# Mechanisms and Examples of Biphasic Dose Response in Low-Level Light Therapy

**Michael R Hamblin PhD**

Ying-Ying Huang. Sulbha K Sharma, Gitika B Kharkwal, Luis de Taboada, Thomas McCarthy, James Carroll

# Disclosures

Consulting fees: Lexington International,
Laser Hair Therapy of North America,
Photothera Inc

Sponsored research: Lexington International,
Laser Hair Therapy of North America,
Palomar Medical Technologies,
Photothera Inc

Scientific advisory board: Lexington International,
Immunophotonics,
BeWell Global Inc

Equipment: Palomar,
Photomedex,
Photothera Inc,
Irradia US

# Outline

- Introduction to mechanisms of LLLT
- Survey of biphasic dose responses in LLLT
- Studies in mouse embryonic fibroblasts
- Studies in mouse primary cortical neurons
- Transcranial LLLT in mouse model of traumatic brain injury
- Conclusions

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*Formerly Nonlinearity in Biology, Toxicology, and Medicine*

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www.Dose-Response.org

**BIPHASIC DOSE RESPONSE IN LOW LEVEL LIGHT THERAPY**

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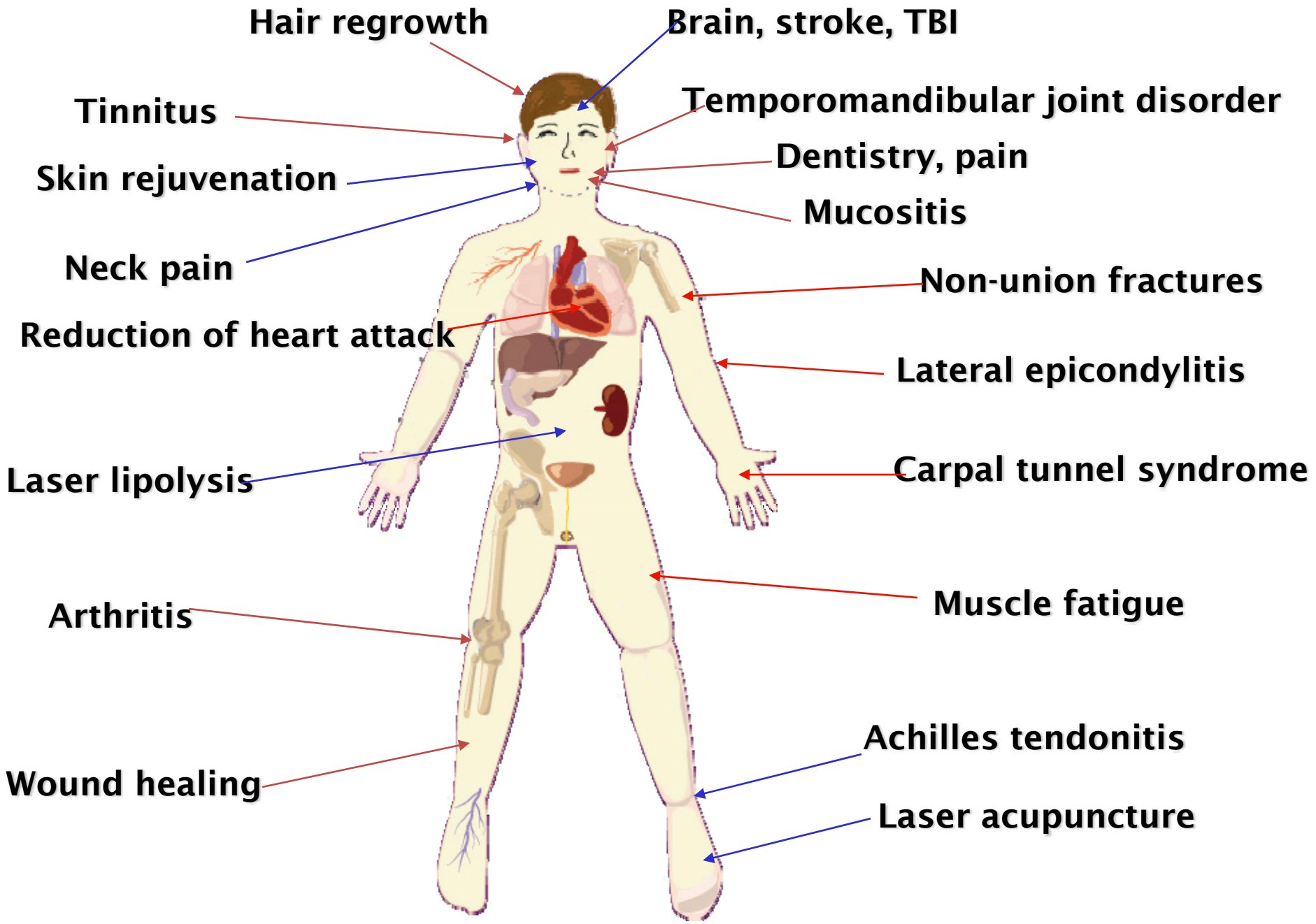
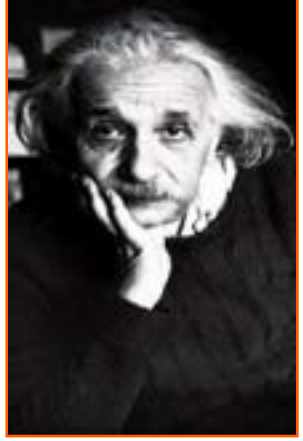
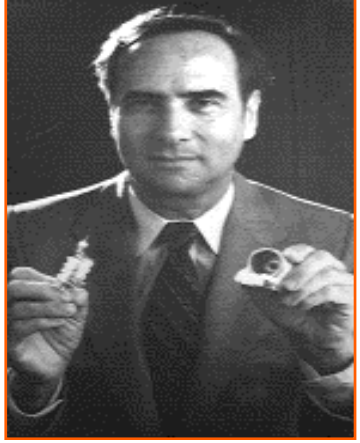
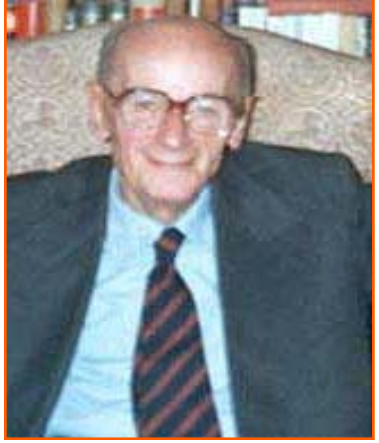
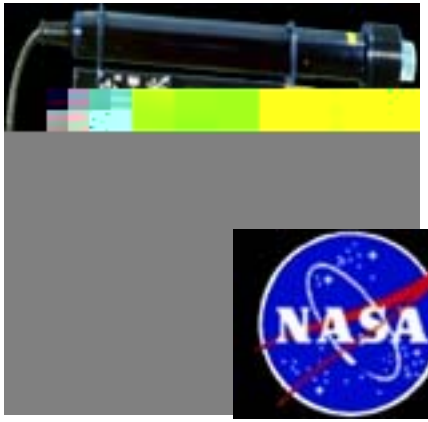
# THOR LLLT Knowledge Matrix

| | | | | | |
|---|---|---|---|---|---|
| **Introduction (context)** | Phototherapy is not new | Not like laser surgery | History & future | Large evidence base | Safe, credible<br>Many applications |
| **Mechanism** | Primary<br>Absorption / molecular rotations and vibrations | Secondary<br>NO ATP ROS<br>membrane permeability | Tertiary<br>Transduction<br>Gene transcription<br>Secretion Signalling | Quaternary<br>Amplification / systemic | Increasing ATP,<br>Reduces oxidative stress and ??? |
| **Clinical Benefits** | Repair | Inflammation | Analgesia | Function<br>muscle, lymph | when LLLT does not work .... |
| **Physics** | Photons<br>Electromagnetic spectrum | Wavelength | Laser vs other<br>Coherence<br>polarisation<br>spectral width | Propagation<br>Depth of target,<br>RATS | What is light<br>How does it behave |
| **Dosimetry** | Wavelength absorption | Irradiance dose rate | Pulse<br>Period on, period off<br>Peak & average power | Tx time<br>How many times<br>Interval | Define medicine<br>Time is the dose |
| **Where** | Injury | Lymph | Nerve<br>Ganglia, trigger points | Blood ?<br>Glands ?<br>Preconditioning ? | Clinical targets |
| **Other Clinical** | Safety | Contraindications | Regulations | Reimbursement | Safe<br>mixed<br>acceptance.health economics |


Hair regrowth
Brain, stroke, TBI
Tinnitus
Temporomandibular joint disorder
Skin rejuvenation
Dentistry, pain
Mucositis
Neck pain
Non-union fractures
Reduction of heart attack
Lateral epicondylitis
Laser lipolysis
Carpal tunnel syndrome
Arthritis
Muscle fatigue
Achilles tendonitis
Wound healing
Laser acupuncture

1916 - Einstein first proposed the phenomenon of stimulated emission
1960 - Theodore “Ted” Maiman built the first working laser
1967 - Prof. Mester - Budapest - discovers laser biostimulation
1983 - UK Physios first use low power laser for sports injuries treatment
1998 - NASA conducted LED therapy wound healing study for space
1999 - US Military nerve regeneration research with THOR lasers
2003 - First FDA clearance for THOR
2005 - Spinal cord regeneration
2006 - LLLT published in Nature and Pain
2007 - Clinical trial of 810-nm laser for Stroke
2008 - WHO Bone & Joint Task Force recommend on neck pain (Spine)
2009 - Lancet review neck pain
2010 - APTA recommends LLLT for Achilles Tendinopathies
2010 - BMJ “strong evidence” for LLLT on frozen shoulder
2010 - Intl Assoc for the Study of Pain, “strong evidence“ Chronic Myofascia
2011 - ???

FDA
U.S. Food and Drug Administration

World Health Organization

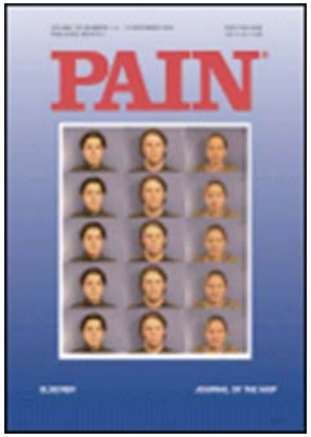
THE LANCET

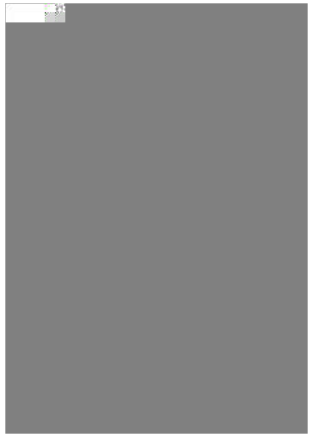
APTA
American Physical Therapy Association

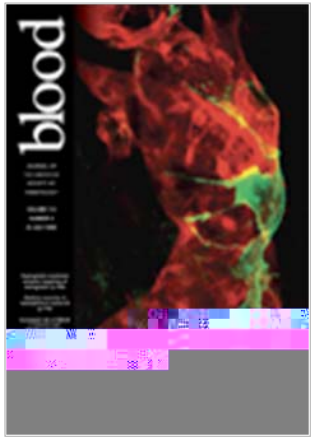
BMJ

IASP

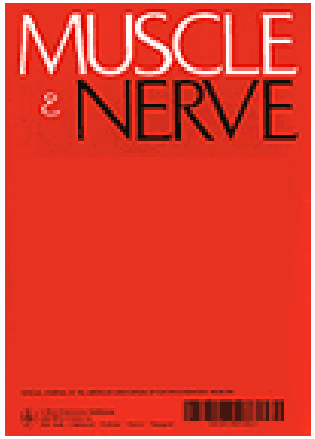
# Low Level Laser Therapy (LLLT)
# Published in the worlds top scientific journals
# Over 200 (RCT) clinical trials
# Over 2000 laboratory studies

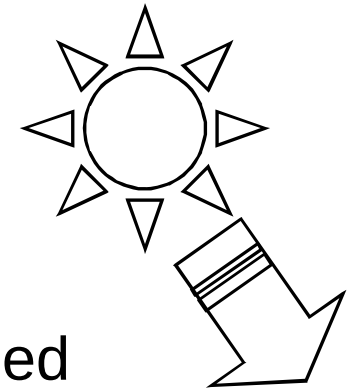




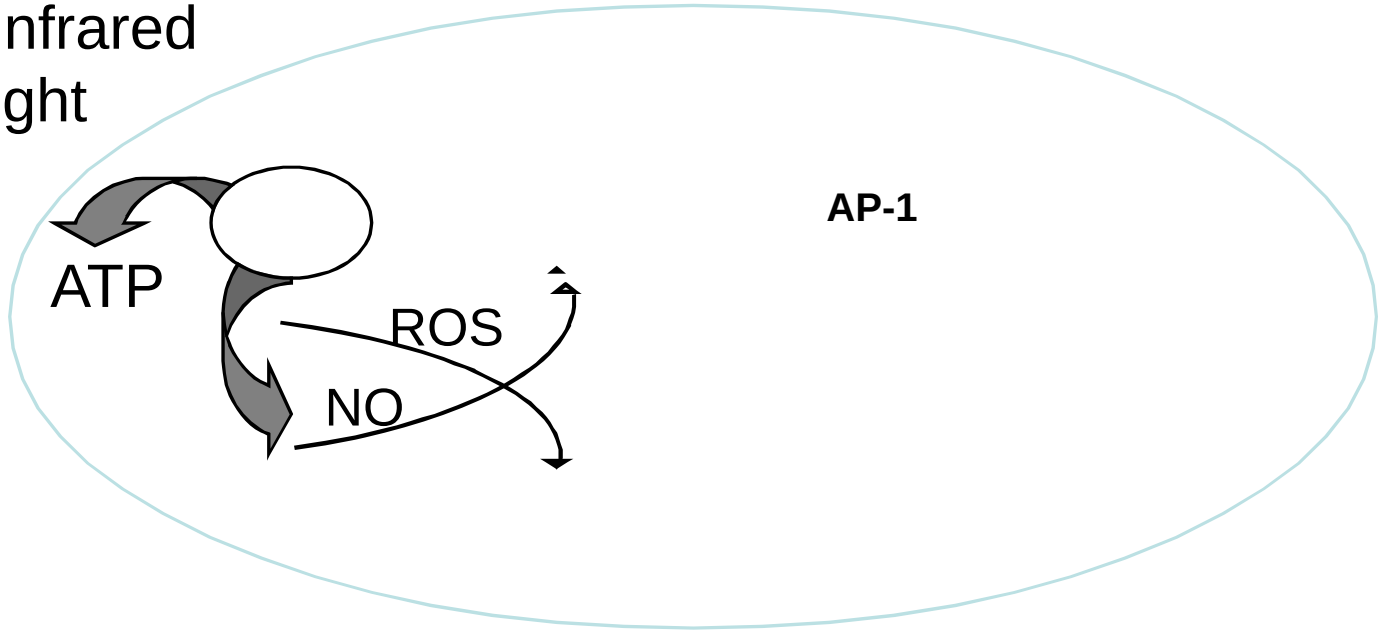




# Mechanisms of LLLT



Red
near infrared
light



# POWER GAMES

There's a fight going on inside all our cells for each breath of air. **Nick Lane** sheds therapeutic light on the implications for cancer and degenerative diseases.

**"The finding that the body could poison one of its own enzymes was initially shrugged off as an imperfection."**

Yet over the past decade, researchers have come to appreciate that cells often use CO, and to an even greater extent NO (nitric oxide), to block respiration. Not only that, but light has striking counter-effects on cytochrome oxidase. And all these suitors to the enzyme turn out to be critical to our understanding not just

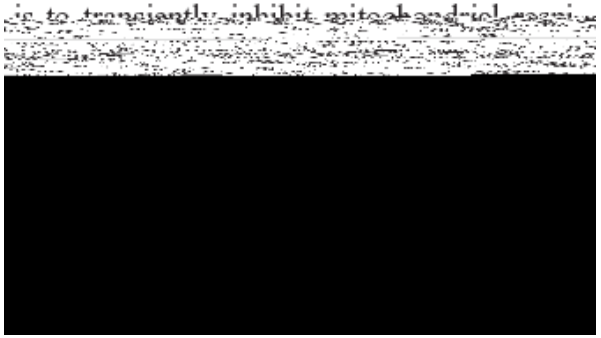
## Nitric Oxide and the Control of Firefly Flashing

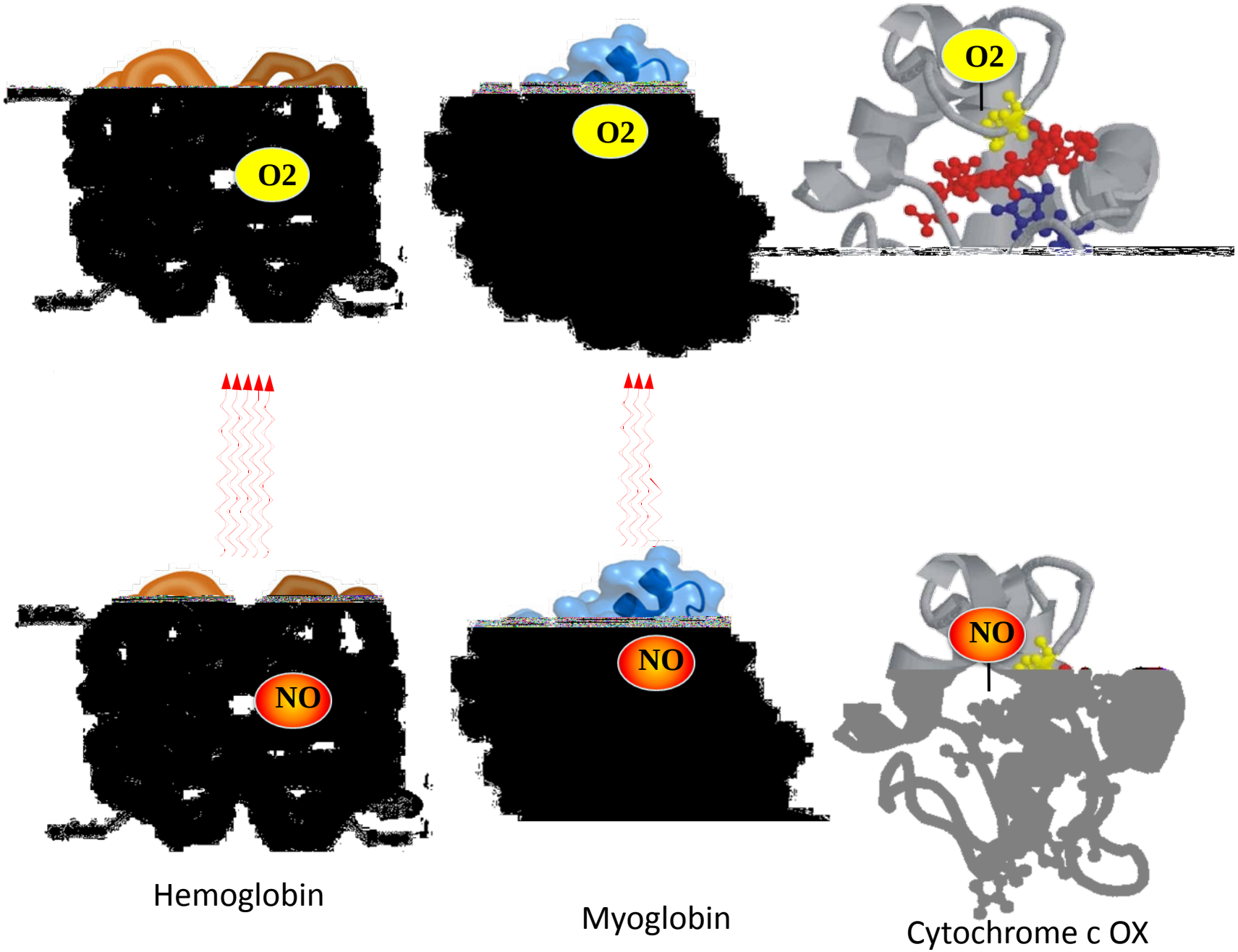
**Barry A. Trimmer,[1]* June R. Aprille,[1] David M. Dudzinski,[2] Christopher J. Lagace,[1] Sara M. Lewis,[1] Thomas Michel,[2,3] Sanjive Qazi,[1] Ricardo M. Zayas[1]**

[1]Department of Biology, Tufts University, Medford, MA 02155, USA. [2]Cardiovascular Division, Brigham and Women's Hospital, and [3]Veterans Affairs Boston Healthcare System, Harvard Medical School, Boston, MA 02115, USA.

