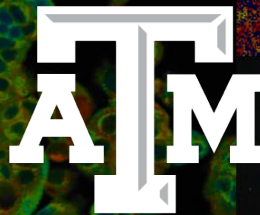


Controlled Manipulation of Cell Function with Infrared Light

Alex Walsh, PhD
(she/her)
Cain Faculty Fellow
Biomedical Engineering
Texas A&M University



TEXAS A&M
UNIVERSITY®

Quad Chart



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

Objective:

- Determine whether short pulses of infrared light stimulate intracellular calcium signaling within non-excitable cells
- Evaluate whether short pulses of infrared light can alter the function of immune cells, fibroblasts, and stem cells
 - Technology development to uniformly and consistently irradiate populations of cells for functional assays
- Bridge to in vivo experiments – does infrared light alter macroscopic behaviors/functions?

Accomplishments:

- Training for 2 Graduate Students (Jocelyn Martinez, Anna Theodossiou), 1 Undergraduate (Emily Nelson)
- Enzyme reaction rate model and experimental testing
- Data of IR induced calcium transients in macrophages
- Design and initial assembly of a uniform illumination multi-well exposure platform

Technical Approach:

- Modeling biochemistry, laser-tissue interactions, imaging endpoints
- Imaging experiments to capture spatial and temporal dynamics of cells responses (metabolic, calcium, etc)
- **Uniqueness:** Imaging tools that focus on capturing dynamics across spatial scales.

DOD Benefit:

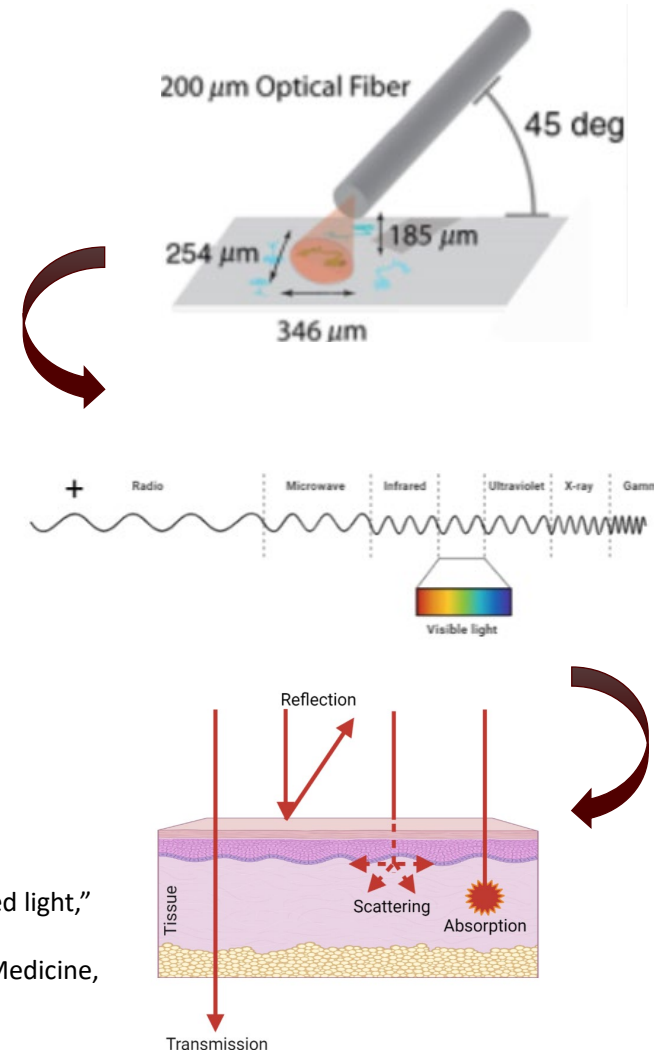
- Characterize biophysical effects of short pulses of infrared light
- Increase our knowledge of fundamental light-matter interactions
- Potential for non-contact interfacing and control of biological function

Infrared Neural Stimulation (INS)



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

- Short pulses of infrared light can stimulate and inhibit action potentials in neurons
 - Biophysical effects are poorly understood
- Method can be beneficial
 - Label-free and noninvasive
 - High spatial specificity
 - Adjustable stimulation depth



Optical Fiber

- Typical diameter: 100 to 400 microns

Infrared Light

- Wavelengths: 1400-2100 nm
- Order: micro- to milliseconds

Photons

- Transmitted
- Reflected
- Scattered
- Absorbed

[1] Chernov, M. and Roe, A. W., "Infrared neural stimulation: a new stimulation tool for central nervous system applications," Neurophotonics, 1(1), 1-4(2014)

[2] Walsh, A. J., Tolstykh, G. P., Martens, S., Ibey, B. L., and Beier, H. T., "Action potential block in neurons by infrared light," Neurophotonics, 3(4), 040501(2016)

[3] Lilijemalm, R., Nyberg, T., and Holst, H. V., "Heating during infrared neural stimulation," Lasers in Surgery and Medicine, 45(7), 469-481(2013)

[4] Richter et al Laser Photonics Rev. 2011

Infrared Light Effects in Neurons



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

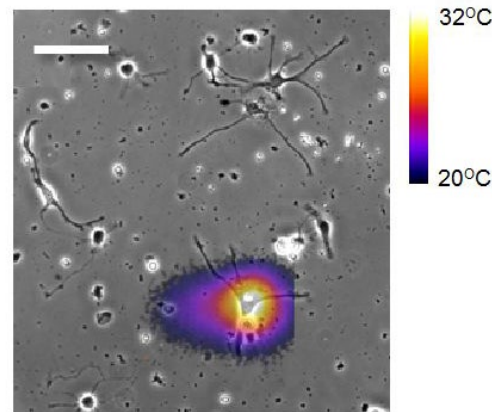
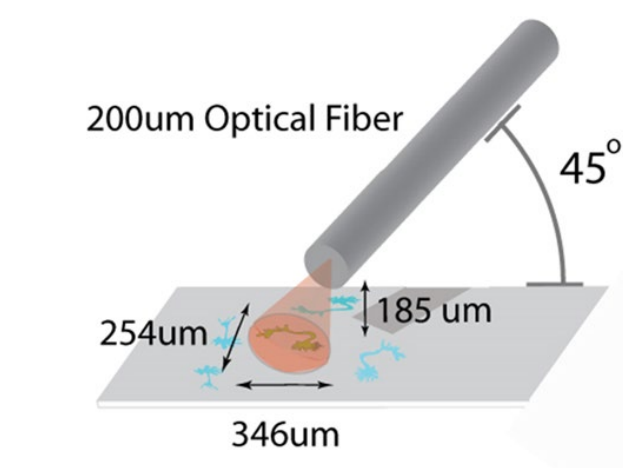
- Short pulses of infrared light can stimulate and inhibit action potentials in neurons
- Method can be beneficial
 - Label-free and noninvasive
 - High spatial specificity
 - Adjustable stimulation depth
- Unknown Photothermal Mechanism

Optical Fiber

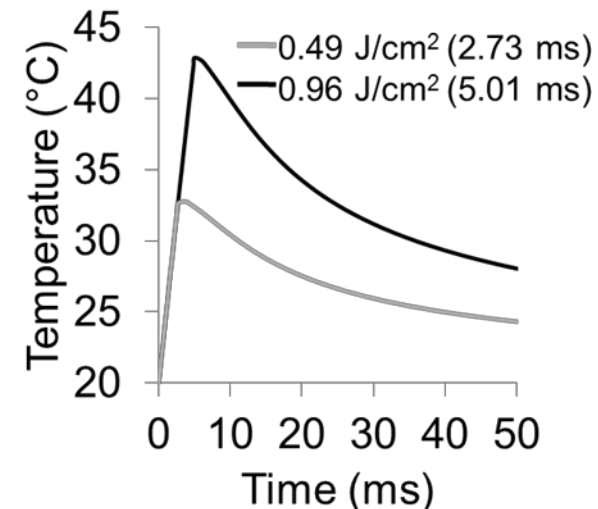
- Typical diameter:
100 to 400
microns

Infrared Light

- Wavelengths:
1400-2100 nm
- Order: micro- to
milliseconds



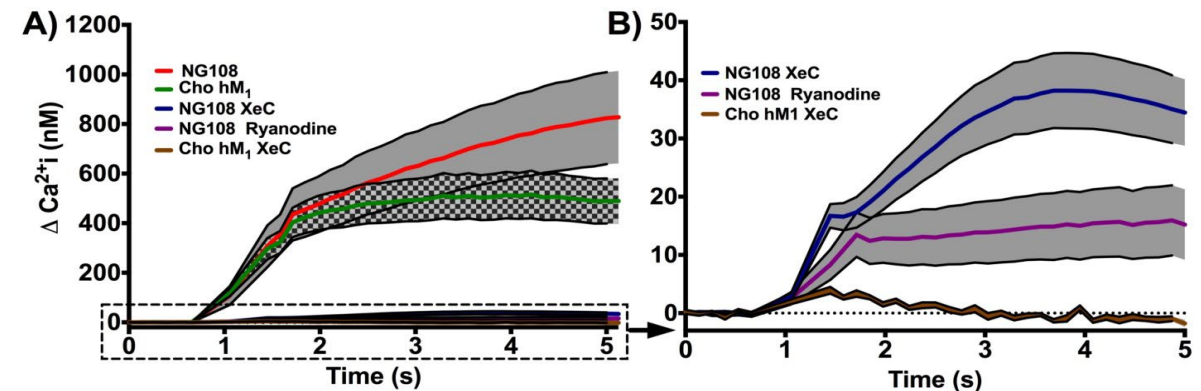
Walsh, et al. Neurophotonics, 2016; 3(4):040501.



Possible Mechanisms



- Direct change of membrane capacitance by a thermal gradient^[1]
- Ion flow through heat activated or stretch activated TRP channels^[2]
- Heat inactivation of ion channels^[3]
- Nanopore formation (and subsequent ion flow)^[4]
- Heat induced increase in membrane fluidity and permeability
- Stimulated calcium-induced calcium release



[1] Shapiro et al. Nat. Commun. 2012.

[3] Wells, et al. Biophys J 2007.

[2] Albert, et al. J Neurophys 2012.

[4] Beier, et al. J Neural Engin. 2014

Tolstikh, et al. Neurophotonics 2017.

Why Study Infrared Stimulation?



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

- Biological mechanism remains poorly understood
- Interaction of physics and biology across multiple spatial and temporal scales
 - Technically challenging to measure responses
 - New imaging and sensing technologies
- Applications:
 - Basic science of biophysical interactions
 - Potential for fundamental neural processing applications
 - Optogenetics without genetics
 - Non-contact interface for electrical stimulation
 - Man-machine interfacing
 - Medical devices (prosthetics, pain control, etc)

