Miniature Microscope for FRET Microscopy

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Client: Professor Matthew Merrins
Advisor: Professor Jeremy Rogers

Abstract

Microscopes are used to study the structure and function of cells, and fluorescence microscopy is one method of observing them. In Fluorescence Energy Resonance Transfer (FRET), one can study intracellular signal transduction and molecular interactions. Our client, Professor Matthew Merrins, currently teaches a human biochemistry course at the University of Wisconsin Madison where his students use a laconic biosensor and FRET to study lactate in pancreatic beta islet cells [1]. Currently, he has only one microscope with these capabilities, limiting the enrollment of his course as well as the lab options. These microscopes typically cost over $100,000; however, they are extremely adaptable. For this course, they will be doing the same lab year after year, so the microscope will only be used for a single purpose. Therefore, the team will design a low-cost microscope for the specific wavelengths of interest.

Background/Motivation

FRET – Fluorescent Resonance Energy Transfer:

- Energy transfer between two light-sensitive molecules (chromophores)
- Donor molecule absorbs energy from light source
- Donor is excited and emits lower energy photons
- Energy transferred to acceptor and lower wavelength emitted
- Client uses Laconic FRET Biosensor

Laconic Biosensor:

- Excitation source of 430 nm
- Donor fluoresces at 470 nm
- Acceptor fluoresces at 535 nm
- Used to measure lactate levels

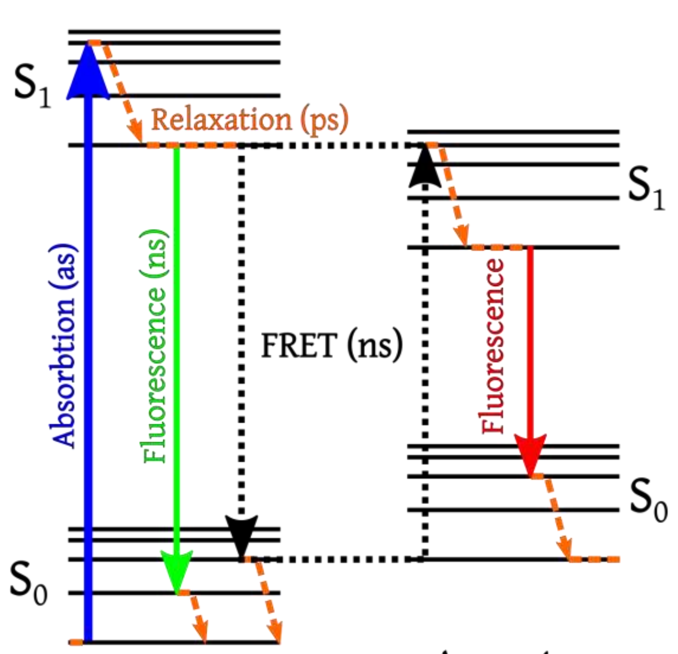

Figure 1. Diagram of FRET concept [2]

Design Criteria

- ***Manufacturability:*** repeatable for manufacturing with limited previous experience
- ***Image Quality:*** significant & detectable change in fluorescence between 470 & 535 nm from 430 nm source
- ***Cost:*** <$4,000 per microscope
- ***Reliability:*** consistent results from experimentation, with similar results to client's microscope
- ***Operability:*** intuitive for student use, easy handling/storage

Testing

Comparison of Imaging Algorithms

- Time Series images acquired with Nikon TI Eclipse
- Imaged islets during changes in concentration of glucose
- Measured FRET Ratio over time with professional software
- Measured FRET Ratio over time with amateur software

Optical Resolution

- Used 200mm tube lens and 0.65NA objective to image test chart
- Calculated resolution from test chart and theoretical resolution

Results

- Data analysis conducted through a paired T-Test of algorithm's respective FRET ratio slopes over time at $\alpha = 0.05$

$$H_0\colon \mu_{Nikon} - \mu_{MATLAB} = 0$$
$$H_A\colon \mu_{Nikon} - \mu_{MATLAB} \neq 0$$

- P-Value = 0.71756
- Retain H_0, suggesting the MATLAB algorithm detected the same relative changes in FRET ratio

