled by experimenters to coincide with excitation illumination and image capture. A custom-printed filter holder will house the needed emission filters and allow fast, compact, and simple swapping of filters by allowing the stepper motor to introduce a small amount of torque that shifts the needed filter into position behind the objective. Movement to the previous filter means that a similar amount of torque is needed in the opposite direction by the stepper motor. In this manner, filter swapping can be accomplished by prompting the stepper motor to rotate in 'positive' and 'negative' directions. Finally, a custom 3D-printed objective holder will serve the dual purpose of providing a place of entry for the filter holder in the beam path of the microscope and an insert for a purchased objective.

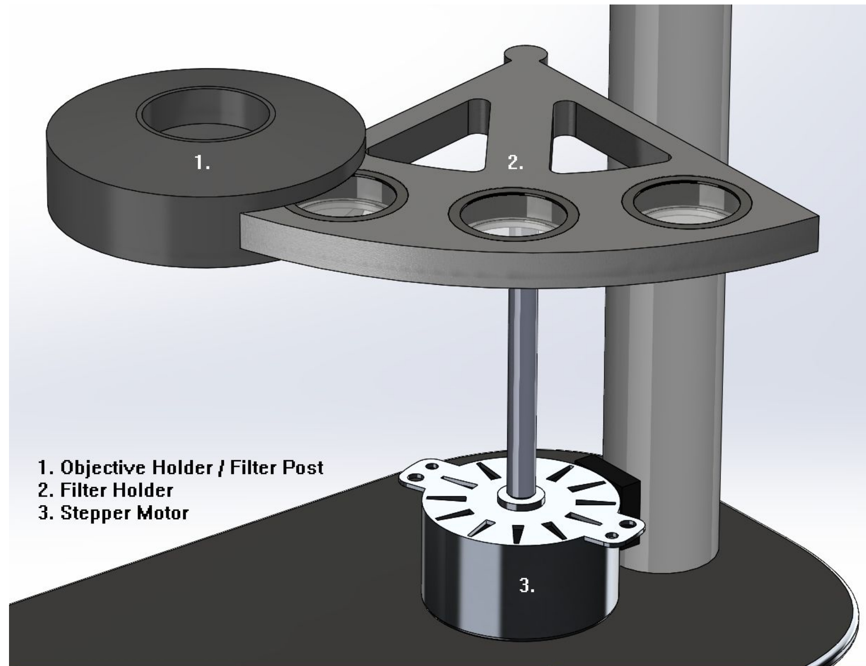


Figure 16. An exploded view of the filter swapping mechanism. This system shares the role of also supporting the objective (not shown). The stepper motor attached to the microscope base will provide the input to switch filters between images.

To give a final general overview, the design can be traced by the path of light from excitation to detection (Figure 17). An excitation source consisting of LEDs and an excitation filter will shine down on the sample in a glass-bottom cell culture plate. This cell culture plate can be slid between the excitation source and the sample platform and be centered based upon the geometry of the sample platform. The sample platform is likewise supported and adjusted by a system arising from the base of the microscope. Excitation light will hit the sample, which will respond by emitting lights of different wavelengths. This emitted light will be captured by an objective, shone through the proper filter from the filter swapping mechanism, collimated through a tube lens, and detected by a camera at the base of the microscope. Images captured by the camera can then be processed by a computer for analysis. This computer likewise controls the camera detection, proper swapping of the filters, and initiation of the excitation light. Further testing will validate or modify this proposed design.

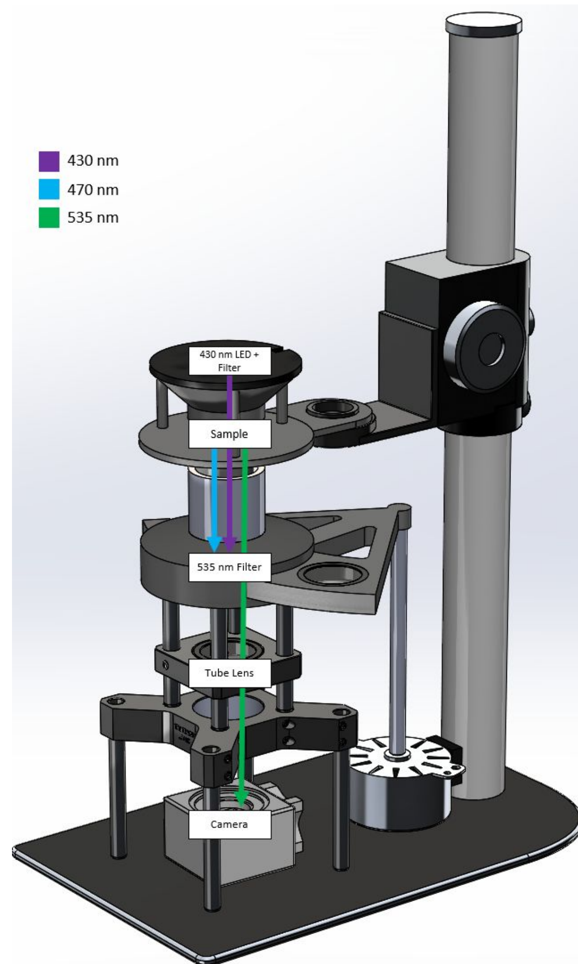


Figure 17. The general beam path of the microscope. 430 nm light will be filtered and excite the sample, which likewise will emit 470 nm and 535 nm light in response. An emission filter (shown as 535 nm) will attenuate the light response to only allow detection of certain wavelengths of light.

4. Experimental Set-up

4.1. Experimental Overview

This semester the team specifically focused on testing the excitation source and camera of the filter-swap design. Without a proper excitation source there would be no image produced. On the other hand, if there is proper excitation, the microscope also needs a camera capable of capturing these images. As a result, the team decided to focus on these two aspects before exploring additional design requirements of the project.

The team first purchased a total of ten LEDs all with 430 nm center wavelength. Using an Oscilloscope and multimeter, the voltage and current were measured for each LED. This was done to make sure none of the LEDs were receiving an obscure amount of current or voltage. Only nine LEDs were used during testing. The results were recorded in a spreadsheet which can be seen in Table 1.

