Miniature Microscope for Fluorescence Imaging

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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

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

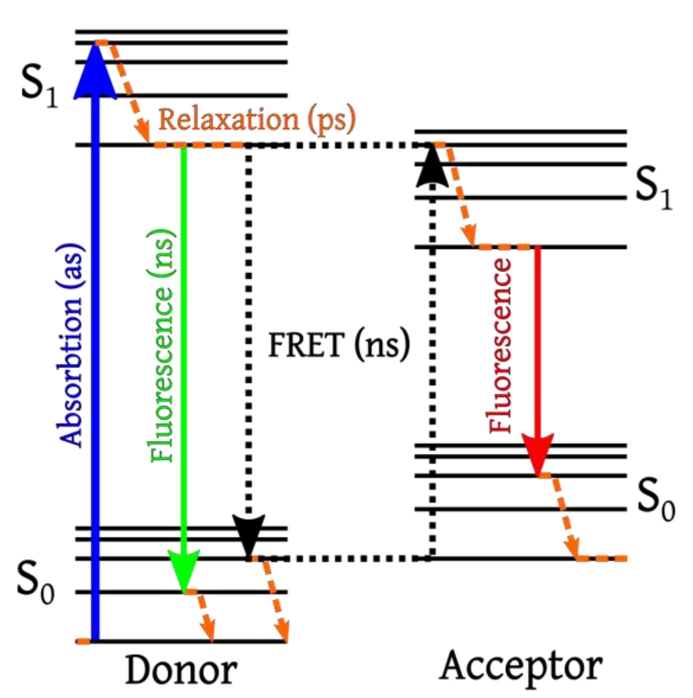

Figure 1. Diagram of FRET concept [2]

Design Criteria

- ***Cost:*** <$2,000 per microscope
- ***Image Quality:*** significant & detectable change in fluorescence between 470 & 535 nm from 430 nm source
- ***Ergonomics:*** intuitive for student use, easy handling/storage
- ***Dependability:*** consistent results from experimentation, with similar results to client's microscope
- ***Manufacturability:*** repeatable for manufacturing with limited previous experience
- ***Modularity:*** interchangeable filters and excitation source

Testing

Initial Prototypes:

- Designed sample excitation source for testing
- Preliminary excitation holder design
- Final excitation holder design

Testing Protocol:

- LED characterization
- Compared cameras
  - DMK 42BUC03 (prototype)
  - RCA-Flash4.00 (client's)
- Compared excitation sources

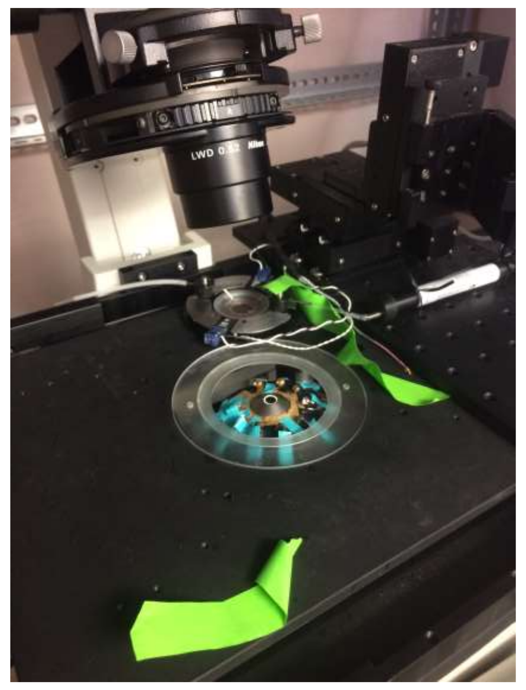

Figure 2. Testing apparatus for light source and camera.

