Miniature Microscope for FRET Microscopy

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Client: Professor Matthew Merrins
Advisor: Professor Jeremy Rogers

Abstract

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

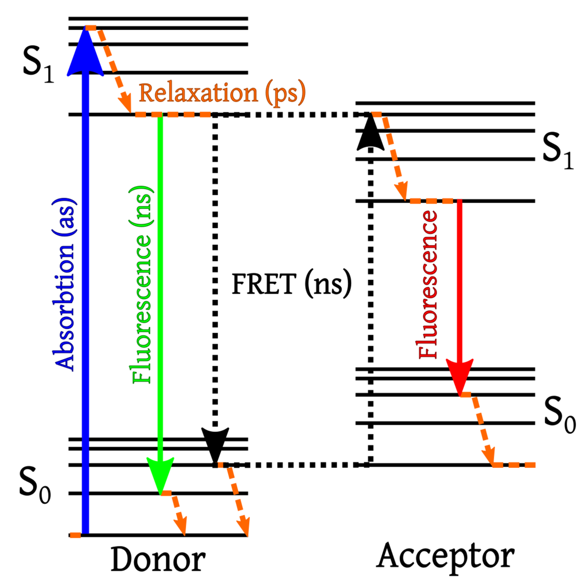

Figure 1. Diagram of FRET concept [2]

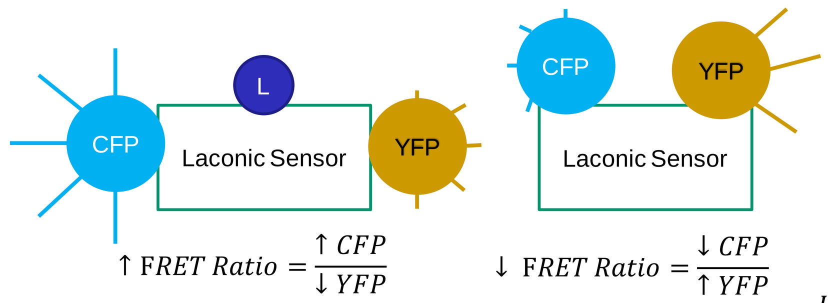

Figure 2. Diagram of the Laconic Biosensor

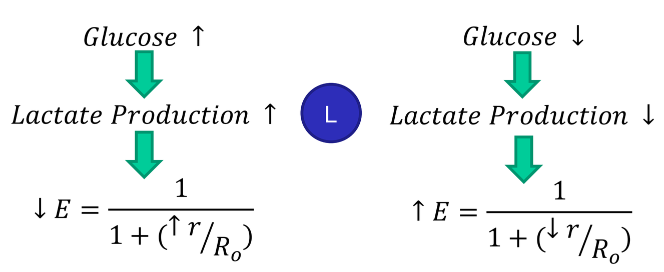

Figure 3. Explanation of how the FRET ratio changes with glucose concentration

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:*** <$10,000 per microscope
- ***Reliability:*** consistent results from experimentation, with similar results to client's microscope
- ***Operability:*** intuitive for student use, easy handling/storage

Testing

Measuring the FRET Ratio

- Imaged islets with 20 mM glucose solution on our prototype microscope
- Determined the FRET ratio by subtracting the background and dividing the 470 nm channel by the 535 channel
- Determined smallest detectable change in FRET ratio

Optical Resolution

- Used 50 mm tube lens and a 40X 0.65 NA objective to image test chart
- Calculated minimum spatial resolution from test chart

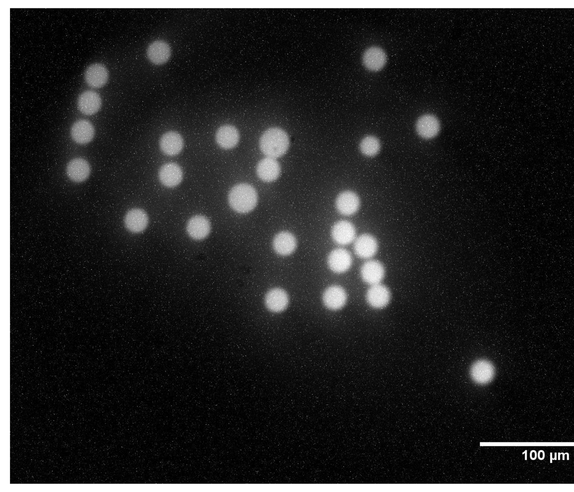

Figure 4. Image of 20um fluorescing microspheres.

