



# AFRL

## DEFINING HOW AN INFRARED-EVOKED FAST THERMAL GRADIENT IMPACTS SYNAPTIC MECHANISMS AT THE DENDRITIC SPINE

BRYAN GAMBOA, RESEARCH COMPUTER ENGINEER, HUMAN EFFECTIVENESS DIRECTORATE

AFOSR, THE HUMAN PERFORMANCE AND BIOSYSTEMS PROGRAM  
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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.



# Air Force Research Laboratory (AFRL)

## JBSA Fort Sam Houston



### **Bioeffects Team Mission**

- Capture and quantify the biological effect of radiation exposures to protect our men and women from harm
- Determine maximum electromagnetic exposure that are safe for US personnel
- Anticipate and alleviate the bioeffects on staff and mission performance



# Neural Modulation with Infrared (IR) Pulsed Lasers

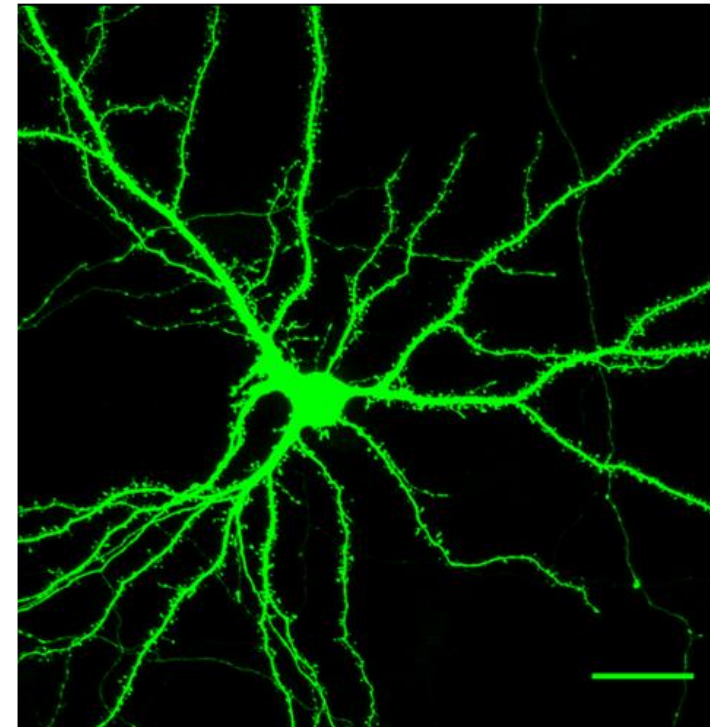
- Pulsed infrared (IR) laser light used to modulate action potentials in neurons in response to a transient targeted deposition of IR energy
- As opposed to other neural stimulation approaches (electrical, optogenetics), IR stimulation is:
  - Label-free
  - Contact-free
  - Artifact-free
  - Non-damaging

504 OPTICS LETTERS / Vol. 30, No. 5 / March 1, 2005

## Optical stimulation of neural tissue *in vivo*

Jonathon Wells, Chris Kao, Karthik Mariappan, Jeffrey Albea, E. Duco Jansen, Peter Konrad, and Anita Mahadevan-Jansen

