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Photobiostimulation in C. elegans as a Model for Low Level Light Therapy

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Abstract
Low-Level Laser Therapy (LLLT) is a developing therapeutic technique that has been gaining recognition in the scientific community in recent years. Previous experiments performed in LLLT research projects have been primarily mammalian and cell culture based. These experiments have produced results showing accelerated tissue repair. In this experiment, we introduce a new model, *Caenorhabitidis elegans,* a free-living soil nematode, to be used in LLLT research by testing the effects of exposure of the organism to various wavelengths and intensities of light commonly used in LLLT. *C. elegans* was shown to respond to photobiostimulation when exposed to specific wavelengths of Infrared light, 920nm-980nm, at an intensity of $5J/cm^2$. These responses include an 18-20% increase in growth rate and overall length and width of each organism. The cellular mechanism behind this acceleration of growth is unclear and as an excellent model for examining the interactions of cells and tissues on a molecular level; the introduction of *C. elegans* into the field of LLLT research will provide valuable insight into the cellular processes that produce this significant change in biochemistry resulting in accelerated tissue repair and growth induced by LLLT.

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Subject Categories
Alternative and Complementary Medicine | Biochemical Phenomena, Metabolism, and Nutrition | Biology | Cell Biology | Developmental Biology | Medical Cell Biology

Photobiostimulation in *C. elegans*

Implementation of LLLT In a New Model

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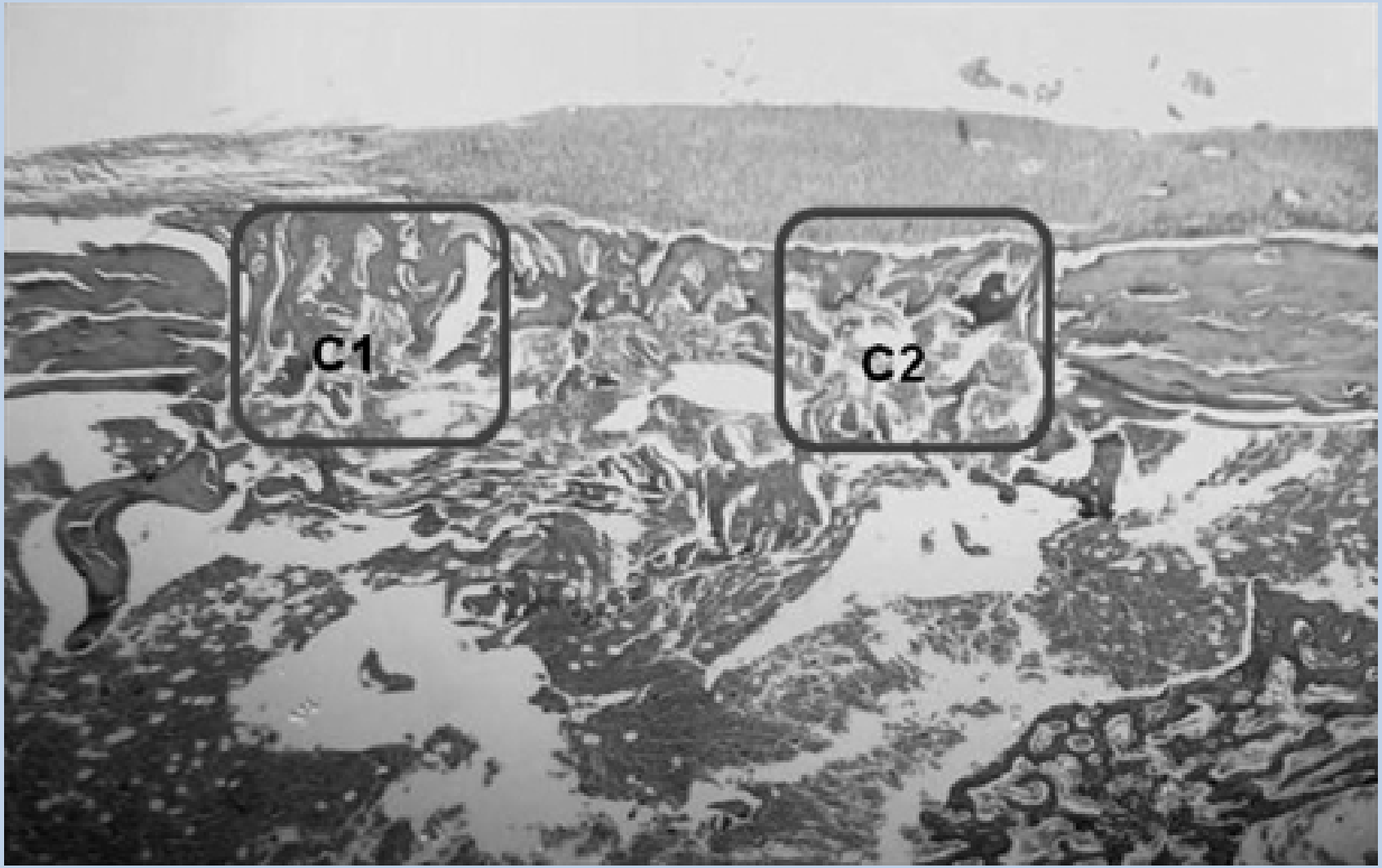
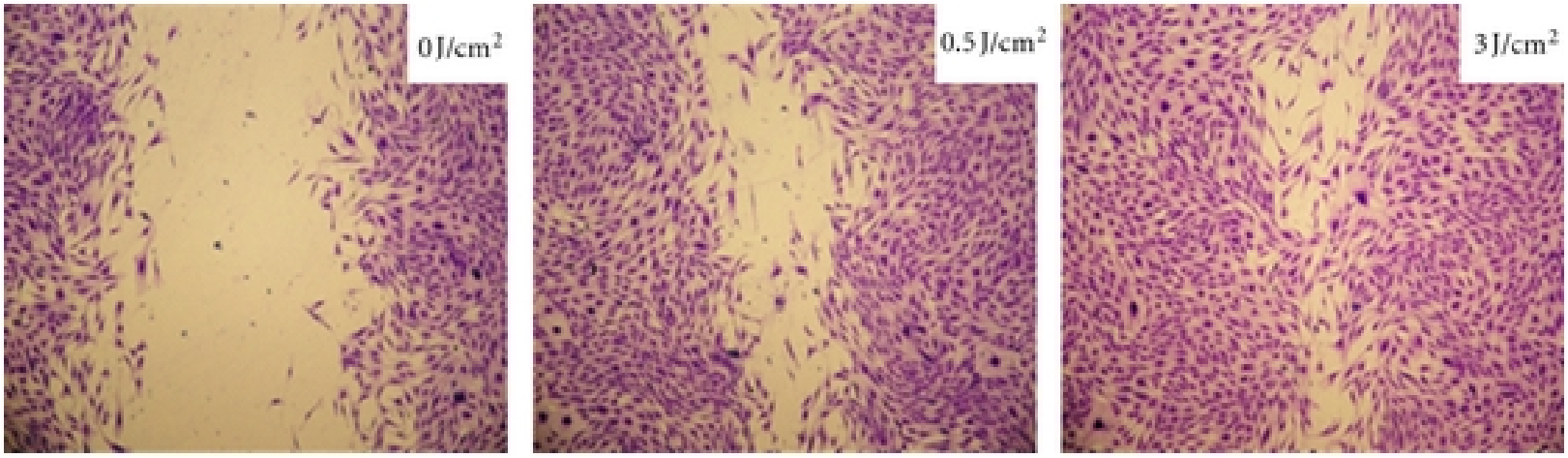
Low Level Laser Therapy



