



AFRL

AFOSR ANNUAL REVIEW

BIOMECHANICAL RESPONSES TO LIGHT

24RHCOR011

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HUMAN EFFECTIVENESS DIRECTORATE - 24 OCTOBER 2024

"The views expressed are those of the authors and do not reflect the official guidance or position of the United States Government, the Department of Defense, the United States Air Force or the United States Space Force."



Objectives:

- Correlate dose-dependent photostimulation and photobiomodulation to intracellular responses & biomechanical signaling
- Transitions for photobiomodulation (PBM) applications
 - Optimization
 - Biomarkers for real-time assessment of efficacy
 - AFRL-STRONG Lab clinical studies
 - Human Performance
 - Industry

THOR Photomedicine (thorlaser.com)
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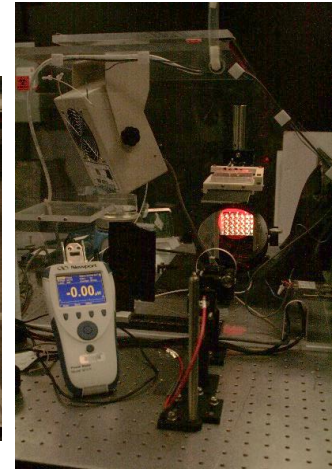
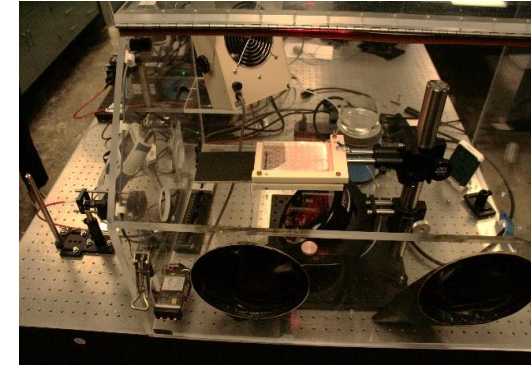


Publications:

- 2 invited chapters in “The Application of Heat in Oncology”
 - Encapsulated 17-yrs of 6.1 transitions of real-time thermography
- 2 invited articles in Frontiers Ophthalmology “special issue”
 - 1st ever unified modeling of photothermal/photochemical damage
 - Collaboration w/ new AFRL ML-RCP recipient (Khan)
 - Addressed dual limits in ANSI Z136.1 Std (concurrent exposures)
 - Summer interns/PhD candidate
- 1 article at “J. Photochem Photobiol” – SOARD Yr-1 data (Bagnato)
- 1 manuscript in preparation for “Science Advances” – SOARD Yr-2 data
 - PBM effects on mitochondria
- 1 manuscript in preparation from 2024 Summer Faculty Program (Arany)
- 1 manuscript in preparation on laser damage across blue λ 's in retina

Technical Approach:

- Optimize photostimulation/photobiomodulation in neurons (rigorous dosimetry & parameterization)
- Assess dose-dependent cellular, biochemical, & biomechanical mechanisms (morphology, tubulin, actin, [ATP], etc.)
- Expose cells to: VIS/NIR; mid-IR, THz
- Using: microscopy, Raman, FT-IR,



Figures taken in TSRL lab

DoD Benefit:

- SOARD transition of multiple LRIR data on in vitro photothermal damage mechanisms to Dr. Vanderlei Bagnato
 - Kick-off December 2023 / Brazil workshop June 2024
 - Drafting white paper #2
- LUCI proposal expected (Gabor/Denton)
- New collaboration with 711 HPW/RHBCP
 - Clinical study of ongoing PBM therapy in STRONG Lab
 - Anticipate mechanistic data to drive biomarker discovery & enhanced real-time reporting of efficacy

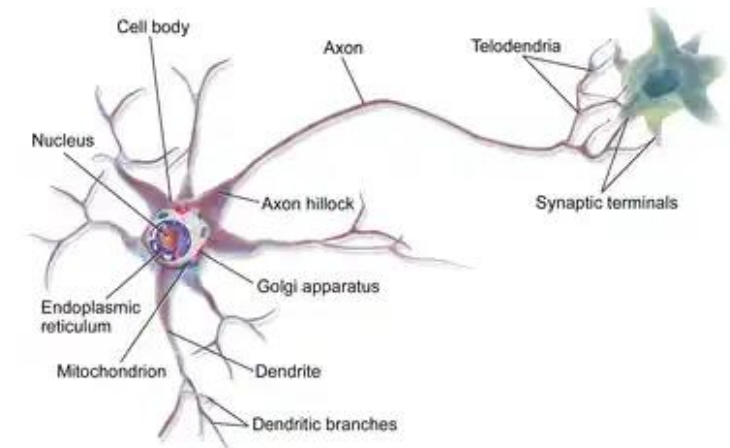


Photobiomodulation

- Characterize photon interactions and responses; lethal/sublethal
- Photobiomodulation (PBM): Stimulatory or inhibitory biological responses to light without adverse effects, typically applied to correct a pathological state
- Organismal/organ level responses
 - Multifaceted response at physiological level
 - Complex dosimetry for individual targets (surface/depths)
 - Overall beneficial effects
 - anti-inflammatory, vasodilation, wound healing, mental acuity/cognitive improvement, helps relieve opioid addiction, amelioration of symptoms in retinal pathologies & Parkinson's Disease
 - Overall inhibitory effects
 - pain relief
- Cellular/subcellular responses
 - Simple systems; dosimetry is easier
 - Photostimulation
 - Growth, metabolic shifts, movement/morphologic



