DEPARTMENT OF
Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

Miniature Fluorescent Microscope

Mid-Semester Report

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1. Abstract

Microscopes are essential for understand the structure of cells, microorganisms, and other molecular structures. Many educational institutions and scientists rely on these devices for everyday research. However, modern microscopes, while available to well-financed labs, are often not an option for a classroom setting and many students in school are unable to use these devices. 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 using a high cost 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 on the market are extremely expensive due to their broad capabilities. 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. Many dynamic processes such as protein-protein interactions can be identified with various FRET biosensors.

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 [2]. In addition, if lactate is not regulated properly, this can lead to risks to a person's health. Frequently, tumor cells have high rates of lactate production when oxygen is present [3]. As a result, many studies have been trying to further understand this process in living cells.

Professor Merrin's 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.

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. In addition, it can be designed for the different fluorophores used. 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 [4].

FIGURE 1: Dino-Lite Fluorescent Microscope [4]. A small hand held microscope that connects to your computer.

The Lumascope 620 uses FRET to image living cells. 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 [5]. 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.

FIGURE 2: The Lumascope 620 [5] 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. This is not ideal for FRET since 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 [6].

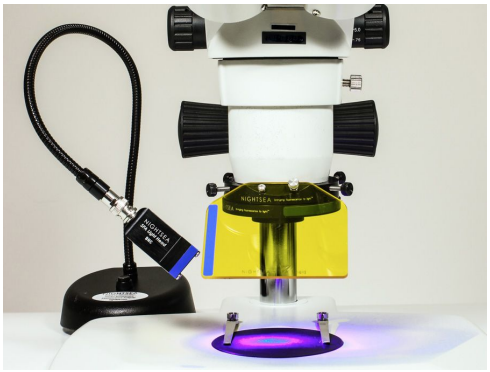

FIGURE 3: The NightSea Model SFA[6] consists of an excitation source and a filter that attaches to stereoscope.

2.4. Product Design Specifications

The final product will be a heavily 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, were 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

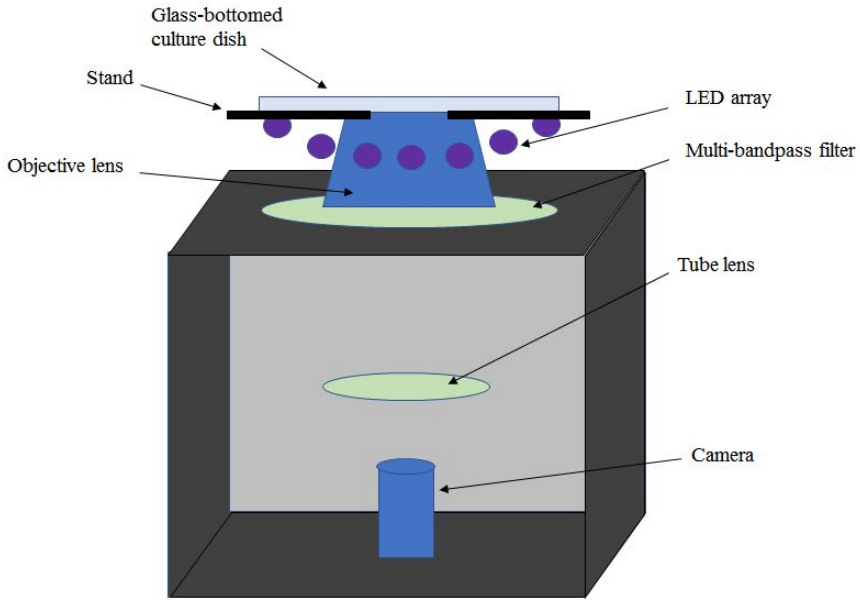

FIGURE 4: Single-Shot Design Schematic. This is the first design idea proposed and it consists of no moving parts.