29 JUNE 2001 VOL 292 SCIENCE

The results reported here document an important role for NO in firefly flash control. It is well established that $O_2$ availability is the immediate biochemical trigger for light production, and we propose that the role of NO is to transiently inhibit mitochondrial respi-




O2
O2
O2
NO
NO
NO
Hemoglobin
Myoglobin
Cytochrome c OX

# Basic mechanisms

Mitochondria are primary photoreceptors

Cytochrome c oxidase activity is increased

NO is dissociated from COX + heme proteins

ATP and cAMP increased

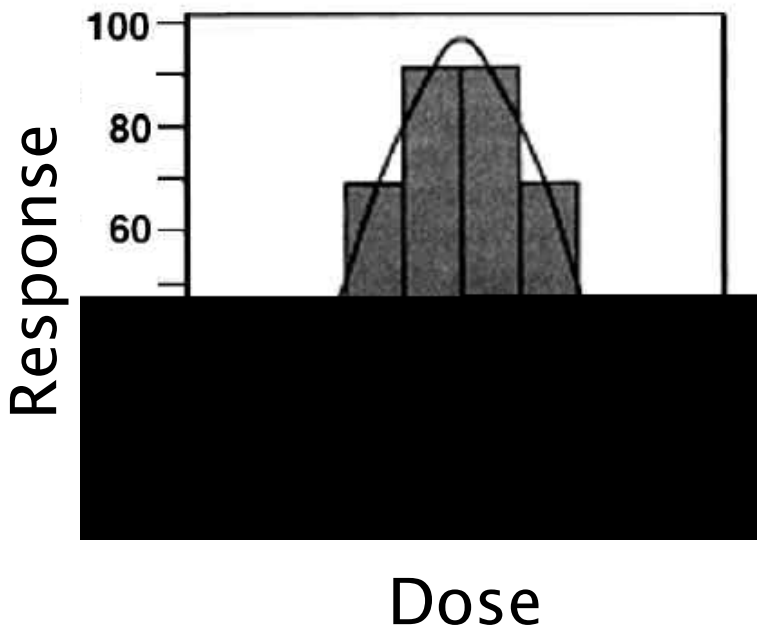
Reactive oxygen species are produced

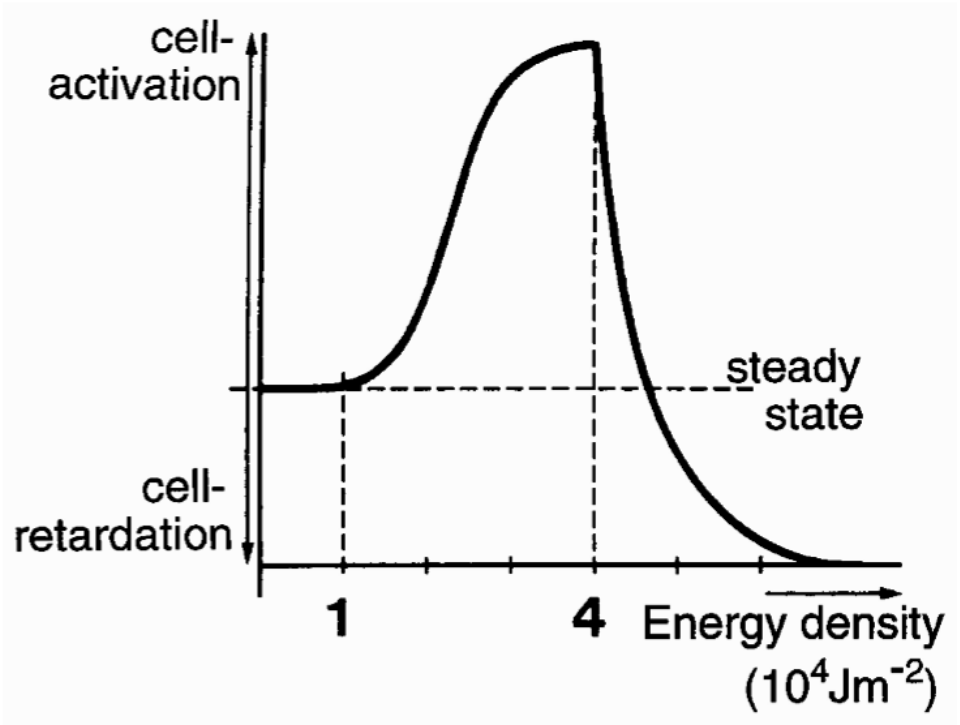
Transcription factor induction

Growth, repair, survival, less inflammation

# Biphasic dose response?

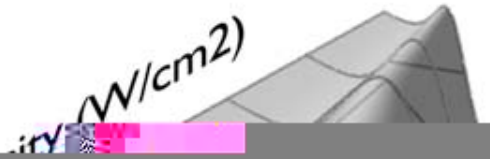
Arndt-Schulz curve




Sommer AP, Pinheiro AL, Mester AR, Franke RP, Whelan HT. (2001) Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system. J Clin Laser Med Surg. Feb;19(1):29-33

# 3D Arndt Schulz model to illustrate 'dose sweet spot'



# How is dose measured?

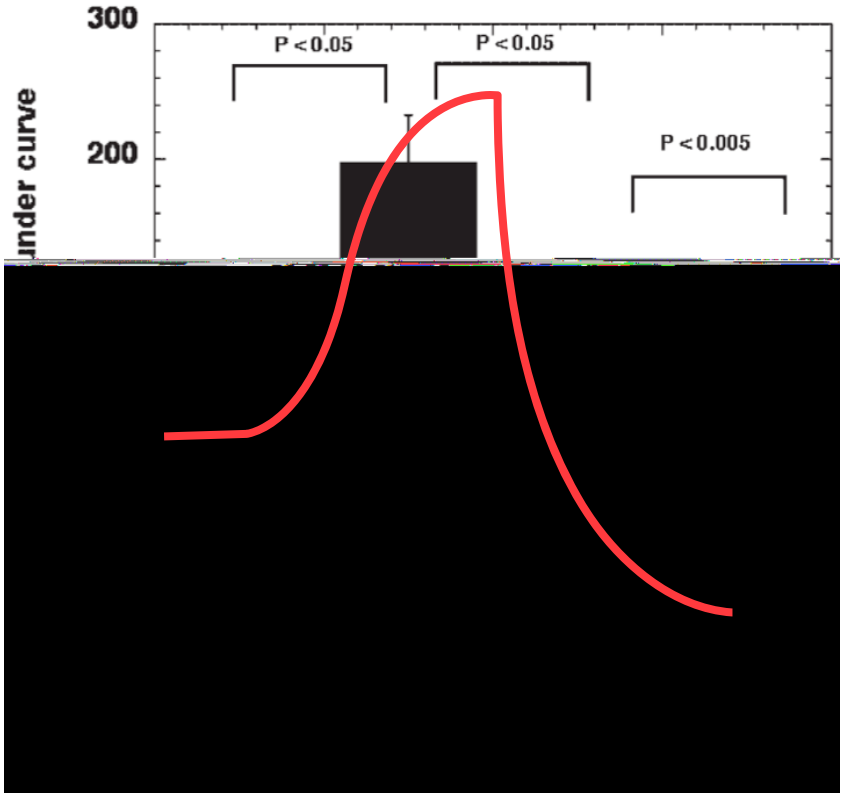
Power (W) x Time (sec) = Energy (Joules)

$$\frac{\text{Power mW}}{\text{Beam Area cm2}} = \text{(irradiance) x time = fluence (J/cm2)}$$

Arguments have been made for total energy, fluence, irradiance and illumination time to be most important parameters in measuring dose

# Stimulation/Inhibition of Wound Healing in Mice

635-nm Laser for wound healing in mice




Lasers in Surgery and Medicine 39:706–715 (2007)

## Low-Level Light Stimulates Excisional Wound Healing in Mice

**Tatiana N. Demidova-Rice, BS,[1,2] Elena V. Salomatina, BS,[1] Anna N. Yaroslavsky, PhD,[1,3] Ira M. Herman, PhD,[2*] and Michael R. Hamblin, PhD[1,3,4**]**

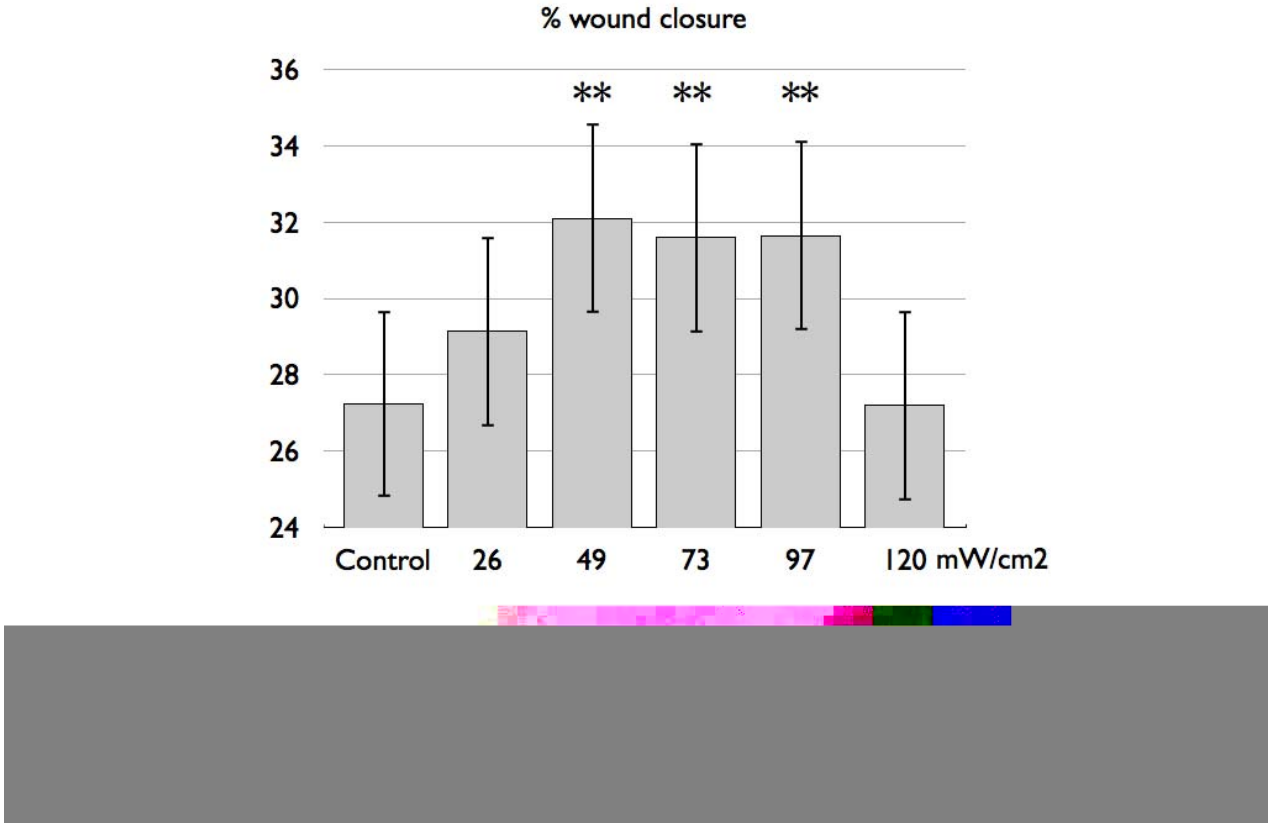
[1]*Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts 02114*

[2]*Graduate Program in Cell Molecular and Developmental Biology, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, Massachusetts 02111*

[3]*Department of Dermatology, Harvard Medical School, Boston, Massachusetts 02115*

[4]*Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts 02139*

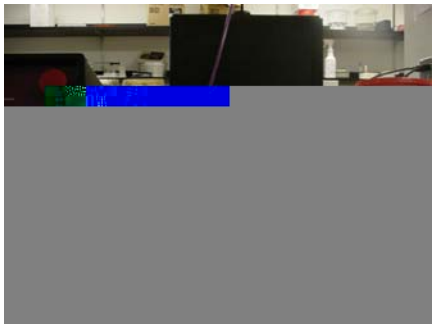
# Wound healing in vitro 980-nm laser - scratch in fibroblast monolayer

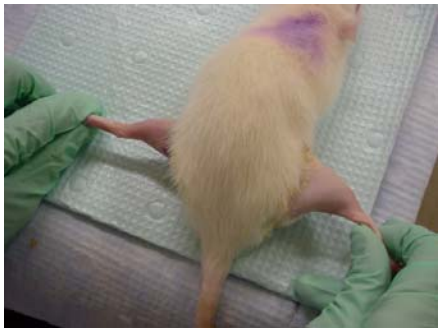



Constant time - irradiance and fluence vary

Mark D. Skopin & Scott C. Molitor
*Effects of near-infrared laser exposure in a cellular model of wound healing*
*Photodermatol Photoimmunol Photomed,* ***25, 75-80.(2009).***

# 810-nm laser for arthritis in rats





| | 3 J/cm2 | 30 J/cm2 |
|---|---|---|
| 5 mW/cm2 | 10 minutes | 100 minutes |
| 50 mW/cm2 | 1 minute | 10 minutes |

Hypothesis: length of illumination is more important than total fluence or irradiance in LLLT effect

Lasers in Surgery and Medicine 39:543–550 (2007)

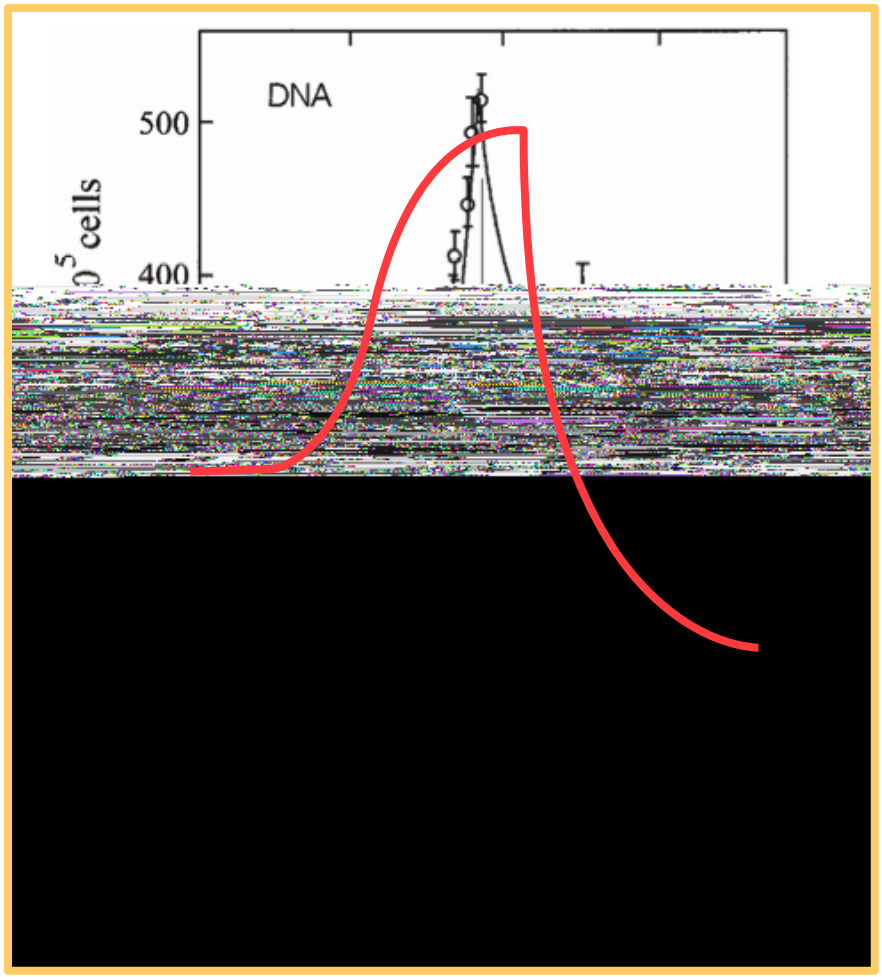
## Low-Level Laser Therapy for Zymosan-Induced Arthritis in Rats: Importance of Illumination Time

Ana P. Castano, MD,[1,2] Tianhong Dai, PhD,[1,2] Ilya Yaroslavsky, PhD,[3] Richard Cohen, MD,[3] William A. Apruzzese, PhD,[3] Michael H. Smotrich, PhD,[3] and Michael R. Hamblin, PhD[1,2,4]*