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Previously Published Data (19RHCOR067 LRIR)

SCOPE

- Title: **Fundamental Effects of Light Absorption on Mitochondrial Function**
- Electron transport chain (ETC) & TCA cycle
- Enzyme activities (Complex III or IV)
- Raman microspectroscopy
- Transient absorption spectroscopy
- Confocal microscopy

PUBLICATIONS

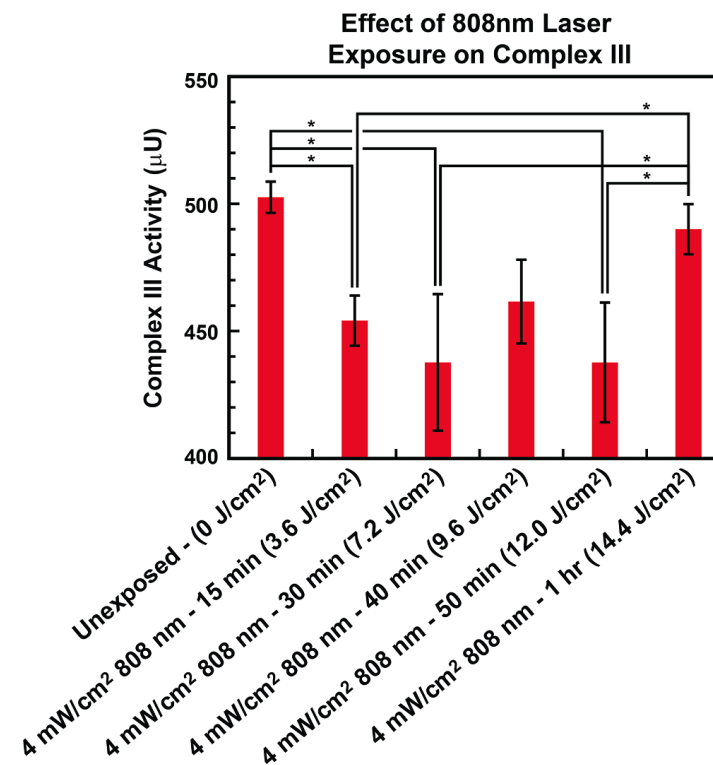
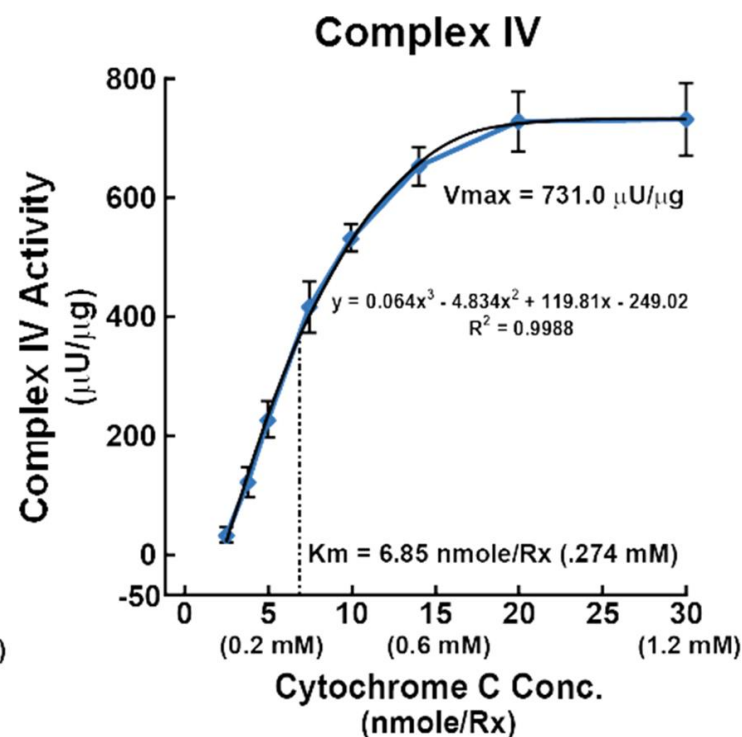
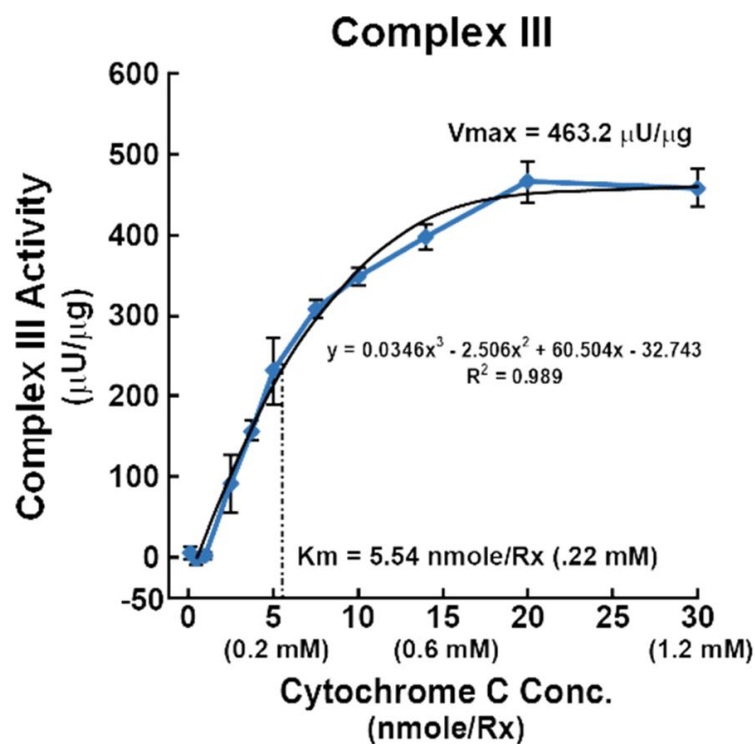
- 10 conference proceedings/podium talks
- 8 journal articles