LED	Voltage (V)	Current (mA)	Power (Watts)	Current (A)
1	3.8	17.11	0.065018	0.01711
2	3.8	14.22	0.054036	0.01422
3	3.8	14.51	0.055138	0.01451
4	3.8	15.71	0.059698	0.01571
5	3.8	15.28	0.058064	0.01528
6	4.2	33.65	0.14133	0.03365
7	3.8	13.01	0.049438	0.01301
8	3.8	10.51	0.039938	0.01051
9	3.8	27.75	0.10545	0.02775

Table 1. LED Characterization. Voltage (V), current (in mA and A), and power (watts) was determined for all nine LEDs with 430 nm center wavelength.

4.2. Initial Set-up with Preliminary LED Sample Holders

Before the team began testing, the team first brainstormed ideas for an LED holder. In order to take an image, the LEDs needed to stay in place around the objective lens. As a result, two prototypes were made and can be seen in Figure 12 and Figure 13 under design ideas. Even though these two designs were both 3D printed, they were not used during the experimental setup with Professor Merrin's microscope.

4.3. Final Testing Apparatus

For the final testing apparatus, the team utilized a circular piece of cardboard. The final setup used nine LEDs attached around the circular piece, which still allowed the lens to fit through the center piece. It could slide over the objective in the gap between the LEDs. The final setup can be seen in Figure 18. For future testing the team plans on using the conical 3D printed design in order to test the excitation source.

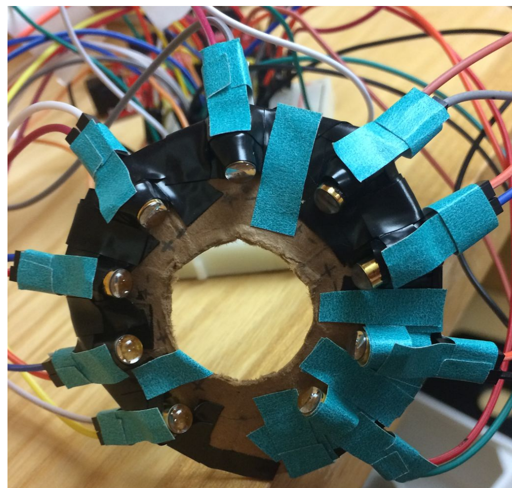


Figure 18. Final Set-up with Nine LEDs. This image represents the LED setup. Since the goal was to determine whether or not the excitation source was bright enough, the two 3D printed holder designs were not used for preliminary testing.

5. Testing and Results

5.1. Preliminary Testing

From the first segment of our testing protocol, the team obtained a picture from the client's current set-up using his excitation source (Lumencor SOLA), excitation and emission filters, and camera (RCA-Flash4.00). The photo was taken with the 535 nm filter. As a test of the quality of the prototype camera (DMK 42BUC03), it was used in conjunction with the client's excitation source, lenses, and filter set. Two images were taken: one with the 470 nm filter and one with the 535 nm filter. The cameras were threaded, so the Flash4.00 could be unscrewed from the microscope base and the prototype camera could be installed instead. The fine adjustment knobs were used to focus the images onto each of the cameras under white light so that they could then be imaged with the proper wavelengths.

Next, the team wanted to test the prototype LED-array excitation source against the light source of the client (Lumencor SOLA). For this test, the client's camera, lenses, and emission filters were used for imaging at both both wavelengths (470 nm and 535 nm) in conjunction with the prototype excitation source. However, the client's emission filter was bypassed in this set-up. These images could be compared with the images previously taken with the client's own camera, filters, and excitation source. The LED-array was placed over the top of the client's objective lens and fit between the objective and the microscope stage such that the angled LEDs illuminated the sample from below (Figure 19).

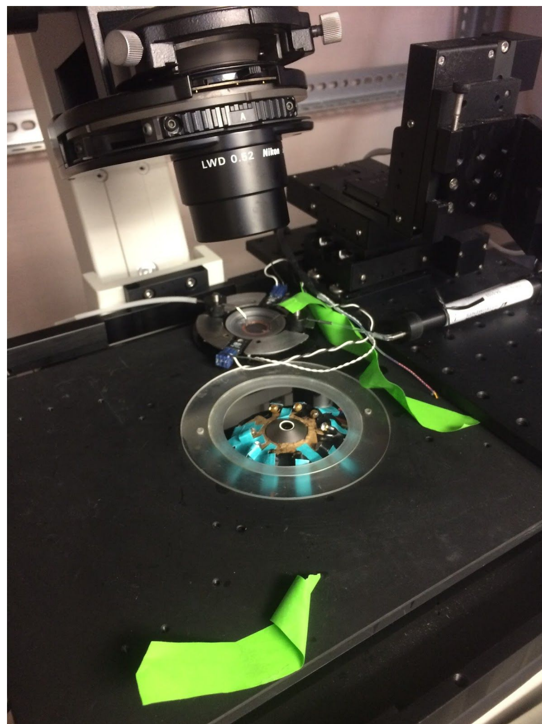
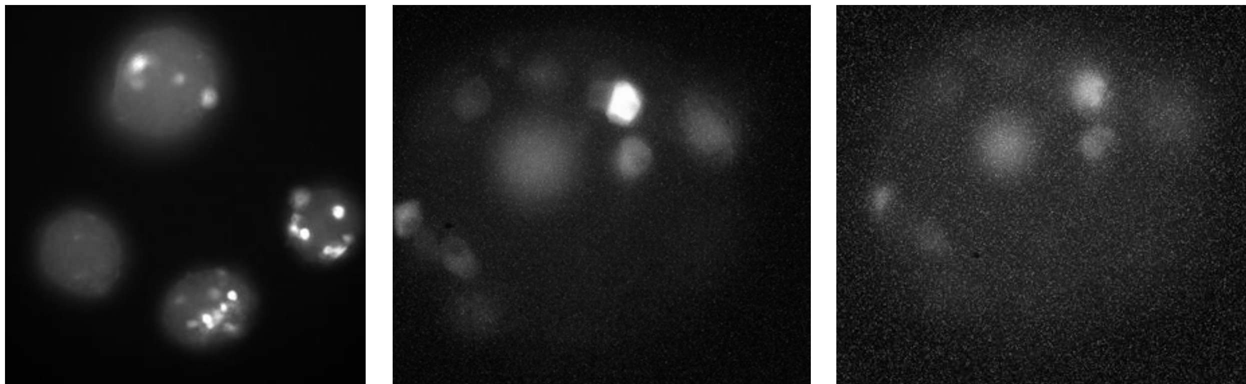


Figure 19. Image of the testing apparatus with prototype LED-array excitation source. Used to test the excitation source with both the client's and prototype cameras.