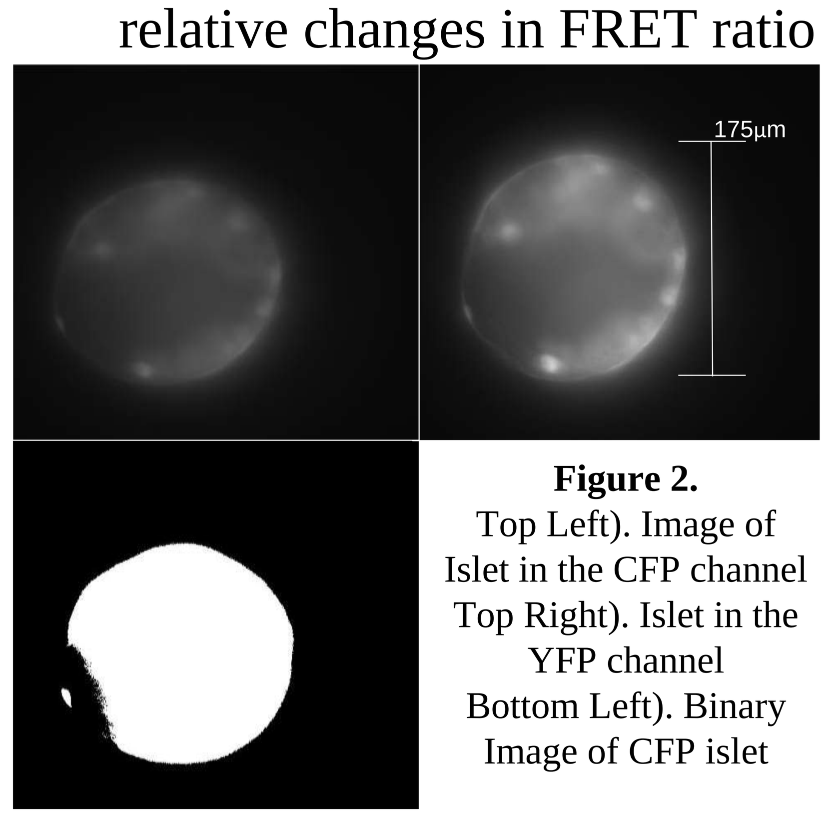

Figure 2. Top Left). Image of Islet in the CFP channel Top Right). Islet in the YFP channel Bottom Left). Binary Image of CFP islet