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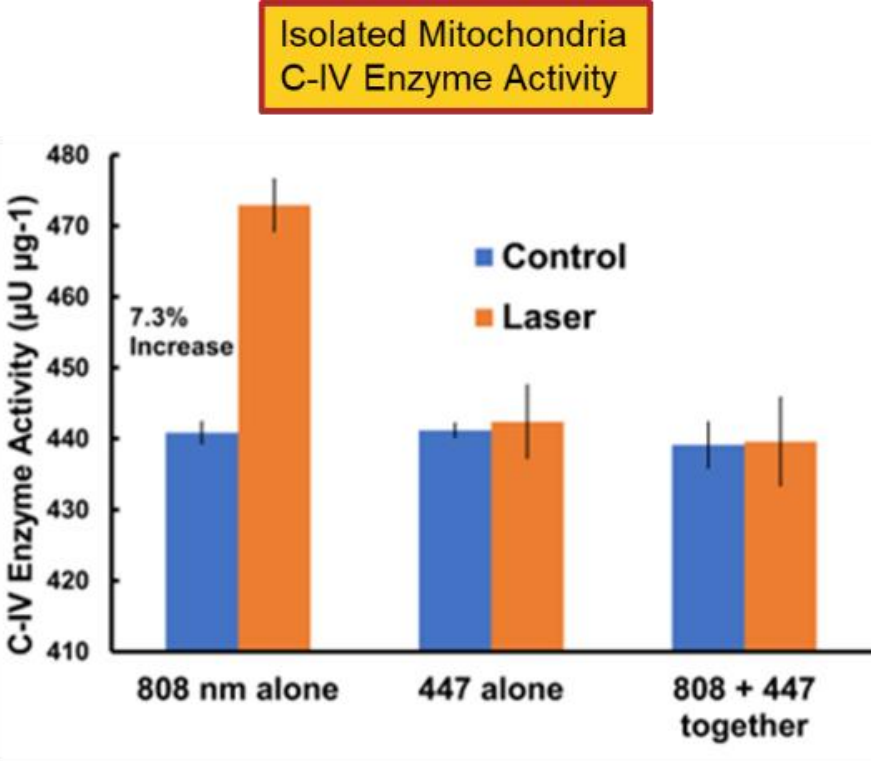
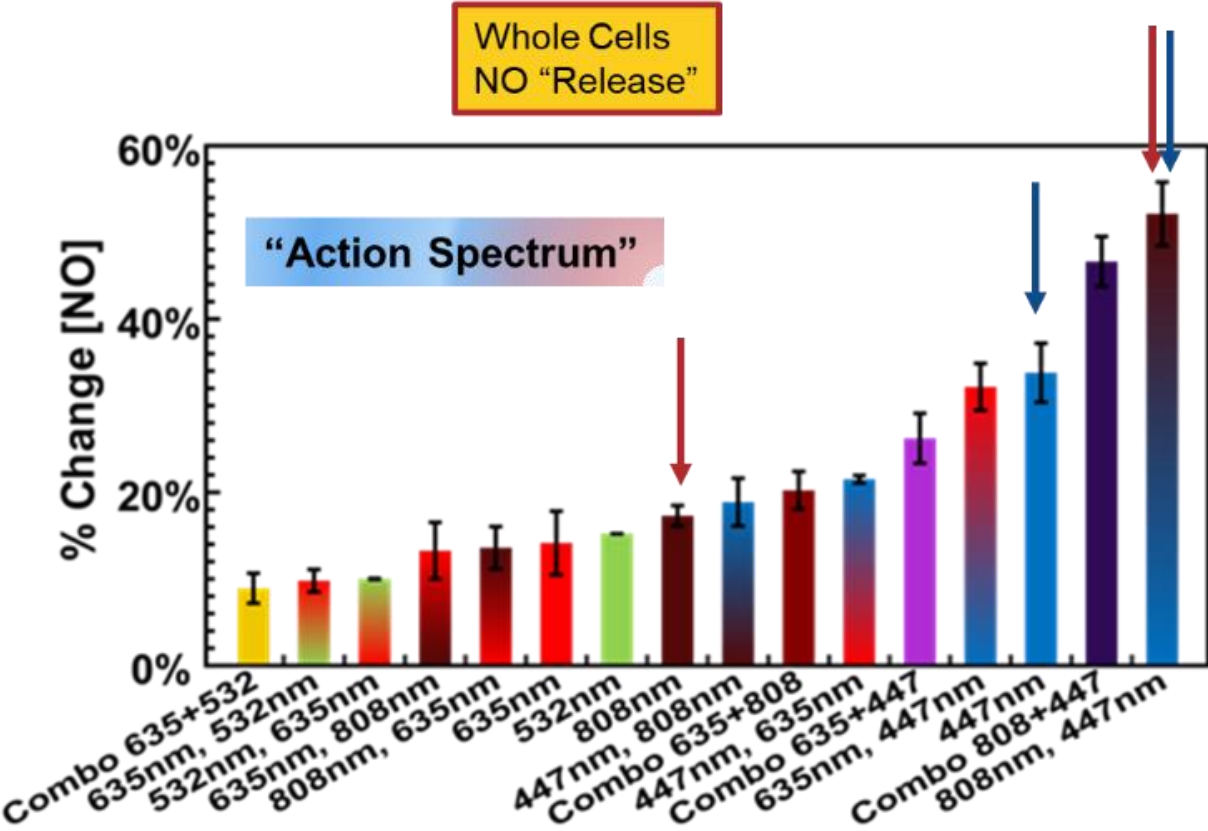
Enzyme Activity (Isolated Mitochondria)