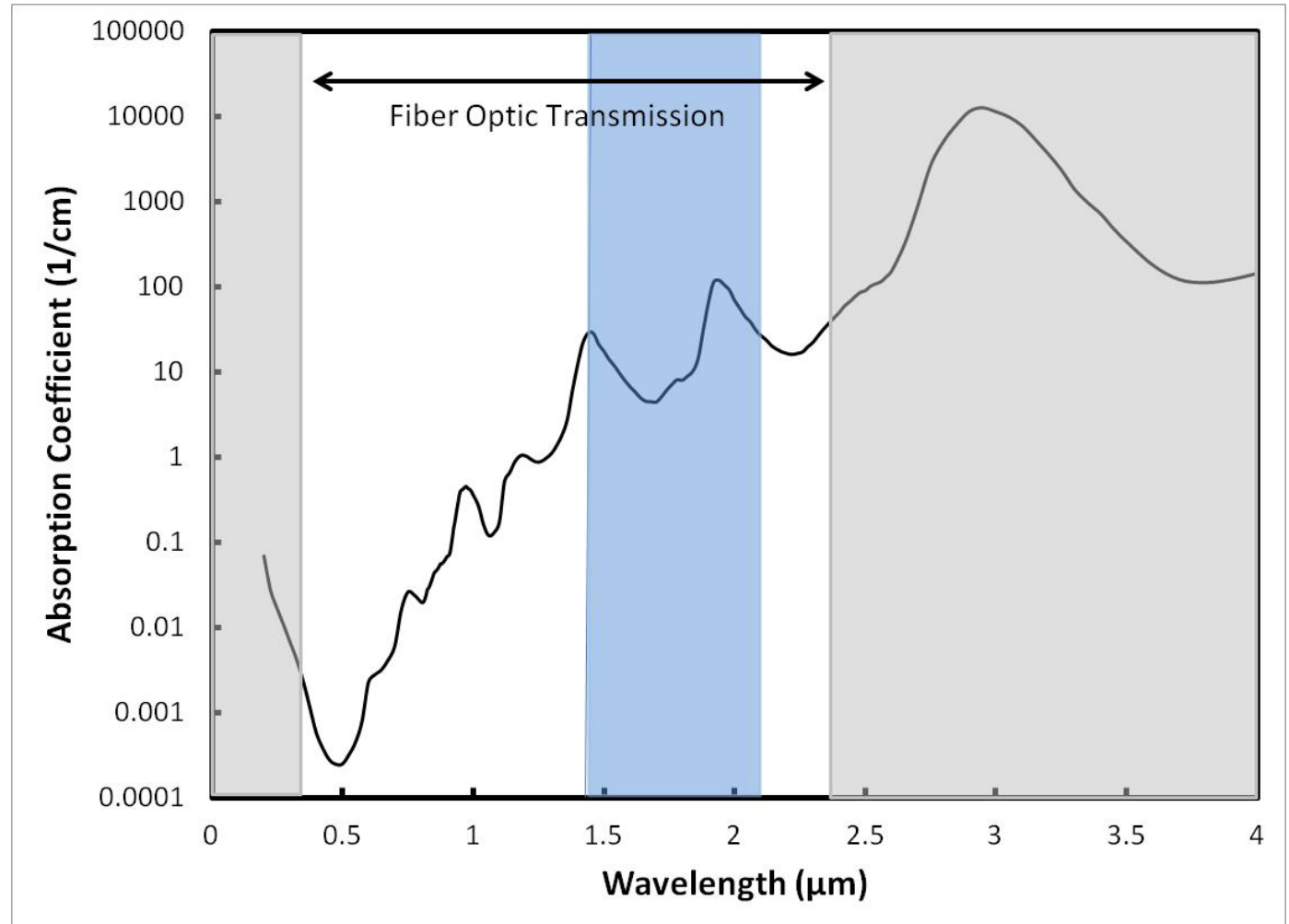
*Departments of Biomedical Engineering and Neurosurgery, Vanderbilt University, 2201 West End Avenue, Nashville, Tennessee 37235*





# Effect Originates from High Absorption by Water

- IR energy highly absorbed by water
- Absorption of IR generates a fast thermal gradient (FTG)

