DEPARTMENT OF
Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

Miniature Microscope for Fluorescence Imaging

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Client:
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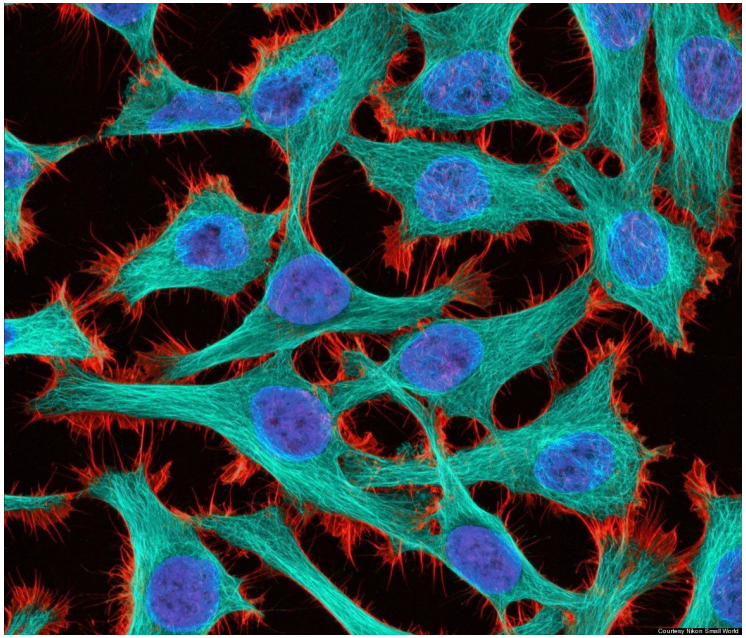
Overview

- Problem Statement
- Background
  - FRET Microscopy
- Competing Designs
- Summary of PDS
- Design Alternatives
- Design Matrix
- Future Work

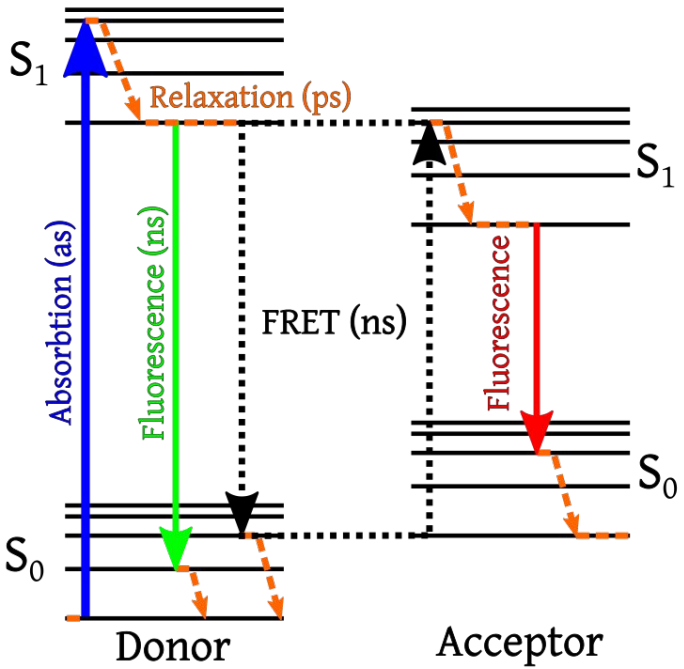


Problem Statement

- Client teaches human biochemistry lab
- Enzyme lactate dehydrogenase
- Fluorescence (Förster) Resonance Energy Transfer (FRET) biosensor
- Microscope excites lactate biosensor using LEDs
- Current microscope is expensive
- Goal is to build a low-cost microscope