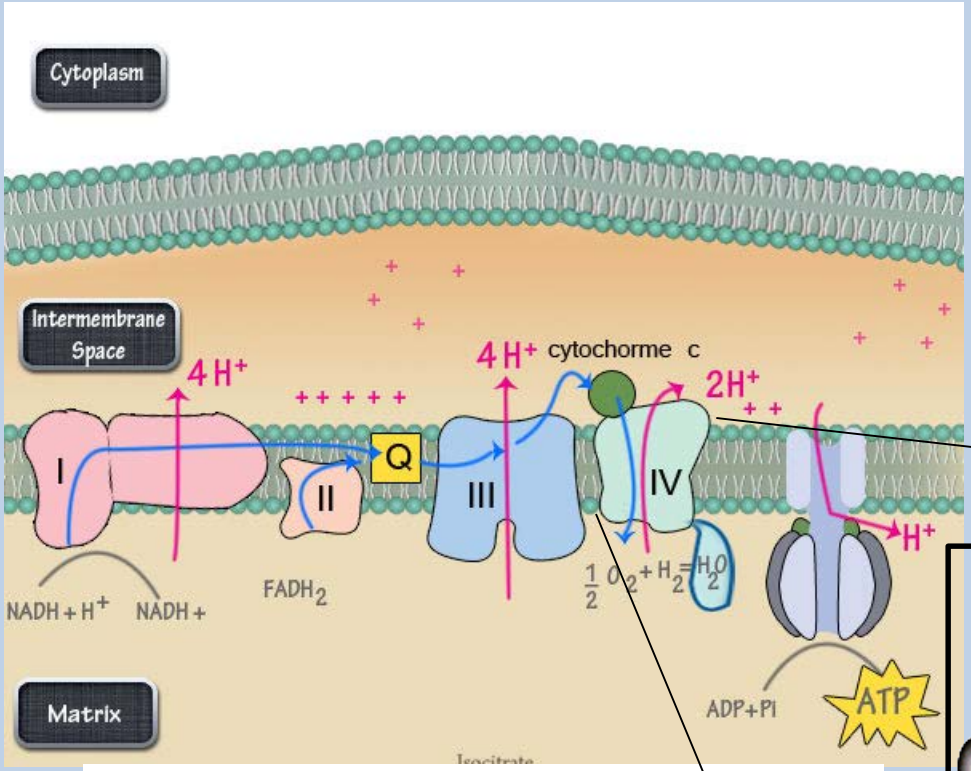
Poliani et.al. Brazilian Journal of Physical Therapy (2010)