Pope, N.J., Denton, M.L., Differential effects of 808-nm light on electron transport chain enzymes in isolated mitochondria: Implications for photobiomodulation initiation. Mitochondrion, 2023, 68, 15-24.



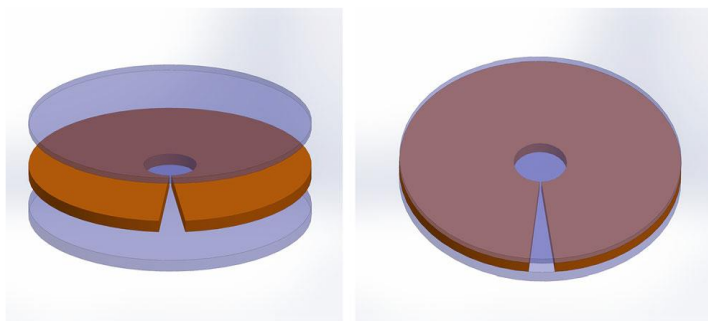
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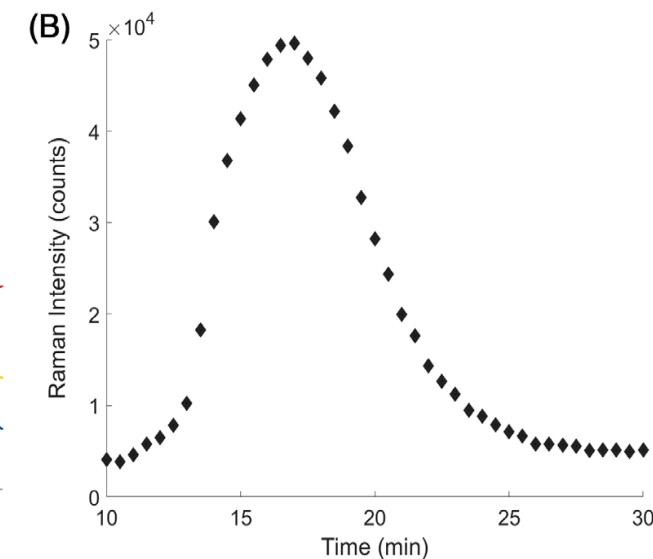
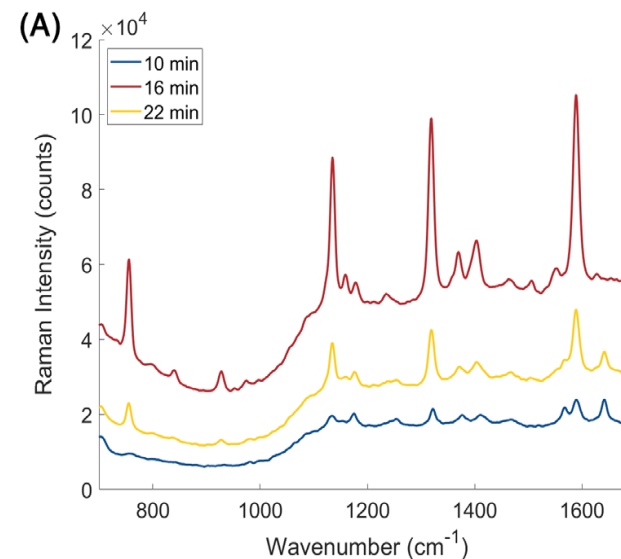
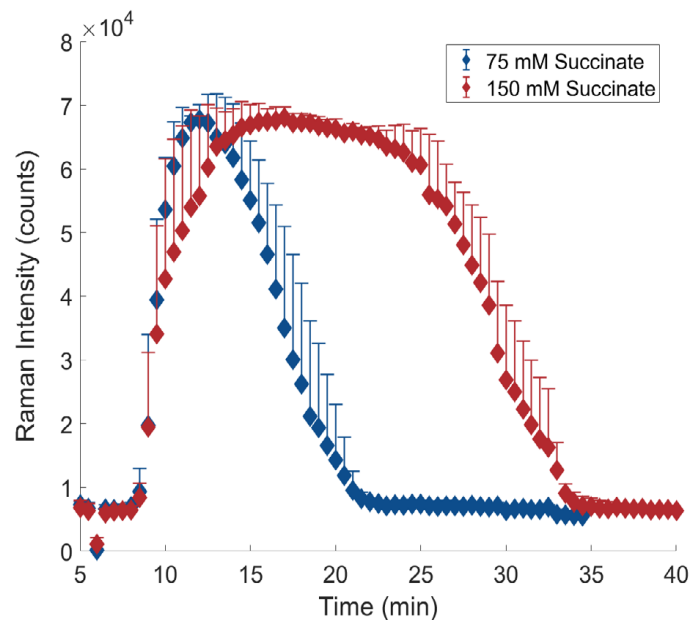
Pope, N.J., S.M. Powell, J.C. Wigle, M.L. Denton, 2020, Wavelength- and irradiance-dependent changes in intracellular nitric oxide level, J. Biomed. Opt. 25(8), p.085001, doi.org/10.1117/1.JBO.25.8.085001.