[1]*Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts 02114*
[2]*Department of Dermatology, Harvard Medical School, Boston, Massachusetts 02115*
[3]*Palomar Medical Technologies Inc., Burlington, Massachusetts 01803*
[4]*Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts 02139*

# • Dose response curve

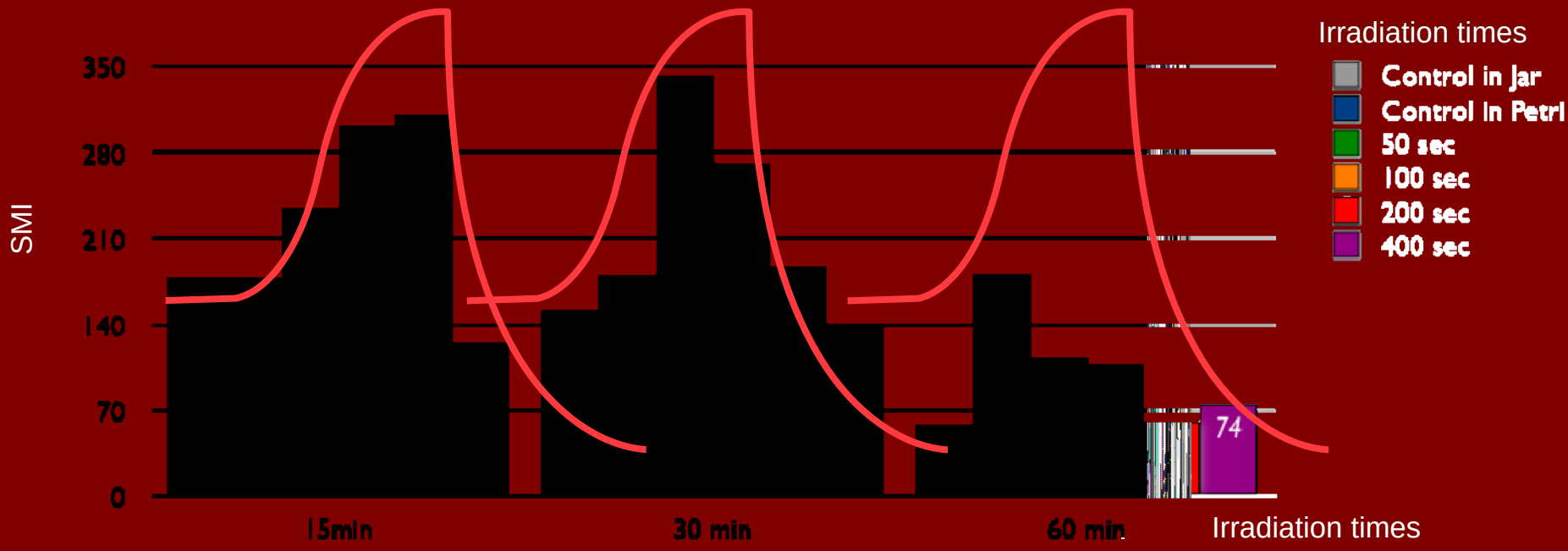
HeLa DNA synthesis
633nm
0.1J/cm2
10 - 1000mW/cm2




increase power
reduce time
(same energy)

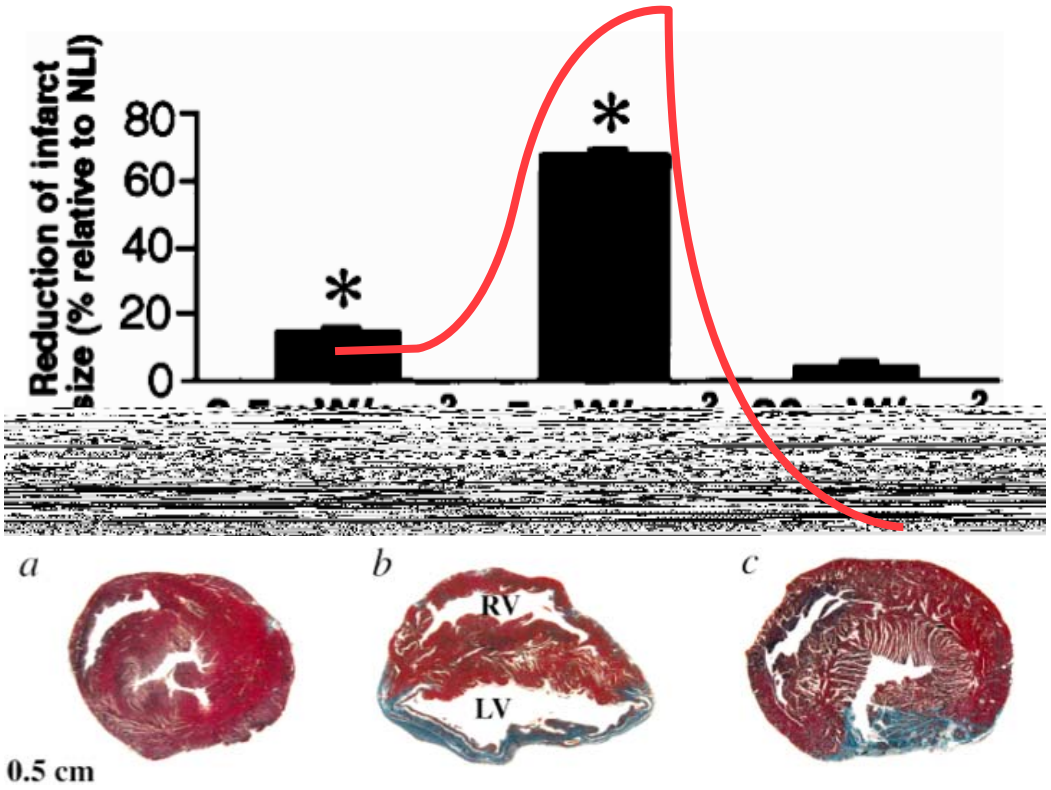
Karu TI, Kolyakov SF. (2005) Exact action spectra for cellular responses relevant to phototherapy. Photomed Laser Surg. Aug;23(4):355-61.

# Motility changes over time
# Fresh Semen
## LED 45mW/cm2




Motility changes over time
with different irradiation times using
104 x LED Cluster mixed 660nm & 850nm
45mW/cm2

# Reduction of infarct size (heart attack) by low-energy laser



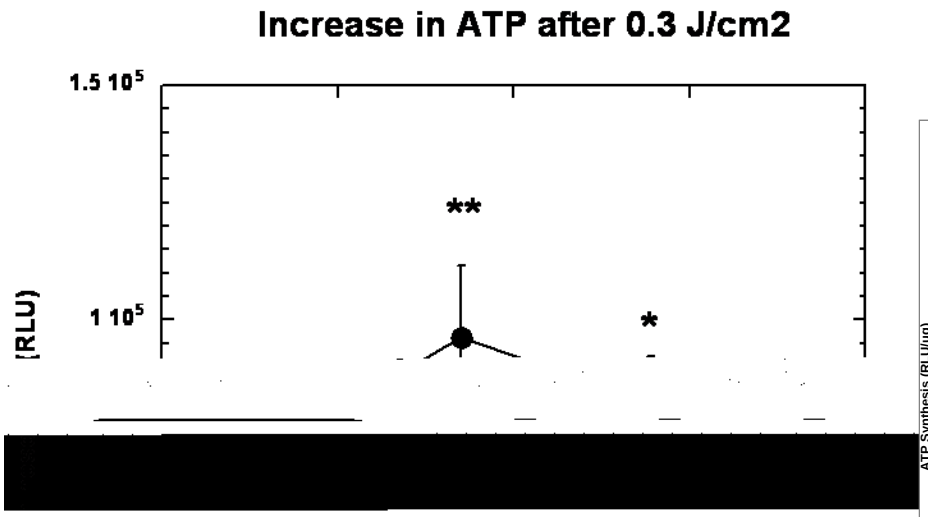

Oron U, et al
Attenuation of infarct size in rats and dogs after myocardial infarction by low-energy laser irradiation. Lasers Surg Med.;28(3):204-11.

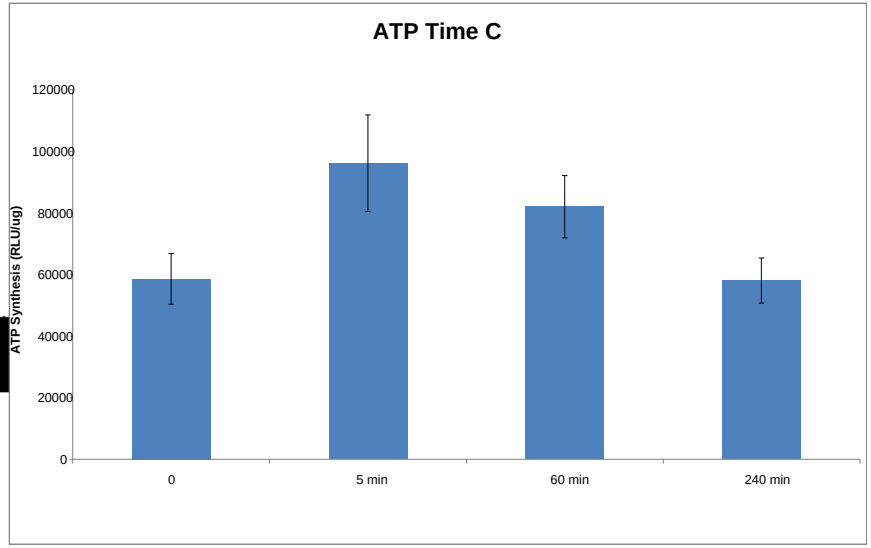
# Does LLLT activate NF-kB?

1. Establish a fibroblast cell line (3T3 protocol) from NF-kB luciferase reporter mice (HLL)
2. Deliver different fluences of 810-nm light from a laser (or other light source)
3. Keep illumination time constant at 5 min (vary irradiance)
4. After various times assay for luciferase expression (NF-kB activation) and cellular ATP

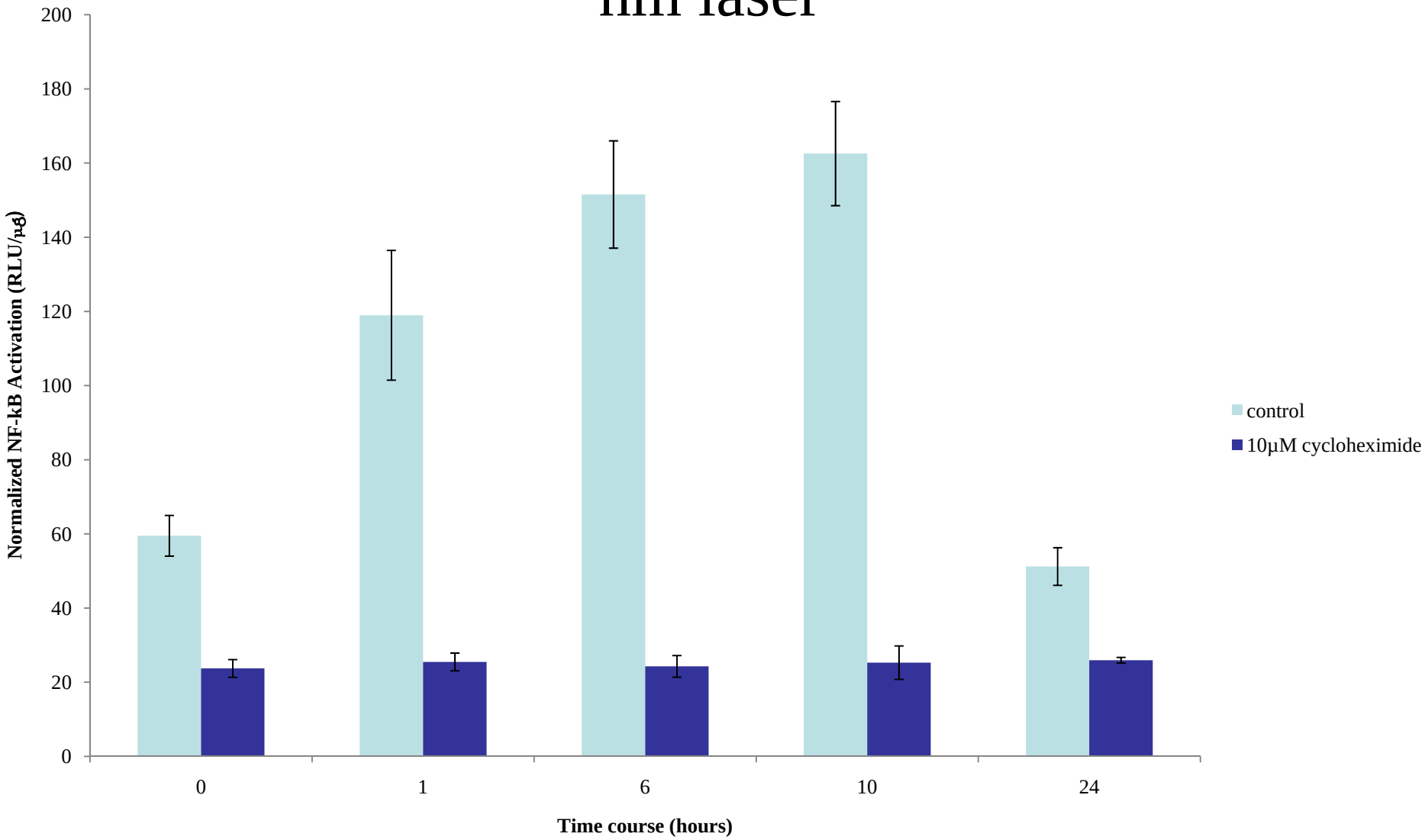
# 1. Effects on ATP production

## 0.3 J/cm$^2$ 810-nm delivered over 5 min

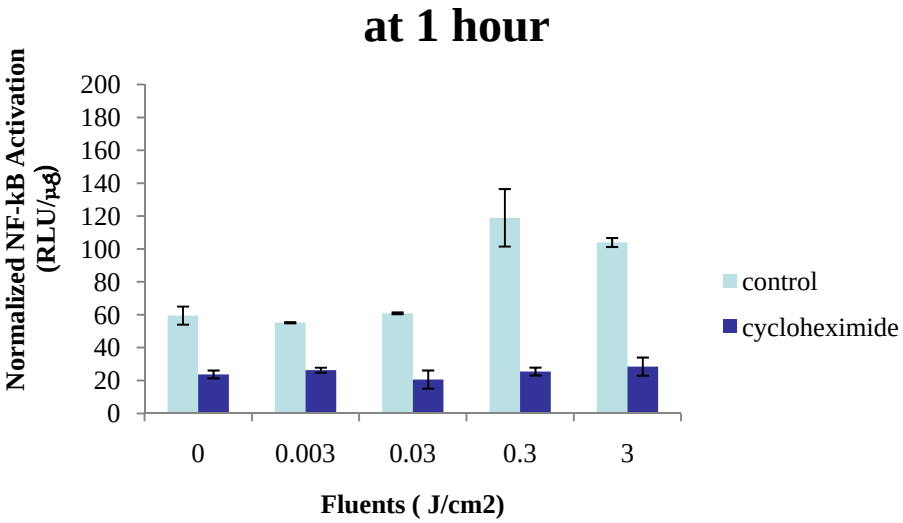


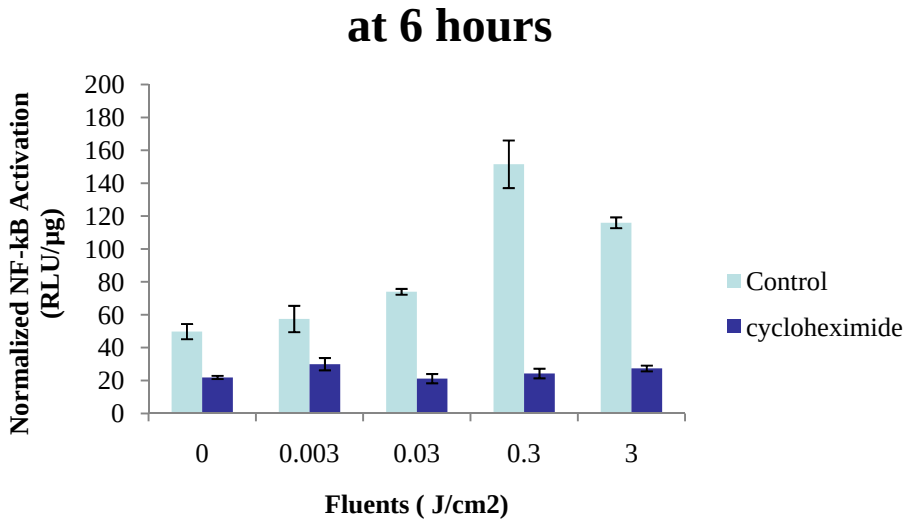



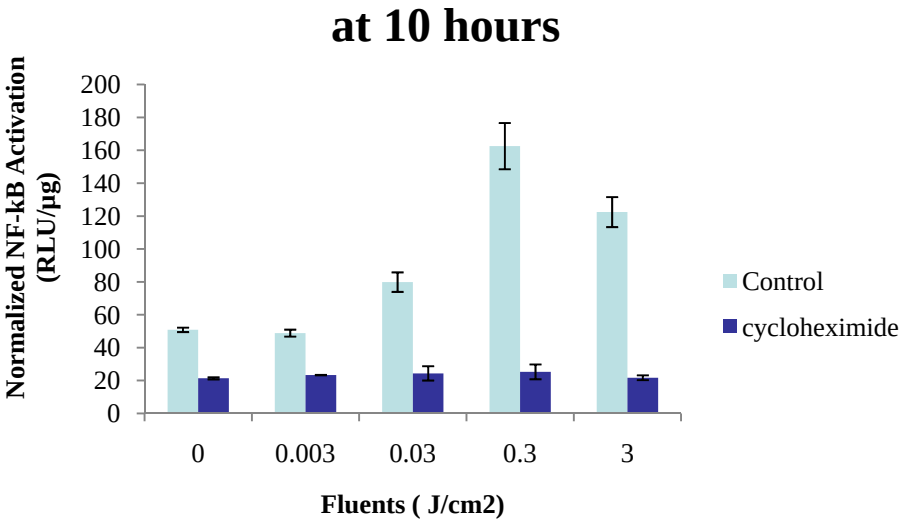
# NF-kB Activation induced by 0.3 J/cm$^2$ 810-nm laser