My Research Progression with IR stimulation



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering



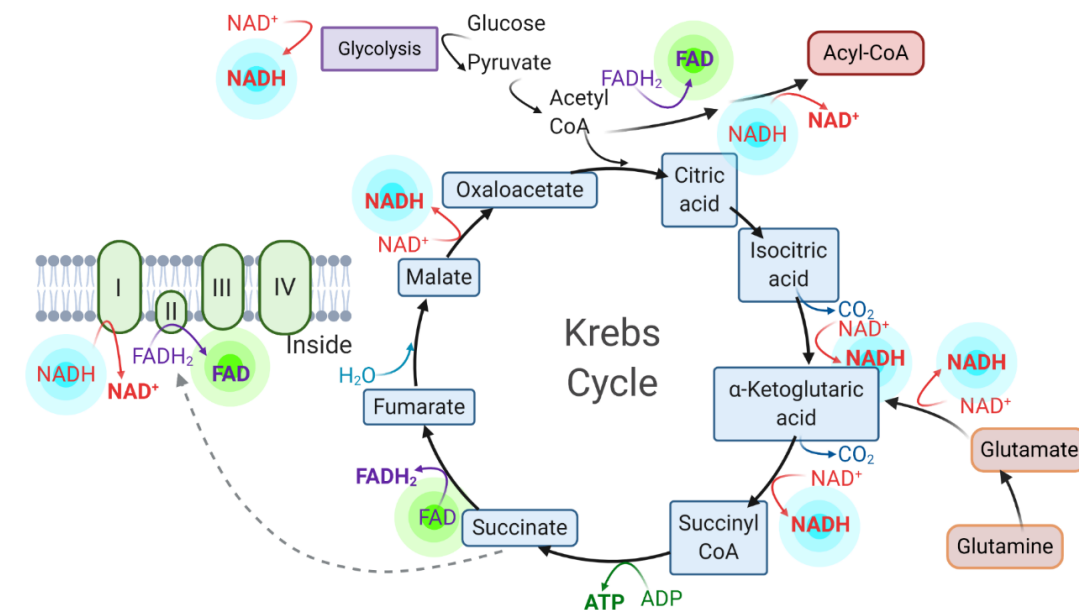
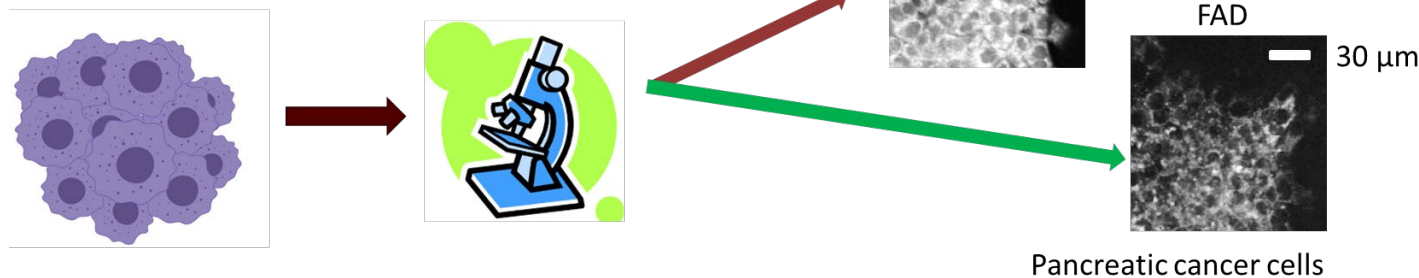
- NRC Postdoc (AFRL)
 - demonstrated ability of IR light to transiently inhibit action potentials in neurons (Walsh, et al. Neurophotonics, 2016)
 - Investigated potential mechanisms: nanoporation, membrane fluidity/permeability, calcium flux (2 SPIE conference presentations + proceedings)
- YIP (TAMU):
 - Model + experimental results on IR-induced thermal gradient effects on metabolism/coenzyme binding (manuscript in preparation)
 - Technology development: Design & characterization of wide-field autofluorescence microscope to capture IR responses (paper in review, J of Biomedical Optics)
 - Training of 2 graduate students (Linghao Hu, Anna Theodossiou) + 2 undergraduates (Sam Morganti, Jocelyn Martinez)
- Current Grant – Controlled manipulation of cell function with IR Light

YIP: Is there a metabolic component to biophysical effects of IR light?



Hypothesis: Short pulses of infrared light interfere with the energy balance of neurons.

- Energy (ATP) is required to activate channels that cause action potentials
- Cellular energy, ATP, is produced from glucose via metabolic reactions
- We can image metabolism via NADH and FAD, co-enzymes of metabolic processes

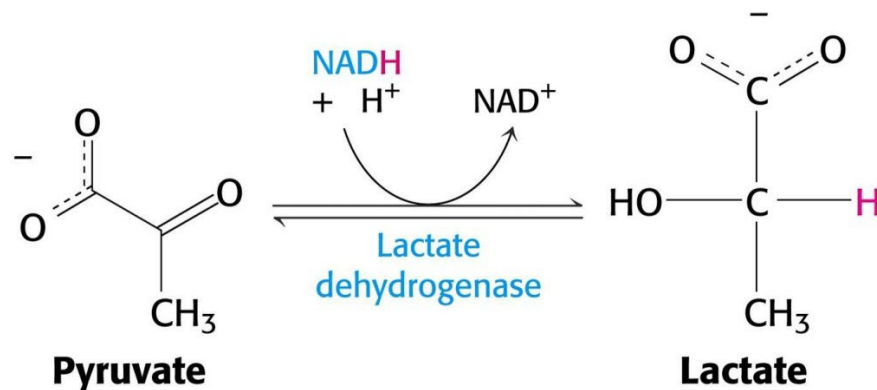


Model + Imaging

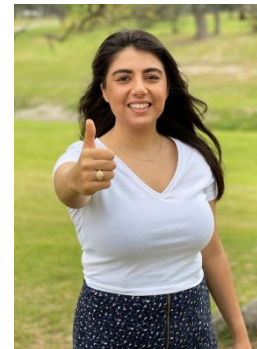


Hypothesis: Short pulses of infrared light interfere with the energy balance of neurons.

- Model:
- Effects of thermal gradient on co-enzyme-enzyme binding.
- NADH:Lactate Dehydrogenase
- validate the model with fluorescence lifetime imaging



Jocelyn
Martinez



Anna
Theodossiou

- Imaging Experiments:
- Measure effect of short pulses of IR light on neuron redox ratio

$$\text{Optical Redox Ratio} = \frac{I_{\text{NAD(P)H}}}{I_{\text{NAD(P)H}} + I_{\text{FAD}}}$$

How fast can we image autofluorescence/metabolism?

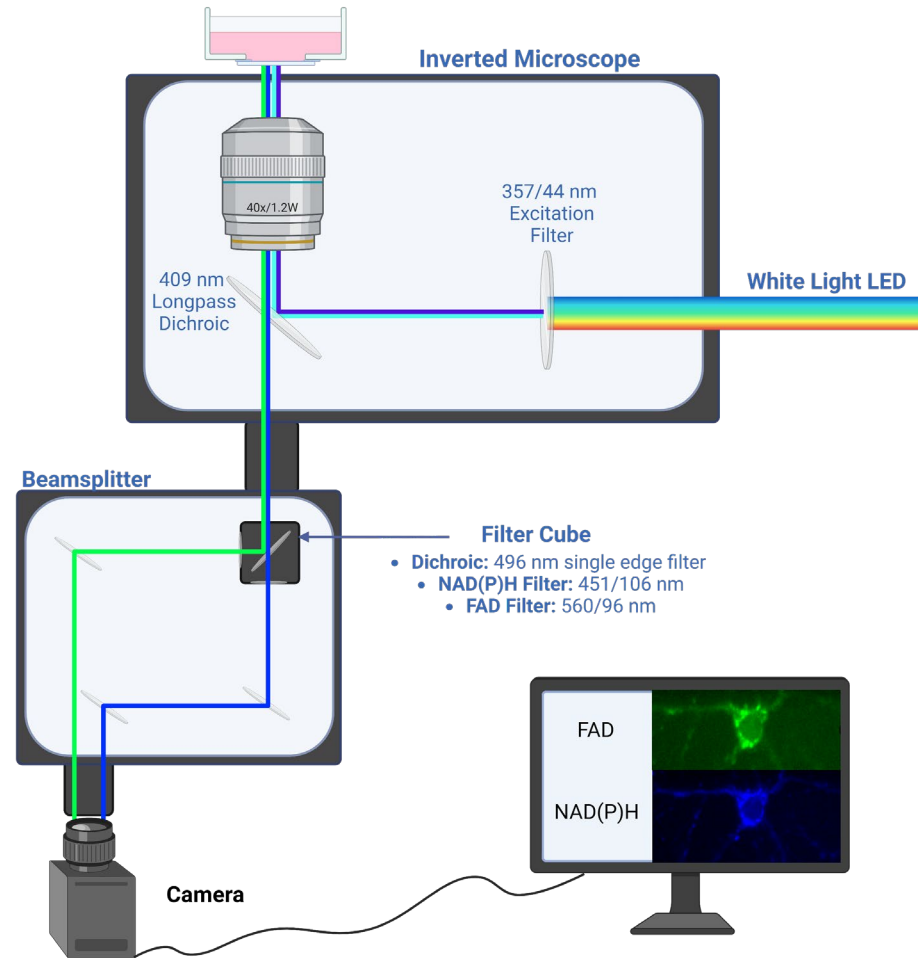


TEXAS A&M UNIVERSITY

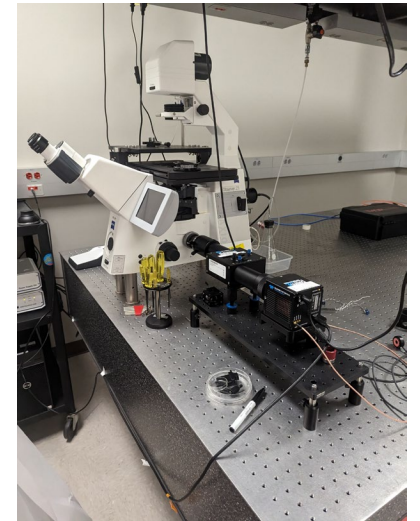
Department of
Biomedical Engineering



Anna
Theodossiou



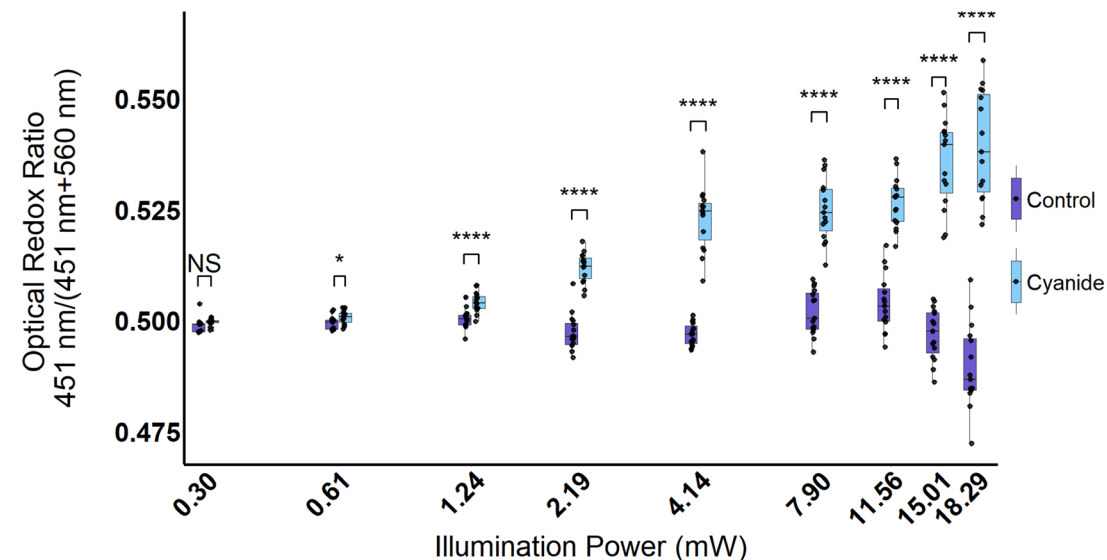
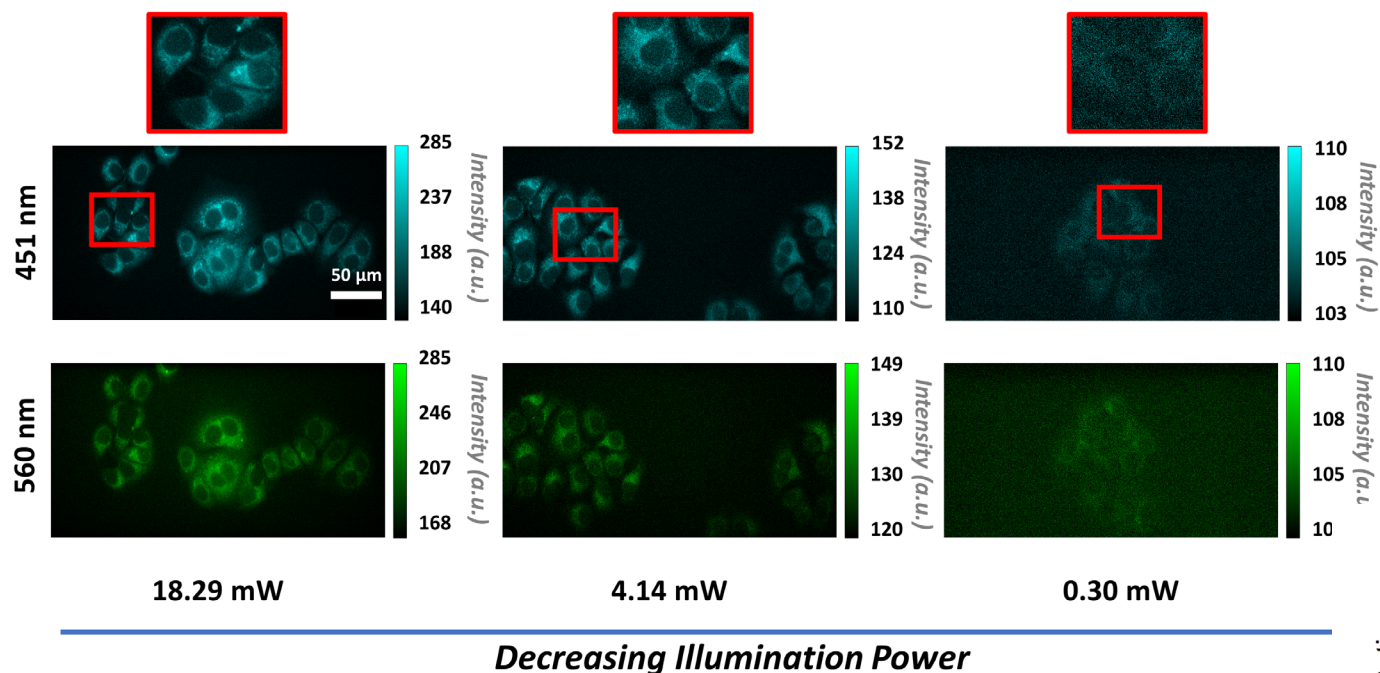
Wide-field fluorescence
microscope