Results

- Smallest detectable change accounting without for fixed pattern noise

$$\frac{100}{FR}\left(\frac{C_{470}-B.G+Noise@500ms}{C_{535}-BG}-\frac{C_{470}-B.G}{C_{535}-BG}\right)=$$

$$\frac{100}{0.6}\left(\frac{80-48+27}{160-48}-\frac{80-48}{160-48}\right)=40.2\%$$

- Accounting for fixed pattern noise

$$\frac{100}{0.6}\left(\frac{80-48+7.5}{160-48}-\frac{80-48}{160-48}\right)=11.2\%$$

- Smallest detectable change 10-bit camera assuming same noise levels

$$\frac{100}{0.6}\left(\frac{320-48+7.5}{643-48}-\frac{320-48}{643-48}\right)=2.1\%$$

- Minimum Spatial resolution is 2.19 µm

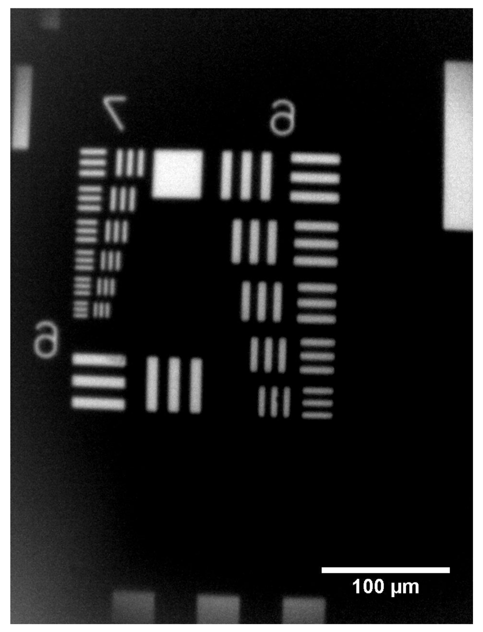

Figure 5. Image of bar chart, used for characterizing microscope system.

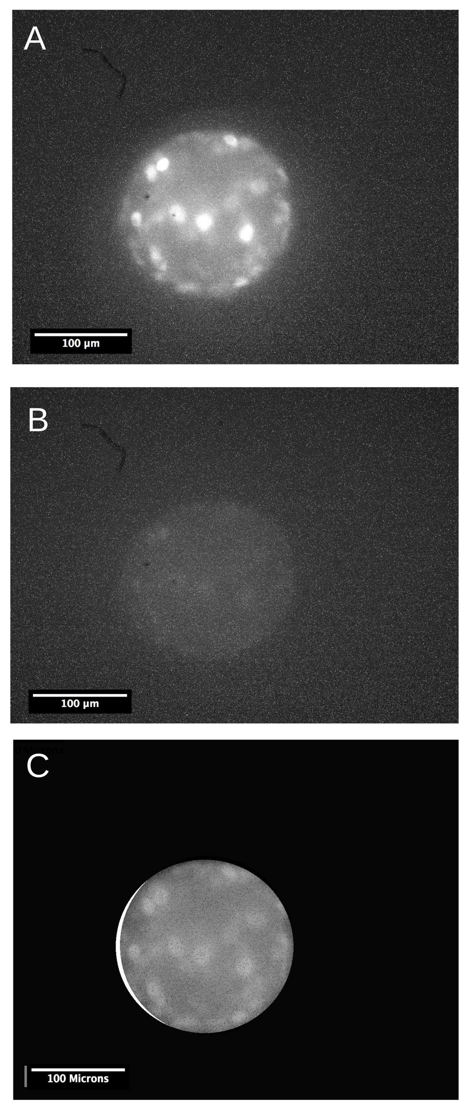

Figure 6. Images taken on the prototype microscope at 500 ms (A) 535nm filter (B) 470nm filter (C) FRET ratio image