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

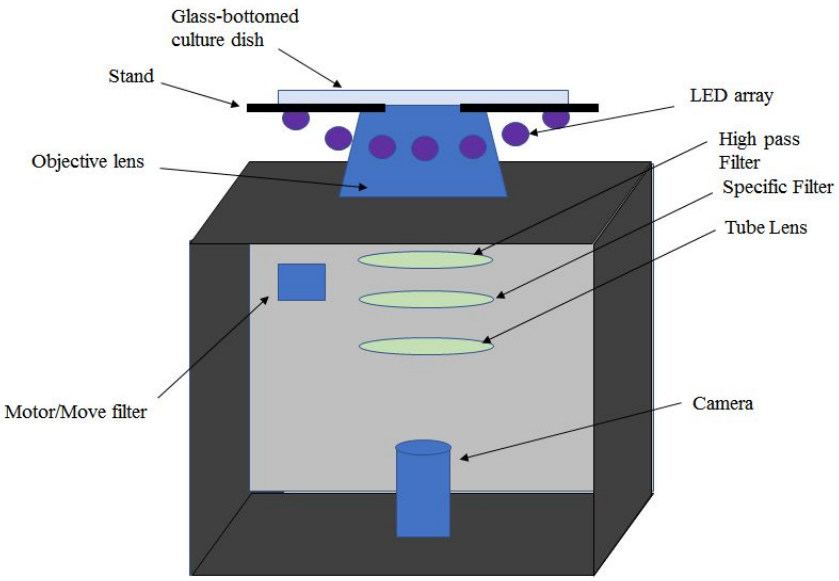

FIGURE 5: Filter-Swap Design Schematic. This is the second design idea proposed and it consists of a motor that swaps out the filters.

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

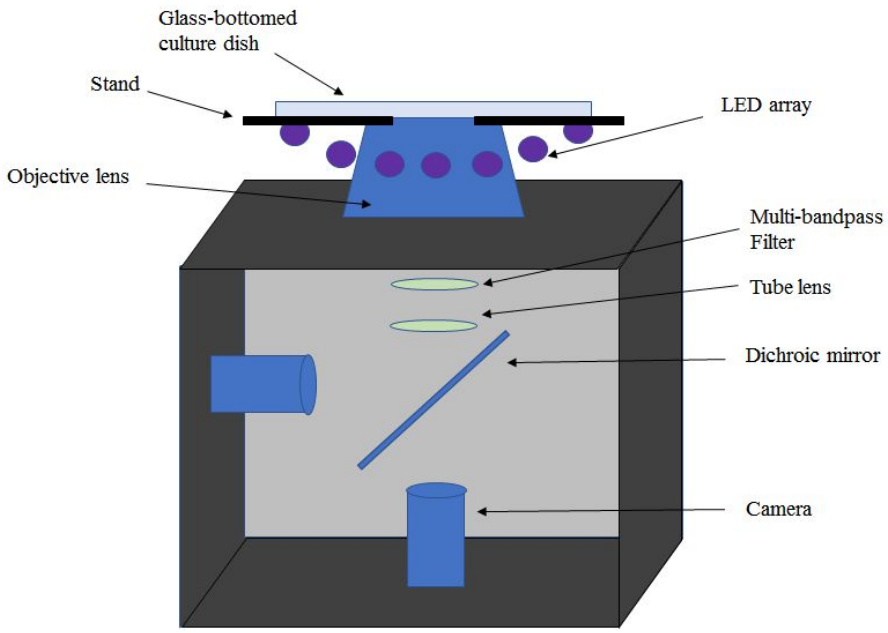

FIGURE 6: Beam-Splitter Design Schematic. This is the third proposed design and it consists of mirror that splits the two wavelengths of interest.

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. Preliminary 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 7: 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 Preliminary 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 that 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.

4. Future Work

A protocol for analyzing acquired images will needed. FIJI image processing software will be used to analyze the images since it is a free software that is developed and maintained by researchers at UW-Madison. Another future task will include properly spacing the components such that the image acquired is a focused image. Approximation for the spacing can be done through lens software provided by Professor Rogers. While spacing approximations are being done, fabrication of the microscope can begin. The stand will allow for components to be adjusted with precision. Once an approximation for proper spacing is acquired and the stand is completed, assembly of the prototype can begin. The prototype will first need to acquire a focused image without the presence of fluorophores. Following this, the team will test with fluorophores, specifically mTFP and Venus. Following successful image acquisition, protocols for analyzing the images can be tested and refined. As a final task the image analysis could potentially be automated thus making the design more ergonomic.

5. 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. The most difficult aspect of this project is being able to meet the client's needs while building the microscope 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.