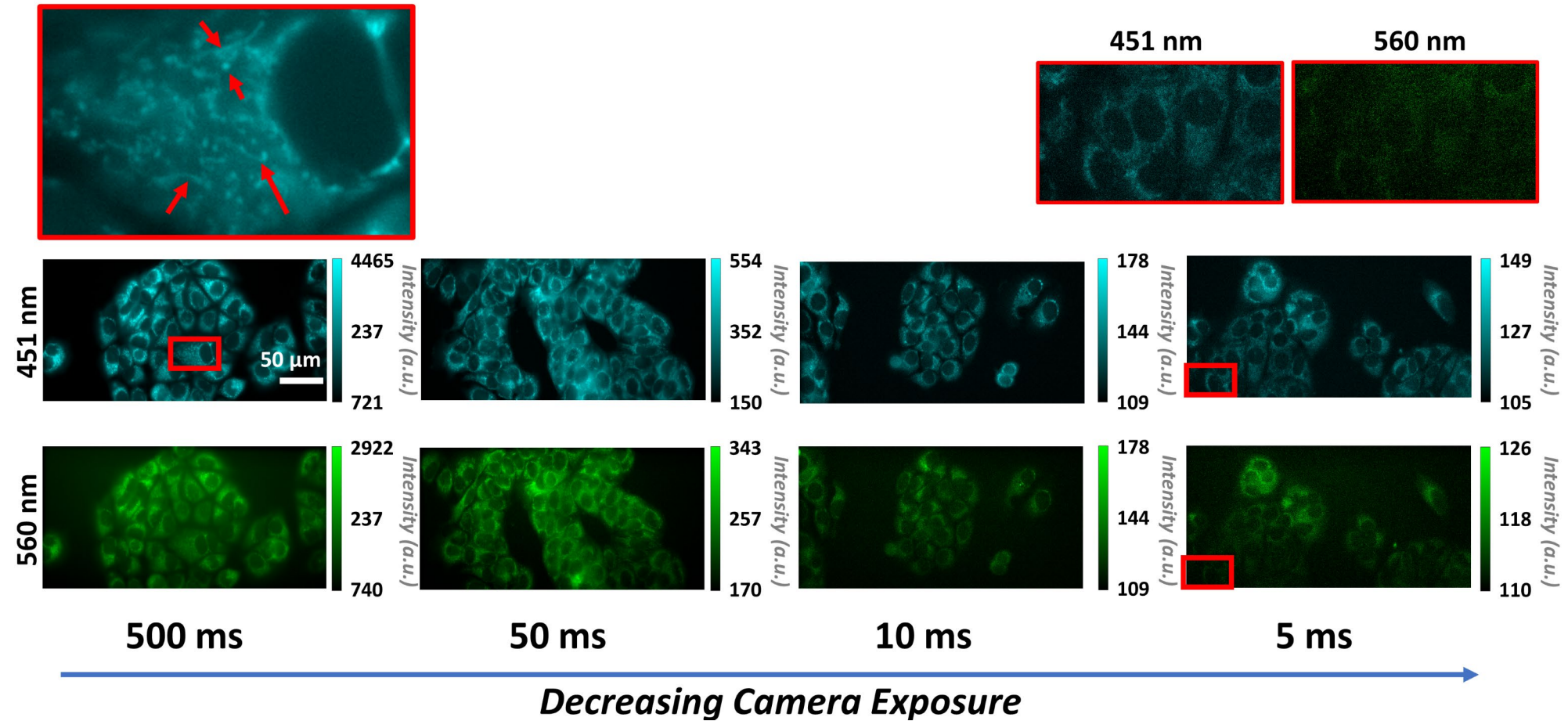


Rapid Imaging of Redox Ratio



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

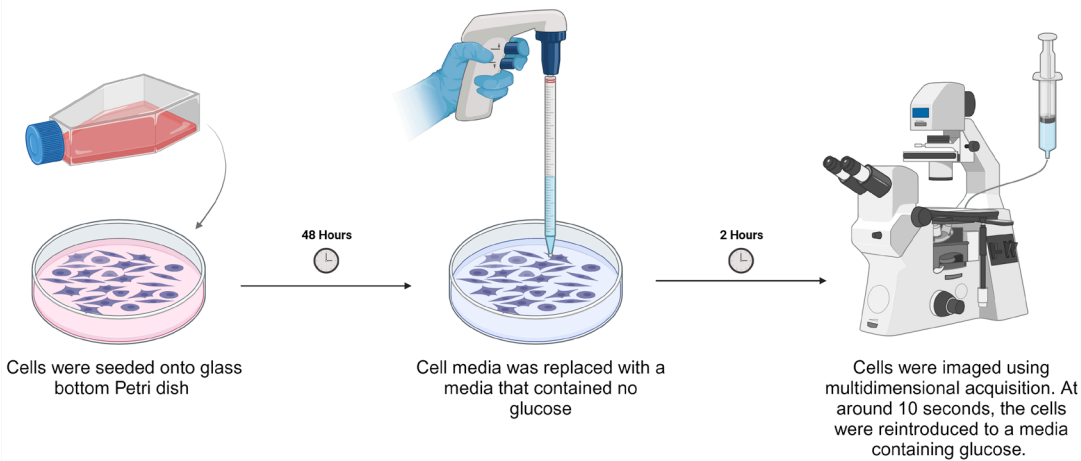




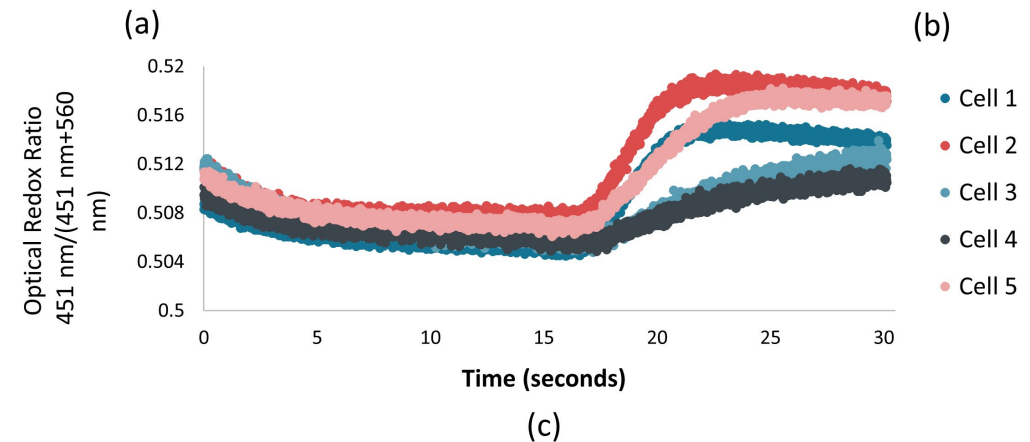
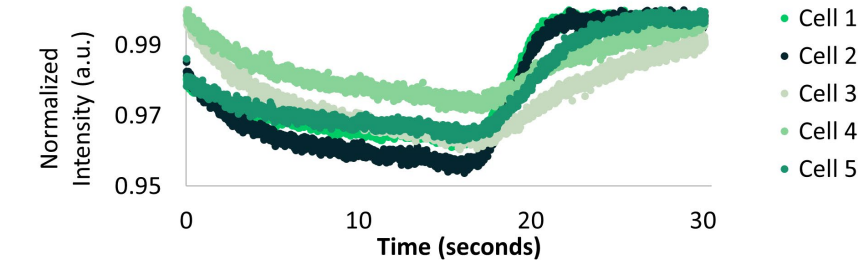
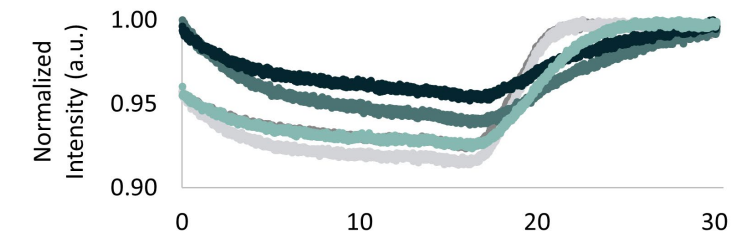
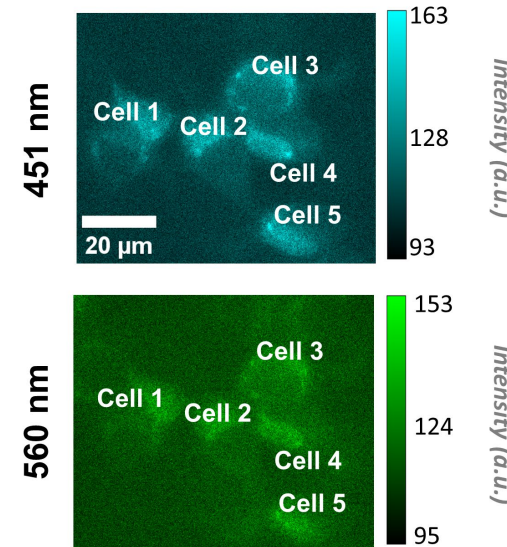
Dynamic Imaging of Metabolism



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering



Major Accomplishment:
Autofluorescence imaging at 100 Hz.

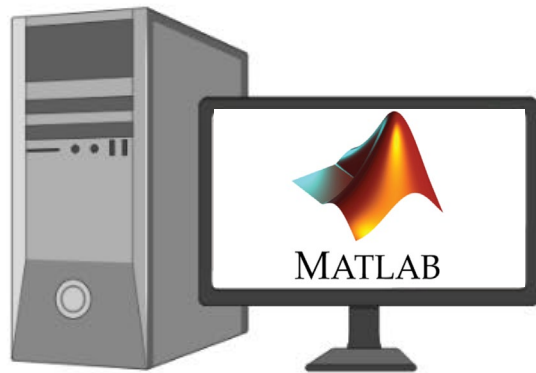


Methods Overview



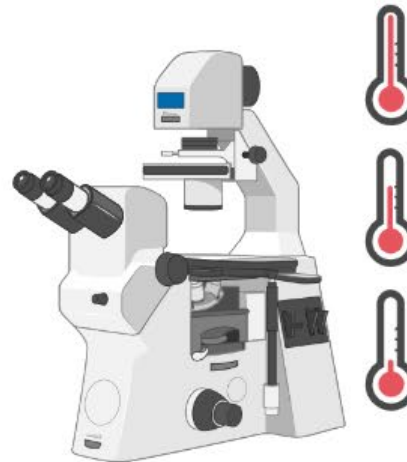
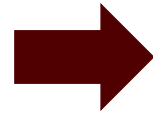
TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering



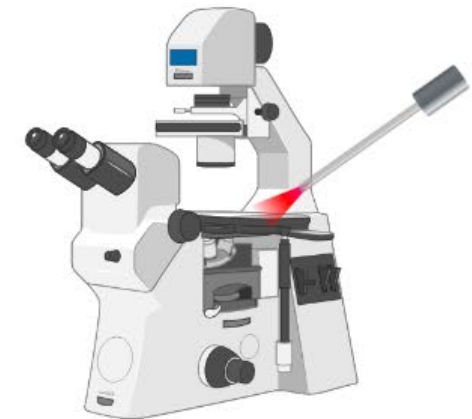
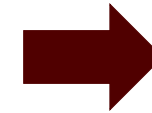
Computational Model

- Import IR-induced thermal gradients
- Simulate the fraction amount of free NADH, α_1



Steady-State Temperature

- Stage-top incubator to mimic thermal gradient temperatures
- Capture FLIM Images
- Quantify the fraction amount of free NADH, α_1 , using SPCImage



Dynamic Temperature

- 1470 nm Laser
 - Pulses: 0-4 ms
- Capture FLIM Images
- Quantify the fraction amount of free NADH, α_1 , with a custom MATLAB code