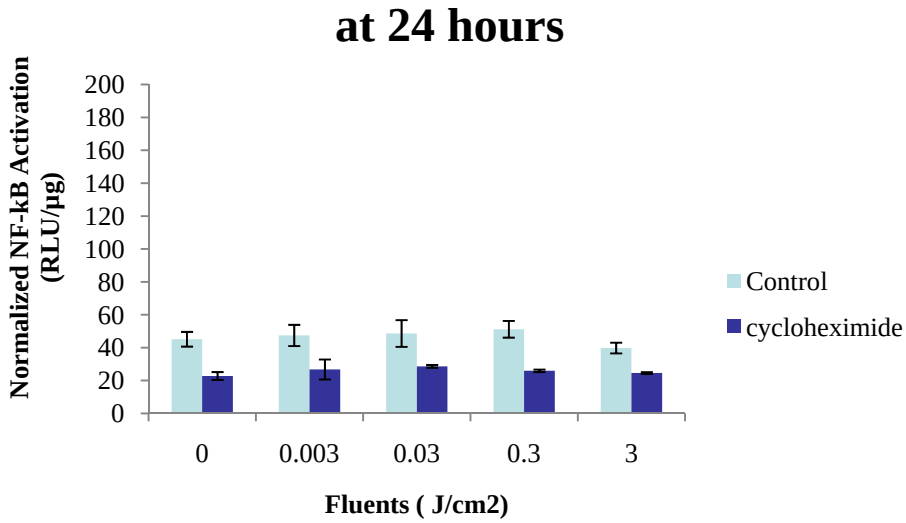


# Laser Induced NF-kB Activation

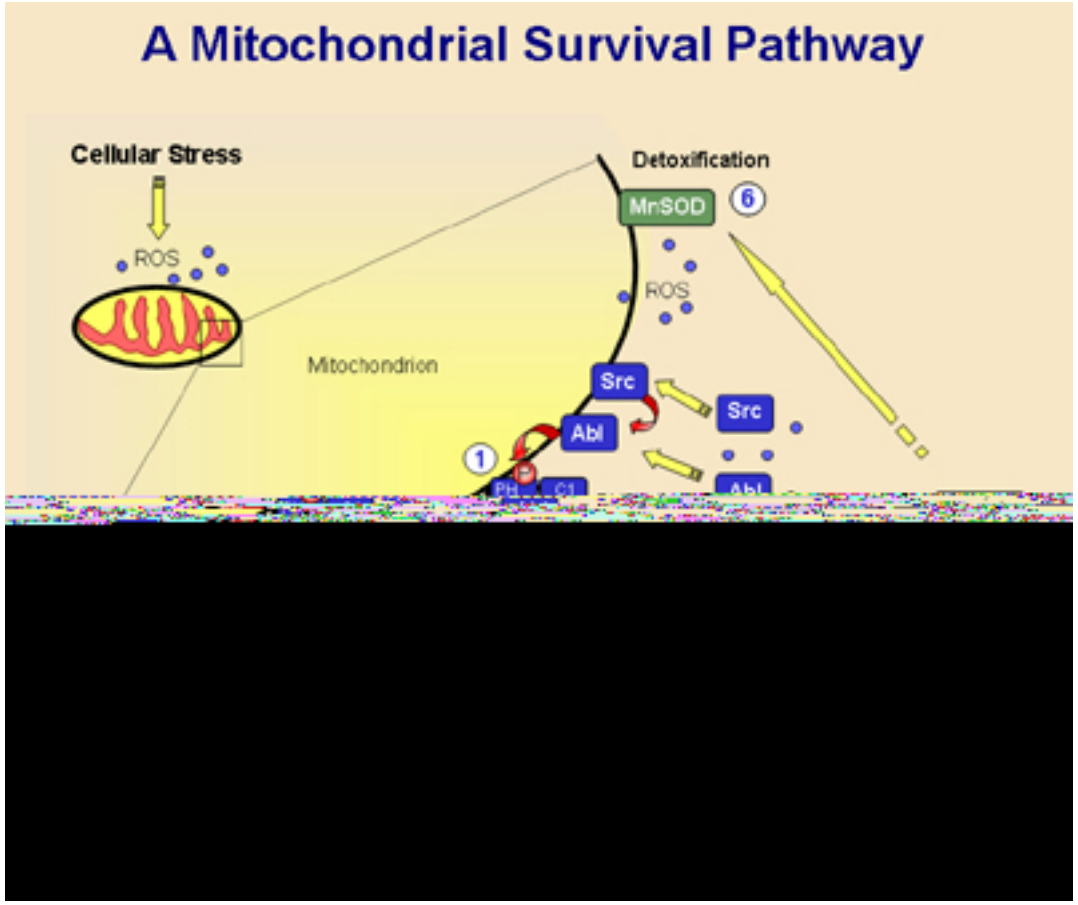


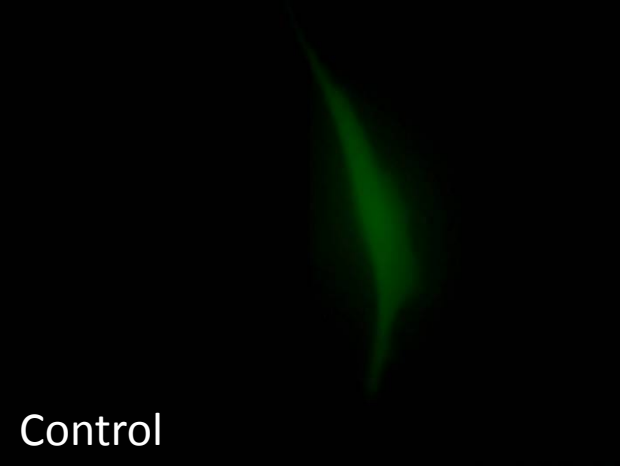



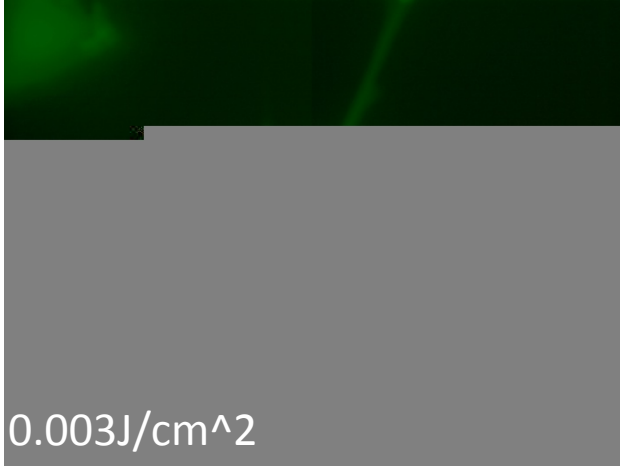
# Mitochondrial ROS induces transcription factor NF-kB

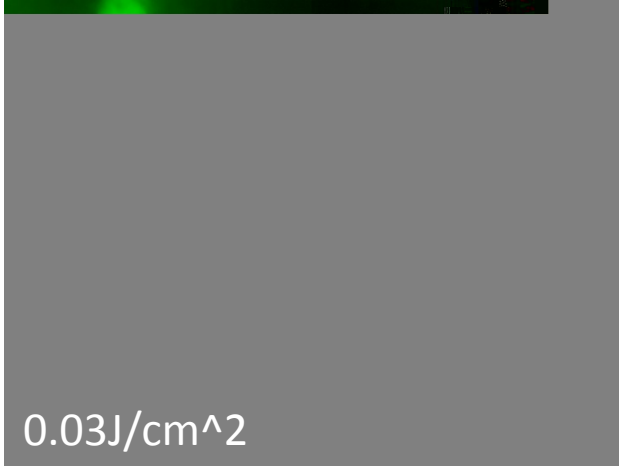


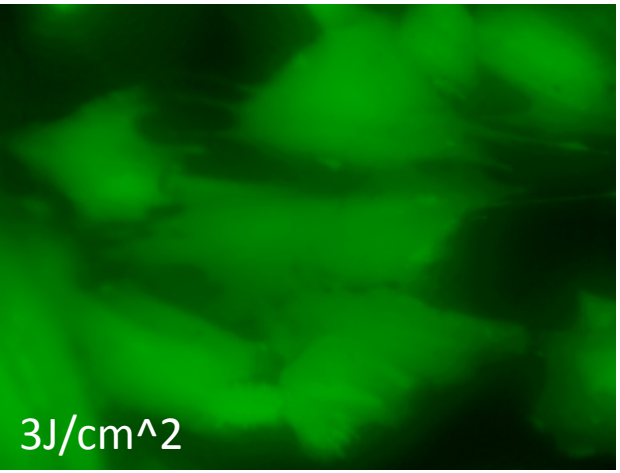
What is mechanism of NF-kB activation?