Finally, the team wanted to see if their prototype camera could be used with the prototype LED-array excitation source. The prototype camera remained installed at the base of the microscope in place of the client's camera and the same arrangement was used for the excitation source above the objective (Figure 19). The image was still focused with the client's white light, but imaging occurred with the client's light source and lenses in conjunction with the prototype light source and prototype camera.

5.2. Preliminary Results

When analyzing the results the team kept the microscope's final purpose in mind. The final product will need to detect the bright spots from the background at both 535 nm and 470 nm and compare the intensity of the images at these spots to determine a FRET ratio. Therefore, Figures 20-24 show photos taken by the client's camera compared to those taken by the prototype camera. To analyze these photos the team will consider how well the bright spots can be detected from the background and compare this to the detection possible with the client's current microscope.



Figures 20-22. Left to right: (20.) Client camera and Lumencor at 535 nm. (21.) Prototype camera and client light source at 535nm. (22.) Prototype camera and Lumencor at 470nm.

The image taken by the client's camera is of a much higher quality than those taken by the prototype camera. For example, the background was a much more uniform color and appeared completely black. On the other hand, the images taken with the prototype camera had backgrounds with a lot of noise in them. The field of view using the prototype camera was also reduced in comparison to the field of view using Professor Merrins' microscope. However, this could be remedied in the final prototype by choosing a different array of objective and tube lenses for the microscope, increasing the field of view. Both images taken by the prototype camera show bright spots where the biosensor was localized, and we could easily see them with the naked eye.

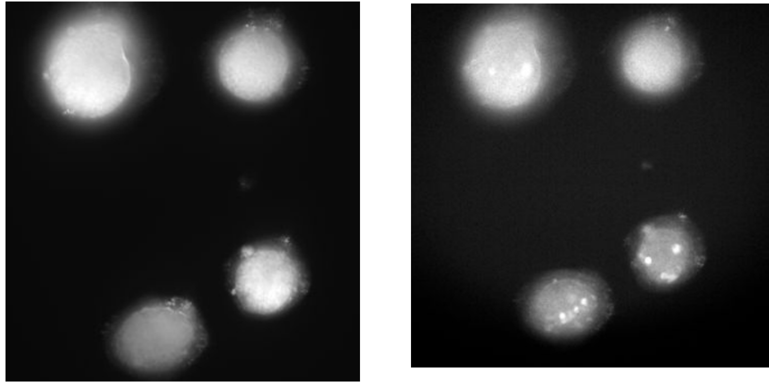


Figure 23-24. From left to right: (23.) Client camera and prototype light source at 470 nm. (24.) Client camera and prototype light source at 535 nm.

These images were taken to evaluate the effectiveness of our prototype excitation source. Both images were taken with our client's camera, lenses, and filters. Both pictures once again show a uniform black background such that the cells and colonies are easy to discern against the background. In the photo taken with the 470 nm filter, there do not appear to be any bright spots within the colonies. However, these bright spots are apparent in the photo taken with the 535 nm filter. Although they are not as distinct as the spots were when using our client's excitation source, the team could still pick the points out.

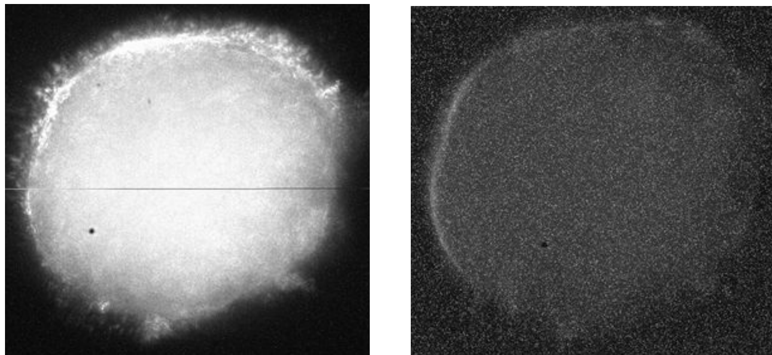


Figure 25-26. From left to right: (25.) Prototype camera and light source at 470 nm. (26.) Prototype camera and light source at 535 nm.

The photos from Figures 25 and 26 were taken with a combination of the team's prototype excitation source and prototype camera. To obtain both of these images, the team needed to increase the exposure time to 1 second. However, there were still no detectable traces of the laconic biosensor at 470 or 535 nm.

5.3. Summary of Results

There were a total of seven tests performed that tested the quality of the two cameras in addition to the quality of the excitation source. The summary of the seven tests can be seen in Table 2. In order validate the design's excitation source, the team first had to validate the camera. As a result, the team compared Professor Merrin's camera (RCA-Flash4.00) to the quality of the prototype camera (DMK 42BUC03). In additional to comparing the quality of the cameras, the team also wanted to test the LED-array excitation source that was designed in comparison to the client's light source (Lumencor SOLA). For analysis, the team used FIJI, which is ImageJ with additional functionality for image processing.

Table 2. Summary of the seven test performed. The team tested the 430 nm excitation source in addition to the quality of the team's prototype camera versus the client's camera.

Test Number	Tests Performed
1	Client camera and light source at 535 nm.
2	Prototype camera and client light source at 535 nm.
3	Prototype camera and client light source at 470 nm.
4	Client camera and prototype light source at 470 nm.
5	Client camera and prototype light source at 535 nm.
6	Prototype camera and light source at 470 nm.
7	Prototype camera and light source at 535 nm.