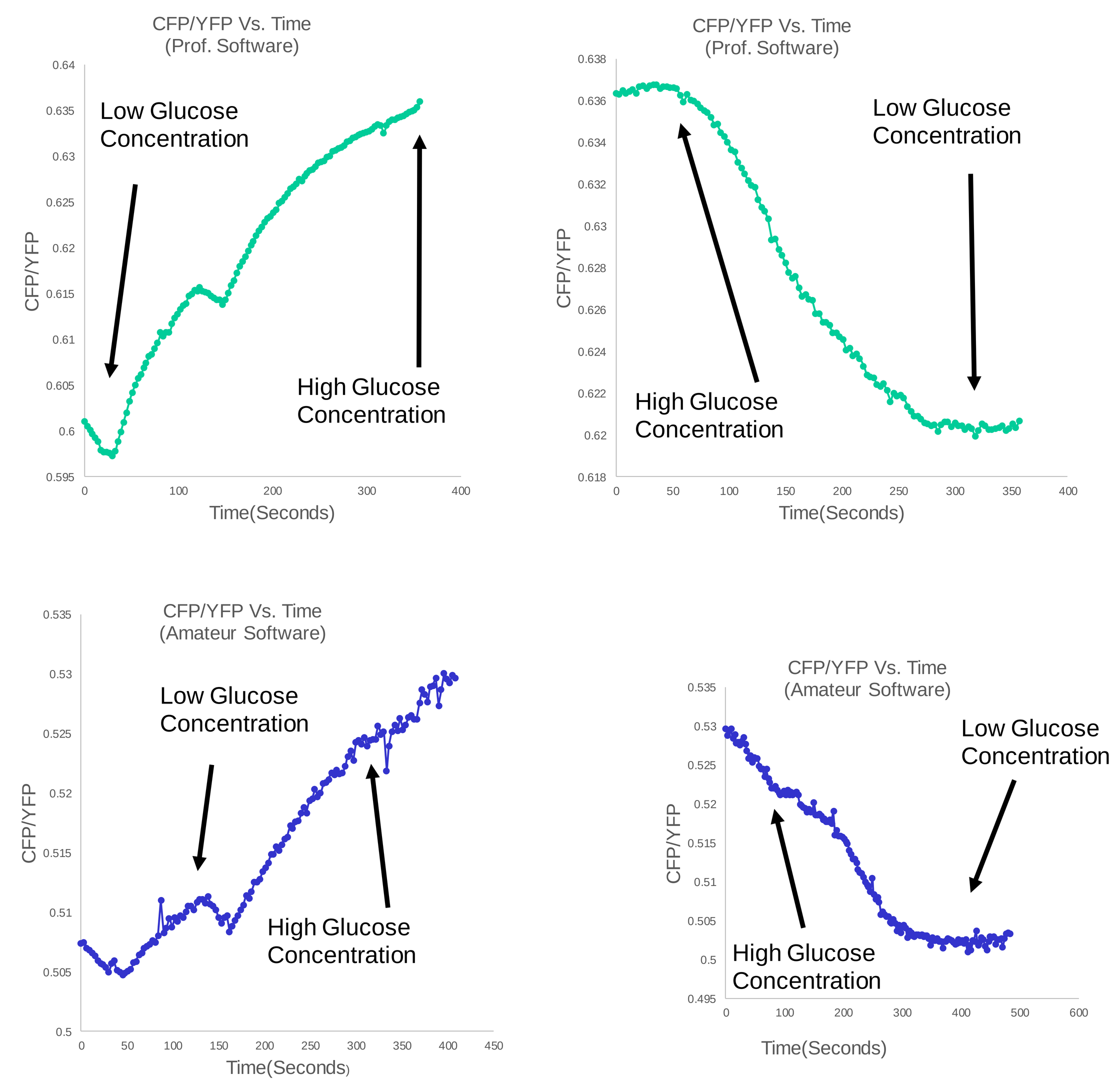

Figure 7. Left Col. Measured FRET Ratio over time going from low glucose to high glucose. Right. Col. Measured FRET Ratio over time going from high glucose to low glucose

Acknowledgements

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Final Design

Optical Components

- Tube lens
- Objective lens
- Emission and excitation filters
- Fold mirror
- Dichroic mirror