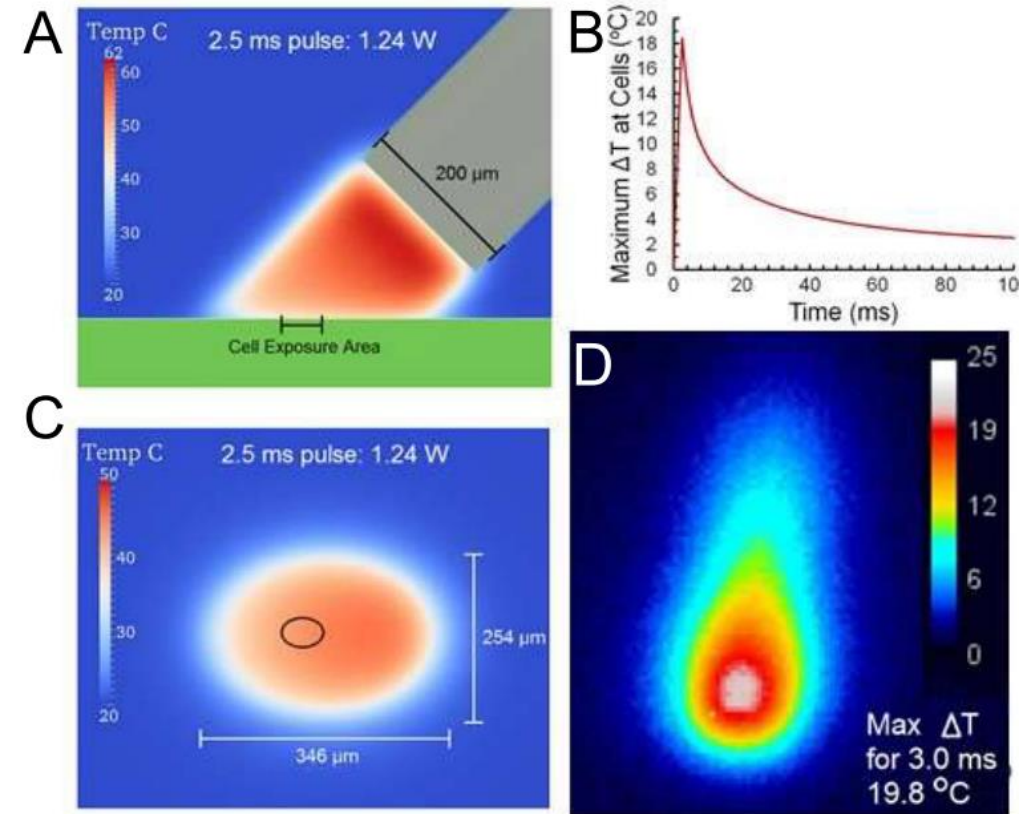
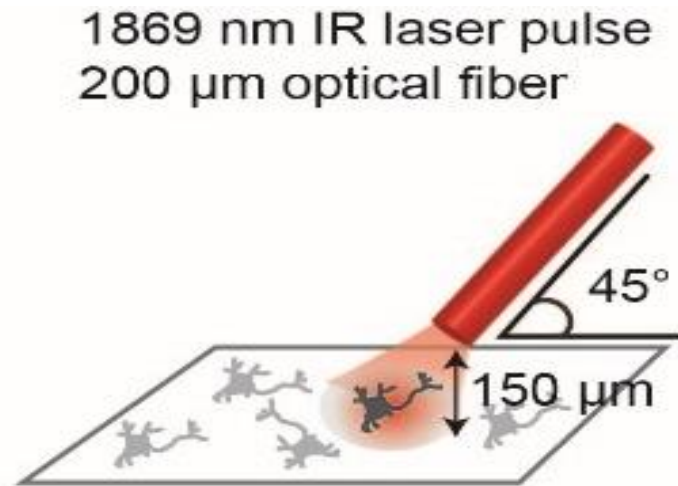


Hale and Query, Appl. Opt 12:555 (1973) Liljemalm, T. *Lasers SurgMed*, Jul 7 2013



# Infrared Neural Stimulation (INS) Mechanism

- INS is a label free optical modulation technique that can elicit or inhibit action potentials
- IR absorption in water induces fast thermal gradient (FTG) that compresses the cell membrane changing the membrane capacitance and voltage