The team performed a two-tailed t-test in order to compare the client's camera with the prototype camera using the client's excitation source, lenses, and filters at 535 nm. This test was performed on Figures 20 and 21. Because the team needed to move the apparatus in order to insert the prototype excitation source, the images were not taken at exactly the same spot. Therefore, the team chose to compare the four brightest spots in one of the colonies. The colony to the far right of Figure 20 was used in analysis. First, an average background intensity was found for each image using FIJI over an area in the corner of each image. Then, the average intensity for each of the four brightest spots was computed by tracing around the outside of the spots. Then, these four intensities for each image were compared in a ratio to the average background of that image. This resulted in ratios ranging from 5 to 20 for each of the spots. Then, these ratios were listed in order of highest to lowest for each photo. This way the brightest spots were compared to each other, then the second brightest, and so on. Using a two-tailed, paired t-test, the team computed a p-value of 0.204 and a 95% confidence interval of -14.86 to 4.83 (Table 3). Based on these statistics, it appeared that there was no significant difference

between the prototype camera and our client's camera in their ability to detect the biosensor fluorescence from the background. Since the microscope will only need to detect fluorescence intensity, overall image quality does not need to be evaluated.

Table 3. Comparison of four brightest colonies using photos taken by the prototype and clients camera to image each picture at 535 nm.

Prototype vs. Client Camera	
p-value	0.2036
95% CI	(-14.86,4.83)
t	1.6205

Figures 23-26 provided additional information that will be vital to the final design of our microscope. Based on a comparison of Figures 23 and 24 to Figure 20, it was clear to the team that the camera is not taking a clean image of the fluorescence of interest at 470 nm with the prototype excitation source. Both had significantly more background than the images with the client's light source, showing the team that the prototype excitation source was not working as well as that of our client's. First, Figures 25 and 26 showed that the prototype excitation source is not bright enough for the prototype because there was no detectable excitation of the fluorophores that was not overpowered by the background light.

Additionally, from the results obtained, there was significant bleedthrough that occurred while imaging at 470 nm. Bleedthrough occurs because of the wide bandwidth of the LED array. A MATLAB analysis was performed to simulate the 430 nm LED spectrum and the excitation curve of mTFP which can be seen in Figure 27. The green curve represents the LED spectrum of the excitation source which peaks at 430 nm. The red curve is the transmission spectrum for the emission filter. As seen in Figure 27, there is overlap between the LED emission spectrum and emission filter transmission spectrum, resulting in a fluorophore signal that is effectively drowned out by the excitation source. In order to prevent bleedthrough, the team decided to purchase an emission filter (seen as the pink transmission spectrum in Figure 27). This filter will block most of the light of the LEDs that is above 440 nm from travelling through to the camera, resulting in an improved ability to image only the fluorescence of the mTFP fluorophore.

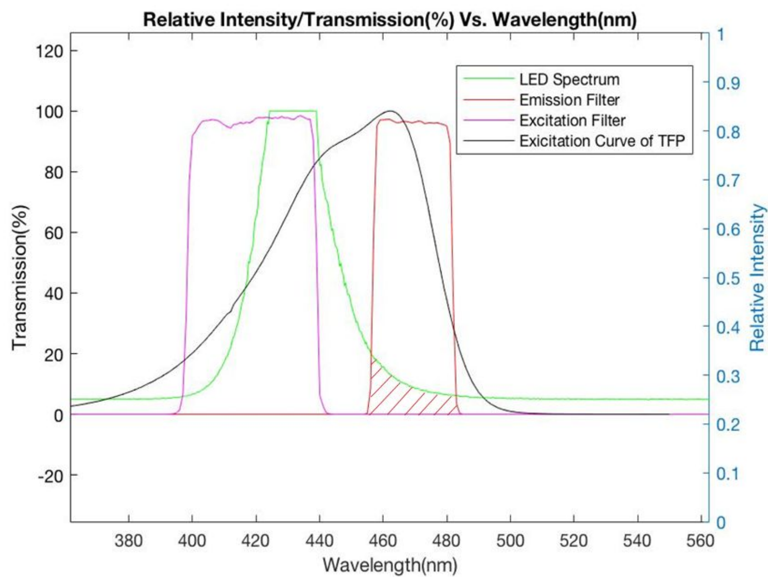


Figure 27. MATLAB analysis of optical components following testing

6. Future Work

The modified excitation source, where the samples are excited from above with a 420 nm filter, needs to be tested with Professor Merrins' setup. If the affordable camera cannot detect both mTFP and Venus proteins enough to find the FRET ratio then further research will need to be done to find LEDs that are bright enough to excite the cells. Following a successful excitation source testing four parallel pathways of development of the microscope should be taken.

The first pathway is determining which tube lens and objective should be used to focus the emitted light to the camera. Potential tube lenses will be tested using an optical simulation software known was OSLO. A list of potential tube lens has already been generated, see Appendix G. The same testing will be done with potential objectives. For a potential list of objectives see Appendix H. A tube lens and objective will be purchased based on the results from OSLO. The objective and tube lens can be tested in the field once the mechanical structure of the microscope is completed

The second pathway is the completion of the structure of the microscope. Once an optical path is finalized, the rest of the microscope will be designed around this constraint. This includes finalizing parts for 3D printing, purchasing components for structure and structural integration, and assembling these parts into one cohesive structure. Parts to be 3D printed include the newly designed sample platform/excitation source, a finalized objective insert and filter post, and the filter holder for the filter swapping mechanism. Consideration and purchase of other needed structural parts will then be accomplished. These parts will then be assembled to produce the final device with a cohesive structure and minimal manufacturing. Mechanical testing would then be performed on all parts of the structure to ensure that the microscope can withstand disturbances from students and other use throughout its implementation.

The third pathway is the development of a system to control all electronics in the final design. A Raspberry Pi will be used to control the LEDs, motor, and camera. The Raspberry Pi is

a 35 dollar computer that fits in the palm of human hand. The computer has general purpose input output pins similar to Arduino. The Raspberry Pi will control the timing of swapping filters, turning the LEDs on, and capturing images. The images will be saved for later image processing. The Raspberry Pi will be used for controlling because it is much more versatile and robust compared to the Arduino at relatively the same price. It may also be connected to a monitor so that students can use this feedback to focus their image with the z-adjustment knob.

The fourth pathway to be taken is creating a program to automatically perform image processing. The image processing will provide all the information needed for Professor Merrins' students to make necessary interpretations of the cell cultures. The image processing will be done on the Raspberry Pi.