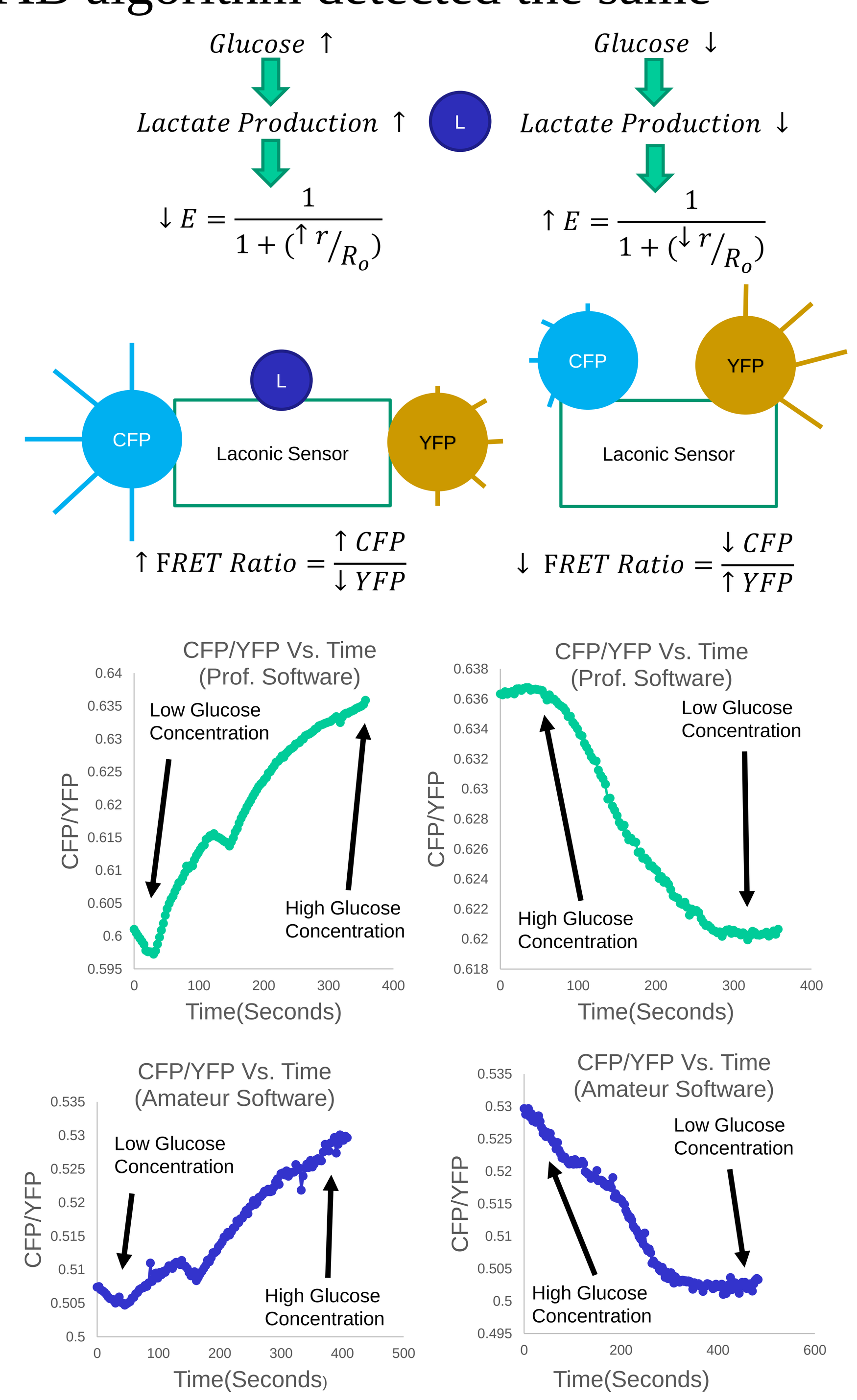

Figure 3. Left Col. Measured FRET Ratio over time going from low glucose to high glucose. Right. Col. Measured FRET Ratio over time going from high glucose to low glucose