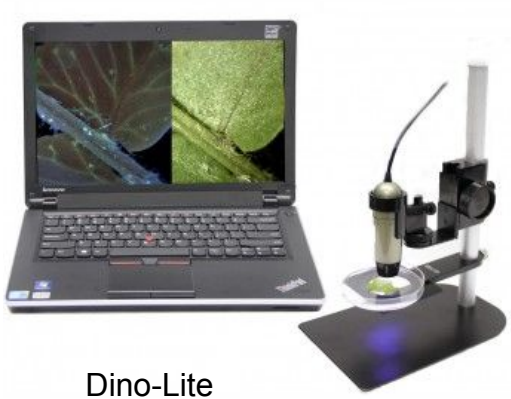
FRET Microscopy- Background

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



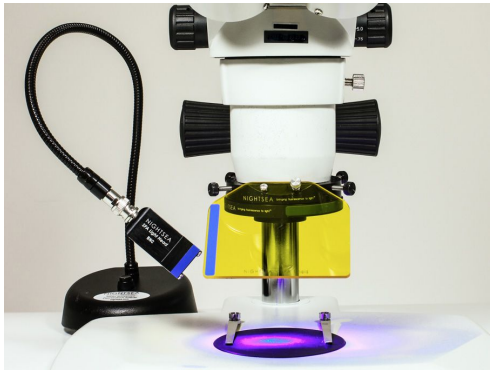
Competing Products

- Dino-Lite
  - Simple fluorescent set-up
  - Not FRET
- Lumascope 620
  - Broad-use FRET system
  - Expensive
- NightSea Model SFA
  - Stereo to fluorescent conversion system
  - No data acquisition



Dino-Lite

Lumascope 620



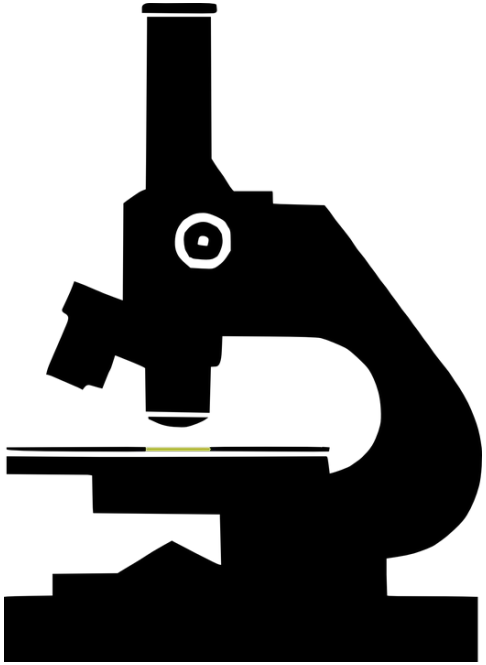
NightSea

Summary of PDS

- **Function**: single prototype FRET microscope
  - Excitation source - 430 nm
  - FRET filters - 470 nm (donor) 535 nm (acceptor)
  - Camera- detect and upload to free software analysis
- **Accuracy**: determine acceptor-donor FRET ratio
- **Ergonomics**: easy and intuitive to use by students
- **Size**: all non-essential microscopy components eliminated (eyepiece, etc.)
  - Base size ~ 20 by 30 cm
  - Height ~ < 45 cm
- **Cost**: < $2,000

