Product Design Specifications
Miniature Fluorescent Microscope

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BME 402
Client: Professor Matthew Merrins
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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 in time lapse using a high cost microscope. This microscope excites the lactate biosensor using a system of high power LEDs and a filter wheel, and the fluorescence is recorded. The project goal to to build a lower-cost, limited alternative to the client's more expensive microscope

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:

- Initial prototype built must be made under $4,000
  - Eventually build multiple devices around $2,000
- Compact and intuitive for student use
- Software used to process images must be free
- Easy to obtain FRET results
- Device should be able to detect yeast cells
- 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 upon stimulation 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) 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. All electronic components should be enclosed.
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 (8" x 14" base), size similar to competing/conventional microscopes. All non-essential 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:* 1 to 10 pounds
K. *Materials:* The device will have an internal circuit and will utilize a single, super-bright white LED, plastics, wires, optical filters, and an Arduino. 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, which will be fabricated out of PLA and ABS.
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 $4,000.
    a. Goal is to make final product around $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 1/30 of current device. A typical

Nikon microscope can cost between $50,000 and $120,000, and the client would like the device to be between $2,000 and $4,000. 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. 3D Printed OPN Scope
   i. This device uses 3D printing to make the outer shell, drawer for the fluorescence filter tude, and tube that holds the eyepiece and light source. A 3D printed device allows the capability to modify the structural parts of the microscope, in addition to making the device extremely cheap to fabricate as compared to customized part manufactured from different companies.
d. 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. Future potential for other teaching labs to incorporate our design but their own sets of filters.