Michael L. Denton, Gary D. Noojin, Nathaniel James Pope, "Assessment of NIR and THz photostimulation at the cellular and subcellular levels," Proc. SPIE 12362, Mechanisms of Photobiomodulation Therapy XVII, 1236203 (14 March 2023); doi: 10.1117/12.2657275

Previously Published Data (19RHCOR067 LRIR)



Sample holder for Raman 50 μ L of mitochondria



Raman microspectroscopy
(reduced cytochrome c)

Lalonde, J.W., G.D. Noojin, N.J. Pope, S.M. Powell, V.V. Yakovlev, M.L. Denton, 2021, Continuous assessment of metabolic activity of mitochondria using resonance Raman microspectroscopy, J. Bio. 14(4), e202000384, DOI: 10.1002/jbio.202000384



Biomechanical Responses to Light (24RHCOR011)

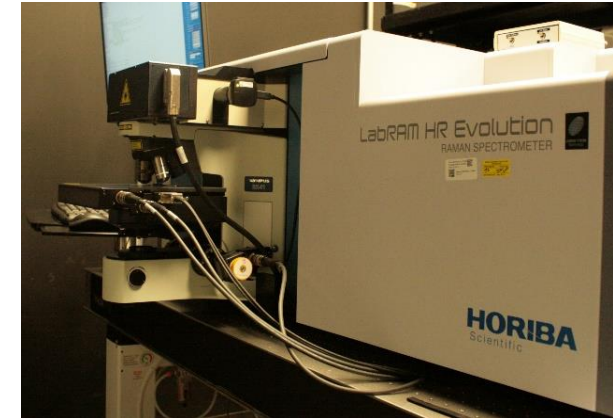
- Objectives
 - Correlate dose-dependent photostimulation to cellular & intracellular responses using *in vitro* models
 - Does the signal begin with biomechanical changes?
 - Actin/tubulin dynamics
 - Mechanotransduction
 - Determine if optimal photostimulation dosimetry provides repair of damaged in vitro model (PBM)
 - Does the signal begin with biomechanical changes?
 - Transition data to clinical PBM applications
 - AFRL-STRONG Lab
 - Targeting enhanced human performance
 - Optimization
 - Biomarkers to assess efficacy in real time
 - Industry



THOR Photomedicine (thorlaser.com) Use of photos approved by James Carroll, CEO THOR Photomedicine.

Biomechanical Responses to Light (24RHCOR011)

- Technical Approach
 - Rigorous dosimetry (λ , power, beam dimensions, irradiance, radiant exposure)
 - Parameterization (assess wide range of laser wavelengths)
 - MIR, THz, VIS/NIR
 - Identify/employ end points of analysis consistent w/ photostimulation
 - mitochondria, cytoskeleton
 - cellular growth, movement, & morphology
 - Changes in Raman signature
 - Selective choice of cell types
 - Retinal pigment epithelial cell cultures
 - Simple neuron cell cultures
 - Co-cultured mixed neural cells
 - Synaptic plasticity