Results

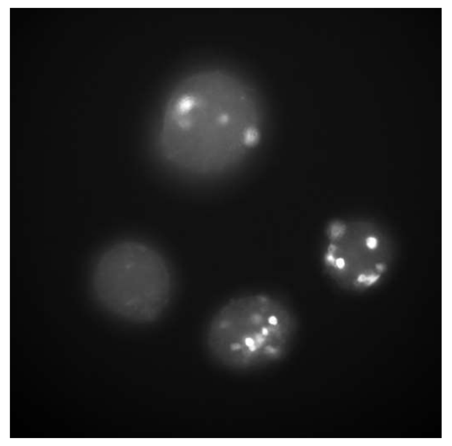

Figure 3. Client camera and light source at 535 nm

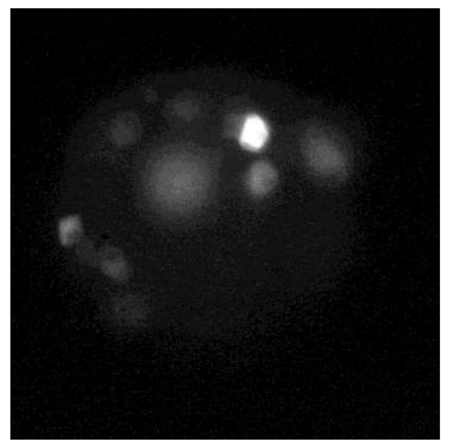

Figure 4. Prototype camera and client light source at 535 nm

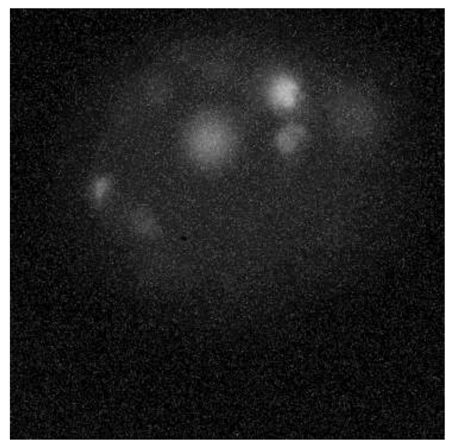

Figure 5. Prototype camera and client light source at 470 nm

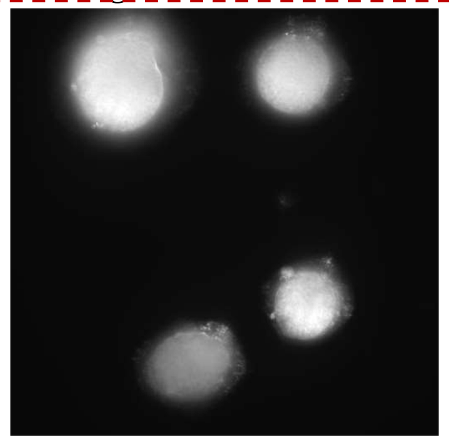

Figure 6. Client camera and prototype light source at 470 nm

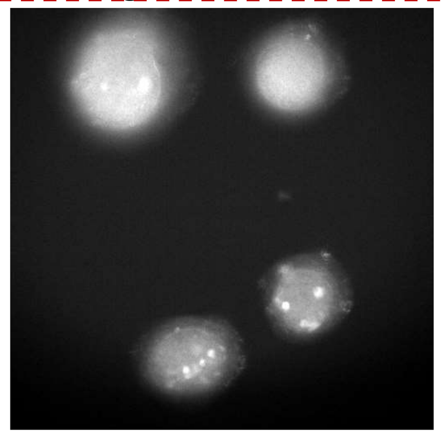

Figure 7. Client camera and prototype light source at 535 nm

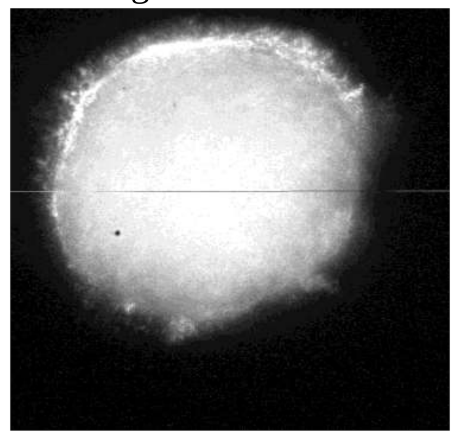

Figure 8. Prototype camera and light source at 470 nm

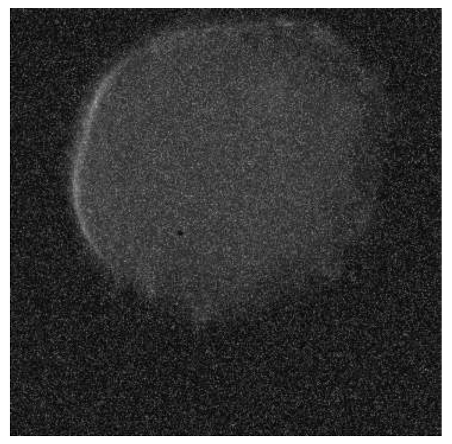

Figure 9. Prototype camera and light source at 535 nm

Prototype vs. Client Camera	
p-value	0.2036
95% CI	-19.69
t -value	1.6205

Table 1. Comparison of four brightest colonies using photos taken by the prototype and clients camera to image each picture at 535 nm