This summer two team members, Kaitlyn Gabardi and John Rupel, plan to work on the project. John's goal is develop a working excitation source, purchase an objective and tube lens, and a control system for the electronics. Kaitlyn's goal will be to create a mechanical structure. Ethan Nethery will be replacing Zach Alden for BME 400 in the fall. Ethan may be assisting with the project over the summer. If he does make contributions, they will be in the development of the control system and/or the image processing. The team will continue to update the cost manufacturing spreadsheet and items purchases, Appendix I, to keep the cost under $2,000.

7. Conclusion

The team decided to work on building a Laconic FRET-based biosensor for their client Professor Matthew Merrins. The goal of this project is to build a single prototype that his students would be able to use in his biochemistry class. The microscope built should have similar features to his lab's current microscope and include an excitation source, a camera, and a series of filters for under $2,000. After further research and various meetings with the team's client and advisor, the team came up with three design ideas: Single-Shoot, Filter-Swap, and Beam-Splitter. After ranking the three designs the filter-swap was chosen as the final design given its low cost to manufacture and how well it met the client's needs.

Building upon this simple design, the team first focused on verifying the prototype camera and the team's excitation source this semester. In preliminary testing, there were a total of seven tests performed that tested the quality of the two cameras in addition to the quality of the excitation source. Using Professor Merrin's light source at 535 nm, a two-tailed t-test was performed in order to compare Professor Merrin's camera with the team's prototype camera. The team concluded that there was no significant difference between the prototype camera and Professor Merrin's camera. As a result, the prototype camera could be used for the final design.

The team additionally found that the prototype excitation source was not bright enough at 535 nm, and that there was significant bleedthrough that occurred at 470 nm. The first fix the team enacted was to increase the power to the LEDs to increase the brightness. In order to address the bleedthrough the team ordered an excitation filter that will filter out the light that was disrupting the signal at 470 nm.

The team is excited to continue this project this summer and for senior capstone design. During this time the team will first establish a working excitation source. Following this the team will also use OSLO to determine which lenses should be purchased for the microscope before assembling the structure of the microscope, including all mechanical elements. Finally, the team will develop programs for image analysis and filter swapping before integrating all of these components into a functioning fluorescent microscope for FRET microscopy.

8. Acknowledgements

The team would like to thank their advisor Professor Jeremy Rogers and their client Professor Matthew Merrins for guiding them through the design process. In addition, special thanks also goes out the the entire BME department for providing helpful resources for this design project.

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10. Appendices

Appendix A.

Product Design Specifications
Miniature Fluorescent Microscope

Team Members: Kaitlyn Gabardi, John Rupel, Kadina Johnston, and Zach Alden

BME 301
Client: Professor Matthew Merrins
Advisor: Professor Jeremy Rogers
Last Updated: April 30, 2017

Problem Statement: The client, Professor Matthew Merrins, teaches human biochemistry lab at the University of Wisconsin-Madison. The course focuses on the enzyme lactate dehydrogenase, which produces lactate from pyruvate. Currently, his lab utilizes Laconic, a Förster Resonance Energy Transfer (FRET)-based biosensor. This biosensor detects the presence of Lactate in healthy, living cells, but the fluorescence must be monitored over a period using a high cost microscope. This microscope excites the lactate biosensor using a system of LEDs and a filter. The fluorescence emission between the two different wavelengths is recorded. Since the current microscope in his lab in extremely expensive, the goal to to build a low-cost microscope specifically targeted to his research.

Function: The final design will be a single prototype device that will allow his students to measure FRET in his human biochemistry class. This device will be similar to his lab's microscope as it will contain an excitation source, two different filters for FRET, and a camera that will capture the images of the specimen in the solution chamber.

Client Requirements:

- Product must be under $2,000
- Compact and intuitive for student use
- Software used to process images must be free
- Easy to obtain FRET results
- Result should be similar to results obtained from client's microscope
- Should be an inverted design
- Interchangeable filters and excitation source
- Device can be repeatedly manufactured with limited engineering experience required
- Microscope should have significant and detectable change in fluorescence between 470 and 535 nm from 430 nm excitation source

Physical and Operational Characteristics:

A. *Performance Requirements:* The designs must be able to accurately measure light intensities at 470 and 535 nm. These readings do not have to be simultaneous but must be close in time. An excitation source of 430 nm should induce this response, which will be recorded by a detector (camera) and uploaded to a freeware image analysis program (ImageJ/Matlab) on a compatible computer for analysis. The lactate level can then be extracted based on the ratio of 470 and 535 intensities.
B. *Safety:* The design should minimize contact between the excitation source and user. This is due to the fact that the excitation source is near the UV light spectrum which is damaging to human skin tissue.
C. *Accuracy and Reliability:* This product should be accurate enough to determine the acceptor-donor ratio. FRET results should be similar to the results obtained from the client's microscope.
D. *Life in Service:* Product itself would last for years and system components should be easily replaced if broken or damaged.
E. *Shelf Life:* Shelf life would be 50 years. Optical filters and CMOS cameras will have lifetime guarantee as long as proper care is given to these components.
F. *Operating Environment:* The design must operate at room temperature.
G. *Ergonomics:* Product should be simple and intuitive for students to use. The image collection and accept/donor ratio calculation should be as simple as possible.
H. *Size:* Able to be used as a typical laboratory station on a lab desk (20 cm by 30 cm base), size similar to competing/conventional microscopes. All nonessential components for analysis should be discarded. Height of microscope < 45 cm.
I. *Power Source:* Device will be powered by a power outlet from the wall, thus eliminating the need for battery replacement.
J. *Weight:* 1lb to 10lbs
K. *Materials:* The device will have an internal circuit and will likely utilize 10 430 nm LEDs, plastics, wires, optical filters, and shift register. A CMOS camera will be used along with a stepper motor for the mobile filter swap. Two emission filters and one excitation filter will be used in order to differentiate the 470 nm and 535 nm fluorescence. The final product will also include 3D printed parts such as the LED holder, filter swap platform, and stand.
L. *Aesthetics, Appearance, and Finish:* Simple aesthetics, appears intuitive to use, and simple finish.