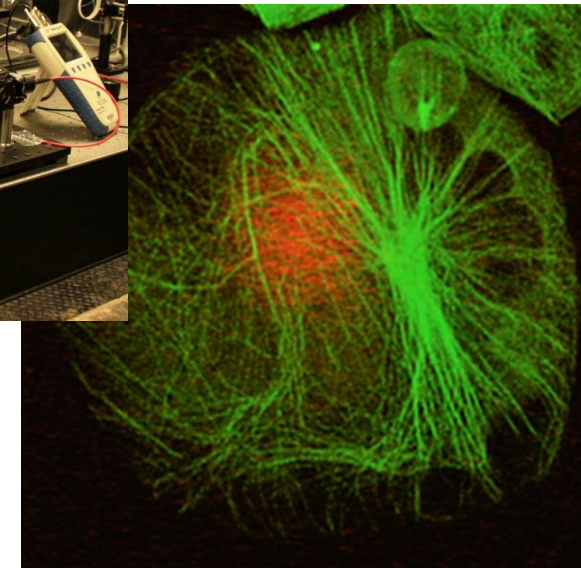
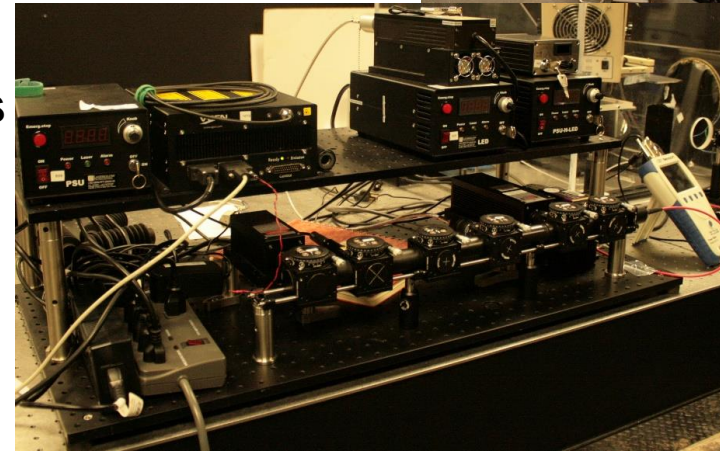
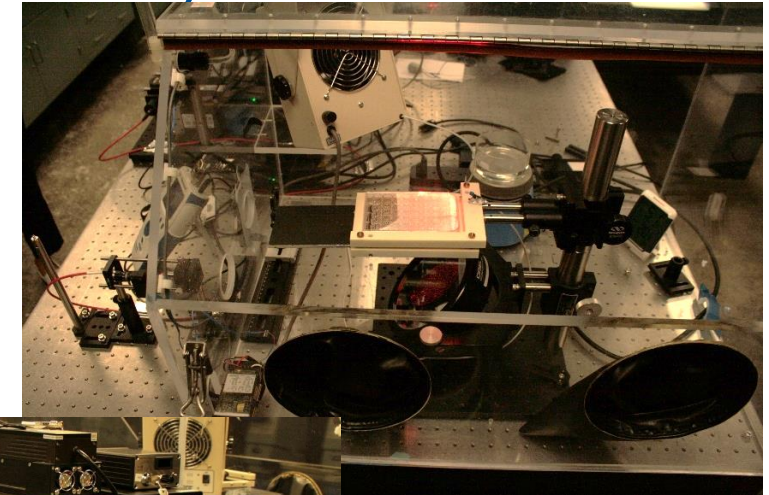
Confocal Raman microscope (UFR)



Terahertz laser (UFR)

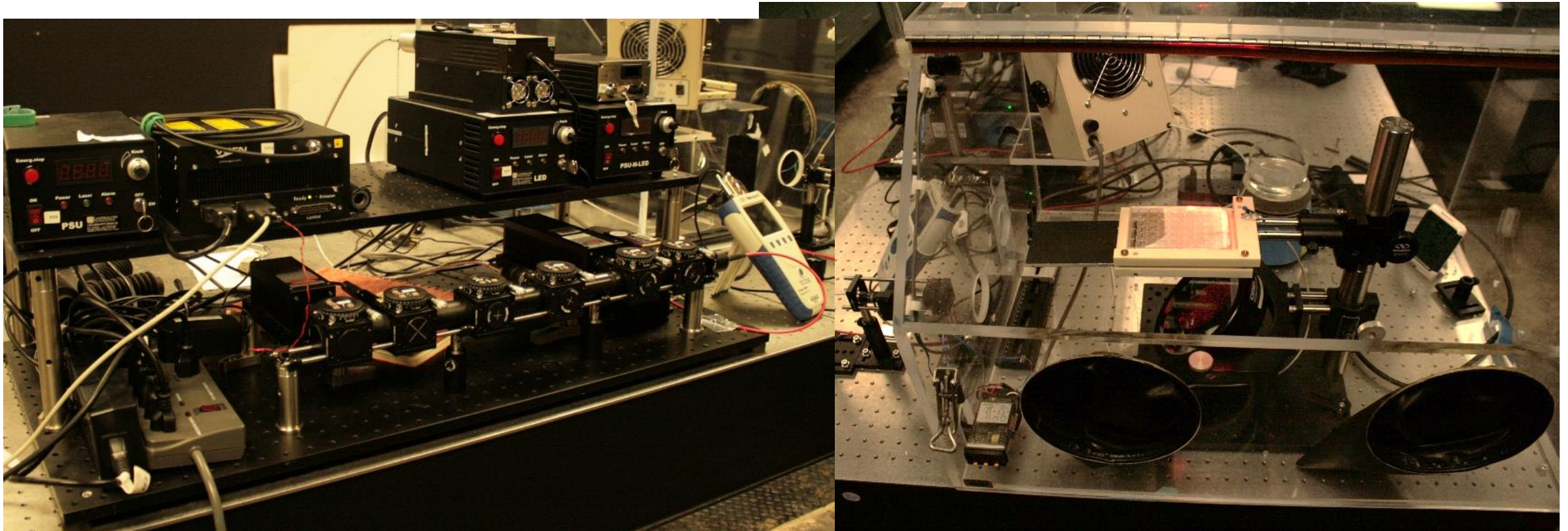
Biomechanical Responses to Light (24RHCOR011)

- VIS/NIR exposures
 - 405 nm, 460 nm, 635 nm, 808 nm, 1070 nm
 - Delivered individually or concurrently
 - Rigorous dosimetry, flat-top beams
 - Slider on bottom provides time course of dose
 - Exposing cells using ATP/growth kit for dosimetry
 - **Feeds into confocal microscopy of single cells**
 - **Biomechanical endpoints**
 - **Actin and tubulin responses**
- Used by Dr. Praveen Arany (U. of Buffalo)
 - 2024 Summer Faculty Fellow (AFOSR SFFP)
 - World leader in PBM research
 - Purified TGF- β 1
 - Joint manuscripts in progress