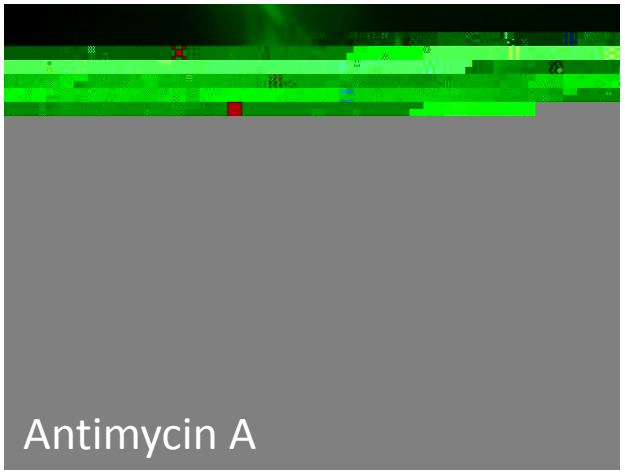
Hypothesis is reactive oxygen species

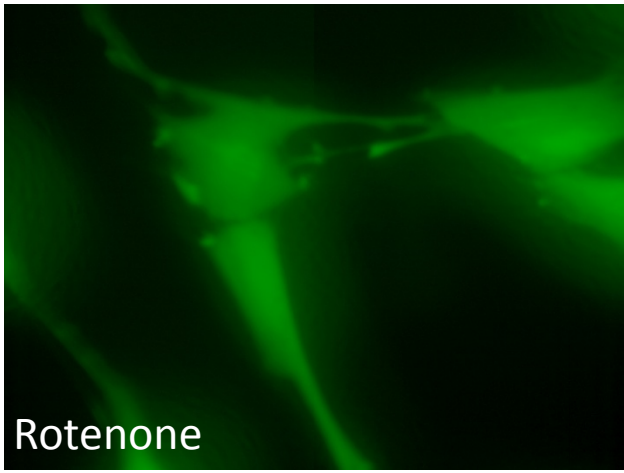


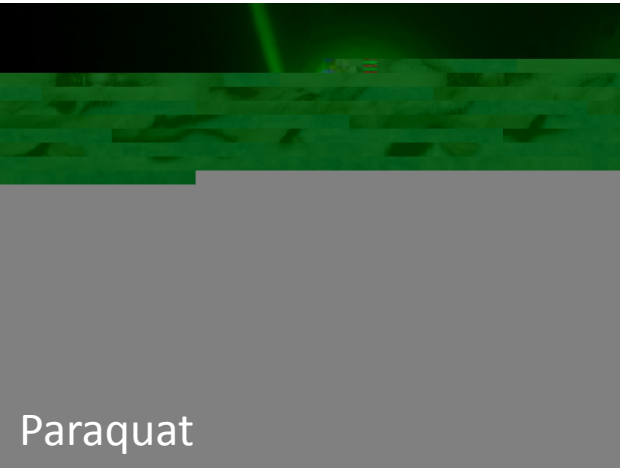



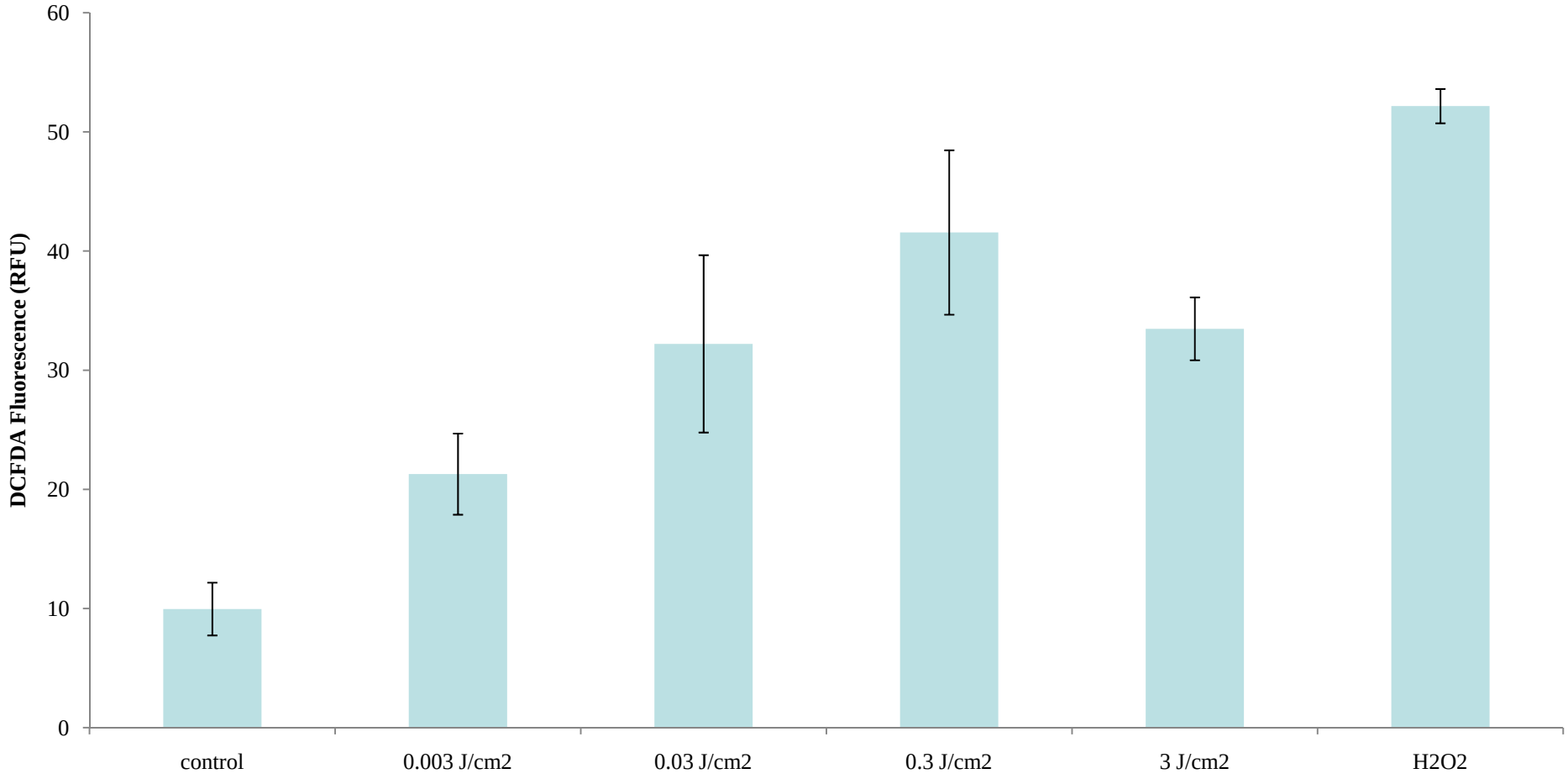



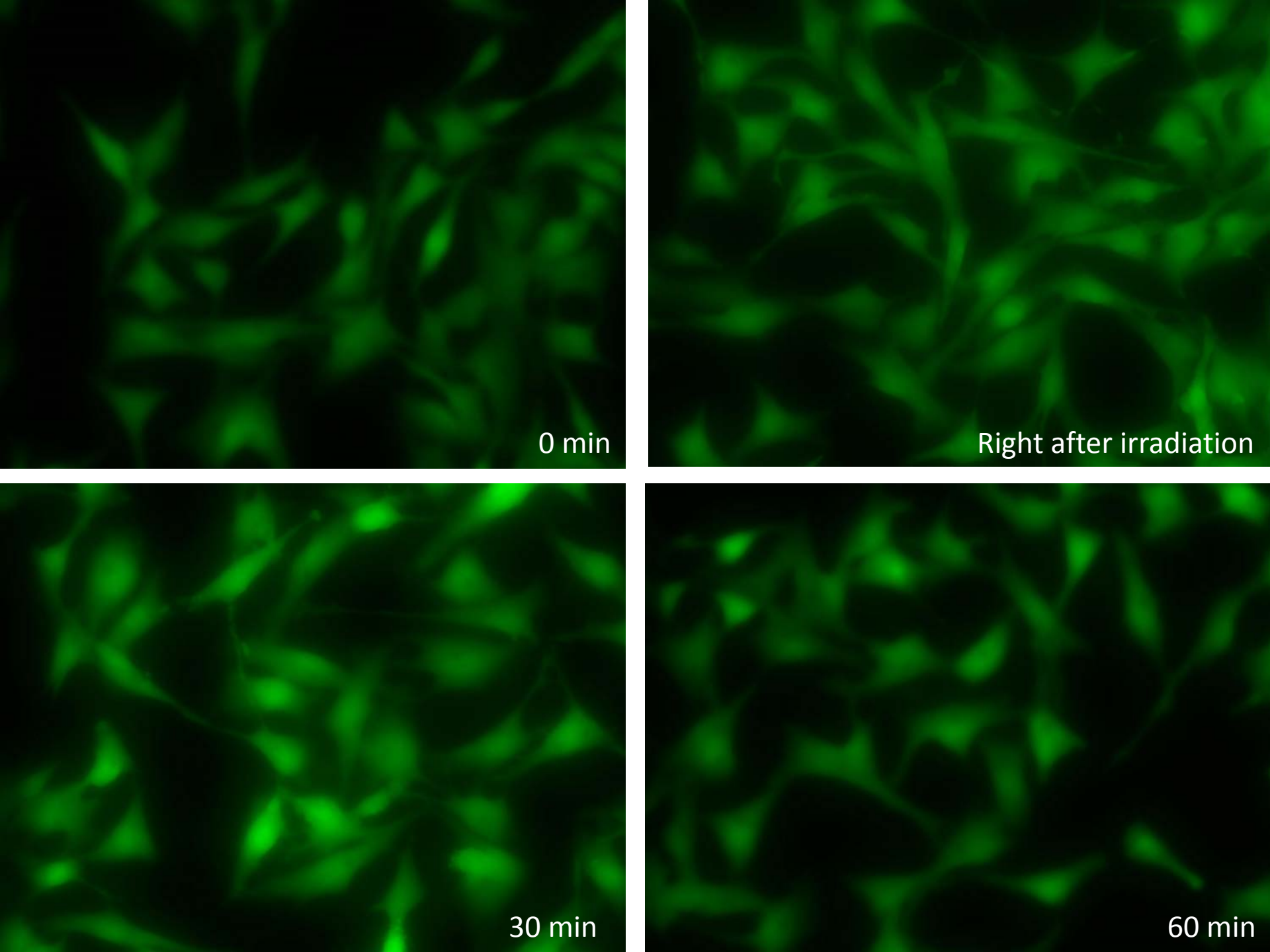



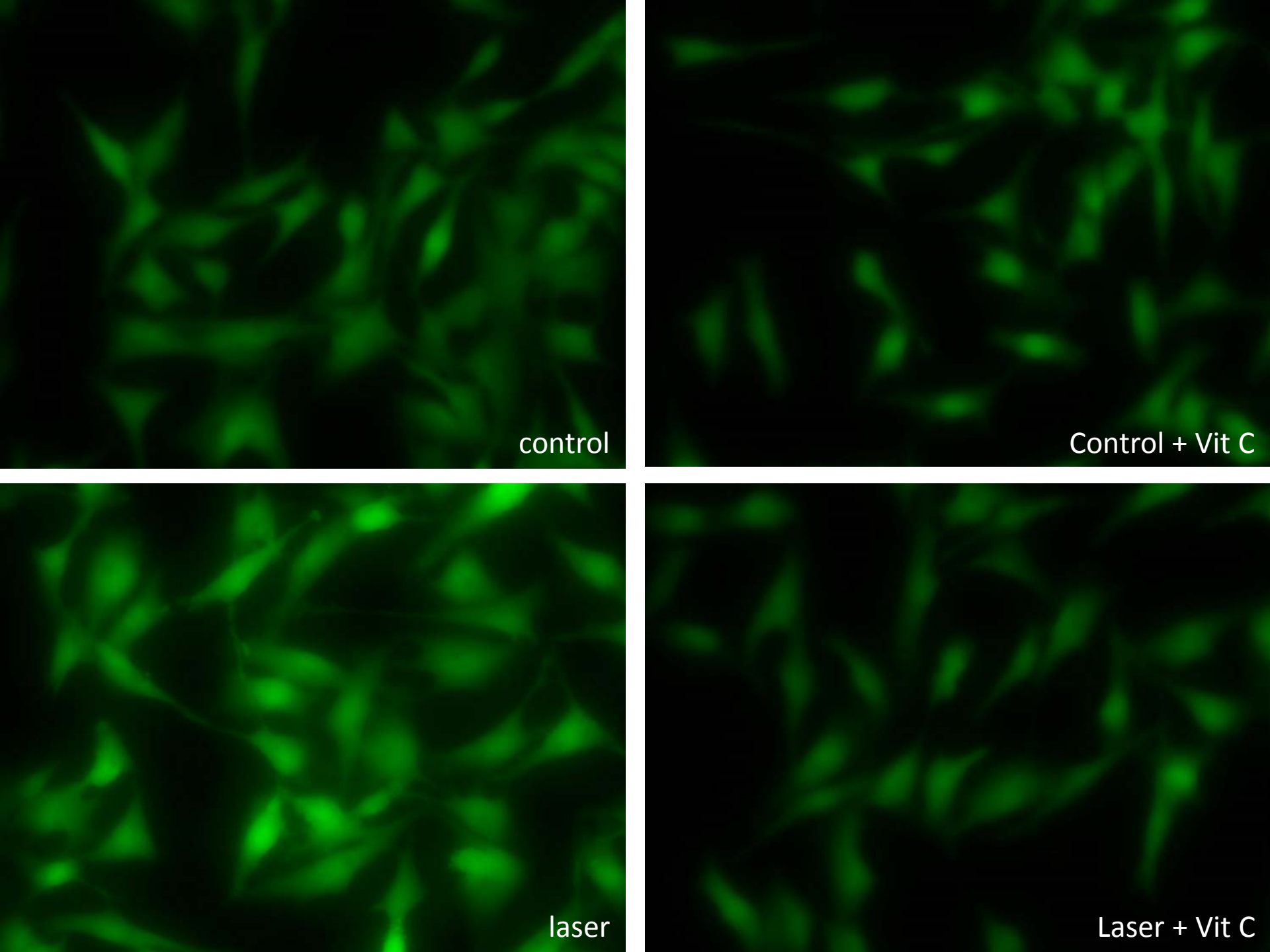




DCFDA
Sensitive to lipid hydroperoxides

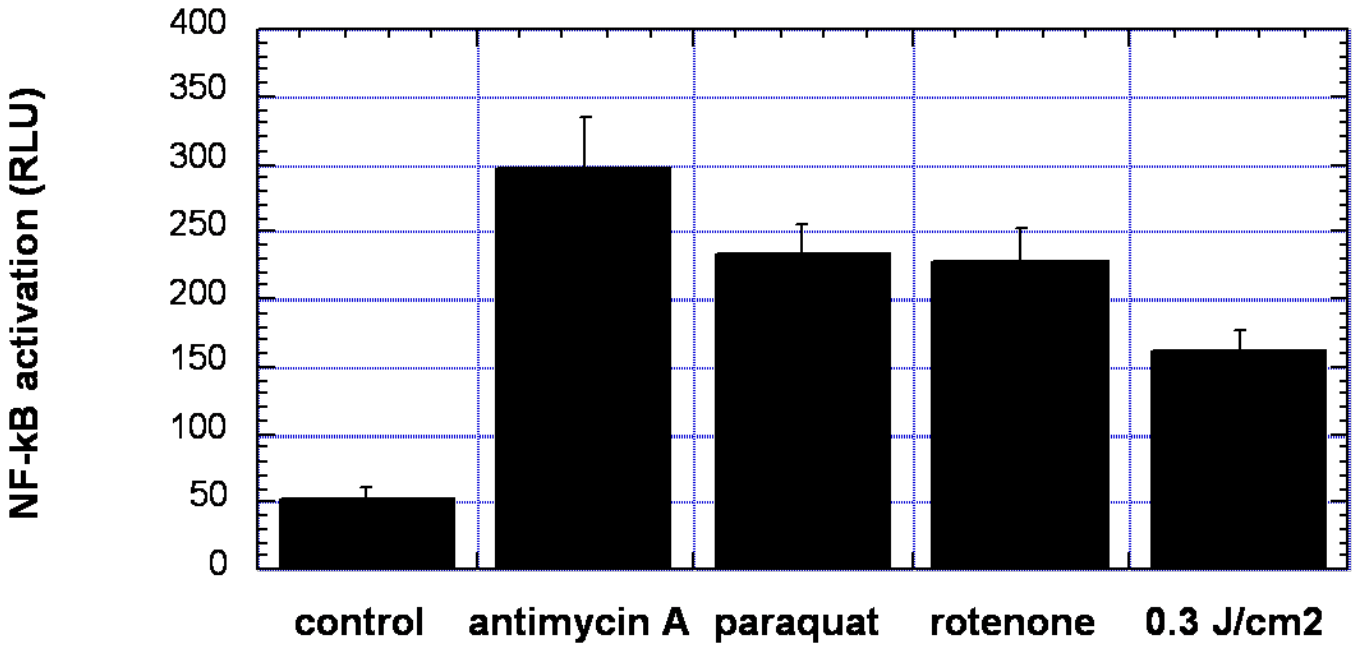
# Quantification of ROS induced by laser



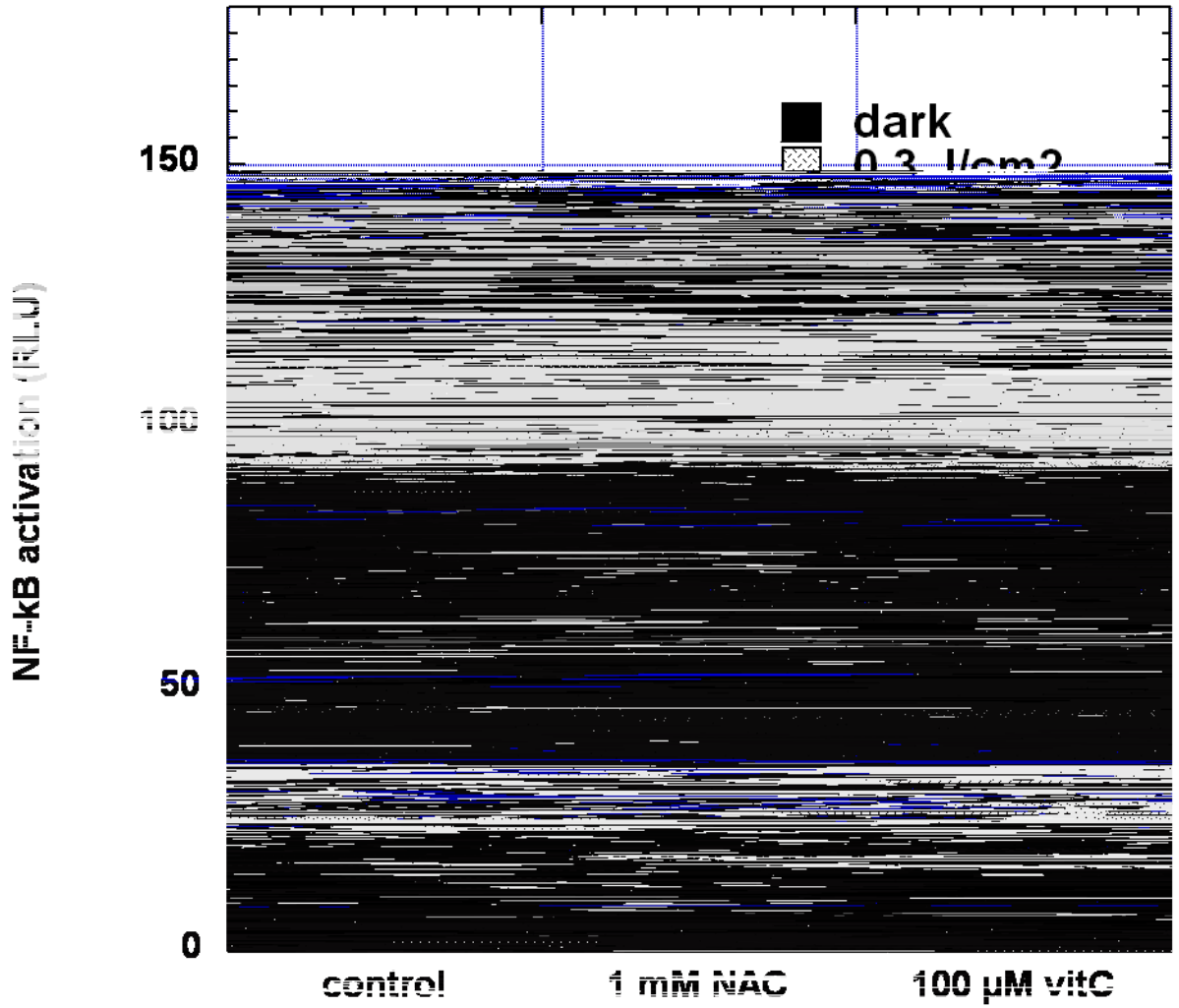

0 min
Right after irradiation
30 min
60 min


control
Control + Vit C
laser
Laser + Vit C

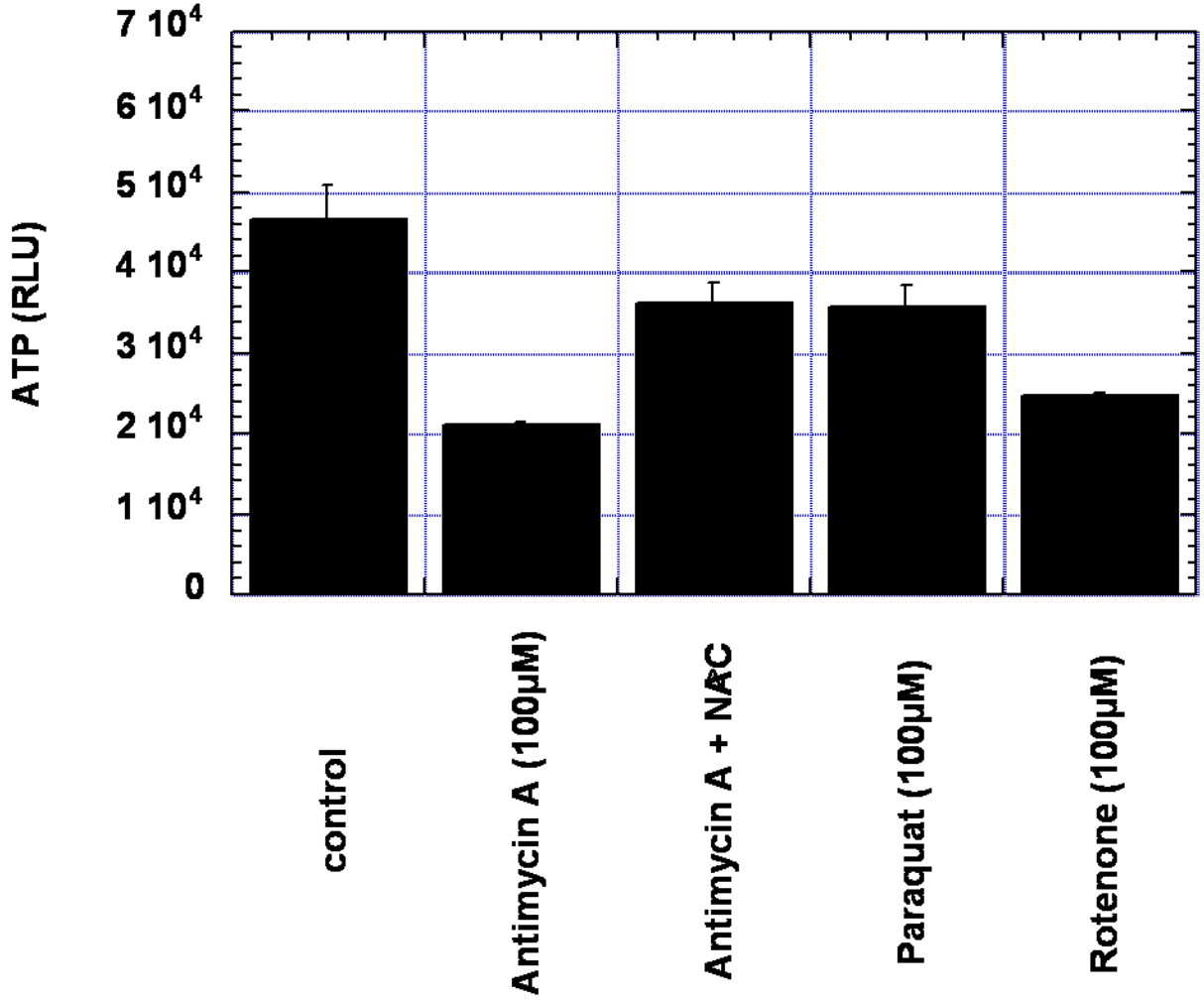
# Do mitochondrial inhibitors increase NF-kB activation?



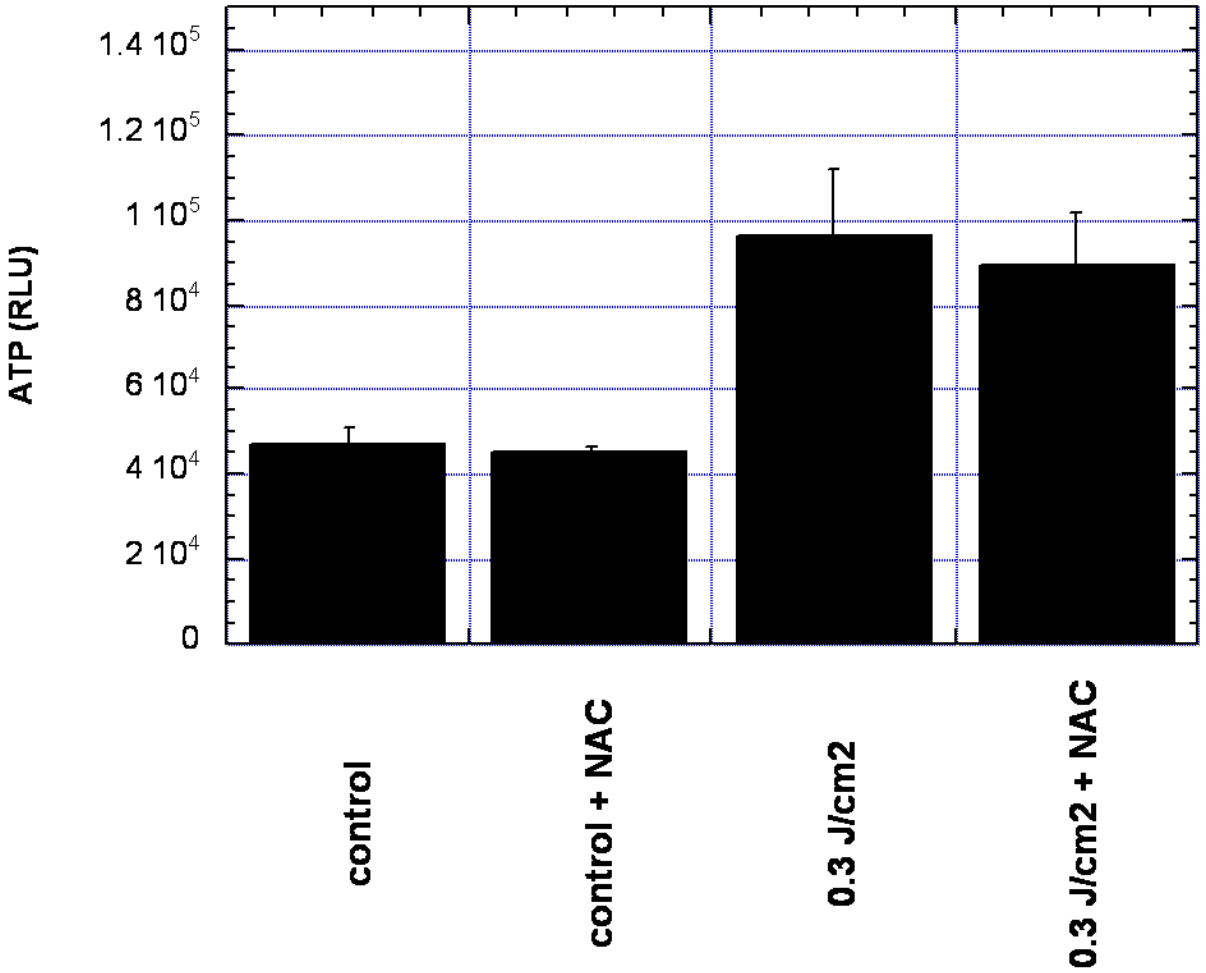
# Do antioxidants prevent NF-kB activation?