Mechanical Components

- 3D printed cell dish
- Z-axis focuser

Software

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

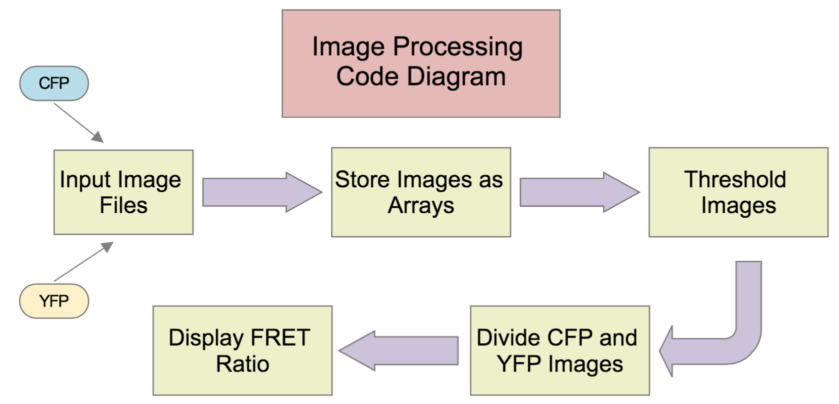

Figure 8. Block diagram covering general protocol of image analysis in software

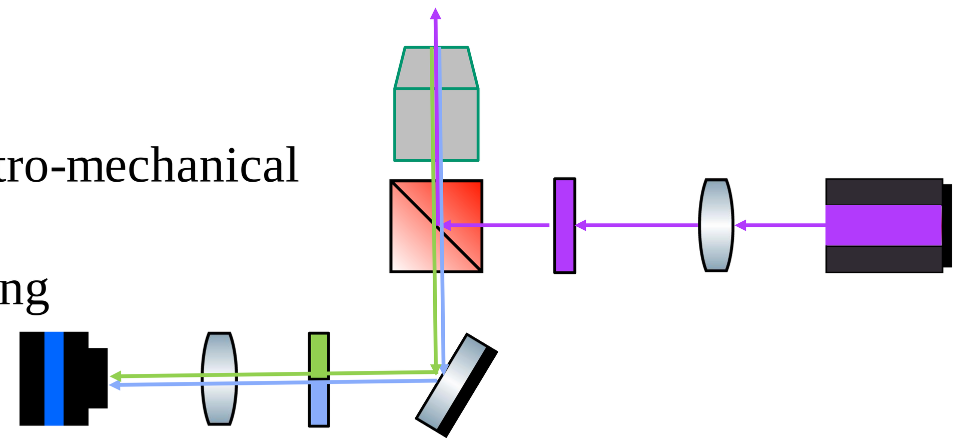

Figure 9. Block diagram covering general protocol of image analysis in software

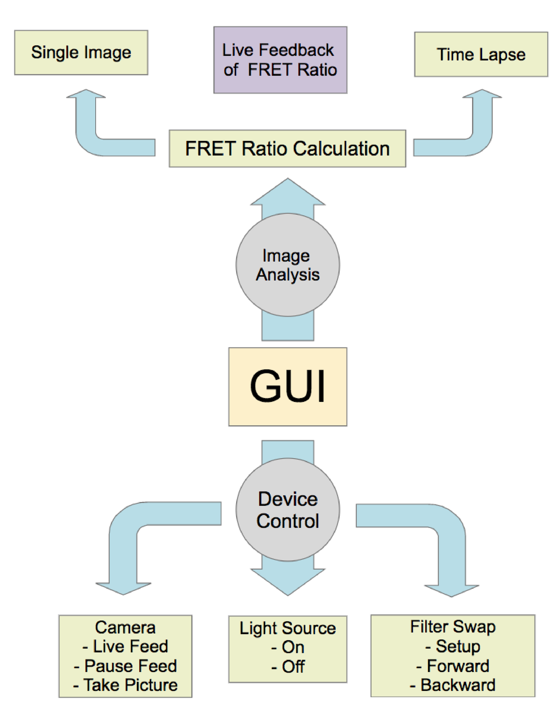

Figure 10. Schematic overviewing the functionality of microscope GUI

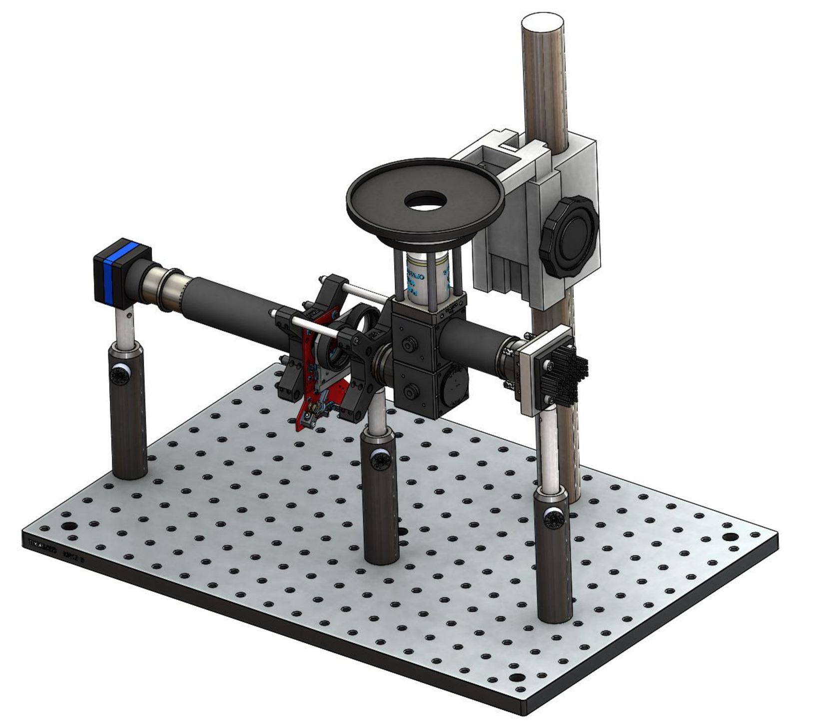

Figure 11. Computer designed rendering of microscope assembly

Future Work

- Testing with the 40X Super Fluor with a NA of 1.3
- Testing improved cameras:
  - ThorLabs – CS2000100M Quantalux
  - Nikon – CoolSnap Dyno
- Update GUI to interface with new camera
- Modulation Transfer Function (MTF) testing to characterize microscope resolution
- Final time-lapse comparison between our prototype and the professional microscope

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