Production Characteristics:

A. *Quantity*: One prototype with ability to be repeatedly fabricated over time with plans to have a total of six to eight devices would be implemented over an 8 semester period.
B. *Target Product Cost:* Max cost is $2,000.

Miscellaneous:

A. *Standards and Specifications*: Should comply with current FRET analysis protocol and/or be adapted into a simple protocol for the client's human biochemistry class teaching lab analysis.
B. *Patient-Related Concerns*: Cost is the highest determinant in design. The functionality should be sufficient for teaching purposes on a budget of 1/60 of current device ($120,000 to $2,000). Resolution is not a key concern, only that the difference in emission intensities can be accurately extracted from experimentation. The data collection is the largest concern, and data analysis should be used by an easily accessible freeware service.

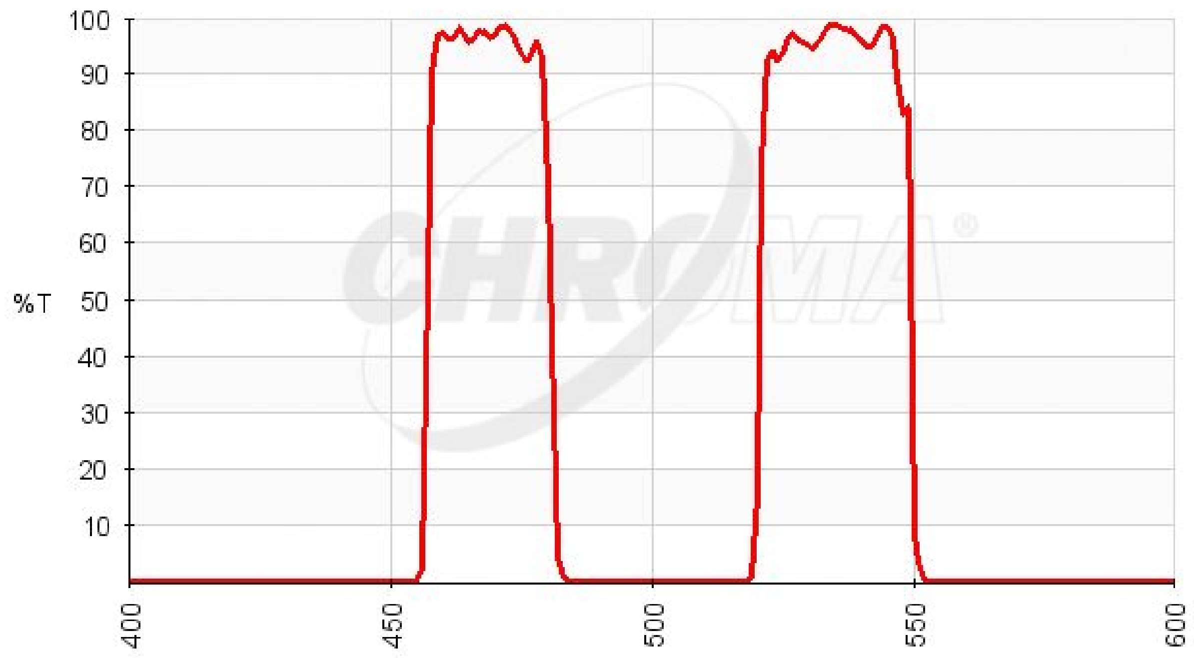
C. *Competition*:

   a. Dino-Lite:
      i. This product is small fluorescence microscope where each type of microscope has a specific wavelength and filter designed for specific fluorophores. They are not ideal for FRET since fret requires the use of two fluorophores.
   b. Lumascope 620:
      i. This product is for professional use. It can be used for a variety of fluorescence microscopy techniques. It is expensive due to its broad capabilities
   c. Nighsea:
      i. This product converts a Stereo microscope into a simple fluorescence microscope. Using an attachable filter and an external light source the microscope can detect light from fluorophores. The lens are designed for specific fluorophores and is not ideal for FRET.

D. *Customer:* Human biochemistry lab (BMC 504) instructor and students.

Appendix B.

Multi-Bandpass Transmission Curve


100
90
80
70
60
50
40
30
20
10
%T
400
450
500
550
600
CHROMA

Appendix C.
Lists for cost of items needed for each design idea.

Parts for Single-Shoot	Cost
Camera	$355
Objective Lens	$143
Multi-bandpass filter	$350
Tube Lens	$150
LEDs	$115
Stand	$100
Circuitry/Power	$50
Box	$20
TOTAL:	**$1283**

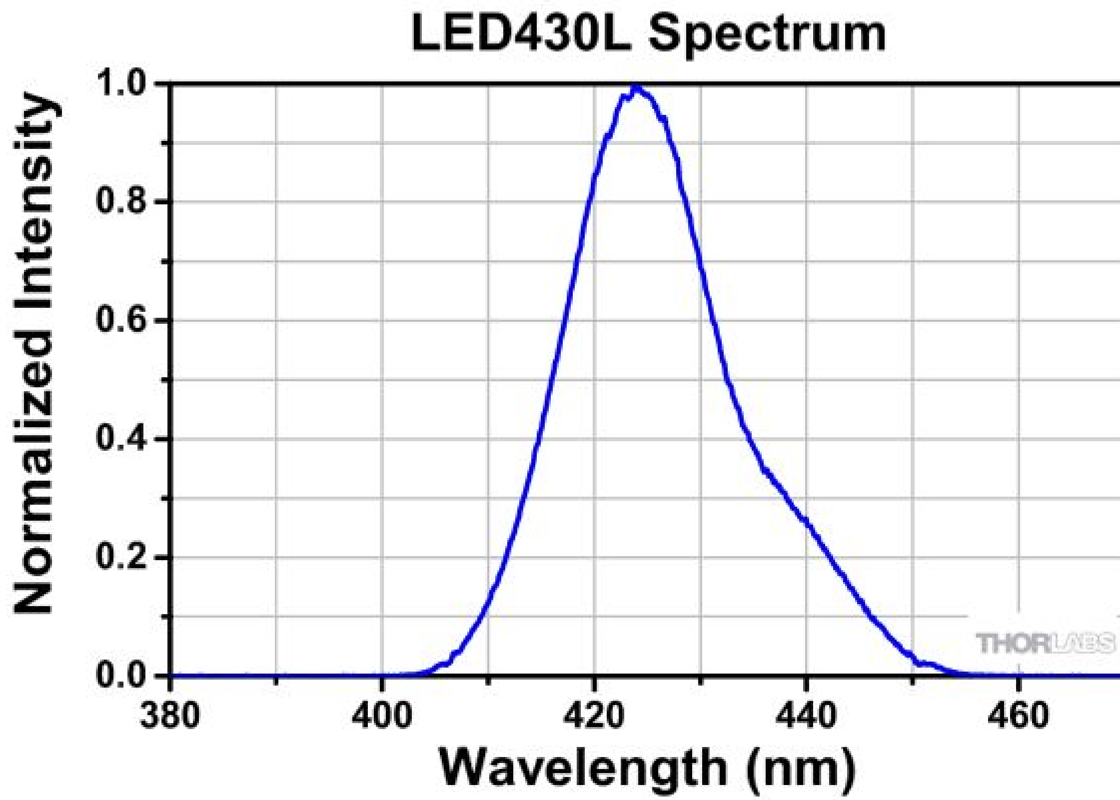
Parts for Filter-Swap	Cost
Camera	$355
Objective	$143
Filters	$340
Move Filters	$10
LEDs	$115
Tube Lens	$150
Stand	$100
Circuitry/Power	$80
Box	$20