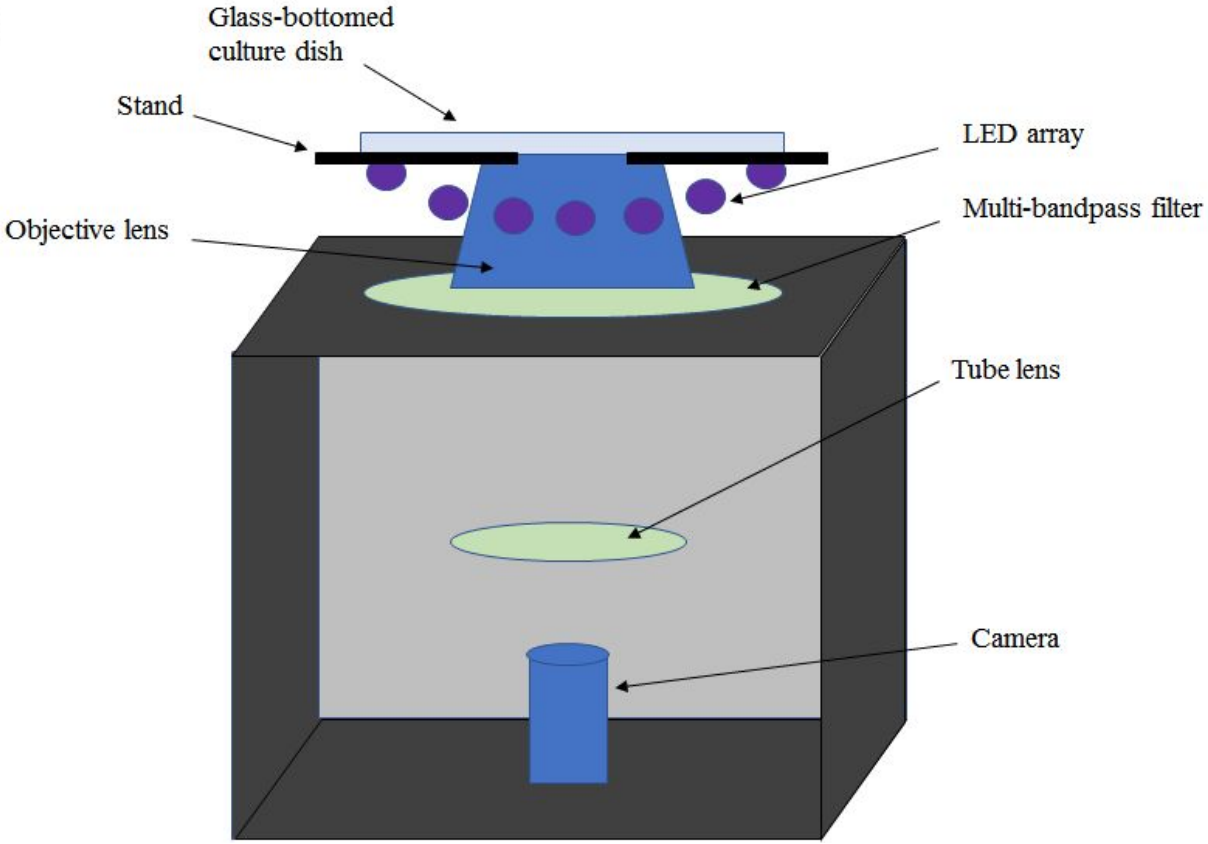
Design Alternatives

- Single-Shoot
- Filter-Swap
- Beam-Splitter



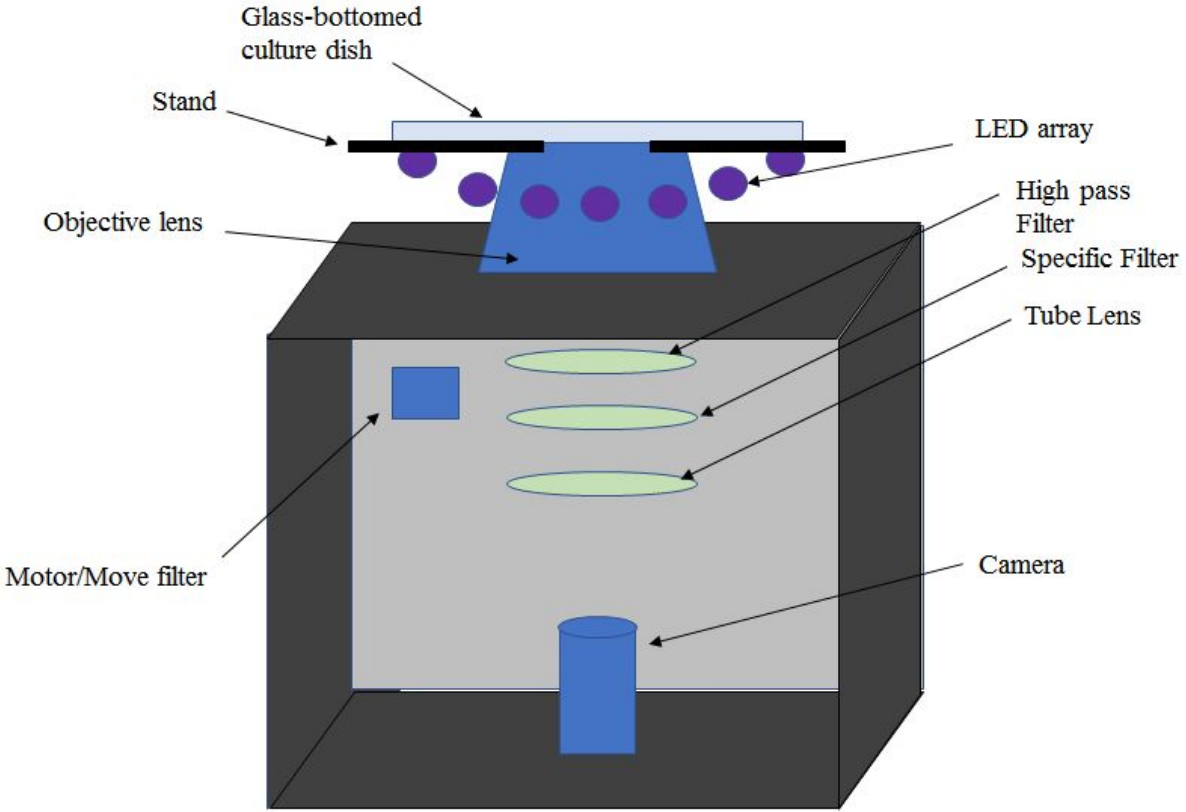
Design 1: Cost Analysis "Single-shoot"