Arrhenius Model

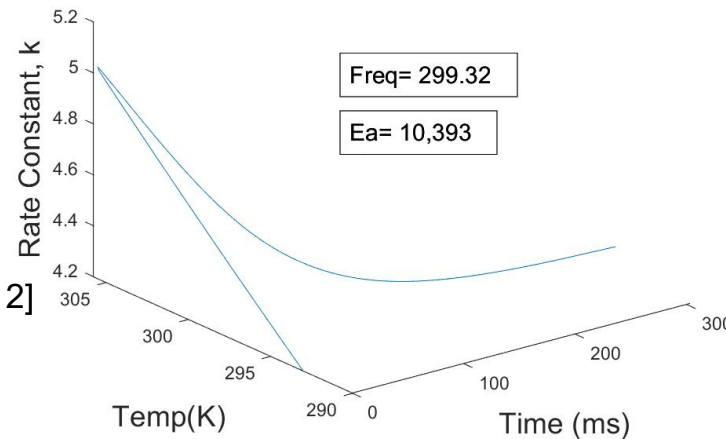


- Utilize the Arrhenius equation of reaction rates to calculate the rate constant, k , as a function of temperature and time for lactate dehydrogenase (LDH)

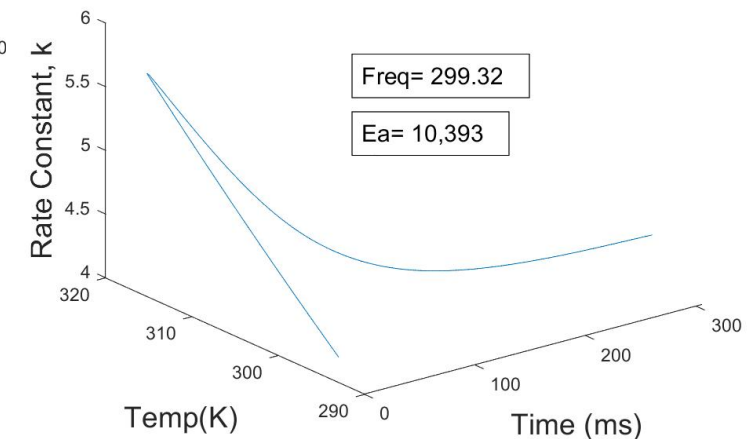
$$k = Ae^{-\frac{E_a}{RT}} \quad [11]$$

- Pre-exponential factor (A)= 299.32^[12]
- Activation energy (E_a)= 10,393 J/mol^[12]
 - Based on a pH of 7.00
- Ideal-gas constant (R)= 8.314 J/K*mol
- Temperature (T)= thermal gradients (K)

LDH Rate Constant, k - 0.49 J/cm² (2.73 ms)



LDH Rate Constant, k - 0.96 J/cm² (5.01 ms)



[11] Place, A. R.; Powers, D. A., Genetic variation and relative catalytic efficiencies: lactate dehydrogenase B allozymes of *Fundulus heteroclitus*, *Proceedings of the National Academy of Sciences* 1979, 76(5), 2354-2358.

[12] Deng, H.; Zhadin, N.; Callender, R., Dynamics of protein ligand binding on multiple time scales: NADH binding to lactate dehydrogenase, *Biochemistry* 2001, 40 (13), 3767-73.

Binding Parameters



- **Step 3:** Set up parameters that establish binding of a 50 μM solution of LDH and NADH
 - NADH concentration= 50 μM
 - Free-to-bound ratio, $\alpha_1 = 0.6$
 - $K_d = 4.2$ (20 $^\circ\text{C}$) ^[13]
 - $S = 4$ binding sites^[12]
 - Enzyme concentration= 13.38 μM

Equation 1	Equation 2	[14]
$[Enzyme] = \frac{[NADH] - (\alpha_1)}{F \cdot S}$	$F = \frac{(\alpha_1) * [NADH]}{(\alpha_1) * [NADH] + k}$	
α_1 = Desired fraction of unbound NADH S = Integer number of binding sites per enzyme molecule F = Fraction of enzyme sites to be occupied	α_1 = Desired fraction of unbound NADH K = Rate constant	

[12] Deng, H.; Zhadin, N.; Callender, R., Dynamics of protein ligand binding on multiple time scales: NADH binding to lactate dehydrogenase. Biochemistry 2001, 40 (13), 3767-73.

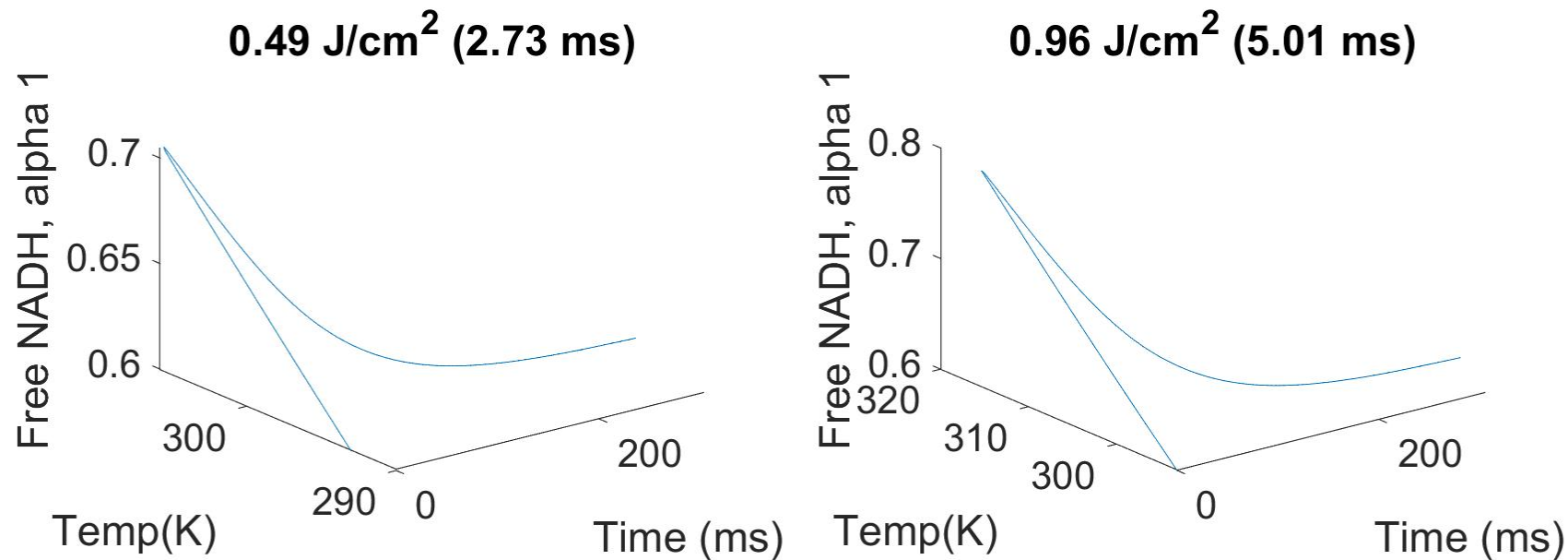
[13] Torikata, T.; Forster, L. S.; O'Neal, C. C., Jr.; Rupley, J. A., Lifetimes and NADH quenching of tryptophan fluorescence in pig heart lactate dehydrogenase. Biochemistry 1979, 18 (2), 385-90

[14] Shanley, J. T.; Favreau, P. F.; Gillette, A. A.; Sdao, S. M.; Morris, M. J., and Skala, M. C., "Protein bound NAD(P)H Lifetime is Sensitive to Multiple Fates of Glucose Carbon," Scientific Reports, (2016)

Fraction of Free NADH, α_1 , Values



- **Step 4:** Calculate the fraction of unbound NADH, α_1



Free NADH, α_1 , increases during exposure

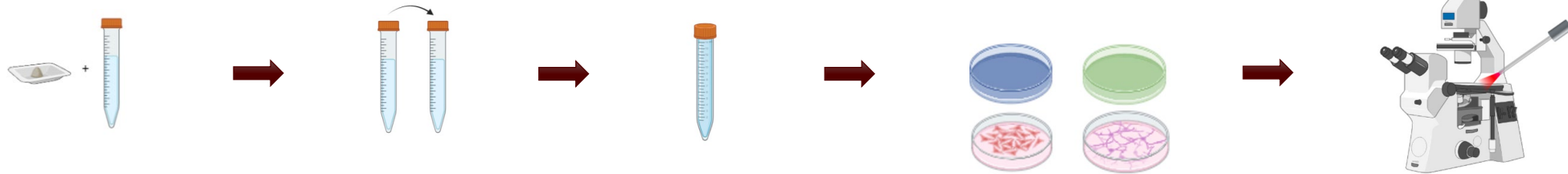
Free NADH, α_1 , decays exponentially after exposure

Dynamic FLIM Experiment



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

- **Purpose:** Collect FLIM images of a 50 μ M solution of NADH and lactate dehydrogenase (LDH), MCF-7 cells, and neuron cells



1000X NADH Stock:

- 354.9 mg: NADH salt
- 10 mL: 1x phosphate-buffered saline (PBS)

100X NADH:

- 100 μ L: 1000X NADH stock
- 900 μ L: 1X PBS

NADH-LDH Solution

- 0.801 mL: LDH
- 10 μ L: 100X NADH
- 189 μ L: 1X PBS

35 mm Dish Preparation

- 100X NADH
- NADH-LDH Solution
- MCF-7 Cells (HTB-22, ATCC)
- Primary neuron cells (Transnet XY, C57EHP)

FLIM Images

- Inverted multi-photon fluorescence microscope (Marianas, 3i)
- 1470 nm laser pulses (LUMICS): 0, 0.5, 1, 2, 3, 4 ms

- **Imaging Specifications**

- 40X water-immersion objective lens (NA 1.1): FOV of 270 μ m
- Titanium: sapphire femtosecond laser (COHERENT: Chameleon) at 750 nm with a laser power of 20 mW to 25 mW
- 1470 nm laser (LUMICS: LuOcean Mini 4)
- Time-correlated single-photon counting (TCSPC) was used (SPC-150N, Becker and Hickl) to capture each 256 x 256 pixel image
- Dwell time: 100 μ s, frame: 1
- Data Analysis: MATLAB

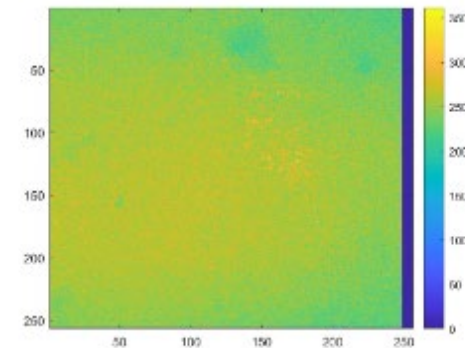
Dynamic FLIM: NADH-LDH Solution



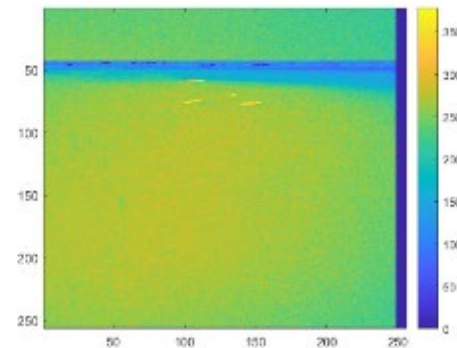
TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

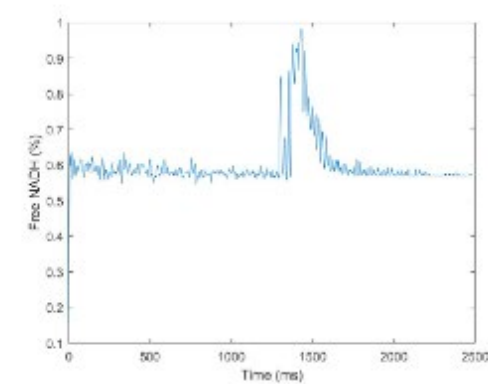
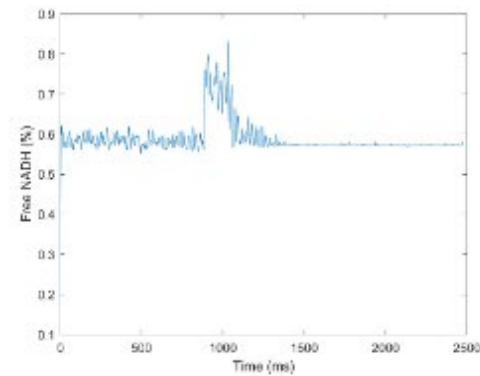
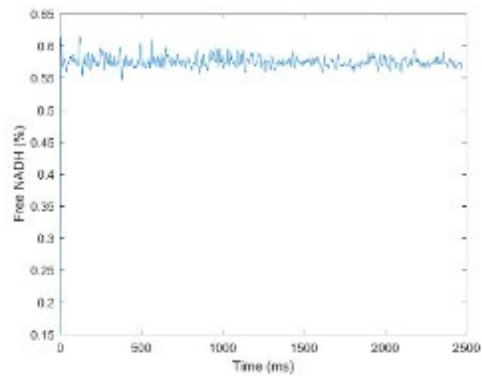
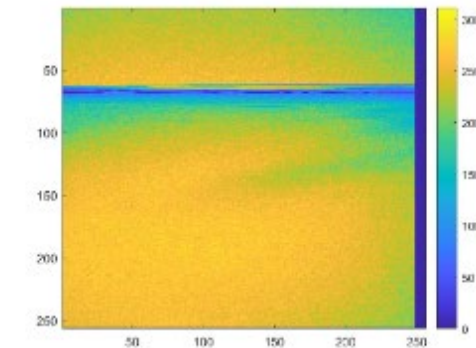
Control