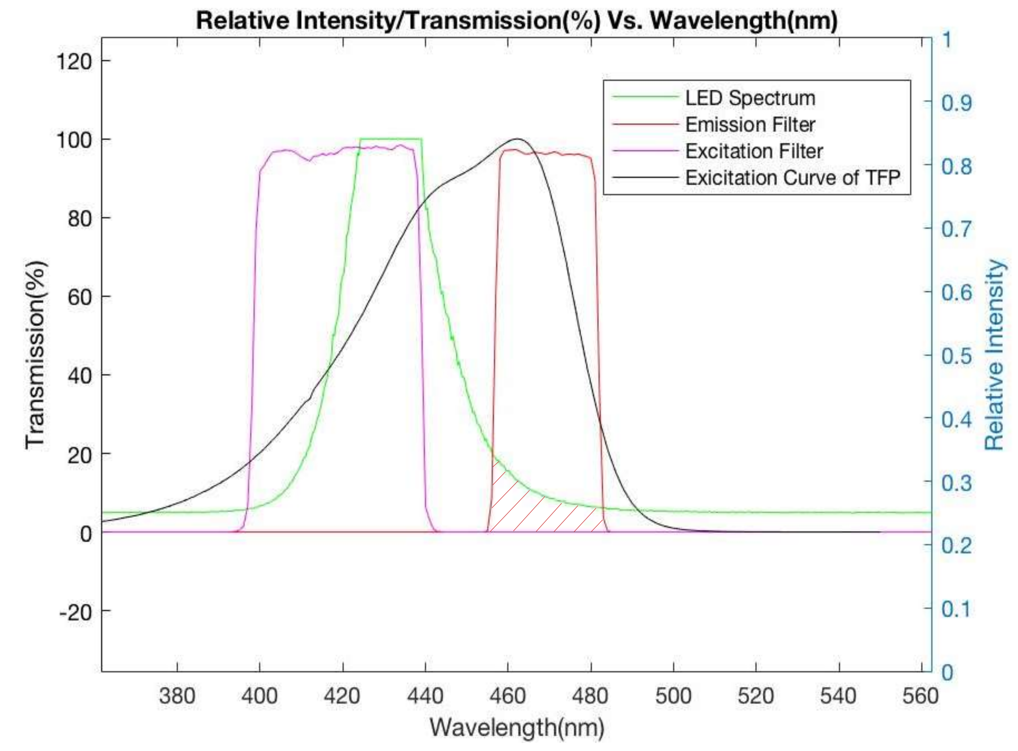

Figure 10. MATLAB analysis of optical components following testing

Acknowledgements

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Final Design

Excitation Source

- Excite cells from above rather than from below
- Add excitation filter to prevent bleed through
- Configure circuit to deliver more current to LED

Mechanical Structure

- Filter swapping mechanism
- Z-adjustment support knob
- Combined excitation source and sample platform
- Vertical alignment of components