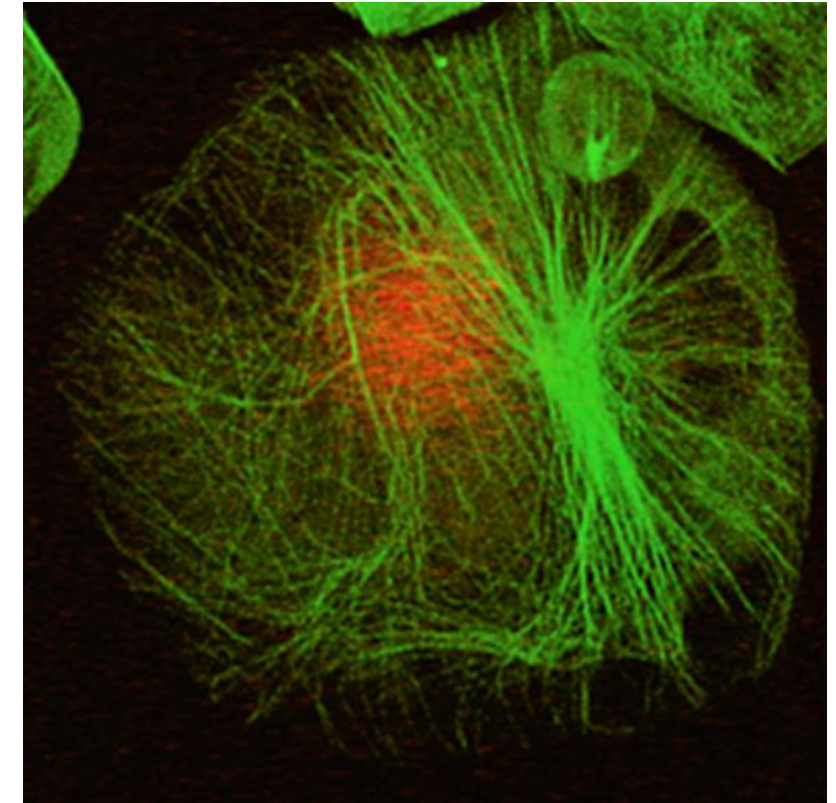
Tubulin-GFP
expressed via
lentivirus

Biomechanical Responses to Light (24RHCOR011)



Biomechanical Responses to Light (24RHCOR011)

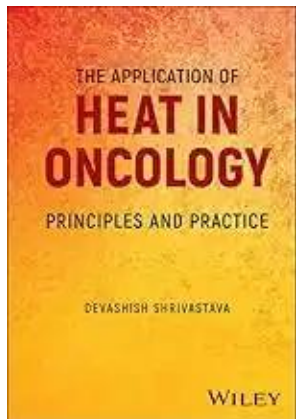
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Tubulin-GFP
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Recent Publications



Chapter 9: *Calibration and Use of Infrared Thermal Cameras in Biological Applications*
Michael L. Denton and Gary D. Noojin
Invited Chapter

Chapter 18: *A Novel Method to Indicate Onset of Photothermal Damage*
Michael L. Denton, Gary D. Noojin, and Elharith M. Ahmed
Invited Chapter

Transition of 17 years of LRIR foundational work on real-time thermography and correlation to cell damage



SOARD Award # FA9550-23-1-0660 (P.O. Dr. Jim Lyke)
Investigation of the photothermal effect during the interaction of electromagnetic radiation with biological tissue
Dr. Vanderlei S. Bagnato, IFSC Institute of Physics of São Carlos



1

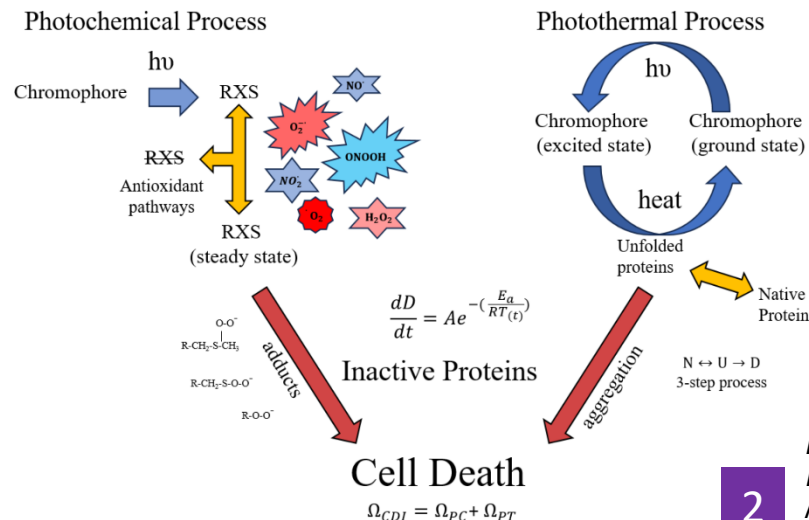
Real-time and post-irradiation effects of 635 nm photobiomodulation *in vitro* on mice liver mitochondrial bioenergetics
In review at J. Photochem Photobiol