0.5 ms



3 ms

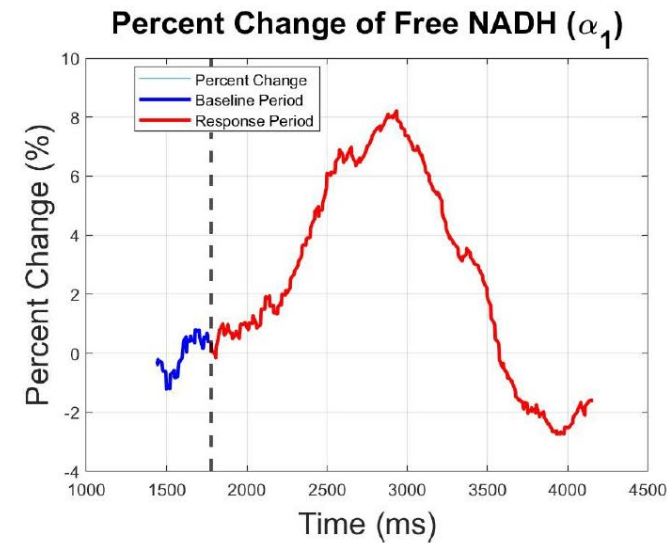
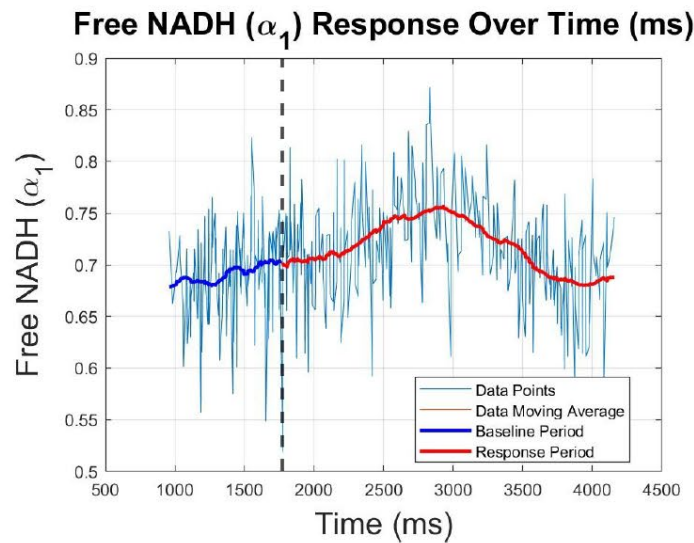
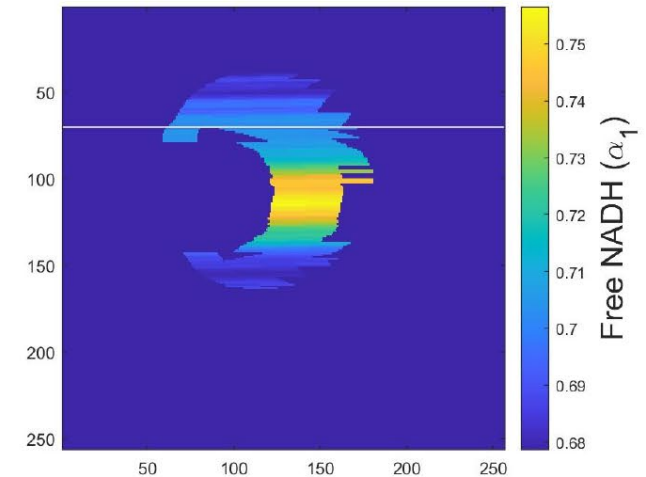
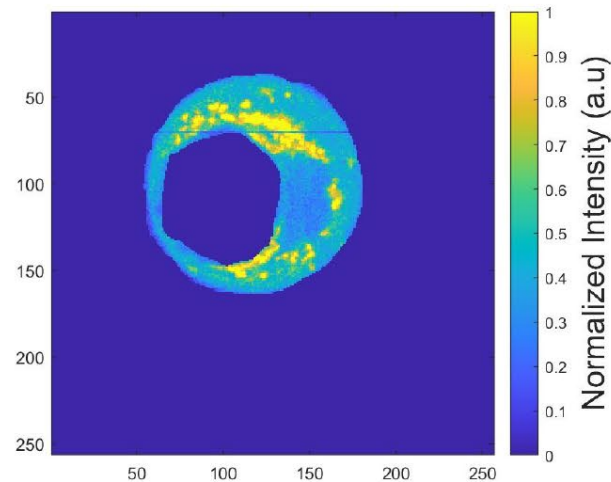
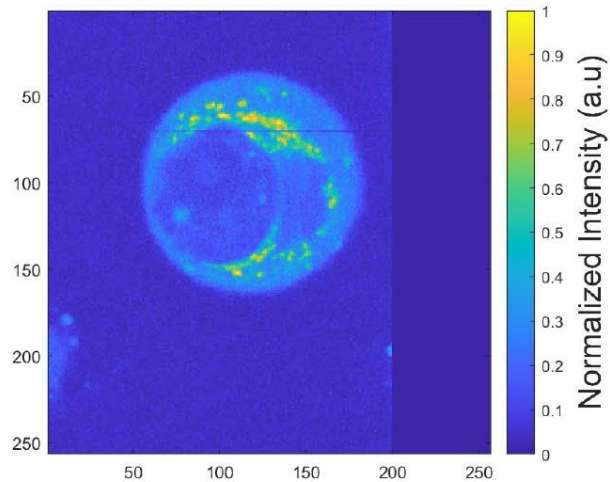


IR Response (4ms), MCF7 Cell



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering



My Research Progression with IR stimulation



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering



- NRC Postdoc (AFRL)
 - demonstrated ability of IR light to transiently inhibit action potentials in neurons (Walsh, et al. Neurophotonics, 2016)
 - Investigated potential mechanisms: nanoporation, membrane fluidity/permeability, calcium flux (2 SPIE conference presentations + proceedings)
- YIP (TAMU):
 - Model + experimental results on IR-induced thermal gradient effects on metabolism/coenzyme binding (manuscript in preparation)
 - Technology development: Design & characterization of wide-field autofluorescence microscope to capture IR responses (paper in review, J of Biomedical Optics)
 - Training of 2 graduate students (Linghao Hu, Anna Theodossiou) + 2 undergraduates (Sam Morganti, Jocelyn Martinez)
- Current Grant – Controlled manipulation of cell function with IR Light

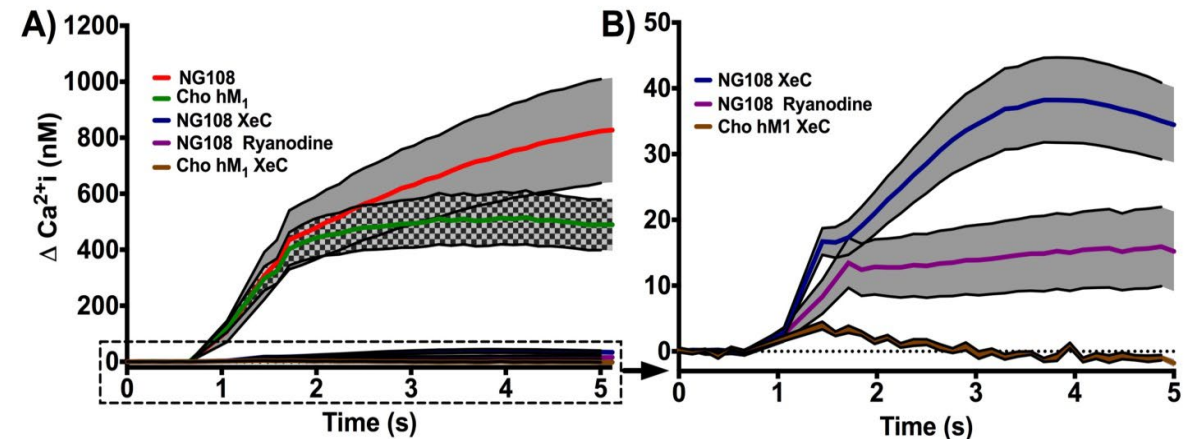
Possible Mechanisms



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

- Direct change of membrane capacitance by a thermal gradient^[1]
- Ion flow through heat activated or stretch activated TRP channels^[2]
- Heat inactivation of ion channels^[3]
- Nanopore formation (and subsequent ion flow)^[4]
- Heat induced increase in membrane fluidity and permeability
- Stimulated calcium-induced calcium release



Tolstykh, et al. Neurophotonics 2017.

[1] Shapiro et al. Nat. Commun. 2012.

[3] Wells, et al. Biophys J 2007.

[2] Albert, et al. J Neurophys 2012.

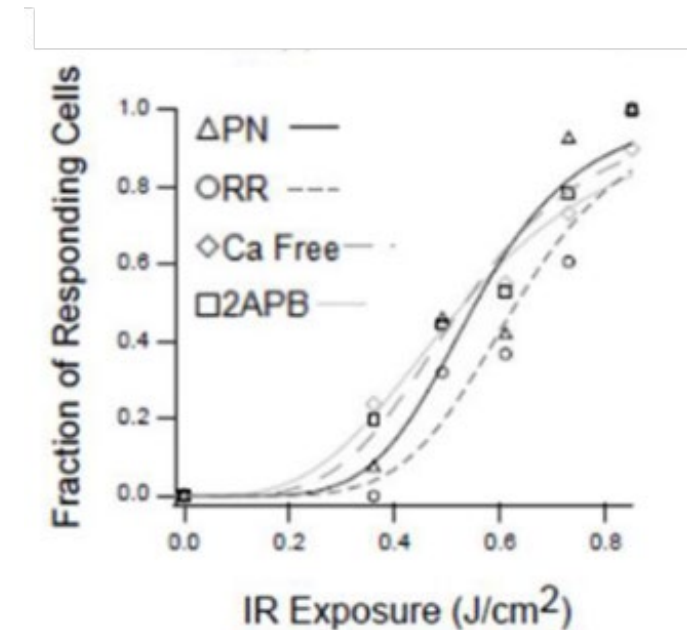
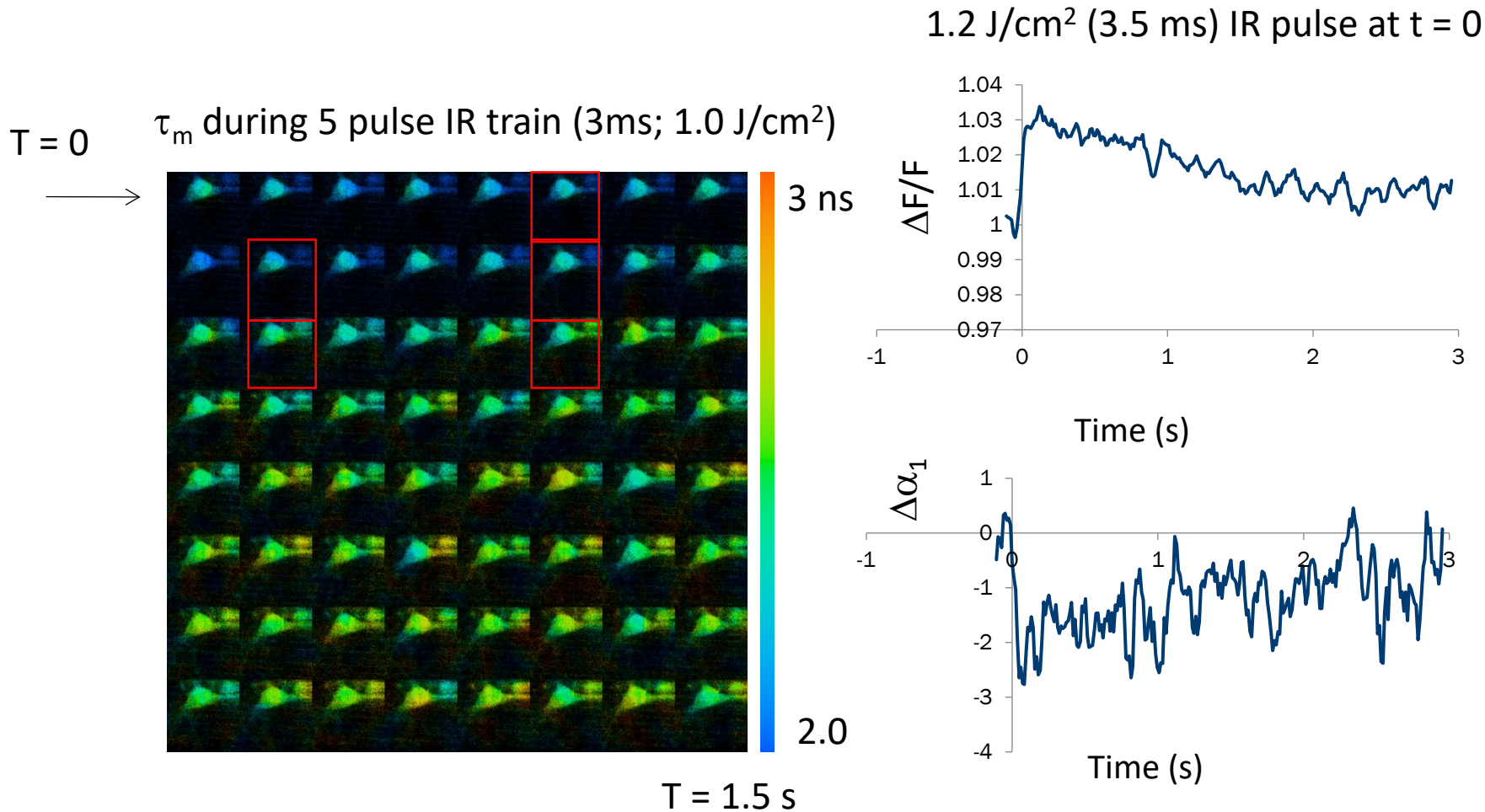
[4] Beier, et al. J Neural Engin. 2014