# What is effect of mitochondrial inhibitors and antioxidants on ATP?

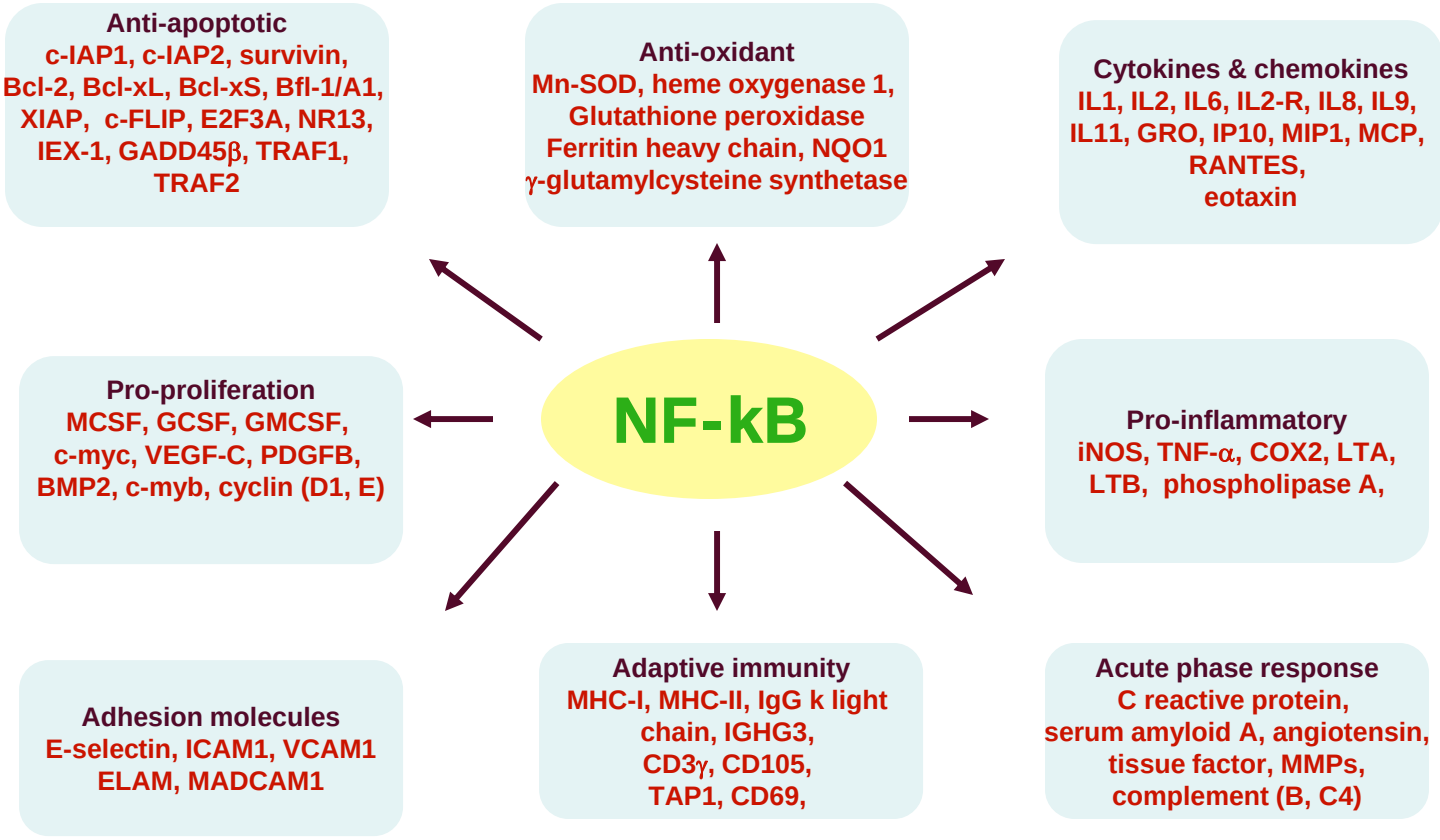


# Do antioxidants abrogate laser induced ATP ?



# NFκB activation by Lasers

# NF-kB target genes



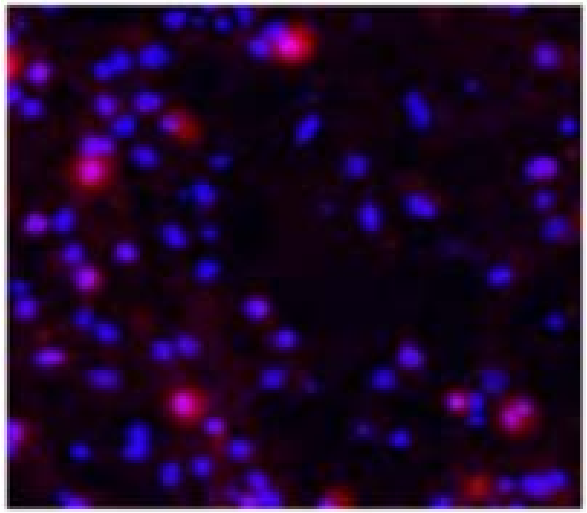
Study mechanisms of 810-nm laser
in mouse cultured primary cortical neurons

Test biphasic dose response for parameters
affected in short term (minutes) by laser

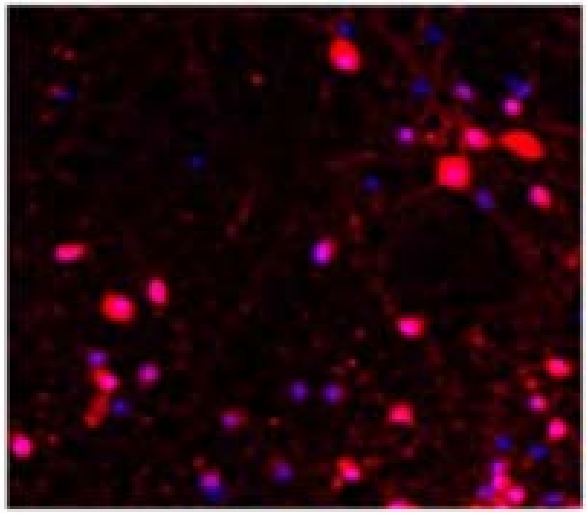
Mitochondrial reactive oxygen species
Intracellular nitric oxide
Mitochondrial membrane potential
Intracellular calcium
ATP

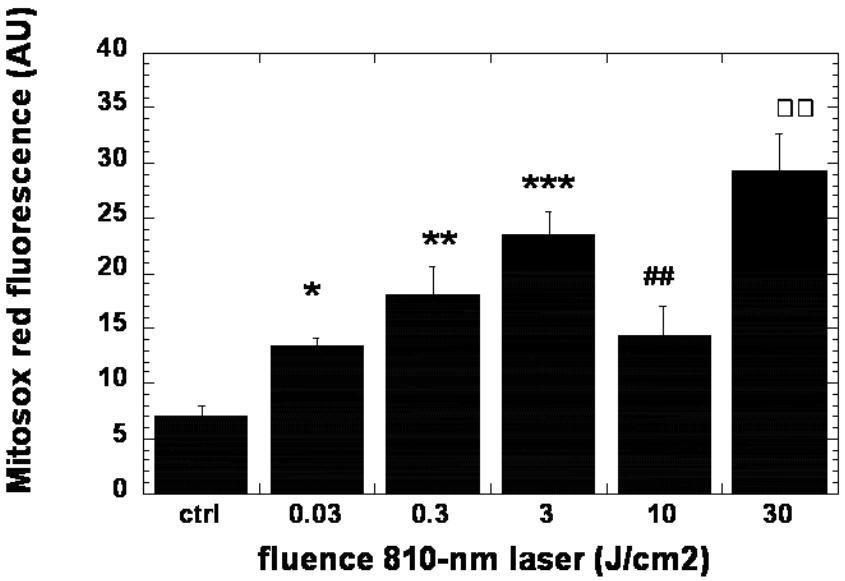
# Intracellular ROS post 810nm laser
## Triphasic dose response

control



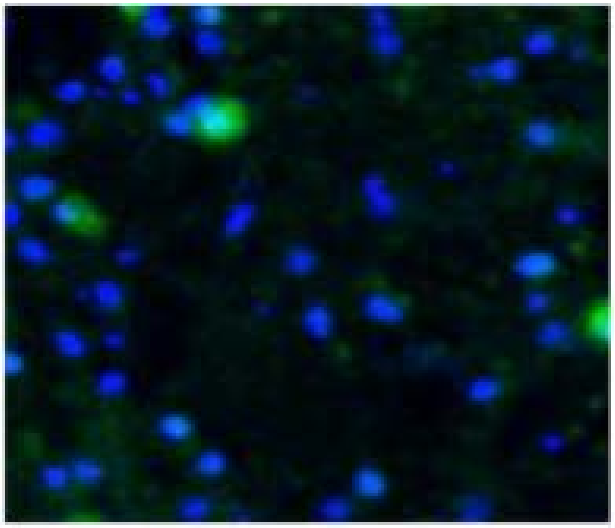
3 J/cm$^2$



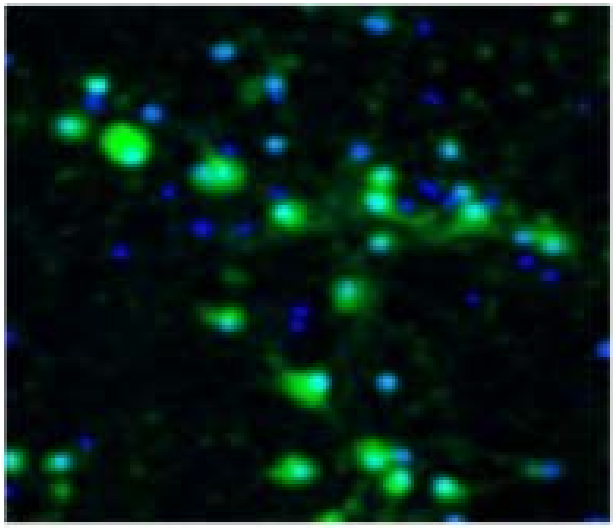


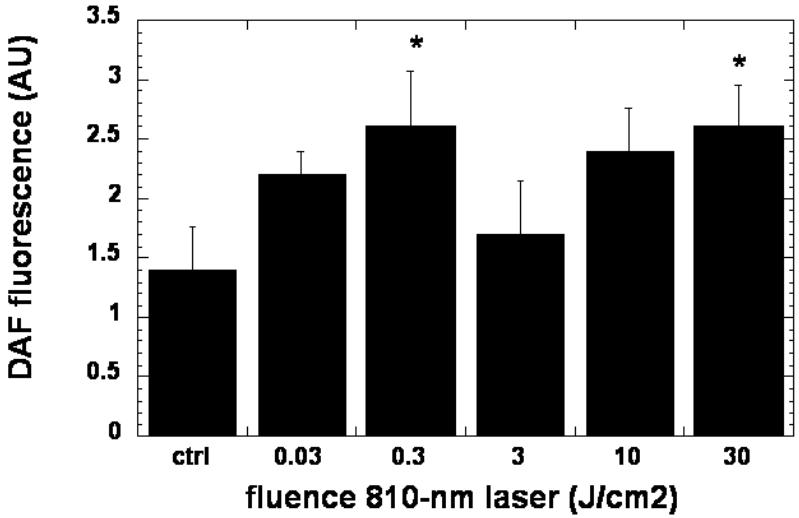
# NO levels post 810-nm laser
## Triphasic dose response?

control



0.3 J/cm$^2$

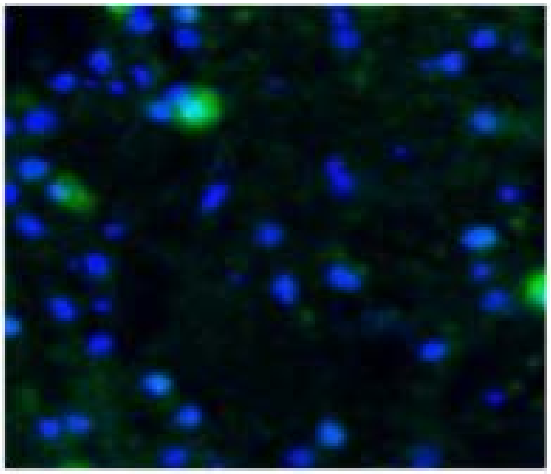




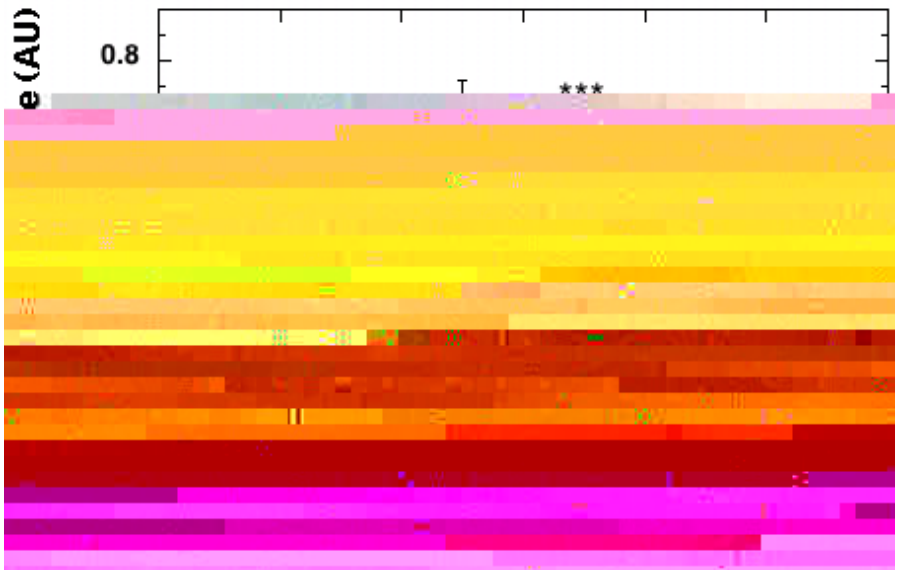
# Intracellular calcium post 810-nm laser
# Biphasic dose response

control

3 J/cm$^2$



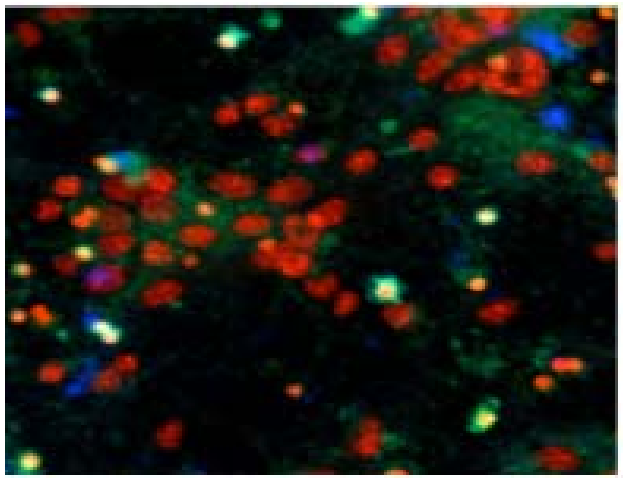


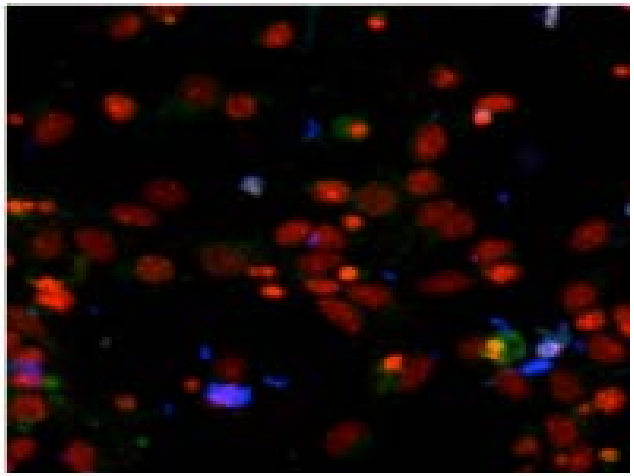


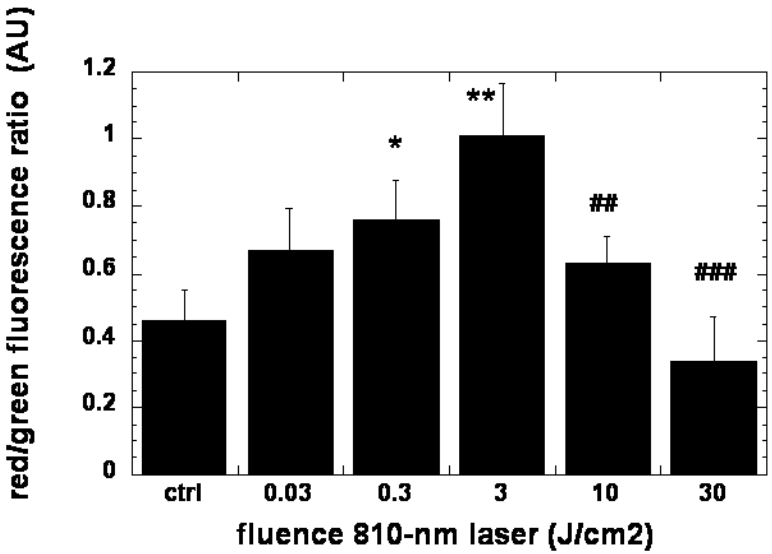
# Mitochondrial membrane potential post 810-nm laser
## Biphasic dose response

control

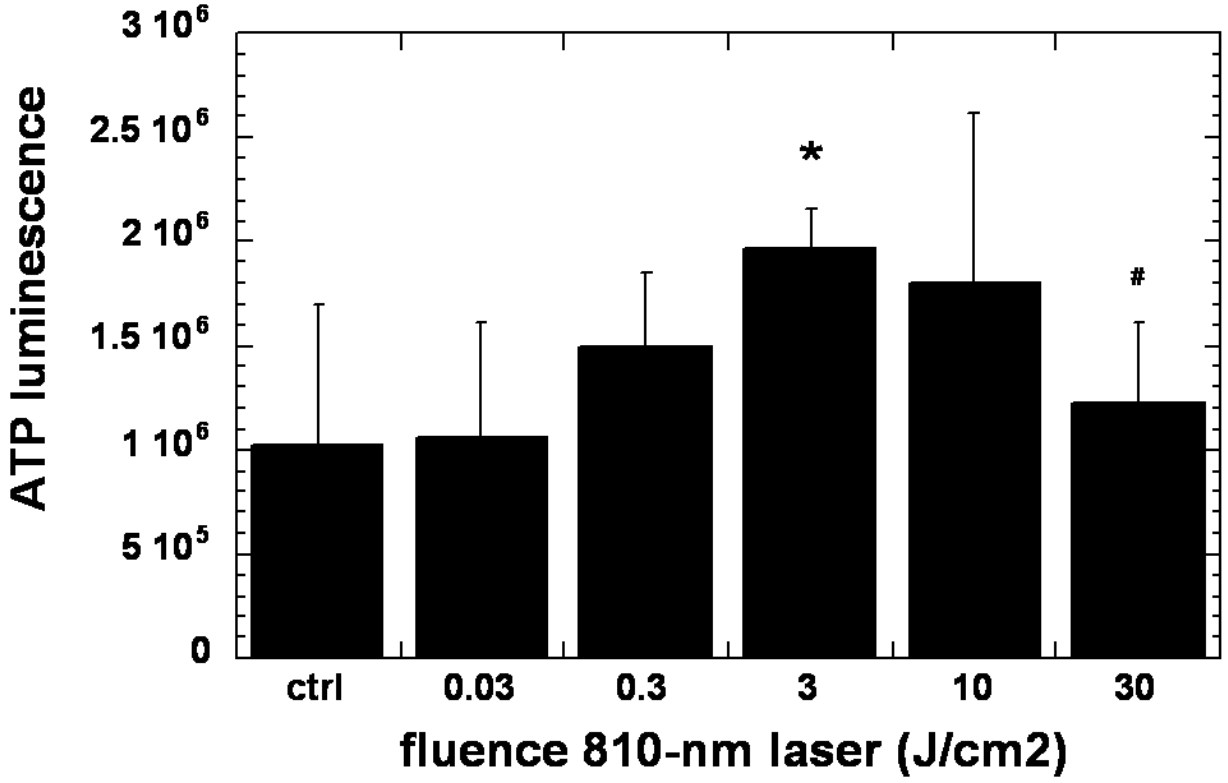
3 J/cm$^2$







# ATP levels post 810 nm laser. Biphasic dose response



- Summary
  - In vitro dose response effects can be divided into 2 groups
  - Group 1 (MMP, Ca2+, ATP) reflects stimulation of mitochondrial function that is biphasic with a maximum around 3J/$cm^2$
  - Group 2 reflects “Janus” signaling mediators ROS and NO – these are triphasic and show 2 peaks - at 3 J/$cm^2$ corresponding to cell stimulation and at 30 J/$cm^2$ presumably corresponding to cytotoxicity