Part	Cost
Camera	$355
Objective Lens	$196
Multi-bandpass filter	$350
Tube Lens	$150
LEDs	$115
Stand	$100
Circuitry/Power	$50
Box	$20
TOTAL:	$1336 Infinite



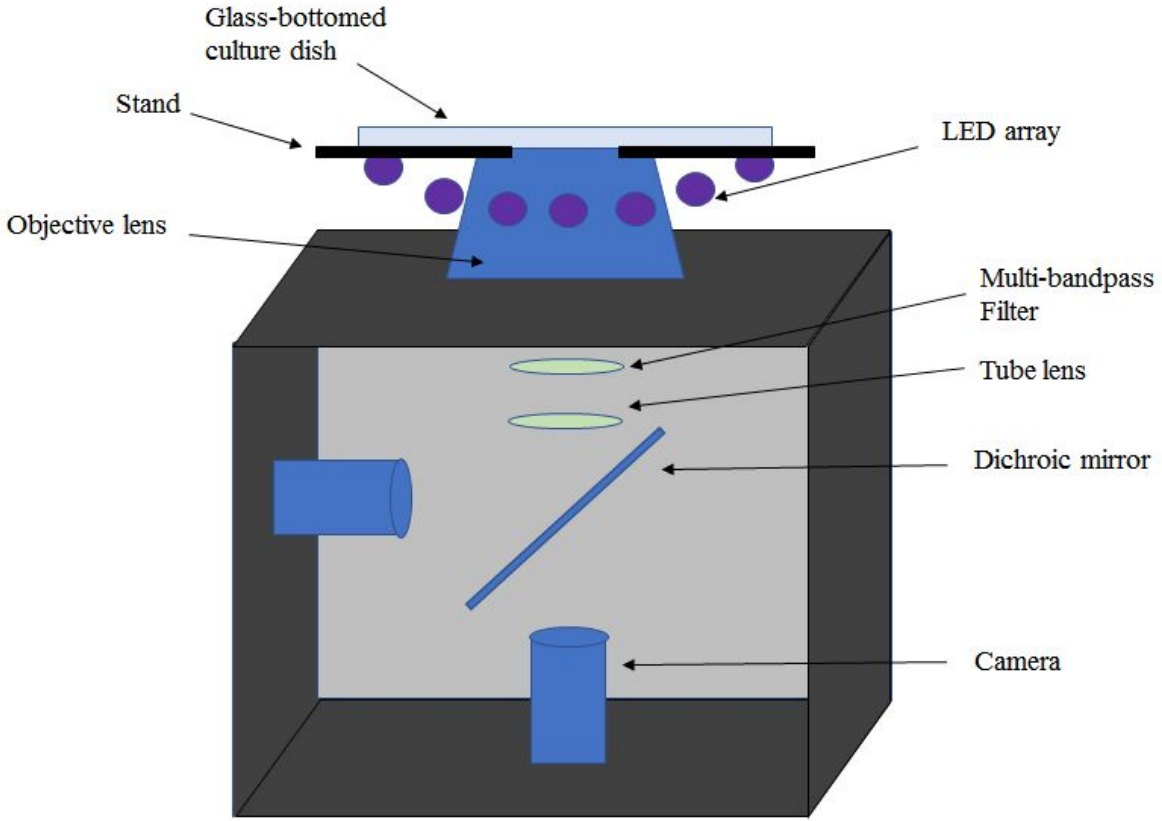
Design 2: Cost Analysis "Filter-swap"