2

Influence of temperature and incubation time on the respiration of permeabilized hepatocytes and mitochondria isolated from mouse liver
Targeted to Science Advances

Frontiers in Ophthalmology Section: Retina

Research Topic: Diagnostic and therapeutic applications of visible and near-infrared light for the retina



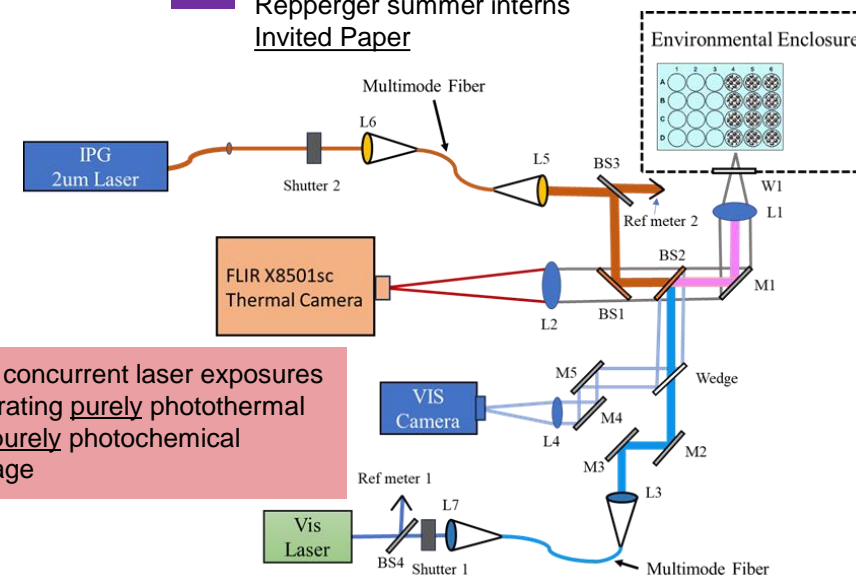
1

Unified Modeling of Photothermal & Photochemical Damage
AFRL, Ft Hays State University, University of North Carolina at Charlotte
Invited Paper

First modeling of both photothermal & photochemical damage

2

Damage Processes in Extended Laser Exposures Using an In Vitro Retinal Model
AFRL, University of the Incarnate Word, Repperger summer interns
Invited Paper



First concurrent laser exposures generating purely photothermal and purely photochemical damage

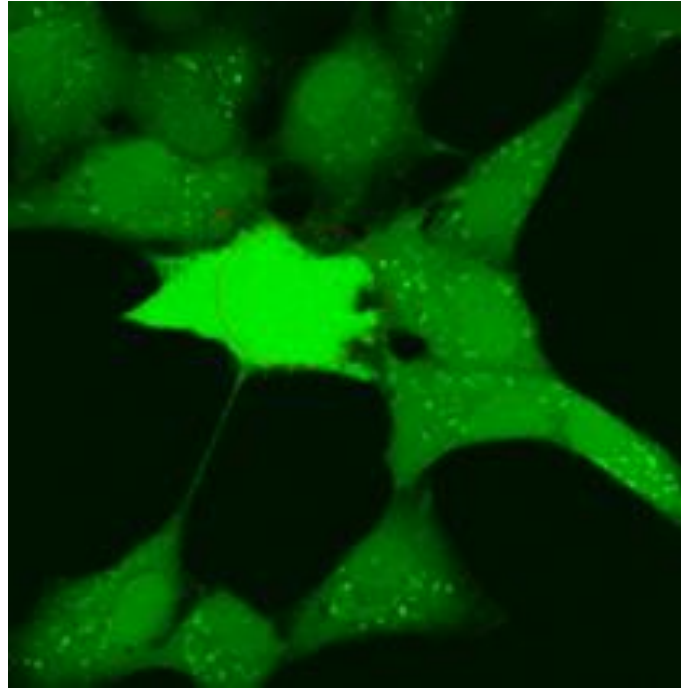


Collaborations

- Dr. Vanderlei S. Bagnato (Brazil/TAMU)
 - World-class scientist (PBM, PDT)
 - Member, National Academy of Sciences
 - SOARD award / multiple joint papers
 - Working a 2nd White Paper for new follow-on proposal
- Dr. Nathaniel Gabor (UC Riverside)
 - Site visit to Riverside in April
 - Expert in wavelength-specific quantum effects in biological systems
 - Discussions on Laboratory University Collaborative Initiative (LUCI) topic
- Dr. Matthais Heyden (Arizona State U)
 - Expert in THz spectroscopy / interfacial H₂O
- Dr. Joshua Hagen (711 HPW/RHBCP)
 - AFRL-STRONG Lab
 - Clinical trials designed to measure enhanced human performance resulting from routine whole-body PBM therapy
- Dr. Praveen Arany (University of Buffalo)
 - Clinician performing basic science to find mechanisms
 - Shown PBM exposures leads to alteration in protein structure (TGF- β 1), which produces cell & physiological signaling

Acknowledgements

- Contractor support
 - Lorinda Aspiras
 - Dr. James Pope
 - Amanda Tijerina
 - Anna Sedelnikova
- NCR Postdoc
 - Dr. Josh Lalonde



- 711 HPW/RHD
 - Gary Noojin
 - Dr. Zach Brawley
 - Dr. Morgan Schmidt

- AFOSR – Dr. Bradshaw



QUESTIONS?