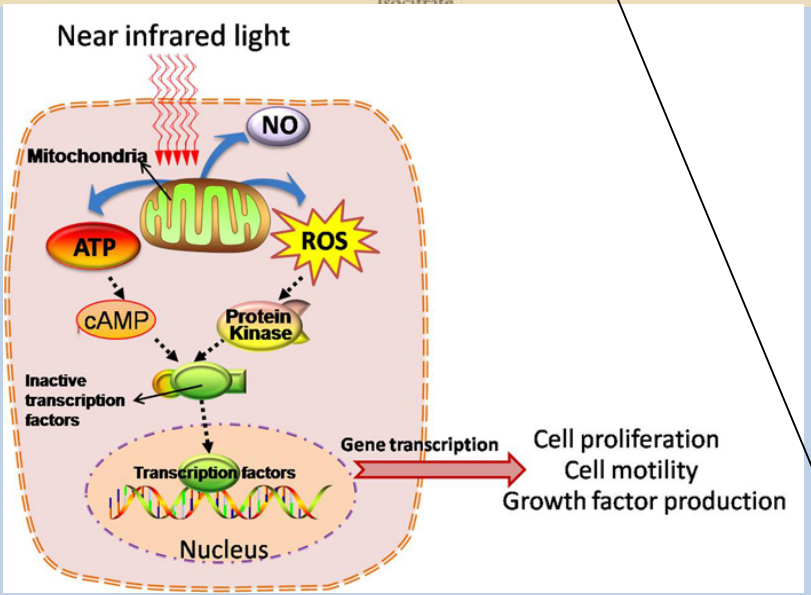
Experimental Precedent

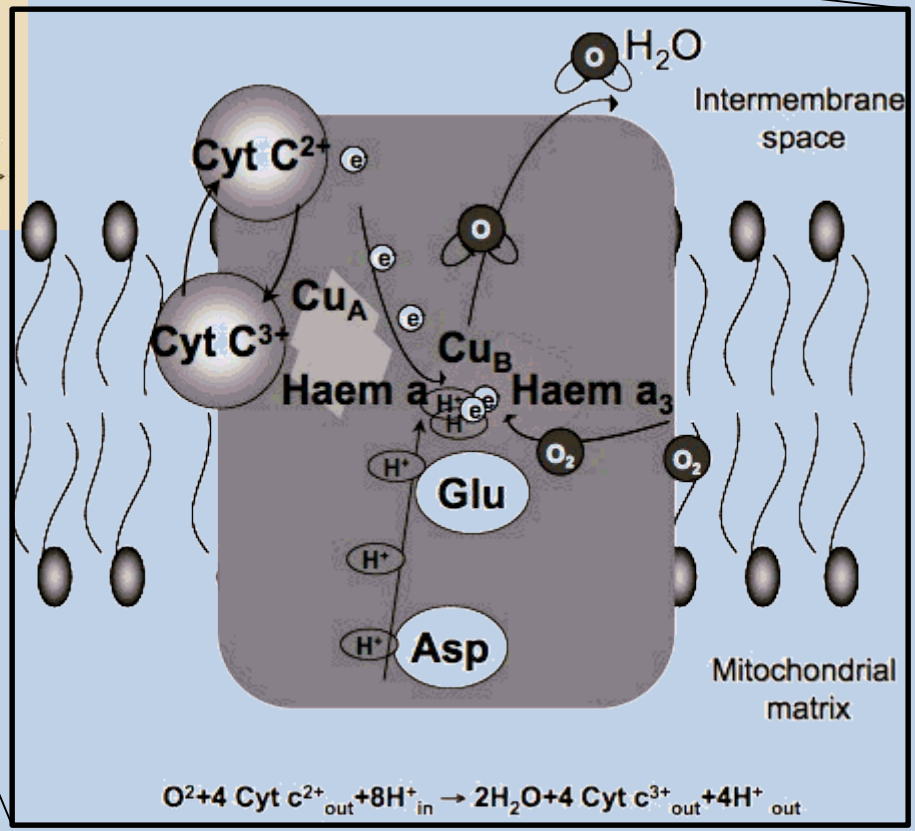


Eesmaeenerjad et. al. Lasers in Medical Science (2012)

Red and Infrared Light Energetically Excite Iron (Fe) in heme group of Cyt C^{2+} in favor of Cyt C^{3+} , a more energetically capable electron transfer state.