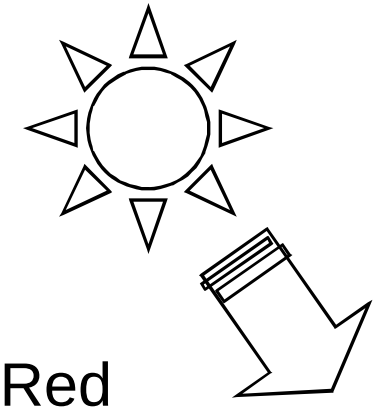
# What is the mechanism for biphasic dose response?

ROS have been shown many times to stimulate at low doses but to be harmful at high doses

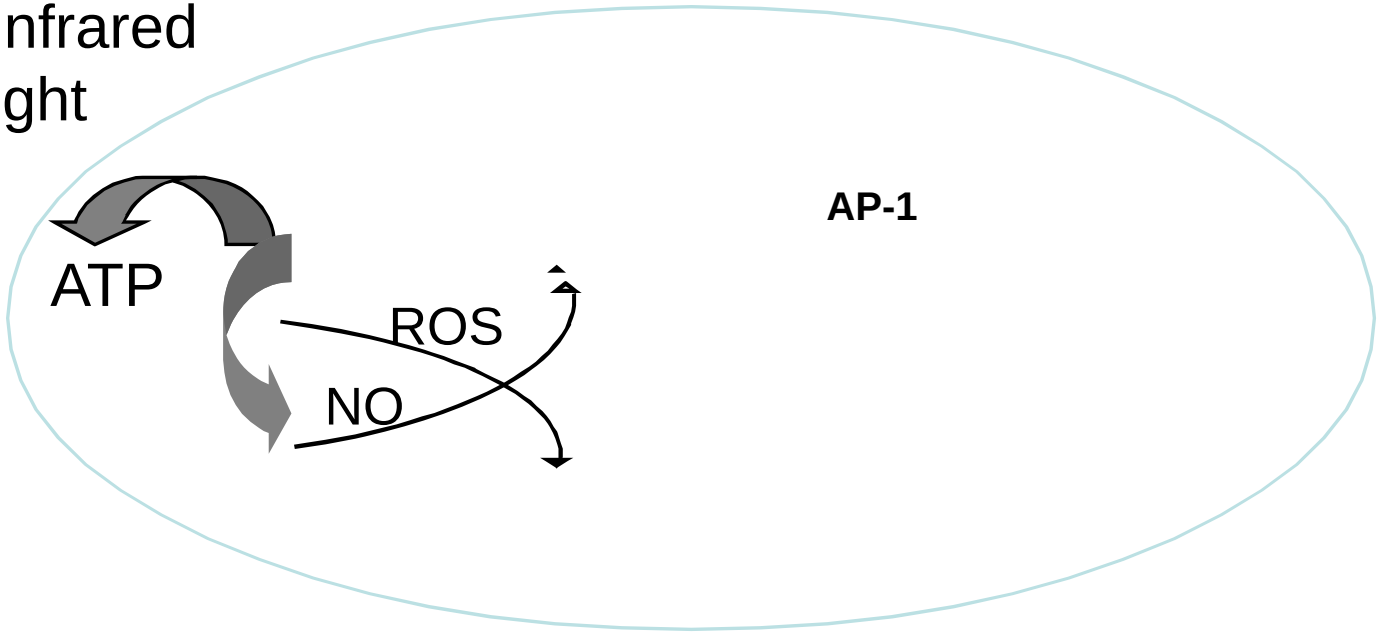
NO (and peroxynitrite) may also have biphasic response: stimulate at low dose and inhibit at high dose

Protective transcription factors may be induced at low dose (NF-kB) and additional different harmful pathways activated at high dose

# Mechanisms of LLLT



Red
near infrared
light



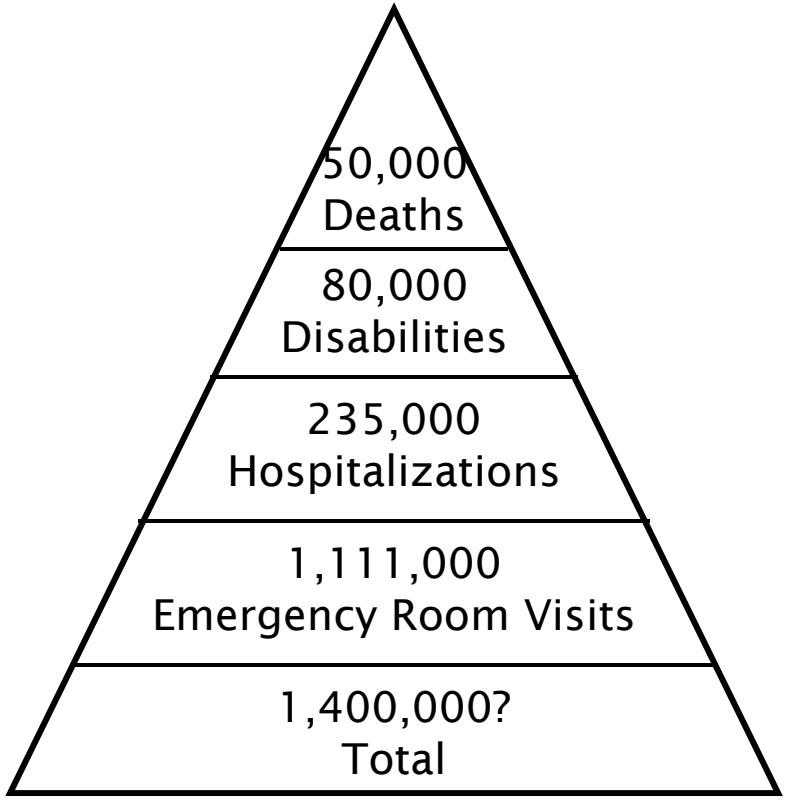
# Transcranial laser (light) therapy for traumatic brain injury

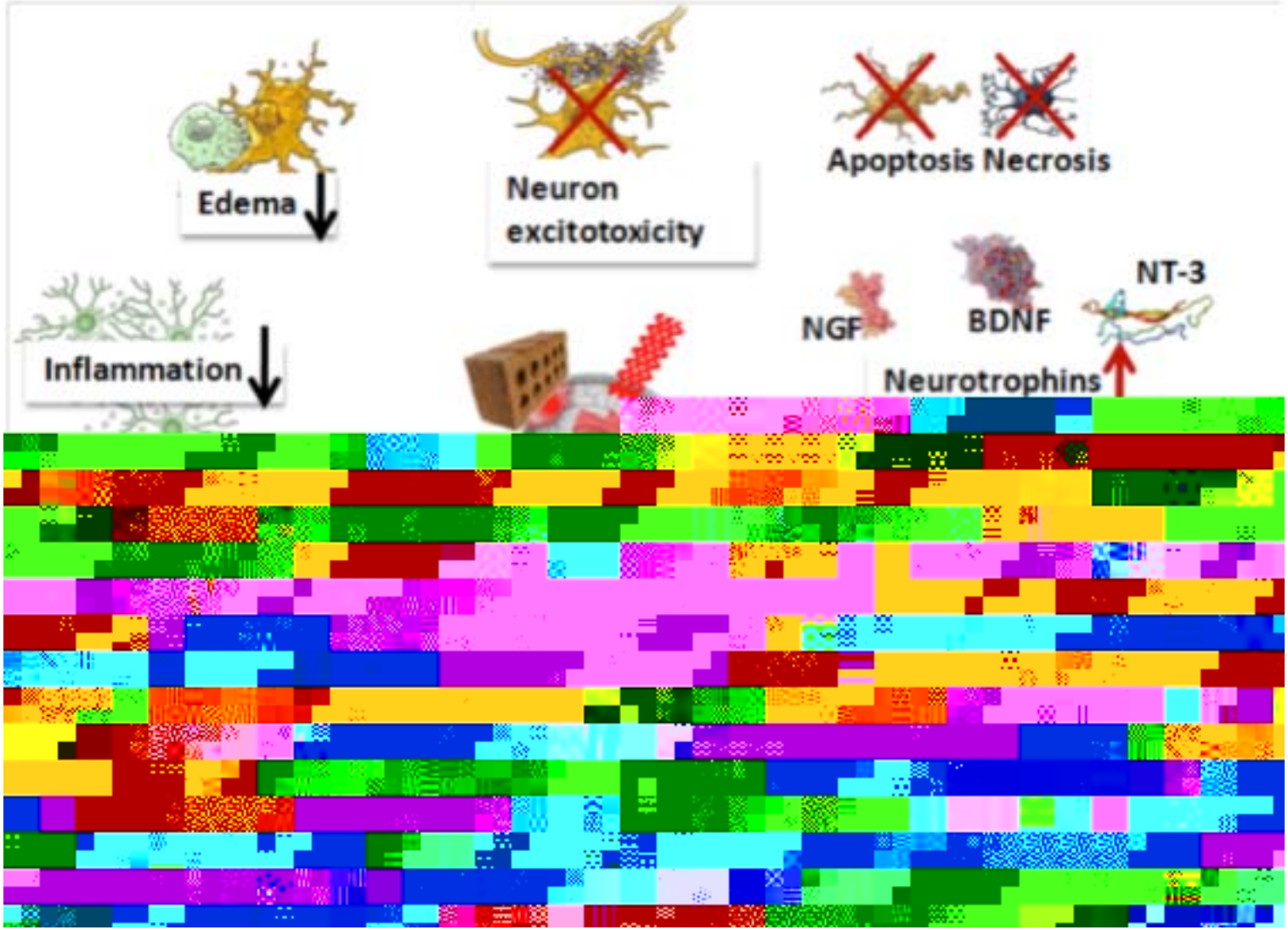
## Military

US: 2003-2007 43,779 cases
28% of patients at Walter-Reed
>$100,000,000
Explosive blast injury,
Overpressure,
Penetrating injury,
Diffuse axonal injury

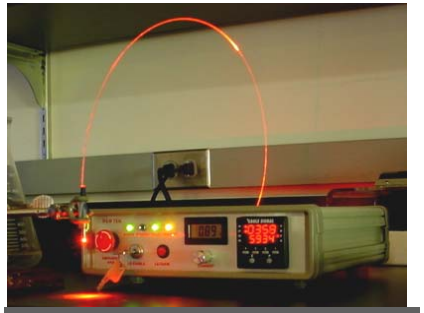
## Civilian

Trauma, MVAs, sports, assaults




Edema
Neuron excitotoxicity
Apoptosis Necrosis
Inflammation
NGF
BDNF
NT-3
Neurotrophins

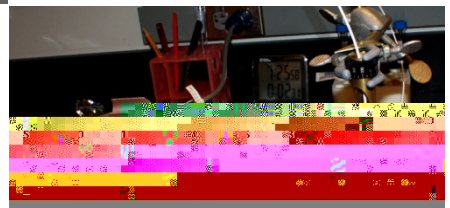
# Four different wavelength lasers



665-nm laser

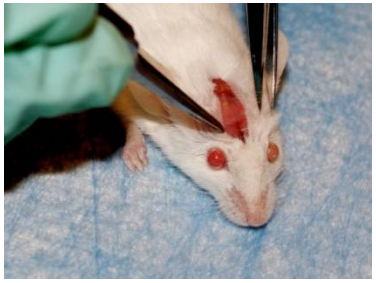
735-nm laser

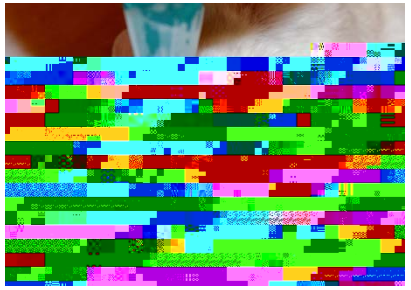
810-nm laser

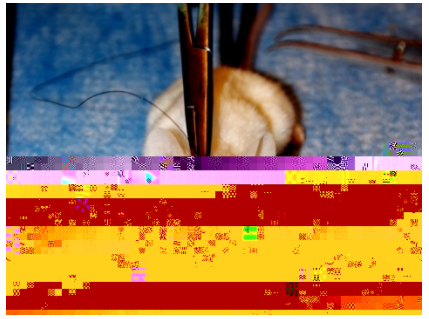


980-nm laser

# Transcranial LLLT for TBI in mouse model (IACUC approved)
# closed head weight drop method based on impact acceleration model of Marmarou (1994)



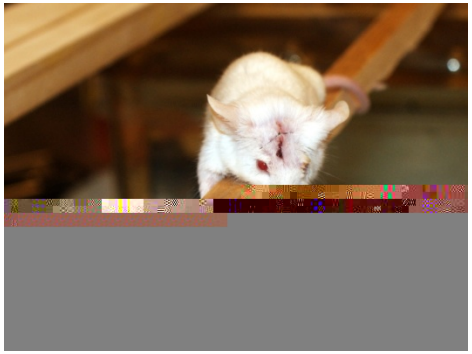


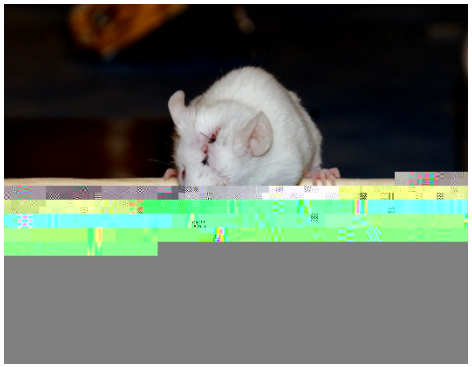


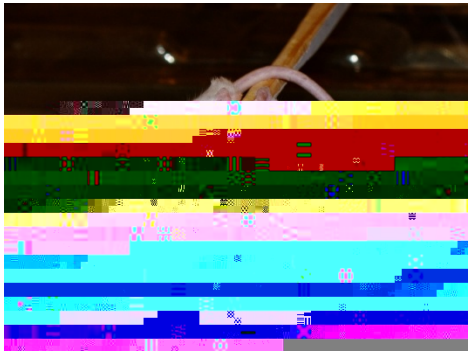
# Neurological performance testing

| Neurological Severity Score(NSS) for Brain-Injured Mice | |
|---|---|
| Presence of mono- or hemiparesis | 1 |
| Inability to walk on a 3-cm-wide beam | 1 |
| Inability to walk on a 2-cm-wide beam | 1 |
| Inability to walk on a 1-cm-wide beam | 1 |
| Inability to balance on a 1-cm-wide beam | 1 |
| Inability to balance on a round stick (0.5 cm wide) | 1 |
| Failure to exit a 30-cm-diameter circle (for 2 min) | 1 |
| Inability to walk straight | 1 |
| Loss of startle behavior | 1 |
| Loss of seeking behavior | 1 |
| Maximum total | 10 |

One point is awarded for failure to perform a task.