- 1951 USAF resolution test chart

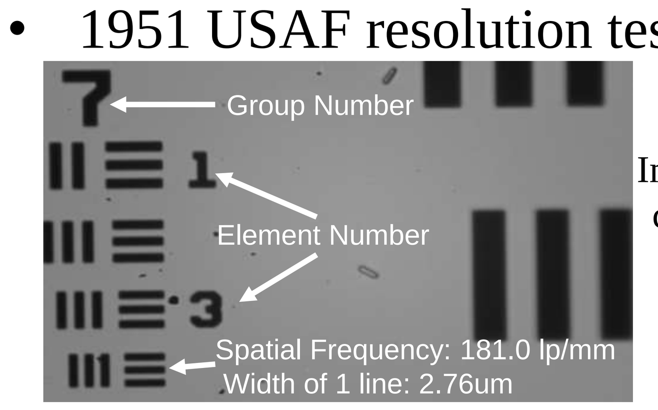

Figure 4. Image of test chart using Imaging Source Camera

Calculation for Resolution

$$Resolution\ (\mu m) = 1000 \Big/ 2^{Group + \frac{element-1}{6}}$$

$$5.5\mu m = \left(2^{7+(4-1)/6}\right)^{-1} * 1000$$

$$Theoretical\ Resolution(\mu m) = \frac{0.61\lambda}{NA}$$

$$0.511\mu m = \frac{0.61 * 0.545\mu m}{0.65}$$

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Final Design

Optical Component

- Tube Lens
- Objective
- Filters

Mechanical Component

- 3D printed parts
- Fold Mirror
- Focuser

Software

- Control of electro-mechanical components
- Image Processing
- Display

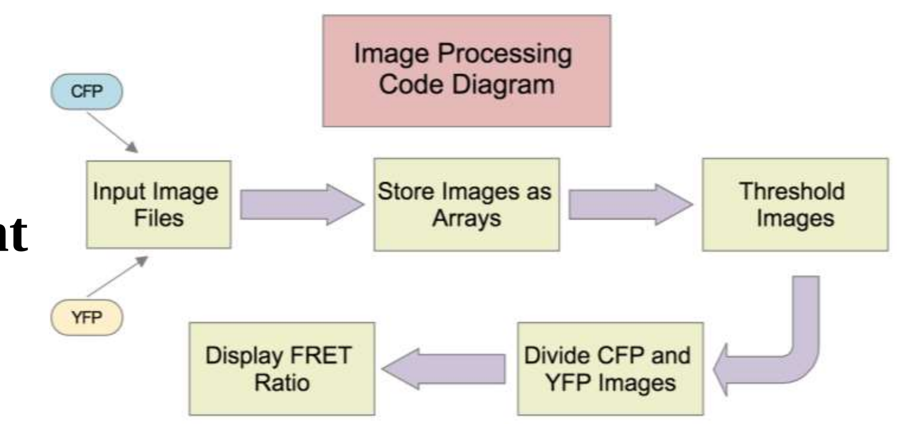

Figure 5. Block diagram covering general protocol of image analysis in software

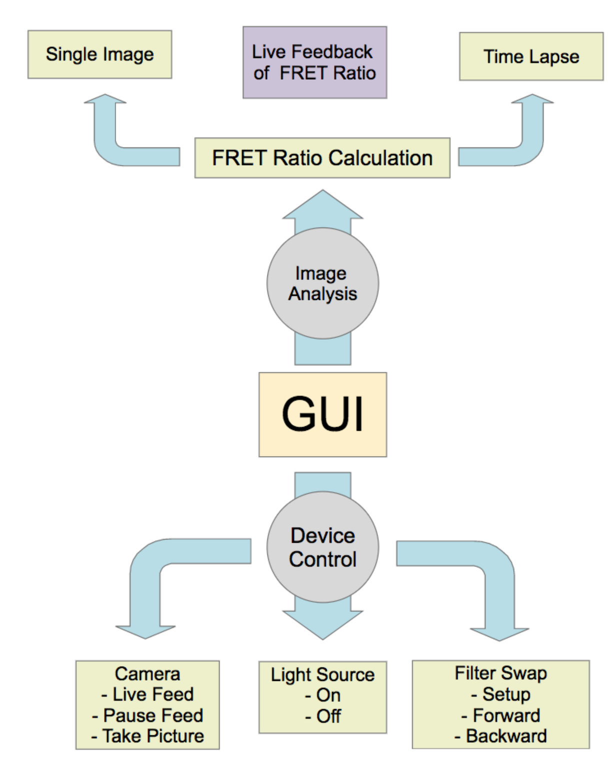

Figure 6. Schematic overviewing the functionality of microscope GUI

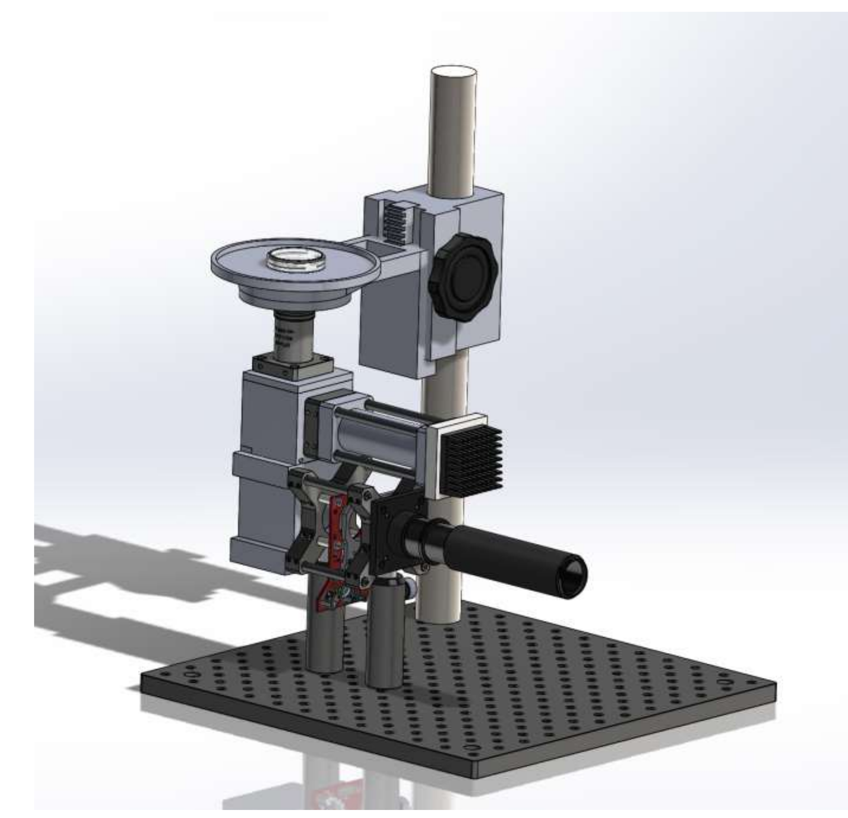
Figure 7. Computer designed rendering of microscope assembly

Future Work

- Refine the GUI and improve its ergonomics
- Make the LED light source more user-friendly and improve its ergonomics
- Replace the circuitry with a PCB board
- Perform a cost analysis to lower the prototype cost below $4,000
- Consider ways to improve factors that affect the microscope's image quality
- Improve the accuracy of the FRET calculations and image processing

References

[1] San Martín A, Ceballo S, Ruminot I, Lerchundi R, Frommer WB, et al. (2013) A Genetically Encoded FRET Lactate Sensor and Its Use To Detect the Warburg Effect in Single Cancer Cells. PLOS ONE 8(2): e57712. doi: 10.1371/journal.pone.0057712
[2] Alex M Mooney (https://commons.wikimedia.org/wiki/File:FRET_Jabolinski _Diagram.svg), "FRET Jabolinski Diagram", https://creativecommons.org/licenses/by-sa/3.0/legalcode