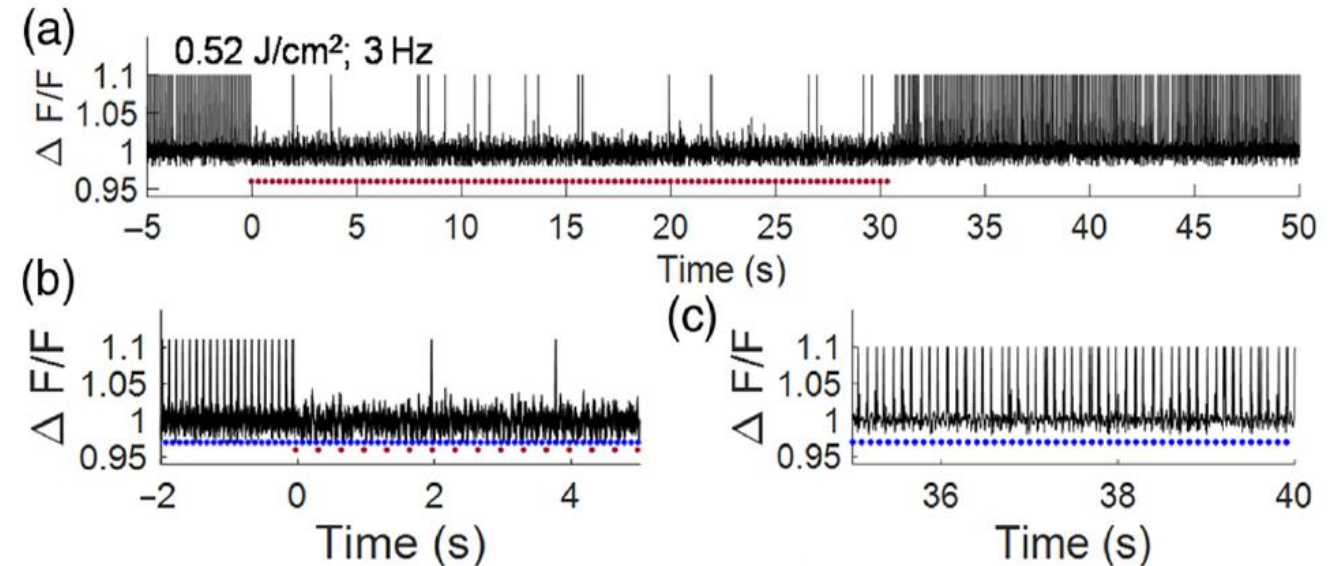


Beier, Hope T., et al. "Plasma membrane nanoporation as a possible mechanism behind infrared excitation of cells." *Journal of neural engineering* 11.6 (2014): 066006



# Modulation of Neurons through IR Induced FTG

- (a) Representative QuasAr2 fluorescence spike plot demonstrating consistent Action Potential (AP) generation prior to Short Infrared Laser Pulses (SILPs), reduced AP activity during the repeated SILPs ( $0.52 \text{ J/cm}^2$ ; 3 Hz) and following the SILP exposure
- (b) Enlarged display of -2 to 5 s data in (a)
- (c) Enlarged display of the 35 to 40 s data in (a) showing recovery of consistent AP



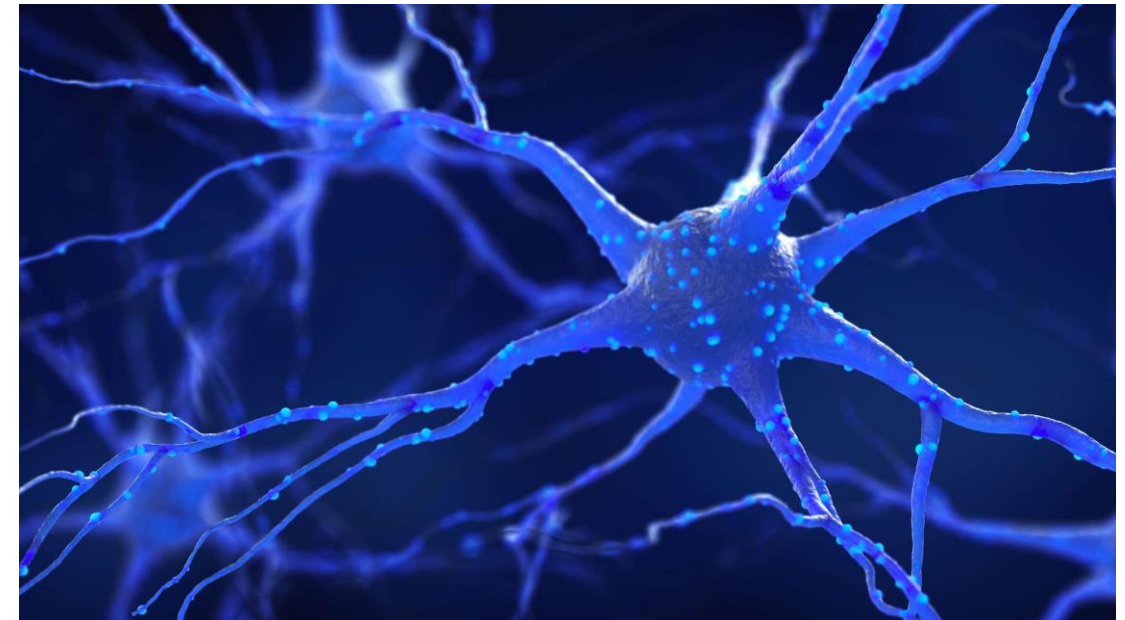
Walsh, Alex J., et al. "Action potential block in neurons by infrared light." *Neurophotonics* 3.4 (2016): 040501