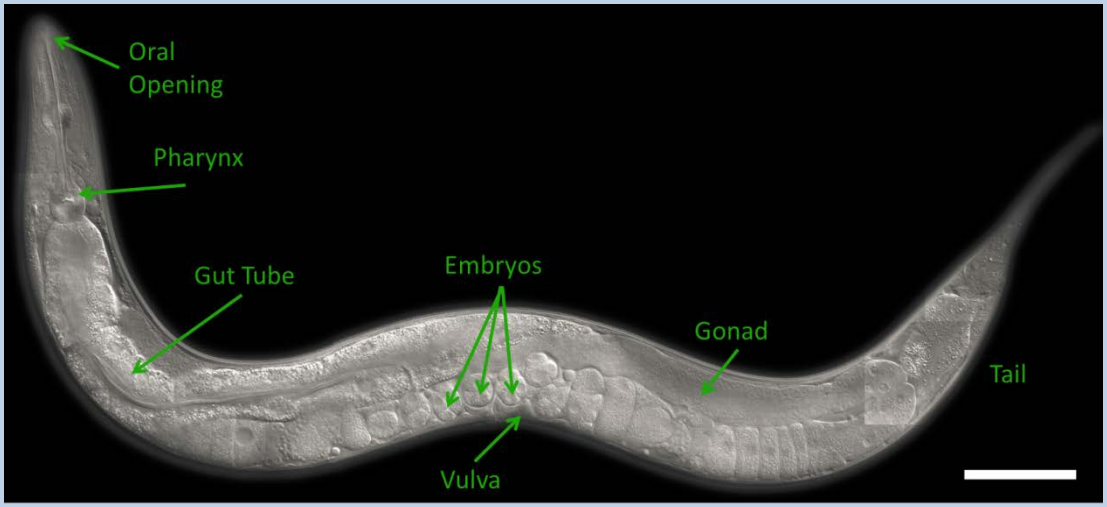






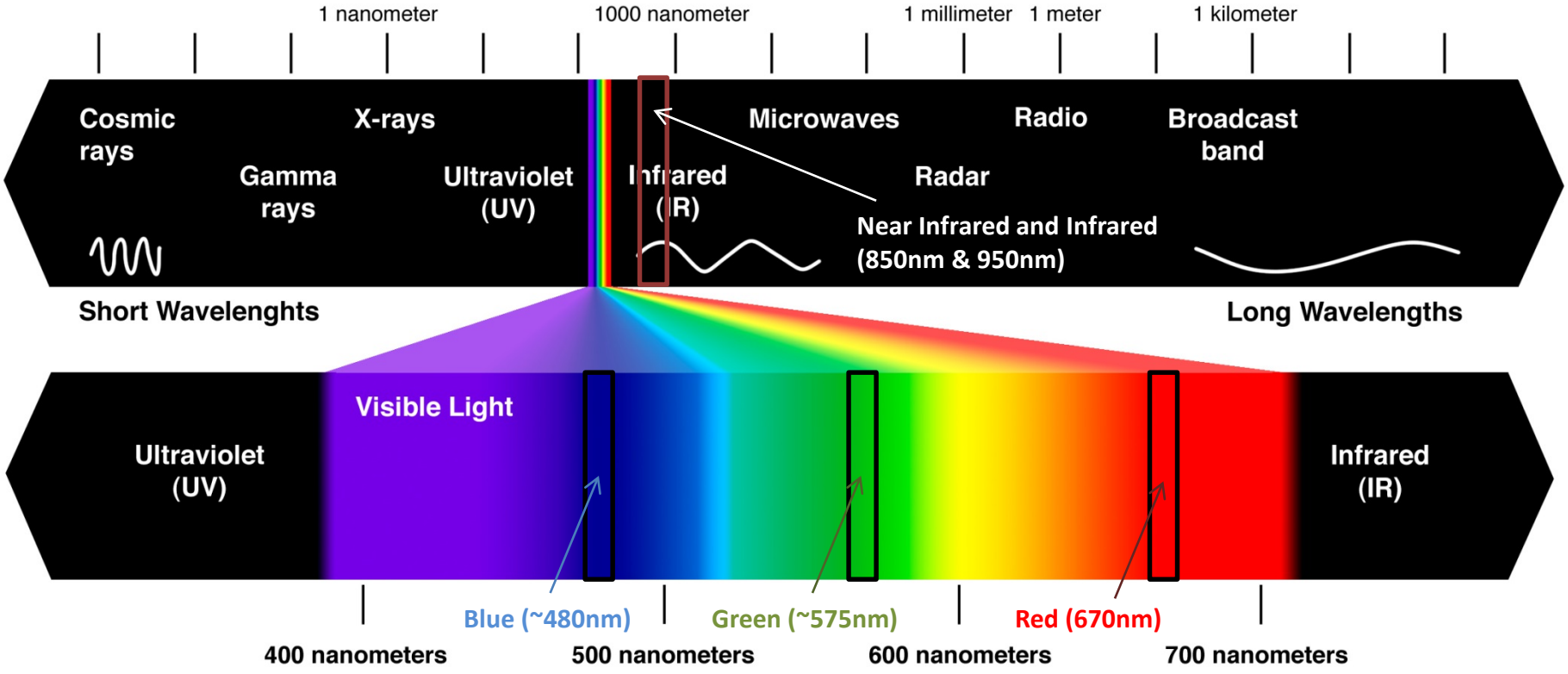
C. elegans as a Model Organism

- 60% homology with human genome, 80-85% with proteome



- Well mapped genetic code, neural network and proteome.
- Easily maintained and manipulated.
- Ideal for studying biomolecular pathways.

Experimental Light Sources



Assay For Photobiostimulatory Effect-Procedure

Setup

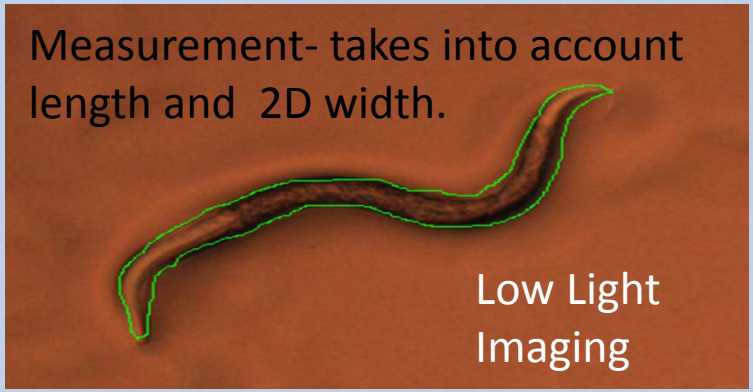
- N2 Phenotype Nematodes Farmed
- Bleach Synchronization (only embryos remain)