Calcium Flux During IR Stimulus



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering



Images acquired at 12 ms frame rate (83Hz); Average of n = 5-6 cells

Calcium as a signaling molecule



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

Hypothesis: If infrared light modulates calcium, we should be able to use infrared light to control the functional behavior of cells dependent on calcium signaling.

- Immune cells
 - Primary intracellular signaling molecule for activation of T cells, polarization of macrophages
- Fibroblasts
 - Stimulates collagen release
- Stem cells
 - Differentiation
- Temporal Patterns to intracellular Ca signaling which is challenging to replicate chemically

Project Goals:



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

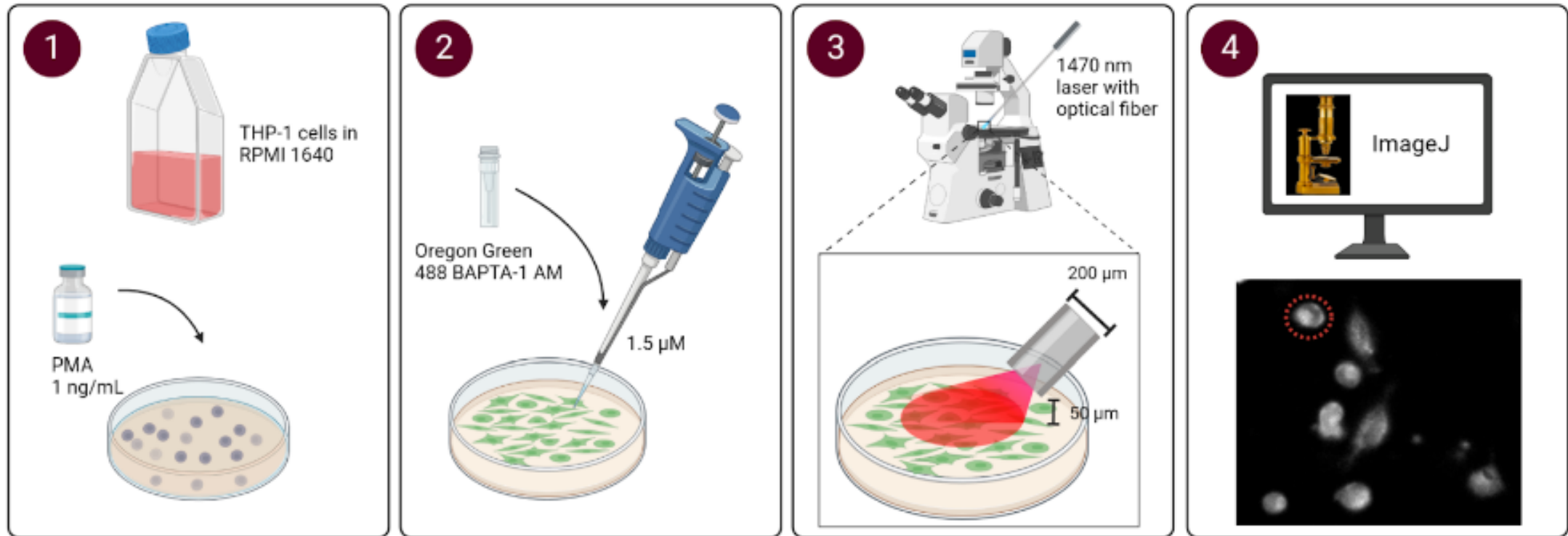
- **Objective 1:** *Quantify the effects of short pulses of infrared light on the calcium signaling of non-excitable cells, including immune cells, fibroblasts, and stem cells.*
- **Objective 2:** *Design, build, and characterize a system for controlled and reproducible IR light exposure of cell populations.*
- **Objective 3:** *Quantify the effects of short pulses of IR light on the functions of non-excitable cells.*
- Bridge cell studies to in vivo studies
 - Collaboration with Dr. Bixler & Dr. Thomas at AFRL
 - In vivo imaging systems for simultaneous IR stimulation while capturing neural activity and animal behavior

Objective 1: Approach



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

Objective 1: *Quantify the effects of short pulses of infrared light on the calcium signaling of non-excitable cells, including immune cells, fibroblasts, and stem cells.*



Laser Characterization

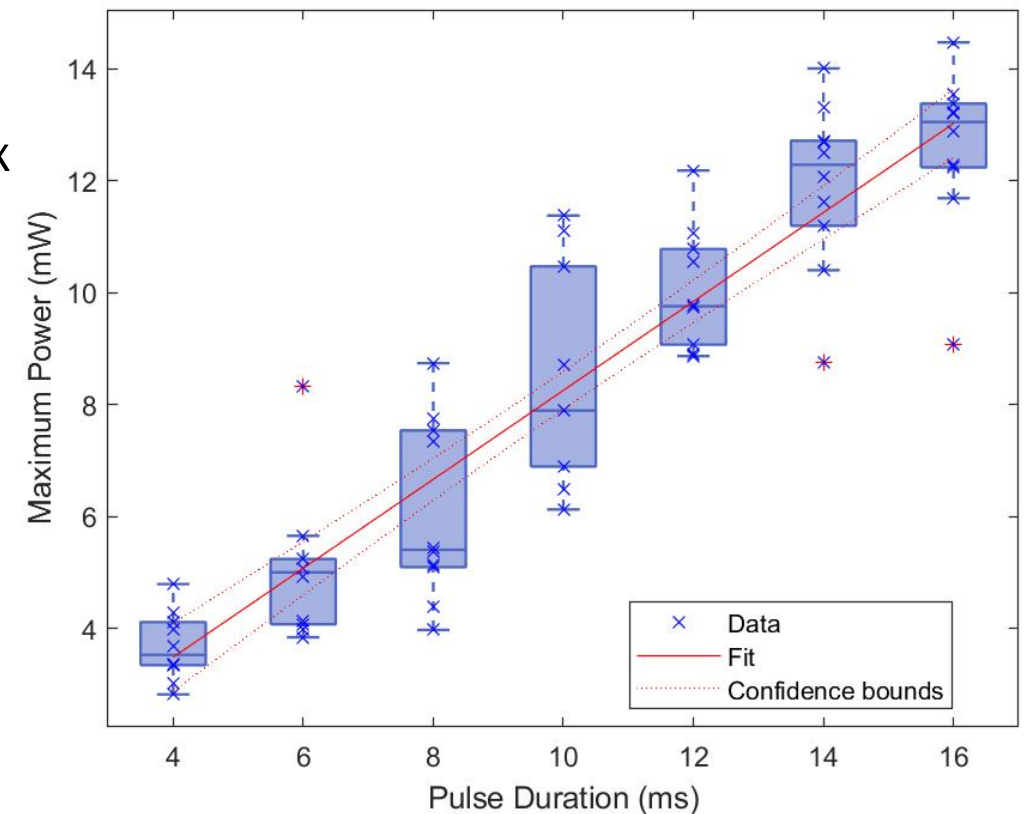


TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

- Fluorescence intensity images of a thermosensitive fluorescent dye: Europium (III) thenoyltrifluoroacetate trihydrate (Eu-TTA)
- Fluorescence excitation: 357 nm
- Stage-top incubator (calibration curve)
- 1470 nm laser: 200 μm above at 45° & a spot size of 200 x 300 microns (Intensity variation with IR pulses)
- Power measurements at 3 Amps current

Power Measurements (3 amps)