# Modulation of Neurons through IR Induced FTG

## Goal:

Determine specific IR exposure parameters (i.e. frequency, pulse duration, amplitude, energy) that enhance or reduce neuronal synaptic activity without damaging the dendritic spine

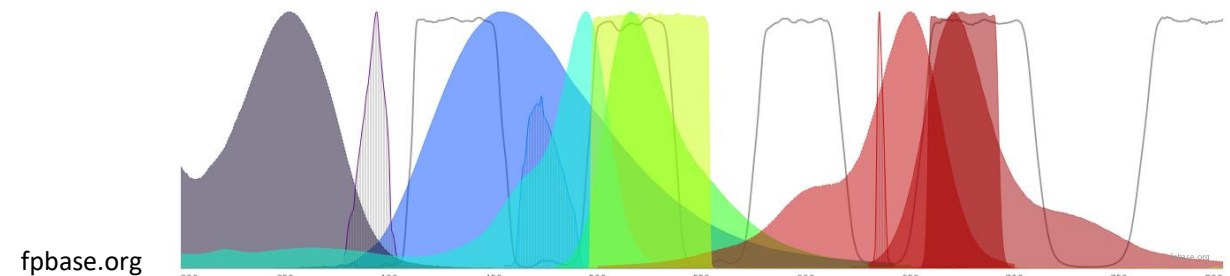
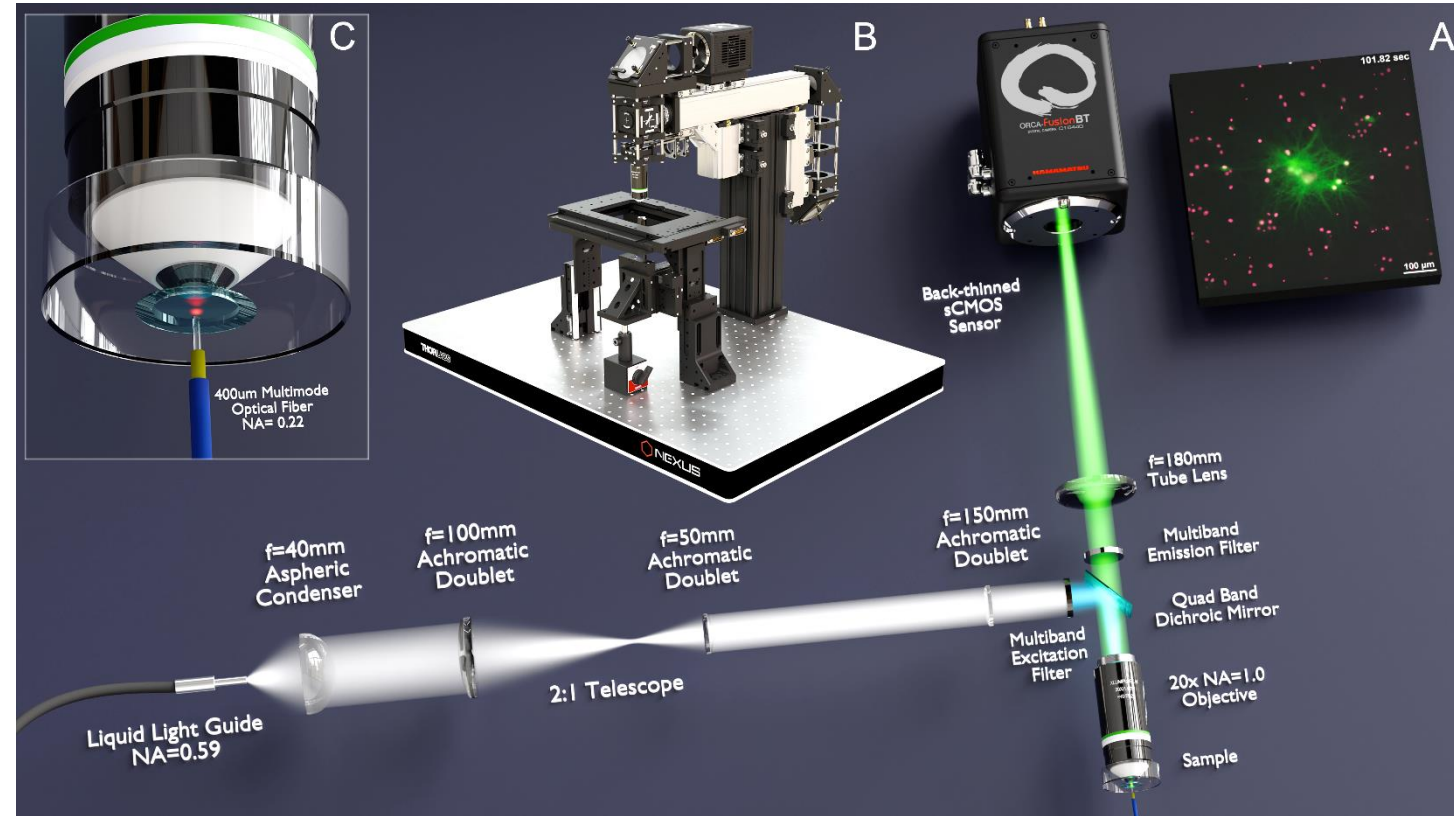
- Specific Aim 1
  - Determine the structural and functional effects of IR-induced FTG exposure on dendritic spines
- Specific Aim 2
  - Develop multiscale biophysical models of FTG exposure on dendritic spines