All three design ideas were ranked in a design matrix under six categories in order to evaluate each of the design. The six categories are as follows: cost, image quality, ergonomics, dependability, and manufacturability. After ranking each design, the second design, Filter-swap, was given the highest score and therefore chosen as the final design. This design was ranked the highest given its low cost to manufacture, and because the design met the client's requirements the best. This is due to the design's capability to change filters. In addition, the monochrome camera has better sensitivity compared to Single-Shoot's color camera. The team's next steps will be to build and test the excitation array.

6. 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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9. Appendix

Appendix A.

Preliminary 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: Feburary 18, 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 product will be a single prototype device that will allow his students to measure FRET with this device. 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 easy to use
- Any software used must be free
- Easy to obtain FRET results
- Should be an inverted design

Physical and Operational Characteristics:

A. *Performance Requirements:* The designs must be able to accurately measure FRET response 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/similar) on a compatible computer for analysis. The lactate level can then be extracted based on the ratio of 470 and 535 intensities. Other modalities, such as a filter at 620 nm, should be considered as transferable to the design.

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.
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 do not degrade quickly if not in constant use.
F. *Operating Environment:* The design must operate at room temperature.
G. *Ergonomics:* Product should be simple 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 station on a lab desk (30 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 LEDs, plastics, wires, optical filters, CMOS camera, and motor.
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. 6 to 8 would be implemented over an 8 semester period.
B. *Target Product Cost:* Max cost is strictly $2,000.

Miscellaneous:

A. *Standards and Specifications*: Should comply with current FRET analysis protocol and/or be adapted into a simple protocol for 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.

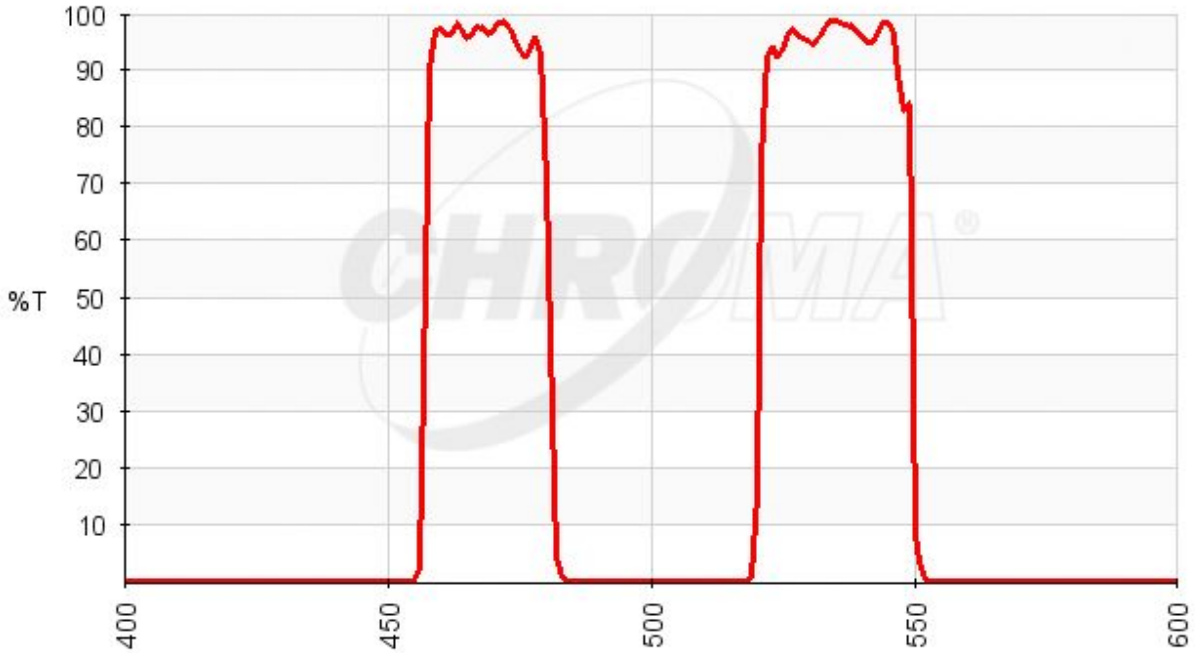
C. *Competition*:

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



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**