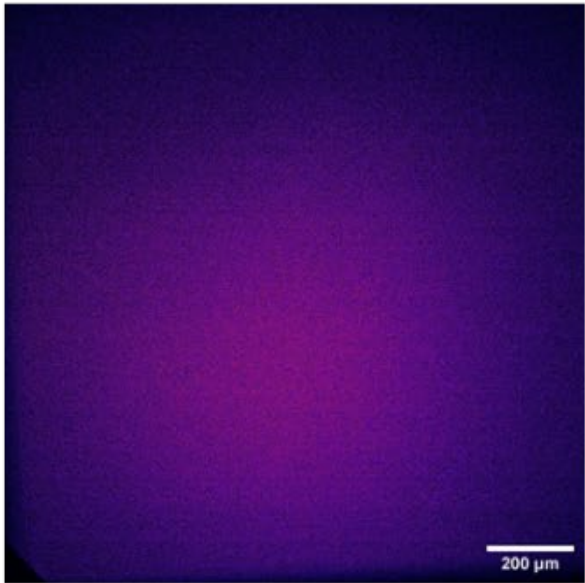


Eu-TTA thermofluorescence response calibration

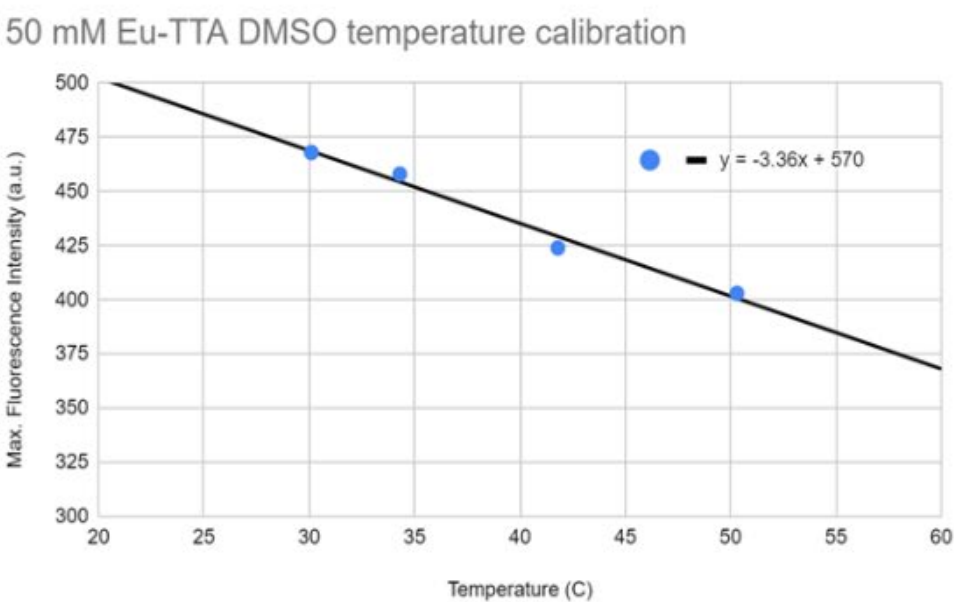


TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

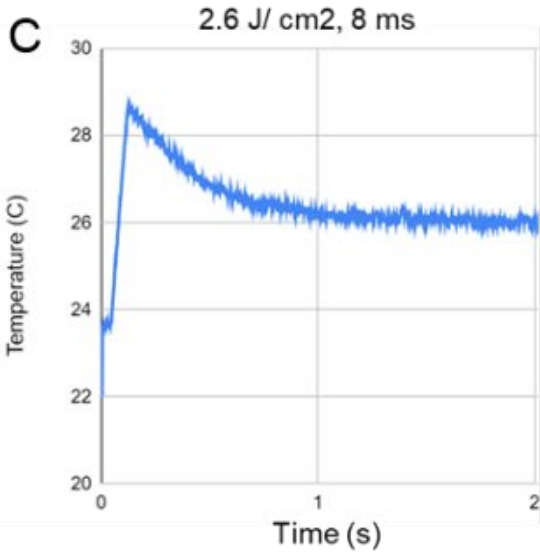
A



B



C

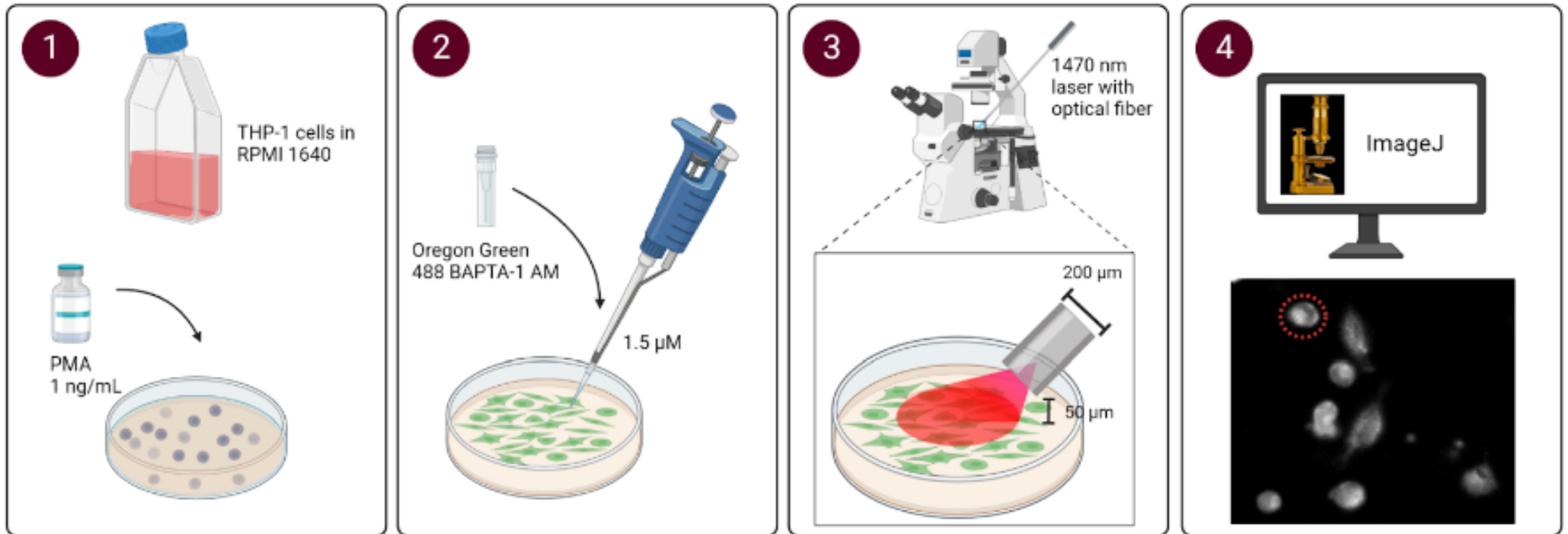


Wavelength (nm)	Pulse Duration (ms)	Power (mW)	Energy (μJ)	Irradiance (J/cm ²)	Degree Change (C)
1470	0	0	0	0	0
1470	0.5	2.903	1.4515	0.004	2.11
1470	1	3.413	3.413	0.010	3.69
1470	2	4.676	9.352	0.028	8.93
1470	3	6.034	18.102	0.054	13.96
1470	4	7.937	31.748	0.095	26.19

Macrophage Stimulation with IR Light



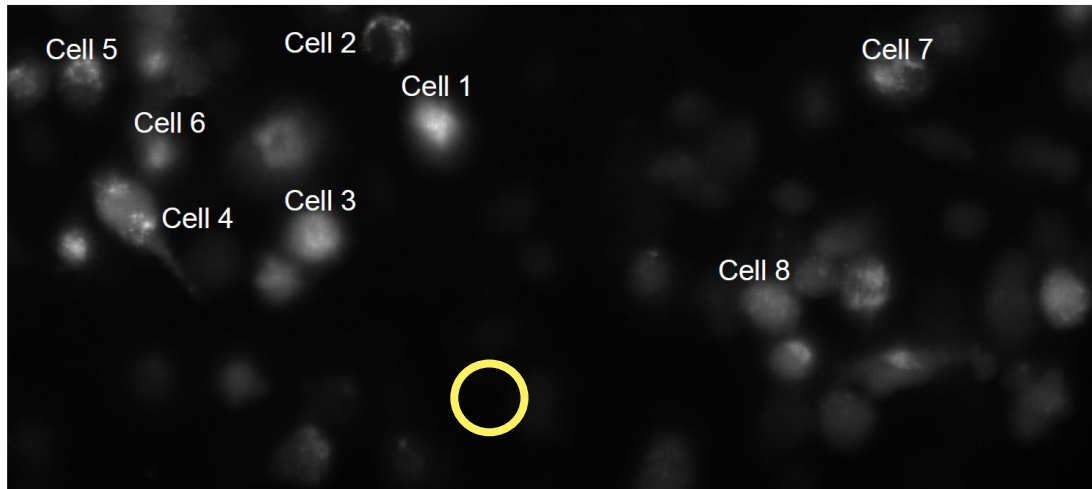
TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering



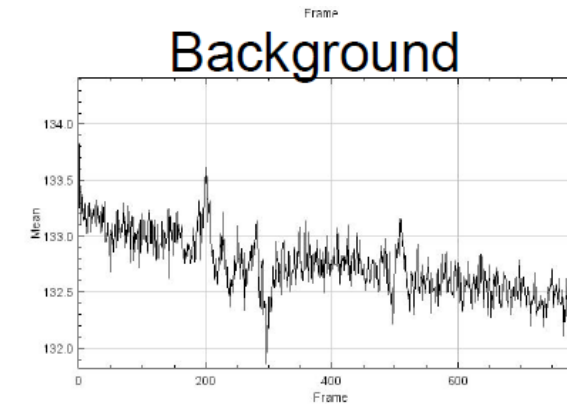


Macrophage Stimulation

Oregon Green BAPTA (Calcium Indicator) labelled
THP-1 Macrophages



4 ms 1470 nm pulse was delivered at $t=3$ s (300 frame)

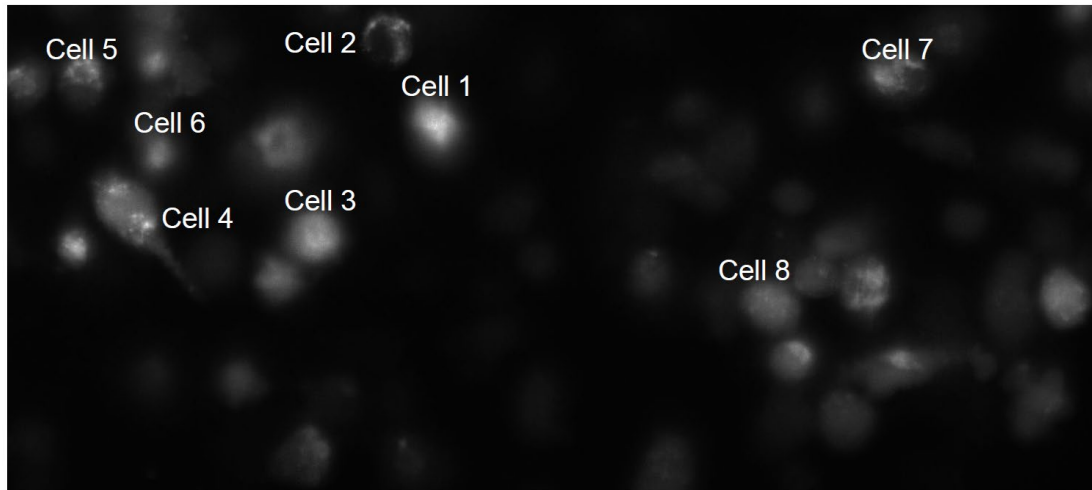


Macrophage Stimulation



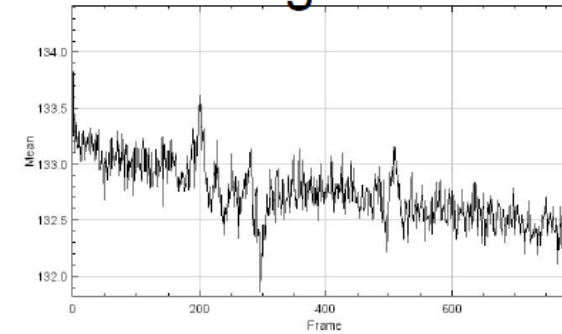
TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

Oregon Green BAPTA (Calcium Indicator) labelled
THP-1 Macrophages

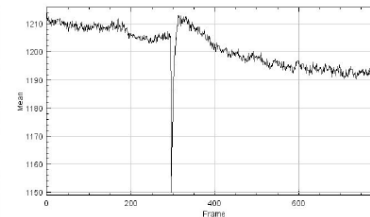


4 ms 1470 nm pulse was delivered at $t=3$ s (300 frame)

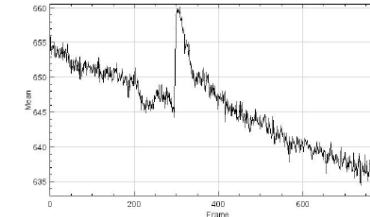
Background



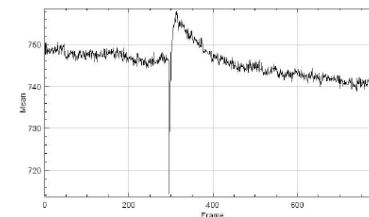
Cell 1



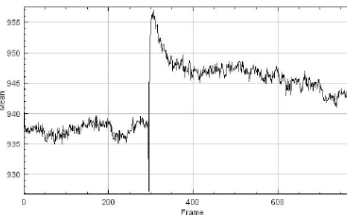
Cell 2



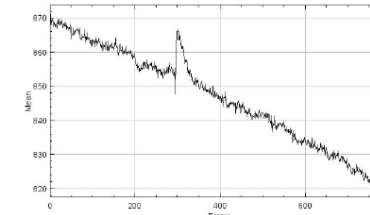
Cell 3



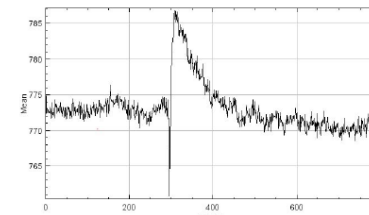
Cell 4



Cell 5



Cell 6

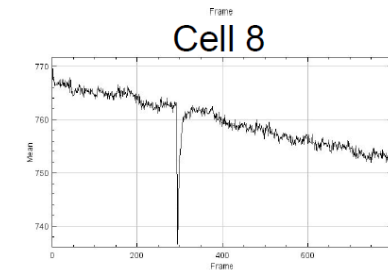
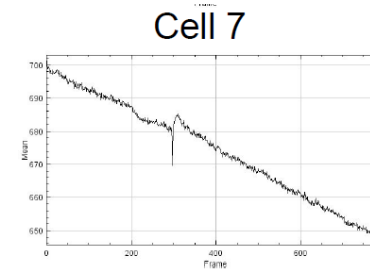
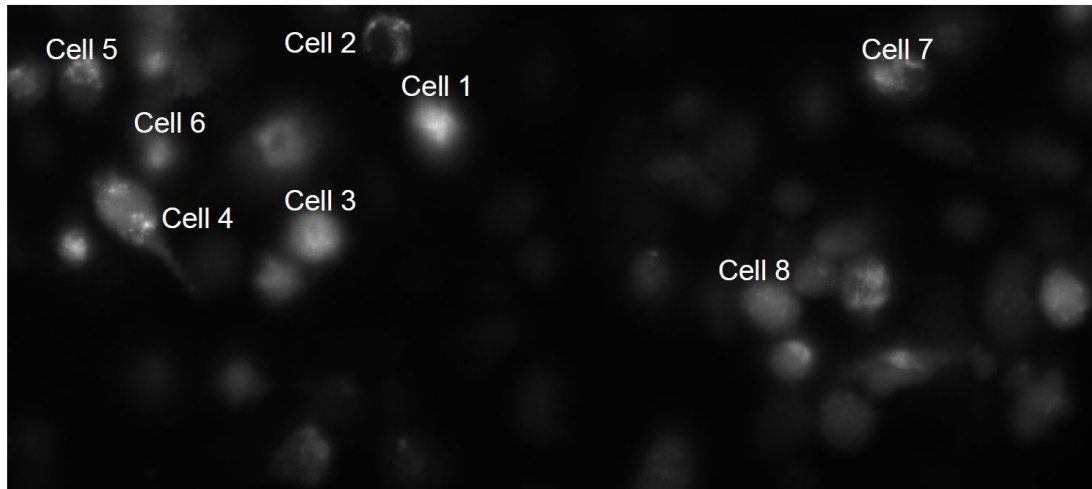