Exposure

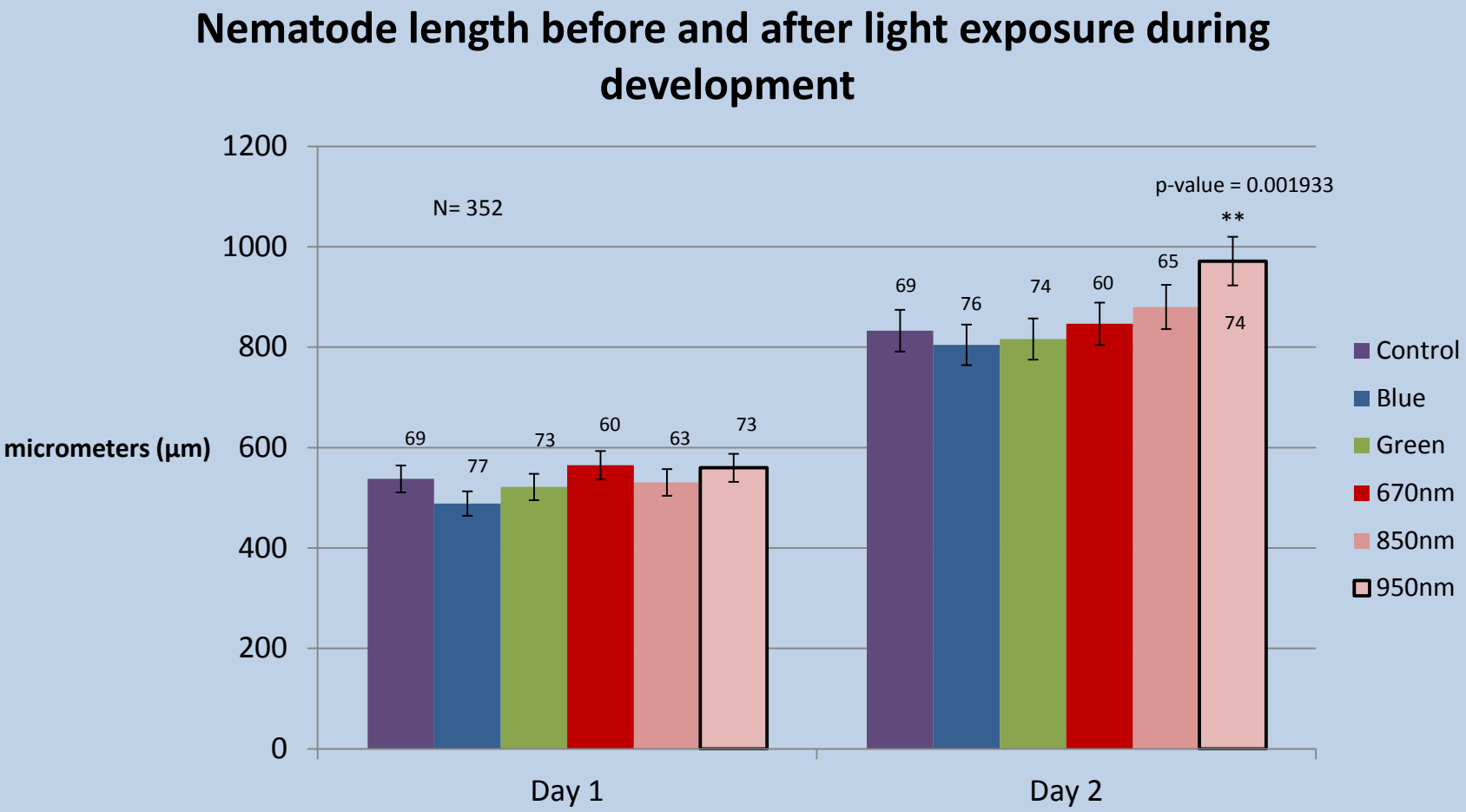
- Embryonic Development @23°C (1 day to maturity) or 16°C (72-84 hours to maturity)
- Exposure to Light (5J/cm^2) per Plate
- Blue, Green, 670nm, 850nm, 950nm, Control (no exposure) (Ultrasound added later)

Measurement

- Image Each Individual in low light @ 24 hrs. in increments Before Exposing
- Measure Length and Total Body Area in pixels and convert to microns.



Assay for Photobiostimulatory Effect



Multiple Systems Measurement

Procedure

Setup

- N2 Phenotype Nematodes Farmed
- Bleach Synchronization (only embryos remain)