<https://www.verywellhealth.com/types-of-neurons-5201172>

# INS Soma Effects

- Widefield Fluorescence Imaging
  - Objective: XLUMPlanFLN 20x 1.0 W
    - **0.288  $\mu\text{m}/\text{pixel}$**
  - Source: Spectra III
  - Camera: Hamamatsu Orca Fusion BT
    - FPS: **50 Hz**
  - Tokai Stage Incubator – 37 °C
- E18 Cortical Neurons
  - 14 - 28 days *in vitro* in Ø35 mm petri dishes with Ø14 mm No. 1.5 CS imaging well
  - Markers
    - Nucleus: **Hoechst** - 500 nM
    - Calcium: **Calbryte-520** - 1.5  $\mu\text{M}$
    - Stimulation fiducial: **Alexafluor 647** - 100 nM
- INS Parameters
  - $\lambda = 1470 \text{ nm}$
  - Pulse Parameters
    - 8 ms, 2 ms, 350  $\mu\text{s}$
  - Pulse Energy: 6 - 9 mJ



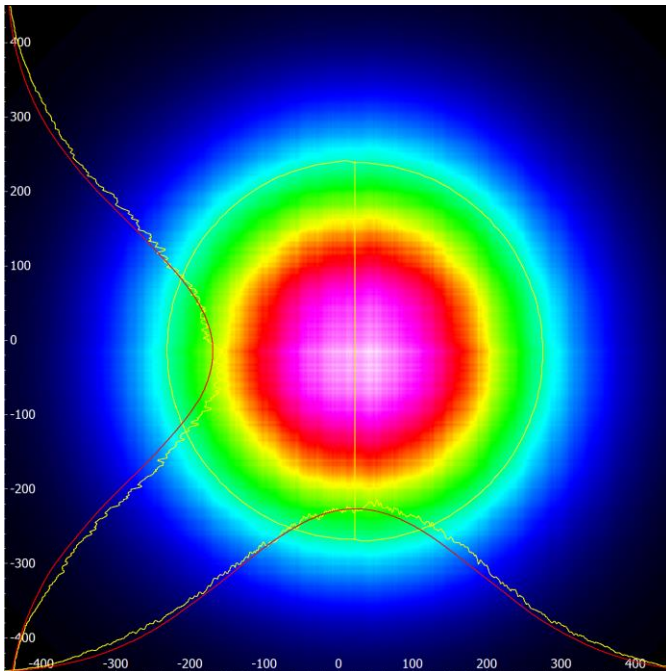


# The Dosimetry Challenge

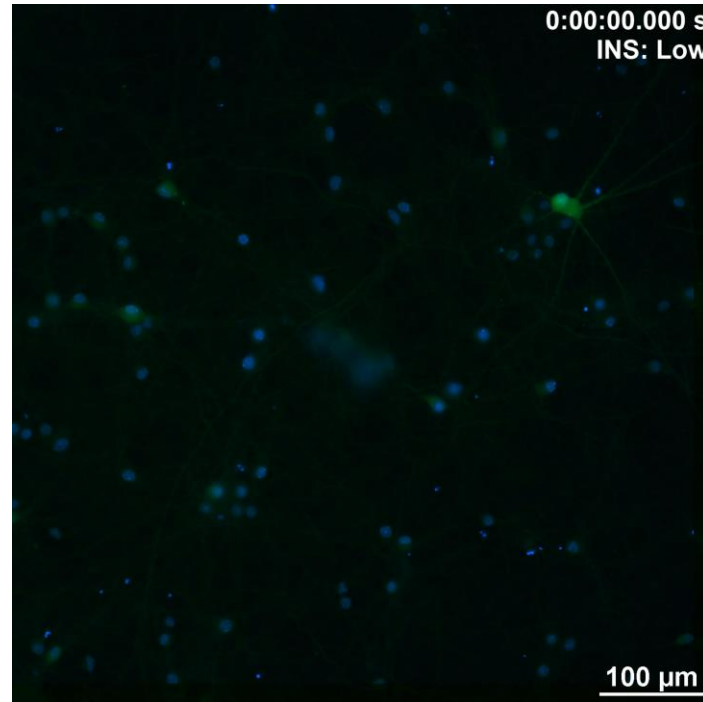
- Energy dose typically reported as Radiant Exposure<sup>1-3</sup>
- Several reactions to stimulus within FOV
- Non-linear energy distribution across field<sup>4,5</sup>
  - Gaussian Beam Shape

$$\text{Radiant Exposure} = \frac{\text{Pulse Energy (J)}}{\text{Beam Spot Size (cm}^2\text{)}}$$