TOTAL:	**$1313**

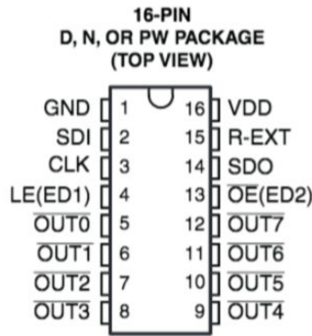
Parts for Beam-Splitter	Cost
Cameras	$710
Objective	$143
Beam Splitter	$113
LEDs	$115
Multi-bandpass filter	$350
Stand	$100
Tube Lens	$150
Circuitry/Power	$50
Box	$20
TOTAL:	**$1751**

Appendix D.

Spectrum of ThorLab LEDs



Appendix. E
Pinout of TLC5917



Pin Functions

PIN		I/O	DESCRIPTION
NAME	NO.		
CLK	3	I	Clock input for data shift on rising edge
GND	1	–	Ground for control logic and current sink
LE(ED1)	4	I	Data strobe input Serial data is transferred to the respective latch when LE(ED1) is high. The data is latched when LE(ED1) goes low. Also, a control signal input for an Error Detection Mode and Current Adjust Mode (see Timing Diagram). LE(ED1) has an internal pulldown.
$\overline{\text{OE}}$(ED2)	13	I	Output enable. When $\overline{\text{OE}}$(ED2) is active (low), the output drivers are enabled; when $\overline{\text{OE}}$(ED2) is high, all output drivers are turned OFF (blanked). Also, a control signal input for an Error Detection Mode and Current Adjust Mode (see Figure 11). $\overline{\text{OE}}$(ED2) has an internal pullup.
$\overline{\text{OUT0}}$ to $\overline{\text{OUT7}}$	5 to 12	O	Constant-current outputs
R-EXT	15	I	External Resistor - Connect an external resistor to ground to set the current for all outputs
SDI	2	I	Serial-data input to the Shift register
SDO	14	O	Serial-data output to the following SDI of next driver IC or to the microcontroller
VDD	16	I	Supply voltage

Appendix. F

Program to Control LEDs

```
/*
Modified From:
SparkFun Inventor's Kit
Example sketch 13

Authors: John Rupel

*/



// Pin definitions:
// The 74HC595 uses a type of serial connection called SPI
// (Serial Peripheral Interface) that requires three pins:

int datapin = 2;
```

```
int clockpin = 3;
int latchpin = 4  ;
int disablePin = 7;
int switchPin = 8;

int switchState = 0;

// We'll also declare a global variable for the data we're
// sending to the shift register:

byte data = 0;


void setup()
{
  // Set the three SPI pins to be outputs:

  pinMode(datapin, OUTPUT);
  pinMode(clockpin, OUTPUT);
  pinMode(latchpin, OUTPUT);
  pinMode(disablePin, OUTPUT);
  pinMode(switchPin, INPUT);
}


void loop()
{
  // We're going to use the same functions we played with back
  // in circuit 04, "Multiple LEDs", we've just replaced
  // digitalWrite() with a new function called shiftWrite()
  // (see below). We also have a new function that demonstrates
  // binary counting.

  // To try the different functions below, uncomment the one
  // you want to run, and comment out the remaining ones to
  // disable them from running.

 switchState = digitalRead(switchPin);

 if(switchState == HIGH) {


  Serial.println("switch on");
```

```
  digitalWrite(disablePin, LOW);
  digitalWrite(13, LOW);
  oneAfterAnother();

 }

 else
 {
  digitalWrite(disablePin, HIGH);
  digitalWrite(13, HIGH);
 }
       // All on, all off

}


void shiftWrite(int desiredPin, boolean desiredState)

// This function lets you make the shift register outputs
// HIGH or LOW in exactly the same way that you use digitalWrite().

// Like digitalWrite(), this function takes two parameters:

//    "desiredPin" is the shift register output pin
//    you want to affect (0-7)

//    "desiredState" is whether you want that output
//    to be HIGH or LOW

// Inside the Arduino, numbers are stored as arrays of "bits",
// each of which is a single 1 or 0 value. Because a "byte" type
// is also eight bits, we'll use a byte (which we named "data"
// at the top of this sketch) to send data to the shift register.
// If a bit in the byte is "1", the output will be HIGH. If the bit
// is "0", the output will be LOW.

// To turn the individual bits in "data" on and off, we'll use
// a new Arduino commands called bitWrite(), which can make
// individual bits in a number 1 or 0.
{
  // First we'll alter the global variable "data", changing the
  // desired bit to 1 or 0:
```

```
  bitWrite(data,desiredPin,desiredState);

  // Now we'll actually send that data to the shift register.
  // The shiftOut() function does all the hard work of
  // manipulating the data and clock pins to move the data
  // into the shift register:

  shiftOut(datapin, clockpin, MSBFIRST, data);

  // Once the data is in the shift register, we still need to
  // make it appear at the outputs. We'll toggle the state of
  // the latchPin, which will signal the shift register to "latch"
  // the data to the outputs. (Latch activates on the low-to
  // -high transition).

  digitalWrite(latchpin, HIGH);
  digitalWrite(latchpin, LOW);
}


/*
oneAfterAnother()

This function will light one LED, delay for delayTime, then light
the next LED, and repeat until all the LEDs are on. It will then
turn them off in the reverse order.
*/

void oneAfterAnother()
{
  //prevents pin on IC from turning off LEDs
  digitalWrite(disablePin, LOW);

  int index;
  // Turn all the LEDs on:
  // This for() loop will step index from 0 to 7
  // (putting "++" after a variable means add one to it)
  // and will then use digitalWrite() to turn that LED on.

  for(index = 0; index <= 7; index++)
  {
    shiftWrite(index, HIGH);
   // delay(delayTime);
```

```
  }

}
```