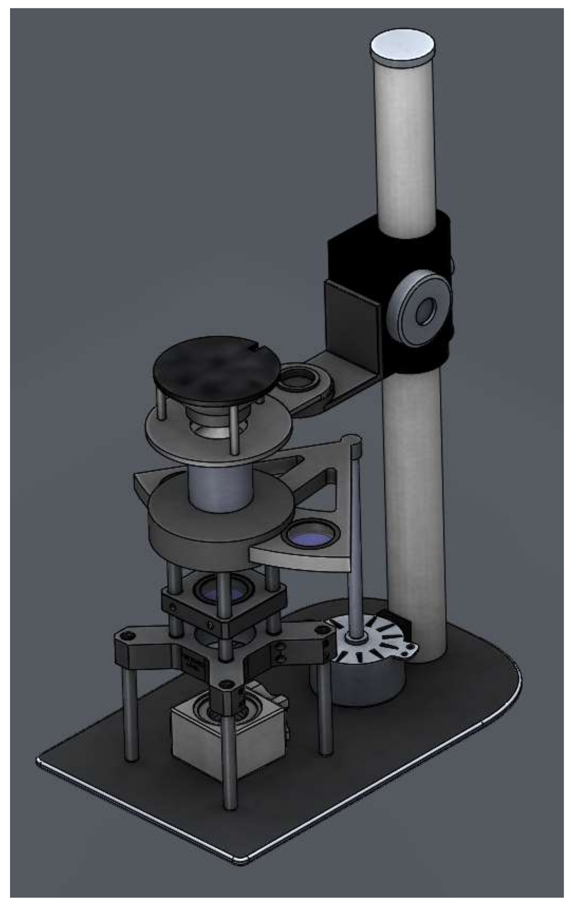

Figure 11. Testing apparatus for light source and camera.

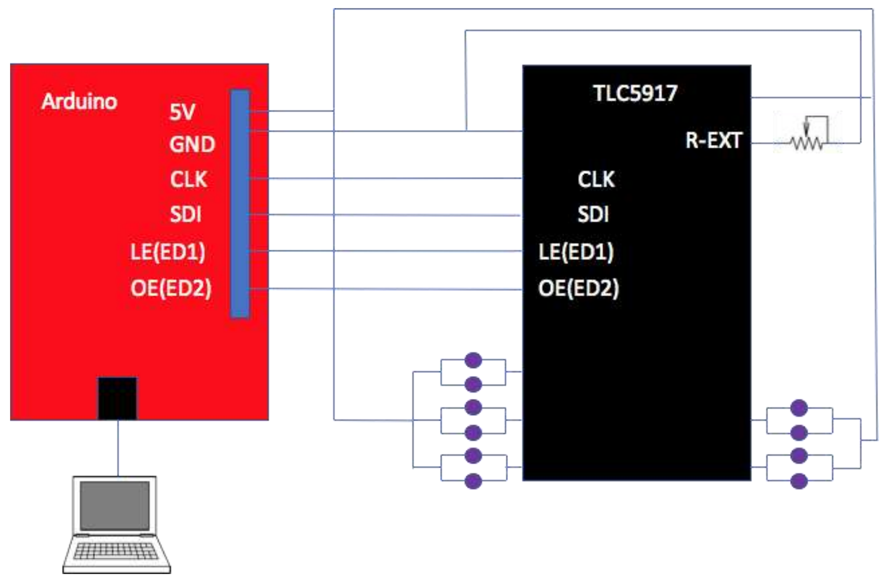

Figure 12. Block diagram of the circuit used in the LED excitation source. The TLC5917 is an 8-bit shift register.

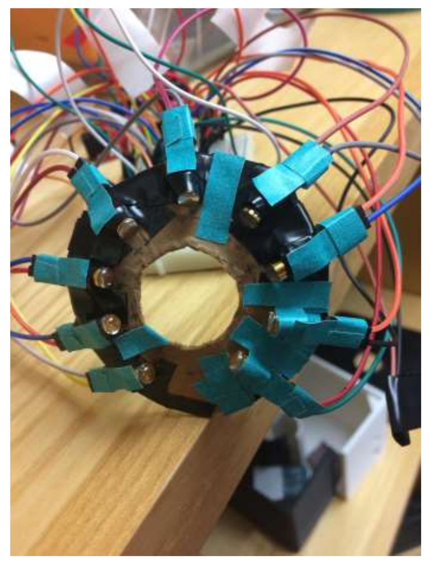

Figure 13. Final Testing apparatus for light source and camera.

Future Work

- Test modified excitation source configuration
- Configure & test full beam-path profile:
  - Objective
  - Emission filters
  - Tube lens
- Finalize filter-swap method:
  - Stepper motor
  - Filter holder
  - Swapping automation
- Complete microscope structure:
  - 3D printed parts
  - Full system integration
  - Integrate with Raspberry Pi

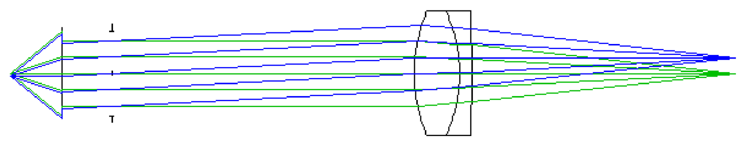

Figure 14. OSLO lens simulator

Figure 15. Rendering of filter-swap mechanism

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