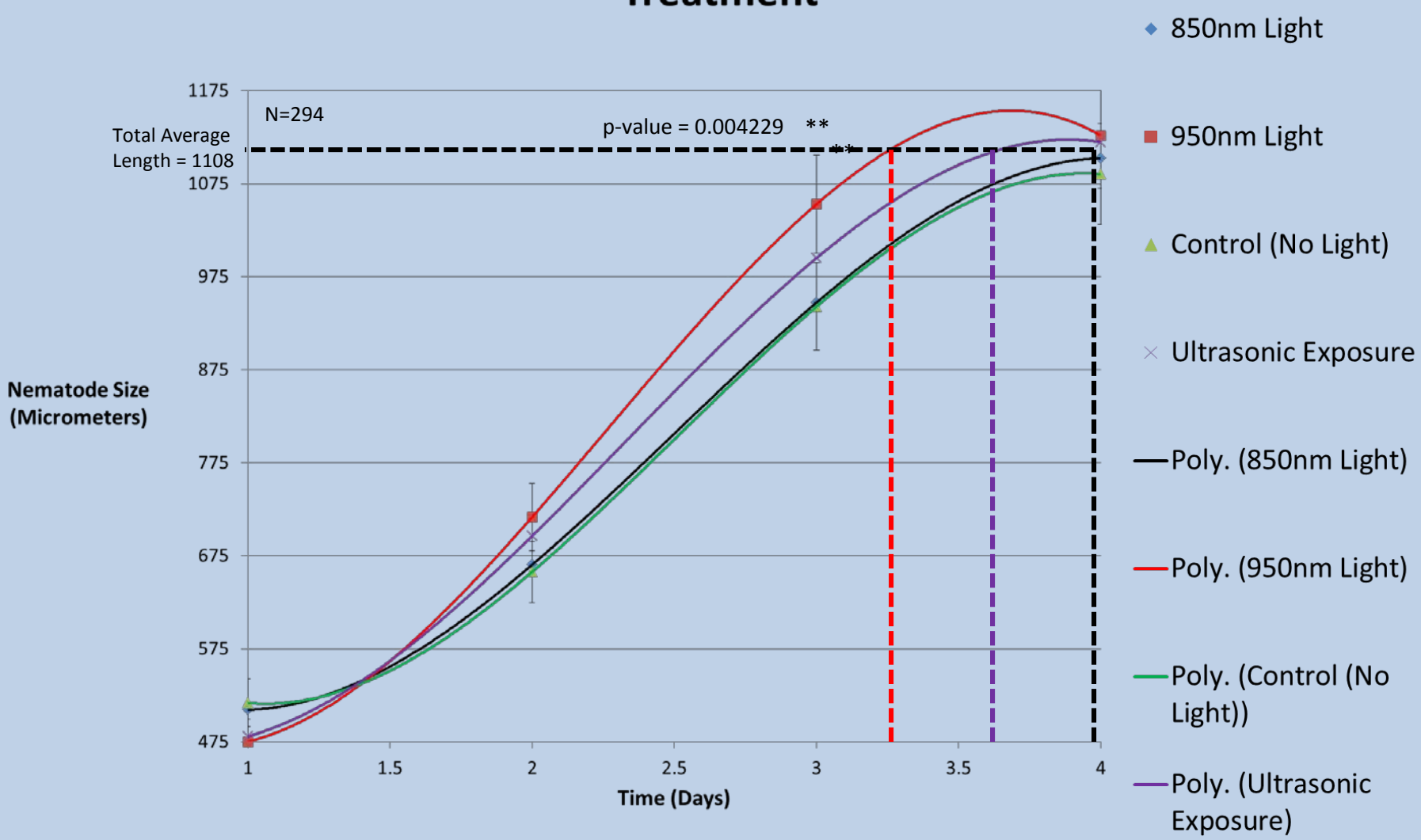
Exposure

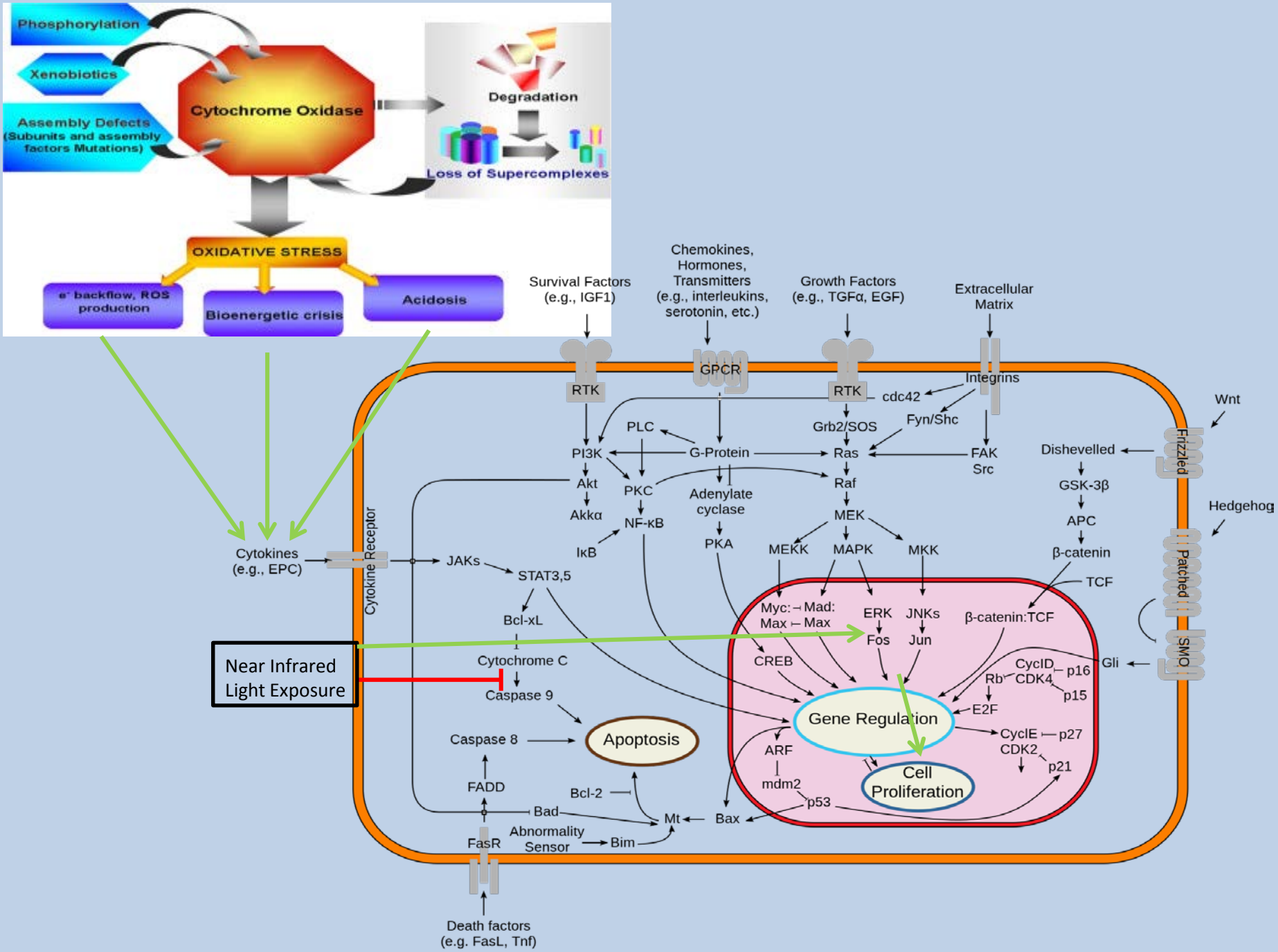
- Embryonic Development @23°C (1 day to maturity) or 16°C (72-84 hours to maturity)
- Exposure to Light (5J/cm^2) per Plate **(**12.5J/cm^2 for Prolonged Development)**
- ~~Blue, Green, 670nm~~, 850nm, 950nm, Control (no exposure) (Ultrasound added later)