Part	Cost
Camera	$355
Objective	$196
Filters	$340
Move Filters	$10
LEDs	$115
Tube Lens	$150
Stand	$100
Circuitry/Power	$80
Box	$20
TOTAL:	$1366



Design 3: Cost Analysis "Beam-Splitter"

Part	Cost
Cameras	$710
Objective	$196
Beam Splitter	$113
LEDs	$115
Multi-bandpass filter	$350
Stand	$100
Tube Lens	$150
Circuitry/Power	$50
Box	$20
TOTAL:	$1804 Infinite



Design Matrix

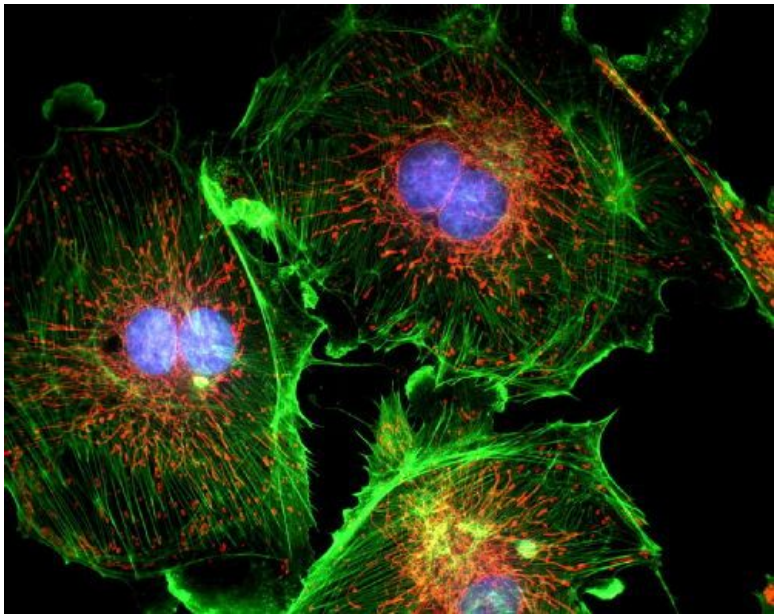
	Single-Shoot	Filter-Swap	Beam-Splitter
Cost (25)	**3/5: 15**	**3/5 : 15**	2/5: 10
Client Input (20)	3/5 : 12	**5/5 : 20**	1/5 : 4
Image Quality (15)	3/5 : 9	4/5 : 12	**5/5 : 15**
Ergonomics (15)	3/5 : 9	3/5 : 9	**5/5 : 15**
Dependability (15)	**4/5 : 12**	3/5 : 9	**4/5 : 12**
Manufacturability (10)	**5/5: 10**	3/5 : 6	3/5 : 6
Total:	67	**71**	62

Potential Pitfalls

- Detector not sensitive enough
- Timing of image acquisition
- Excitation Source bleed through
- Intensity of light source

Conclusion and Future Work

- Fabricate and test excitation source
- Properly space components
- Test prototype with mammalian cells



Questions?

References

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

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