Macrophage Stimulation



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

Oregon Green BAPTA (Calcium Indicator) labelled
THP-1 Macrophages



4 ms 1470 nm pulse was delivered at $t=3$ s (300 frame)



Objective 1 Summary

Objective 1: *Quantify the effects of short pulses of infrared light on the calcium signaling of non-excitabile cells, including immune cells, fibroblasts, and stem cells.*

Findings:

- Characterization of laser illumination and thermal gradients for given pulse widths
- Damage threshold
- Reproducible stimulation of THP-1 macrophages
- Optimization of protocols for isolation and culture of primary human immune cells

Next Steps:

- Repeat experiments on THP-1 macrophages to determine dose-dependence
- Repeat with primary macrophages
- Learn protocols for fibroblast and stem cell culture



Objective 2

Objective 2: *Design, build, and characterize a system for controlled and reproducible IR light exposure of cell populations.*

Approach:

- *Integrate optics for beam expansion into wide-field microscope for imaging verification of IR light exposures*
- *Model in zemax*
- *Build and validate*
- *Integrate stage scanning for automated exposures of well-plates*



Zemax Simulation of IR Spot

Geometric Image Analysis

400 μm core diameter

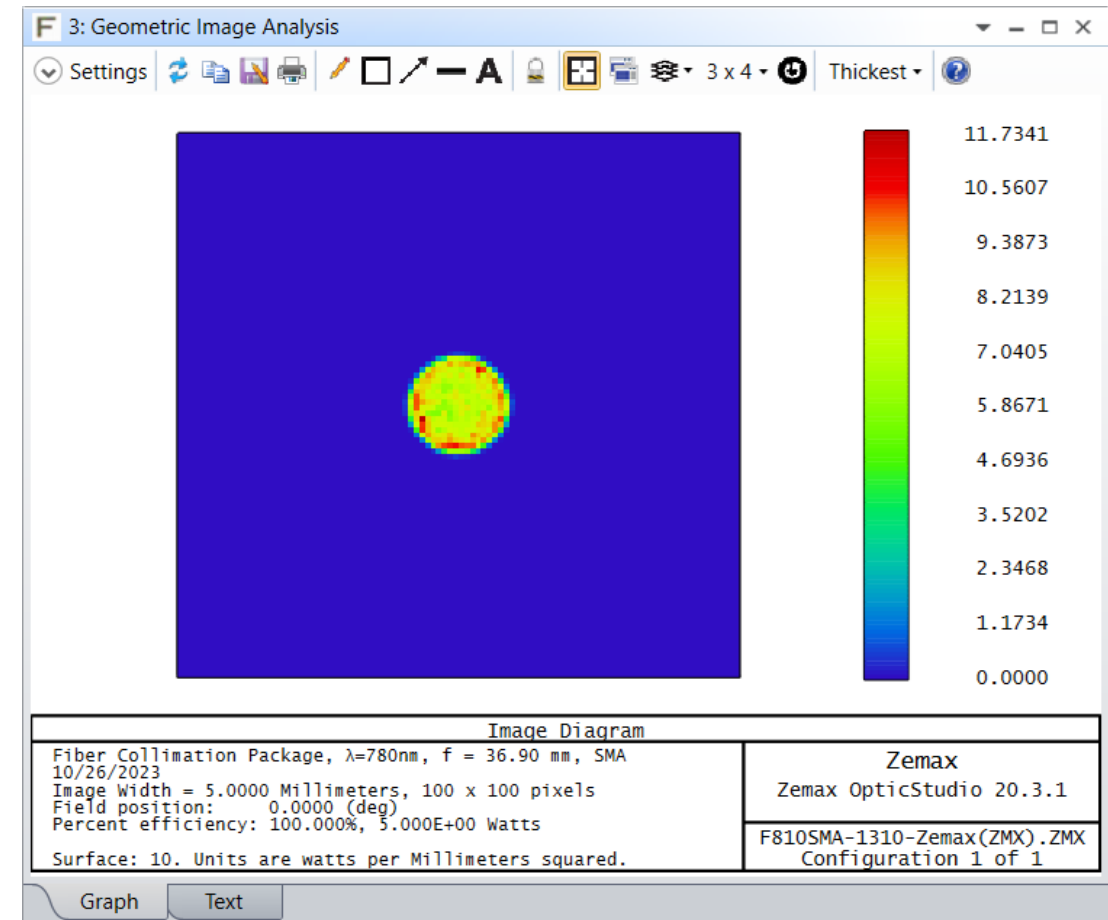
1.2 mm IR spot diameter

Simulated irradiance:

5 W input \rightarrow 11 W/mm²

Experimental irradiance:

8 W (input to optics out of fiber) \rightarrow
10 W/mm² (at sample)*** some
additional power loss expected due
to reflections and transmission
losses

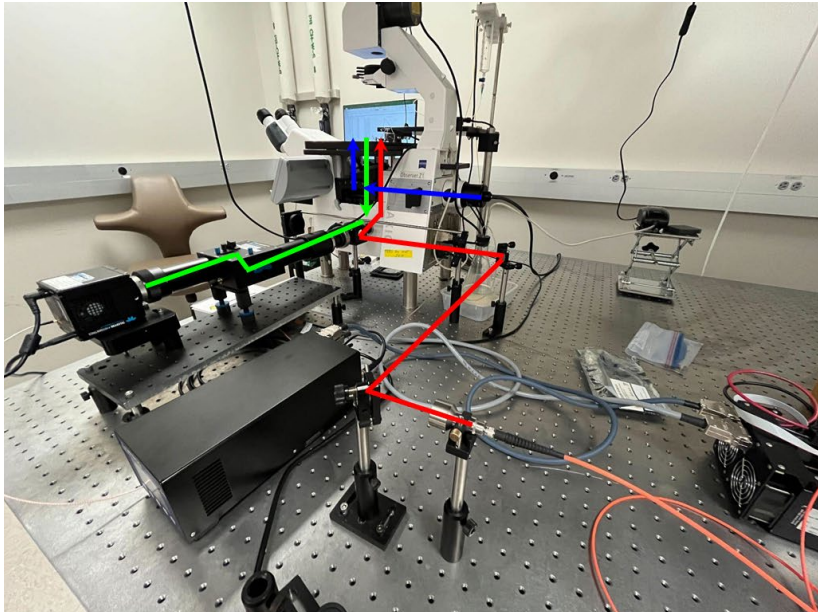


Solving IR stimulation throughput challenge



TEXAS A&M UNIVERSITY
Department of
Biomedical Engineering

- Assays of cell function/behavior typically require the pooling of substrates from a large number of cells
 - Flow cytometry, mRNA, Western blot of protein expression, etc
- IR stimulation of populations of cells



— 1470 nm
— 357 nm exc.
— 570 - 640 nm em.



Oscar Benavides



Objective 2 Summary

Objective 2: *Design, build, and characterize a system for controlled and reproducible IR light exposure of cell populations.*

Progress:

- Designed and assembled optical hardware
- Custom code for X-Y scanning control in progress

Next steps:

- Validation of spot size
- Optimization of IR light pulse doses with thermal dye, optical lensing imaging, power meter measurements



Objective 3

Objective 3: *Quantify the effects of short pulses of IR light on the functions of non-excitabile cells.*

Progress:

- Optimization of macrophage phenotyping protocols with control chemical stimulation
 - Single cell level → immunofluorescence
 - Population → qPCR

Next steps:

- Perform immunofluorescence on macrophages after IR pulse exposure (grided dishes)
- Use exposure platform (Objective 2) to test multiple IR doses and evaluate changes in macrophage exposure.
- Find and optimize protocols for function of fibroblasts and stem cells.



Accomplishments/Products Year 1

- Training for:
 - Anna Theodossiou (Graduate student)
 - Jocelyn Martinez (Graduate student)
 - Oscar Benavides (Post-doc)
 - Emily Nelson (Undergraduate student)
 - Ines Ibrahamsson (Undergraduate student, Repperger Intern)
- Presentations:
 - Walsh AJ. “Label-free Optical Technologies to Image (and Potentially Alter) Cellular Metabolism” Vanderbilt University Biophotonics Seminar, Nashville. February 2024.
- Articles
 - In Review: Theodossiou A, Martinez J, Walsh AJ, “Fast Autofluorescence Imaging to Evaluate Dynamic Changes in Cell Metabolism”, Journal of Biomedical Optics.
 - In Advanced Preparation: Martinez J, Theodossiou A, Walsh AJ, “Pulsed Infrared Light Effects on Coenzyme Binding” Targeted Journal: Biophysical Journal, Fall 2023.

Acknowledgments



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering



Current Lab Members: Oscar Benavides, Jocelyn Martinez, Anna Theodossiou, Elizabeth Bullard, Blanche Ter Hofstede, Lakhvir Singh, Tasmi Tabassum, Amanda Galloway, Daniela De Hoyos, Melody Yeh, Erin Stout, Emily Nelson, Ines Abrahamsson, Shubha Holla, Sophie Romero, Farah Andleeb, Karla Ortega, Malik Salami, Michael Sibille, Sam Morganti, Aditya Krishna, Anaya Bawiskar

Past Members: , Linghao Hu (PhD), Nianchao Wang (MS), Vidhya Shree Ravi (MS), Elizabeth Cardona, Johanna Webb, Samantha Morganti, Uyen Nguyen, Joseph Afreh, Sarah Salem, Addison Threet, Sam Mathew,

IR Collaborators: Joel Bixler (AFRL), Bob Thomas (AFRL), Anna Sedelnikova



FA9550-20-1-0078
FA9550-21-1-0280
FA9550-23-1-0441
FA9550-23-1-0537

<https://qoil.engr.tamu.edu/>

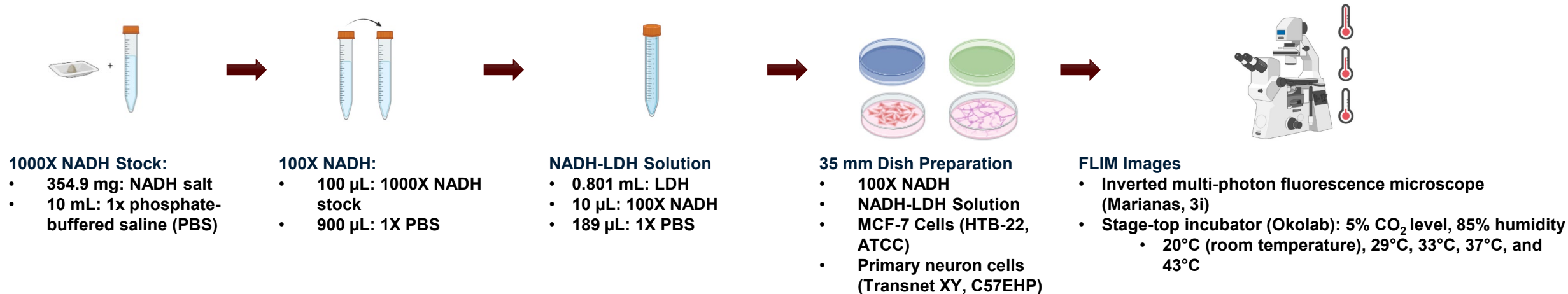
Steady-State FLIM Experiment



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

- **Purpose:** Collect FLIM images of a 50 μM solution of NADH and lactate dehydrogenase (LDH), MCF-7 cells, and neuron cells



- **Imaging Specifications**
 - Eight different positions for each of the temperature values
 - 40X water-immersion objective lens (NA 1.1): FOV of 270 μm
 - Titanium: sapphire femtosecond laser (COHERENT: Chameleon) at 750 nm with a laser power of 20 mW to 25 mW
 - Time-correlated single-photon counting (TCSPC) was used (SPC-150N, Becker and Hickl) to capture each 256 x 256 pixel image
 - Dwell time: 50 μs , frame: 5, capturing time: 60 s
 - Data Analysis: SPCImage

Steady-State Results



TEXAS A&M UNIVERSITY

Department of
Biomedical Engineering