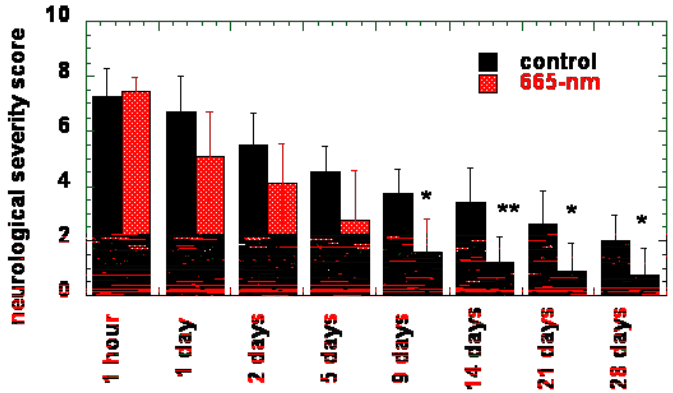


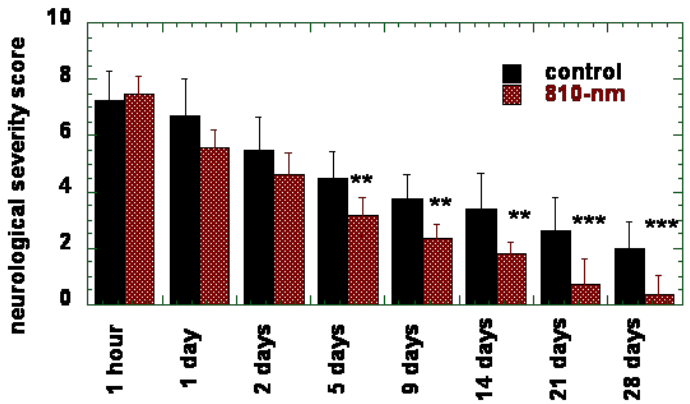


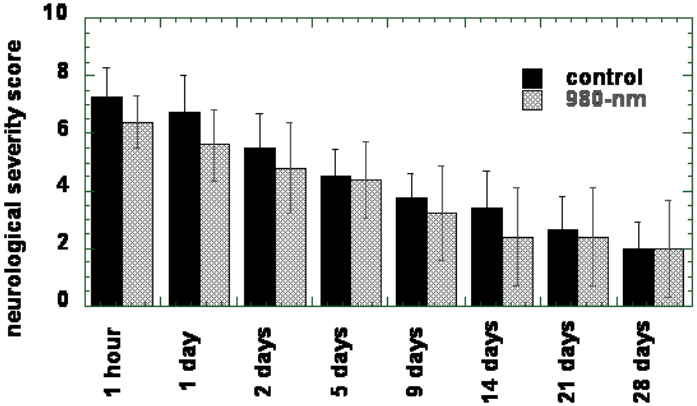
# Transcranial LLLT for TBI in mouse model

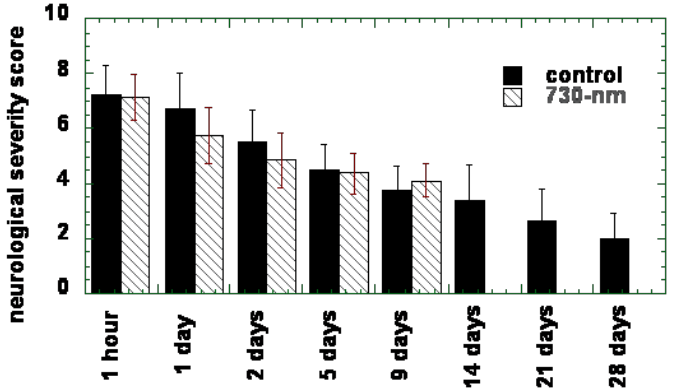
Mice were performance tested 1 hour post-TBI
Mice received a single exposure to laser
on top of head at 4 hours post-TBI
Fluence was 36 J/cm$^2$ delivered at 150 mW/cm$^2$
over 4 min with spot size 1-cm diameter
Estimated light penetration to dura is 3%
Subsequent performance testing for 4 weeks

# Transcranial LLLT for TBI in closed head model



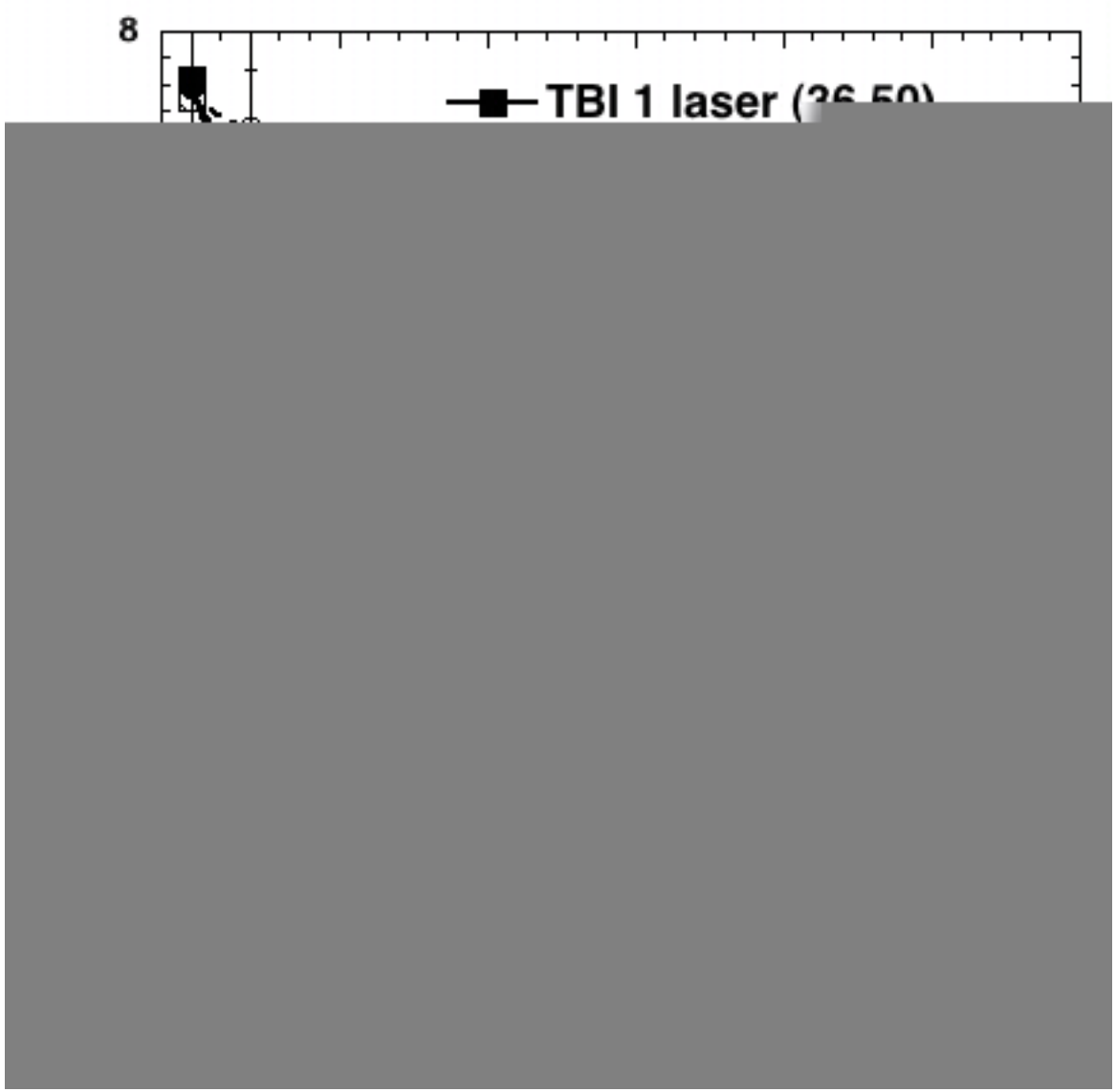



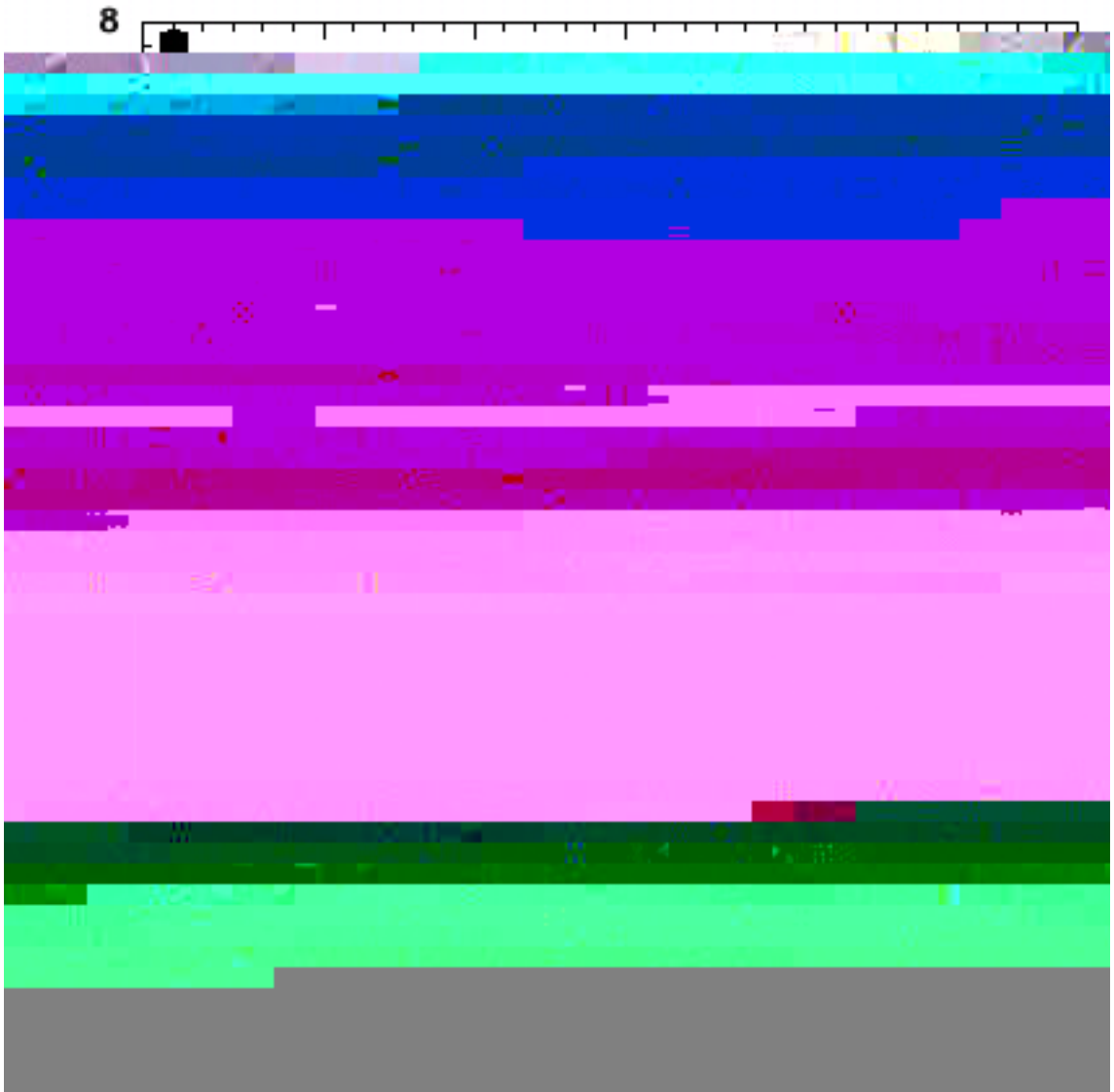



Both 665-nm and 810-nm work but not 730-nm or 980-nm

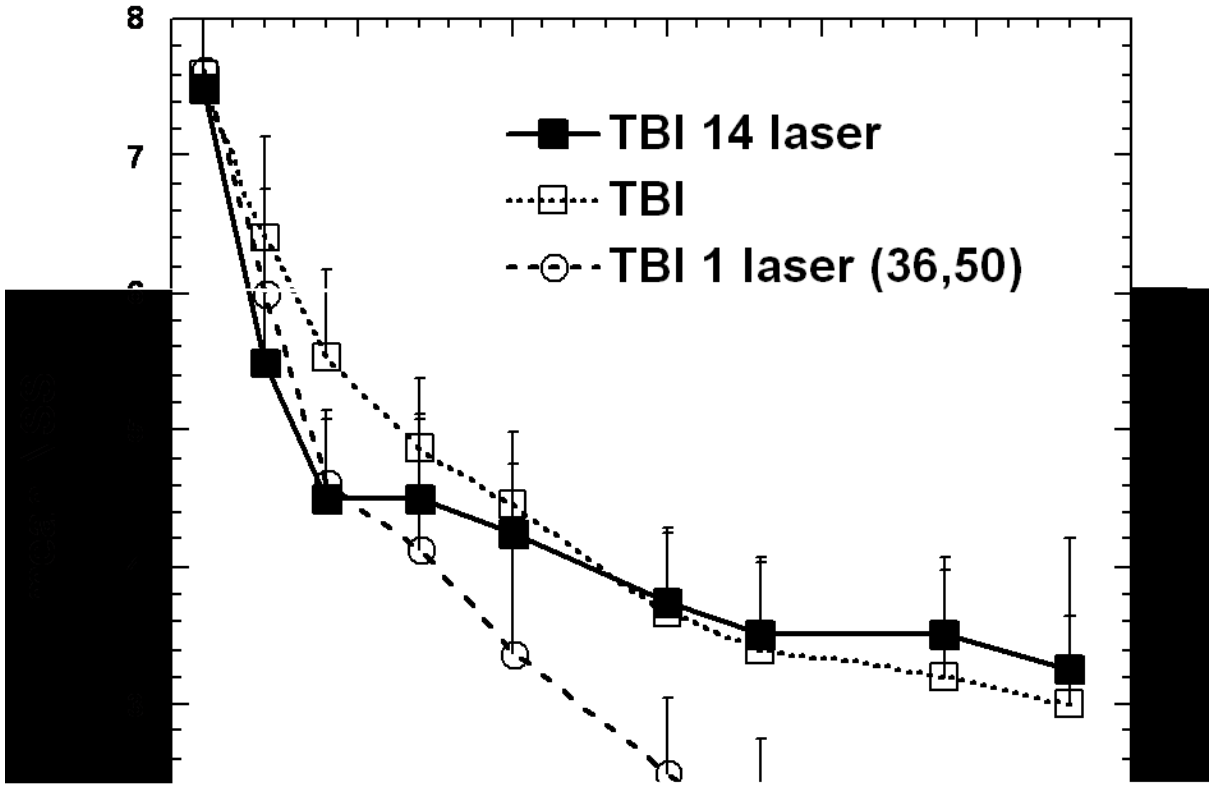
# A single laser Tx of 36 J/cm$^2$ at 50 mW/cm$^2$ at 4 hour is best



# A single laser Tx of 36 J/cm$^2$ at 50 mW/cm$^2$ pulsed at 10 Hz is better than CW or 100 Hz



# A single Tx is better than 14 daily Tx



# Conclusions

Mitochondrial mediators show a biphasic dose response
"Janus" mediators show a triphasic dose response
In vivo wound healing shows biphasic dose response
Transcranial laser for TBI shows biphasic dose response for:

- irradiance
- treatment repetition
- pulse structure

# LLLT for Depression and PTSD

**Behavioral and Brain Functions**

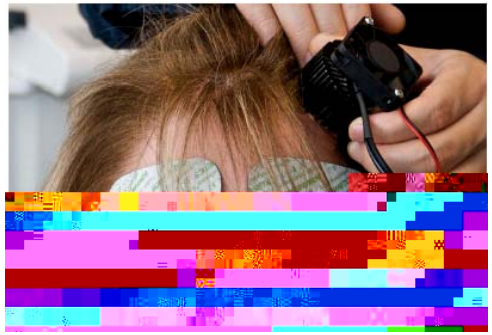
BioMed Central

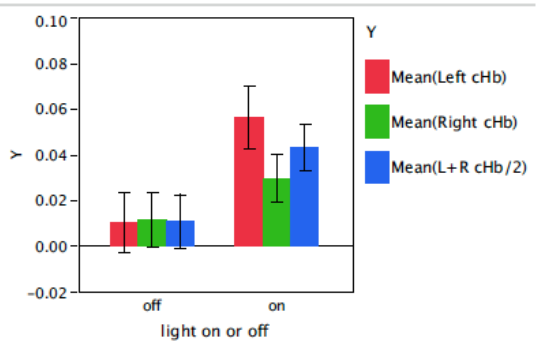
Research

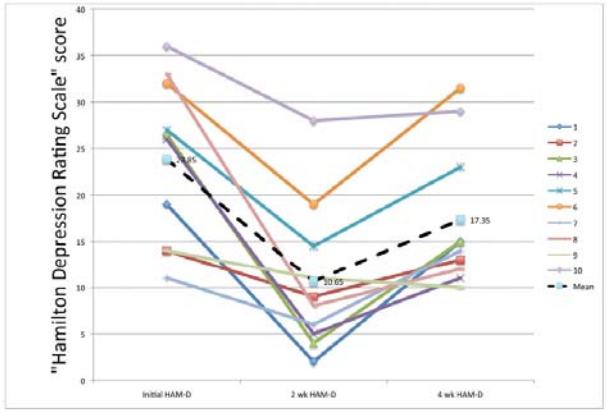
Open Access

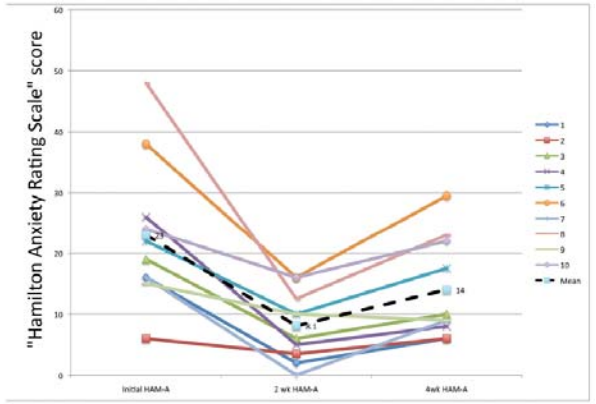
**Psychological benefits 2 and 4 weeks after a single treatment with**

[redacted]





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# Improved Cognitive Function After Transcranial, Light-Emitting Diode Treatments in Chronic, Traumatic Brain Injury: Two Case Reports

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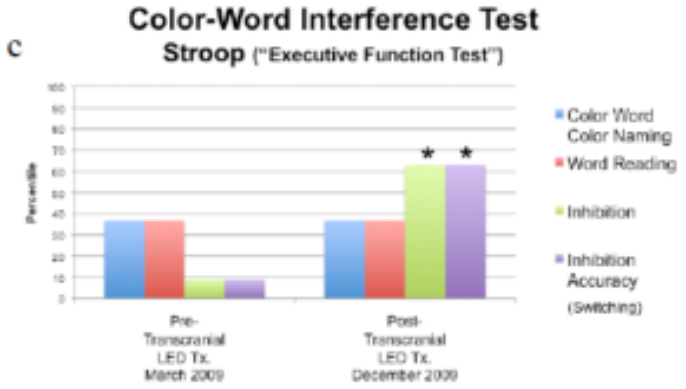
Maxine H. Krengel, Ph.D.,[1,2] Michael R. Hamblin, Ph.D.,[4,5,6] and Jeffrey A. Knight, Ph.D.[1,2,7]


* +2 SD Improvement, inhibition, inhibition accuracy

**P2, Pre- and Post- LED Tx., *Neuropsychological Test Results***
**Post- LED Testing,Post- 9 months, nightly, transcranial LED Tx.**

**P2, Pre- and Post- LED Tx., *Neuropsychological Test Results***
**Post- LED Test, Post- 9 months, nightly, transcranial LED Tx.**

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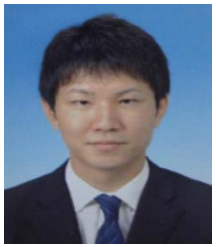
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