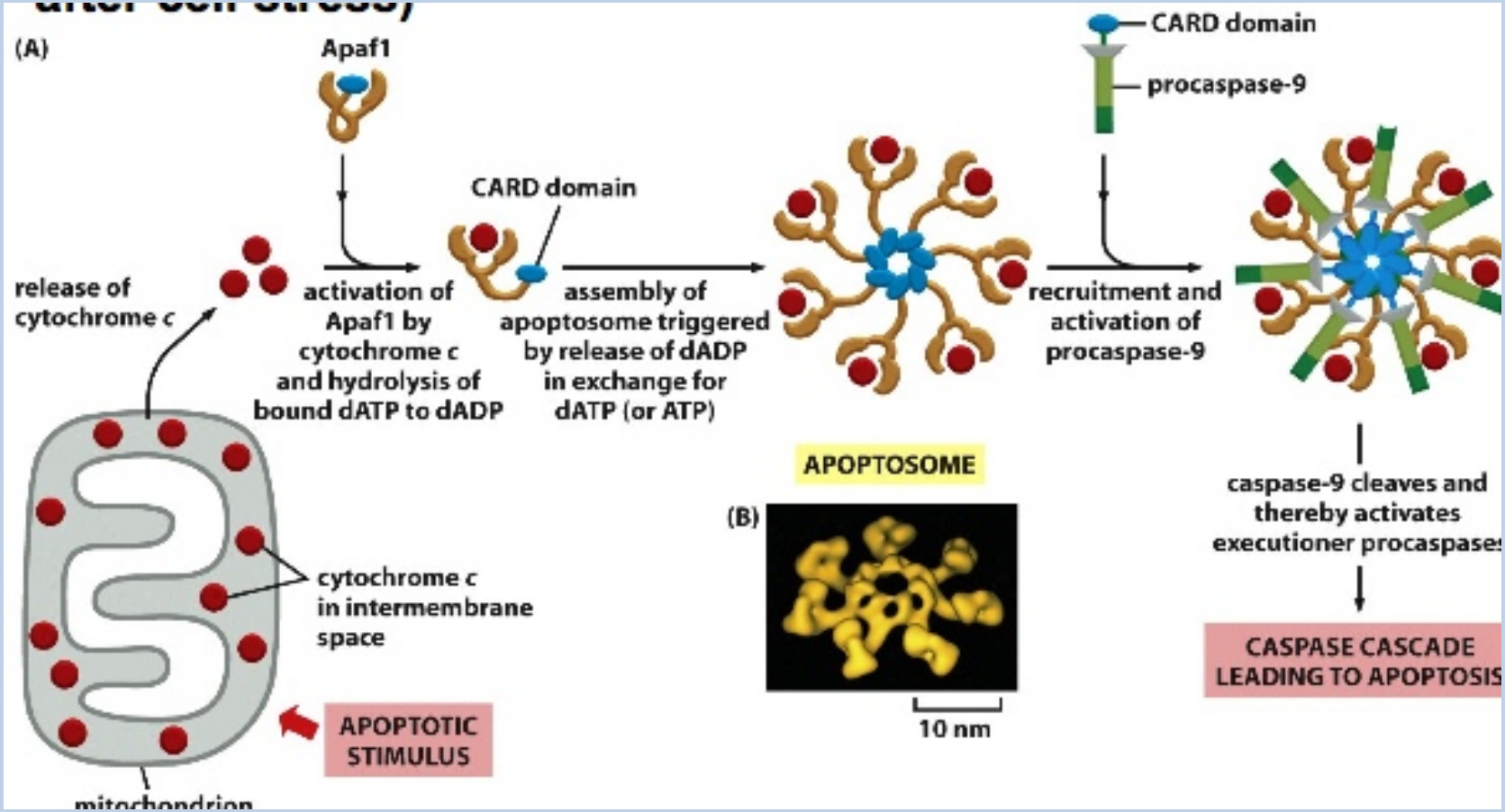
Measurement

- Image Each Individual in low light @ 24 hrs. in increments Before Exposing
- Measure Length and Total Body Area in pixels and convert to microns.