Appendix G

Potential Tube lenses

	PARAMETERS												
Doublets	**Focal Length**	**Diameter**	**Working Distance**	**Center T1**	**Center T2**	**Center T3**	**Edge T**	**R1**	**R2**	**R3**	**R4**	Outer Material	Inner Material
https://www.edmundoptics.com/optics/optical-lenses/achromatic-lenses/25mm-dia.-x-60mm-fl-mgfsub2sub-coated-achromatic-doublet-lens/	60	25		9	2.4	N/A	8.78	31.94	-29.95	-1029.79	n/a	SSK8	SF10
https://www.newport.com/p/PAC16AR.15	60	25		5.75	6.5	N/A	6.54	36.25	-28.67	-76.58	n/a	N-FK5	F2

Appendix H

Potential Objectives

Type of Objective	**Cost**	**Magnification**	**Numerical Aperature**	**Focal Length**	**where to buy**
finite conjugate	**186**	**20**	**0.4**	**9.0mm**	**https://www.newport.com/p/M-20X**
finite conjugate	**196**	**40**	**0.65**	**4.5mm**	**https://www.newport.com/p/M-40X**
Finite conjugate	**110**	**20**	**0.4**	**8.33mm**	**http://www.edmundoptics.com/microscopy/finite-conjugate-objectives/international-standard-microscope-objectives/33438/**
Finite conjugate	**160**	**40**	**0.65**	**4.40mm**	**http://www.edmundoptics.com/microscopy/finite-conjugate-objectives/nikon-achromatic-finite-conjugate-objectives/59936/**
infinite conjugate	**133**	**20**	**0.4**	**9mm**	**https://www.newport.com/f/infinite-conjugate-objective-lenses**
infinite conjugate	**143**	**40**	**0.65**	**4.5mm**	**https://www.newport.com/f/infinite-conjugate-objective-lenses**

Appendix I.

Current breakdown of budget

Miniature Microscope Material's Purchased by Professor Merrins				
Part Number	Description	Supplier	Qty	Price/Unit
DMK 42BUC03	Prototype camera (DMK 42BUC03)	oem cameras	1	$349.00
AT420	25 mm excitation filter, 420 nm, bandwidth 40 nm	Chroma	1	$200.00
LED430L	LED with a Glass Lens, 430 nm, 8 mW, TO-18	Thorlabs	10	$11.50
TOTAL BUDGET SPENT				$664.00
Green represents items purchased spring 2017.				
Red represents items purchased in summer 2017				

Projected budget

Miniature Microscope Material's Cost for Manufacturing					
Part Number	Description	Supplier	Link	Qty	Price/Unit
K67-US	Stepper Motor (5 sets) + ULN2003 Driver Board + Better Dupont Wire 40pin Male to Female Breadboard Jumper Wires Ribbon Cables	Kuman	https://www.amazon.com/gp/product/B01IP7IOGQ/ref=oh_aui_detailpage_o01_s00?ie=UTF8&psc=1	1	$14.99
DEV-11021	Arduina Uno- R3	Sparkfun	https://www.sparkfun.com/products/11021	1	$24.95
MS35B	Dino-Lite MS35B Versatile Table Top Stand with 10" WD	Dino-Lite	http://www.microscope.com/digital-microscopes/dino-lite/stands/dino-lite-ms35b-rigid-industrial-pole-stand.html#gref	1	$99.00
ER2-P4	Cage Assembly Rod, 2" Long, Ø6 mm, 4 Pack	Thorlabs	https://www.thorlabs.com/thorproduct.cfm?partnumber=ER2-P4	2	$23.18
CP02	Threaded 30 mm Cage Plate, 0.35" Thick, 2 Retaining Rings, 8-32 Tap	Thorlabs	https://www.thorlabs.com/thorproduct.cfm?partnumber=CP02	1	$16.40
LCP02	30 mm to 60 mm Cage Plate Adapter, 8-32 Tap	Thorlabs	https://www.thorlabs.com/thorproduct.cfm?partnumber=LCP02	1	$39.00
DMK 42BUC03	Prototype camera (DMK 42BUC03)	OEM Cameras	http://www.oemcameras.com/dmk-42buc03.htm	1	$349.00
AT420	25 mm excitation filter, 420 nm, bandwidth 40 nm	Chroma	https://www.chroma.com/products/parts/at420-40x#tabs-0-main-2	1	$200.00
MF535-22	YFP Emission Filter, CWL = 535 nm BW = 22 nm	Thorlabs	https://www.thorlabs.com/thorproduct.cfm?partnumber=MF535-22	1	$238.00
MF479-40	CFP Emission Filter, CWL = 479 nm, BW = 40 nm	Thorlabs	https://www.thorlabs.com/thorproduct.cfm?partnumber=MF479-40	1	$238.00
LED430L	LED with a Glass Lens, 430 nm, 8 mW, TO-18	Thorlabs	https://www.thorlabs.com/thorproduct.cfm?partnumber=LED430L	10	$11.50
TOTAL COST					$1,403.88
Green represents items purchased in spring 2017					
Red represents items team plans to purchase					