Average Size of Nematodes over Four Days based on Treatment

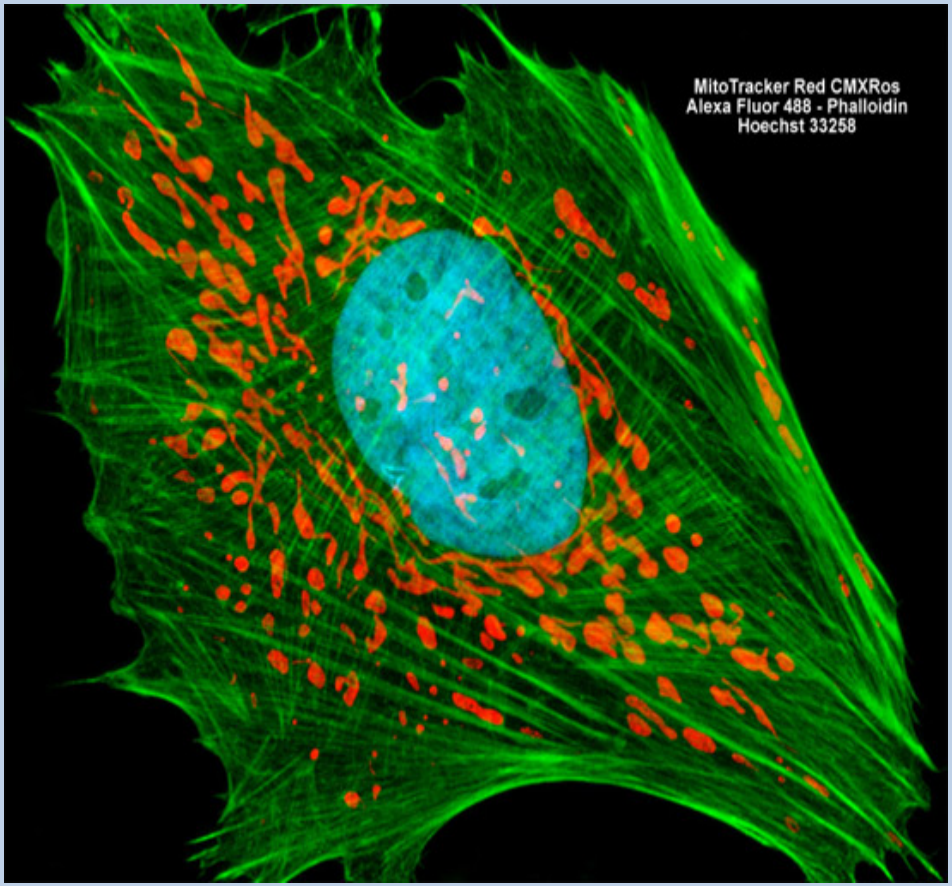



Phosphorylation
Xenobiotics
Assembly Defects (Subunits and assembly factors Mutations)
Cytochrome Oxidase
Degradation
Loss of Supercomplexes
OXIDATIVE STRESS
e⁻ backflow, ROS production
Bioenergetic crisis
Acidosis
Survival Factors (e.g., IGF1)
Chemokines, Hormones, Transmitters (e.g., interleukins, serotonin, etc.)
Growth Factors (e.g., TGFα, EGF)
Extracellular Matrix
RTK
GPCR
RTK
Integrins
cdc42
Fyn/Shc
Grb2/SOS
PLC
PI3K
G-Protein
Ras
FAK
Src
Akt
PKC
Adenylate cyclase
Raf
Akkα
NF-κB
MEK
IκB
PKA
MEKK
MAPK
MKK
Cytokines (e.g., EPC)
Cytokine Receptor
JAKs
STAT3,5
Bcl-xL
Cytochrome C
Caspase 9
Caspase 8
Apoptosis
FADD
Bcl-2
Bad
Mt
Bax
FasR
Abnormality Sensor
Bim
Death factors (e.g. FasL, Tnf)
Near Infrared Light Exposure
Myc:⊣Mad:
Max⊢Max
ERK
JNKs
Fos
Jun
CREB
β-catenin:TCF
Gene Regulation
E2F
Rb
CycID
CDK4
p16
p15
CycIE
CDK2
p27
p21
ARF
mdm2
p53
Cell Proliferation
Wnt
Frizzled
Dishevelled
GSK-3β
APC
β-catenin
TCF
Hedgehog
Patched
SMO
Gli


(A)
Apaf1
CARD domain
procaspase-9
release of cytochrome c
activation of Apaf1 by cytochrome c and hydrolysis of bound dATP to dADP
CARD domain
assembly of apoptosome triggered by release of dADP in exchange for dATP (or ATP)
recruitment and activation of procaspase-9
APOPTOSOME
(B)
10 nm
caspase-9 cleaves and thereby activates executioner procaspases
CASPASE CASCADE LEADING TO APOPTOSIS
cytochrome c in intermembrane space
APOPTOTIC STIMULUS
mitochondrion

Future Directions

- **Visualizing mitochondrial morphology**
  - Visualizing modified Oxidative Phosphorylation *in vivo*.
- **Suppression of mitochondrial mutants**
  - Hone the range of study to enhance understanding of biochemical pathway
  - Identifying the protein or proteins responsible



Works Cited

- Dixit, S., Maiya, A., Umakanth, S., and Borkar, S. (2013). Photobiomodulation of Surgical Wound Dehiscence in a Diabetic Individual by Low-Level Laser Therapy Following Median Sternotomy. Indian Journal of Palliative Care *19*, 71–75.
- Esmaeelinejad, M., Bayat, M., Darbandi, H., Bayat, M., and Mosaffa, N. (2014). The effects of low-level laser irradiation on cellular viability and proliferation of human skin fibroblasts cultured in high glucose mediums. Lasers in Medical Science *29*, 121–129.
- Fillipin, L.I., Mauriz, J.L., Vedovelli, K., Moreira, A.J., Zettler, C.G., Lech, O., Marroni, N.P., and González-Gallego, J. (2005). Low-level laser therapy (LLLT) prevents oxidative stress and reduces fibrosis in rat traumatized Achilles tendon. Lasers in Surgery and Medicine *37*, 293–300.
- Hamblin, M.R., and Demidova, T.N. (2006). Mechanisms of low level light therapy. pp. 614001–614001–12.
- Kushibiki, T., Hirasawa, T., Okawa, S., and Ishihara, M. (2013). Regulation of miRNA Expression by Low-Level Laser Therapy (LLLT) and Photodynamic Therapy (PDT). International Journal of Molecular Sciences *14*, 13542–13558.
- Oliveira, P., Sperandio, E., Fernandes, K.R., Pastor, F.A.C., Nonaka, K.O., and Renno, A.C.M. (2011a). Comparison of the effects of low-level laser therapy and low-intensity pulsed ultrasound on the process of bone repair in the rat tibia. Brazilian Journal of Physical Therapy / Revista Brasileira de Fisioterapia *15*, 200–205.
- Oliveira, P., Sperandio, E., Fernandes, K.R., Pastor, F.A.C., Nonaka, K.O., and Renno, A.C.M. (2011b). Comparison of the effects of low-level laser therapy and low-intensity pulsed ultrasound on the process of bone repair in the rat tibia. Brazilian Journal of Physical Therapy / Revista Brasileira de Fisioterapia *15*, 200–205.
- Posten, W., Wrone, D.A., Dover, J.S., Arndt, K.A., Silapunt, S., and Alam, M. (2005). Low-Level Laser Therapy for Wound Healing: Mechanism and Efficacy. Dermatologic Surgery *31*, 334–340.
- Rhee, C.-K., He, P., Jung, J.Y., Ahn, J.-C., Chung, P.-S., Lee, M.Y., and Suh, M.-W. (2013). Effect of low-level laser treatment on cochlea hair-cell recovery after ototoxic hearing loss. J. Biomed. Opt *18*, 128003–128003.
- Sievert, S.M., Scott, K.M., Klotz, M.G., Chain, P.S.G., Hauser, L.J., Hemp, J., Hügler, M., Land, M., Lapidus, A., Larimer, F.W., et al. Genome of the Epsilonproteobacterial Chemolithoautotroph Sulfurimonas denitrificans.