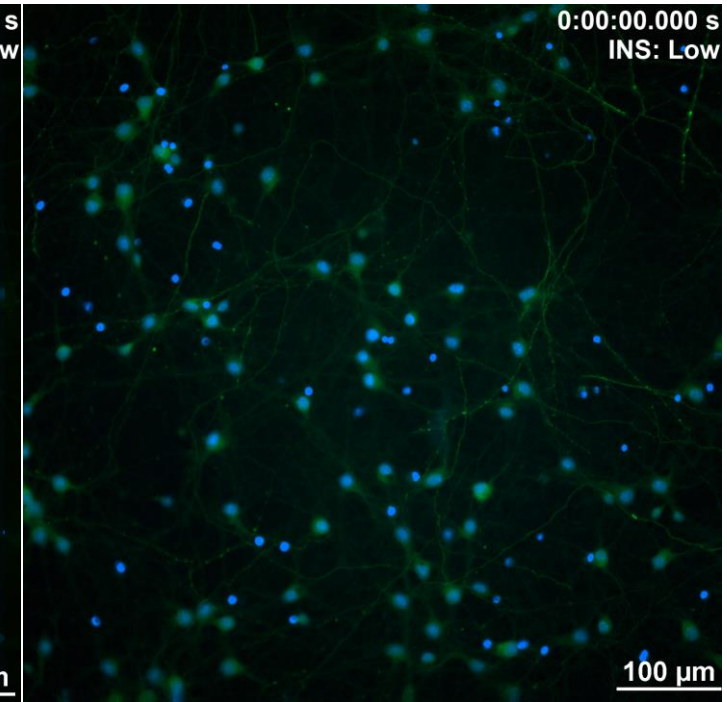
## Infrared Beam Profile



2 ms, 10 mJ, 5.65 J/cm<sup>2</sup>



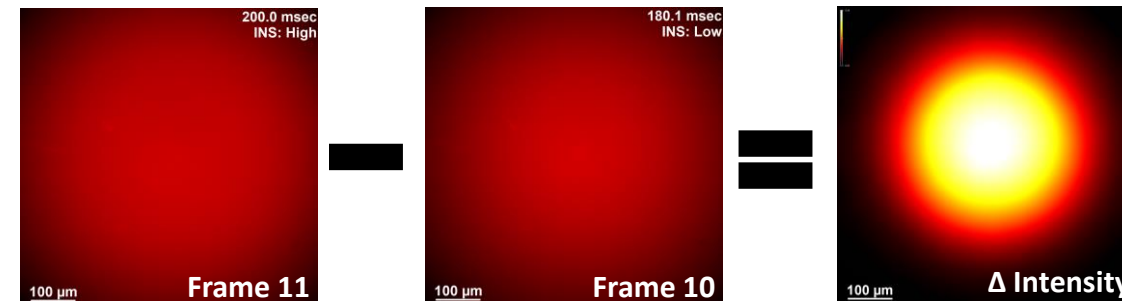
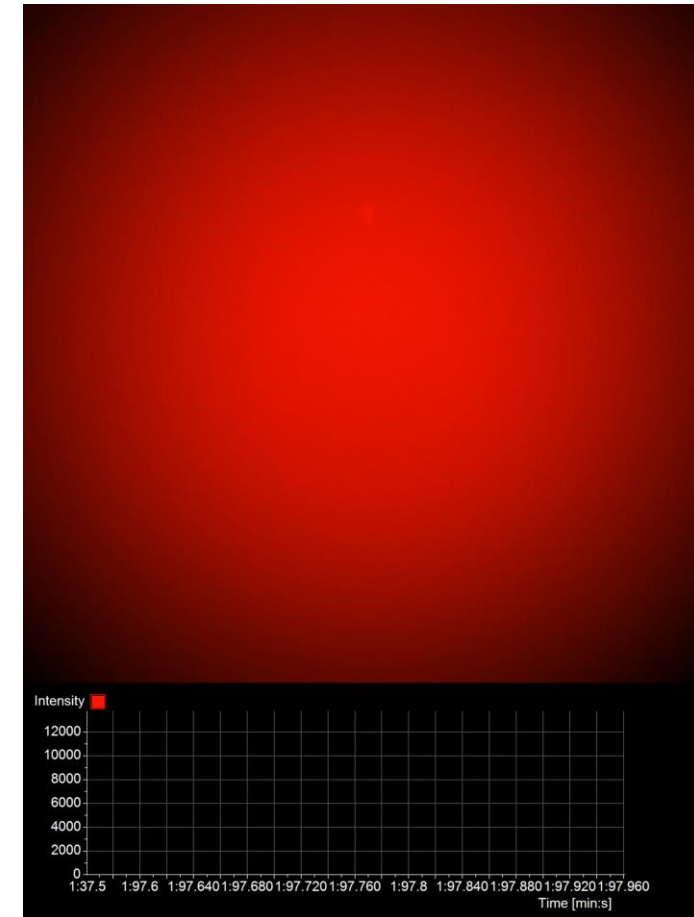
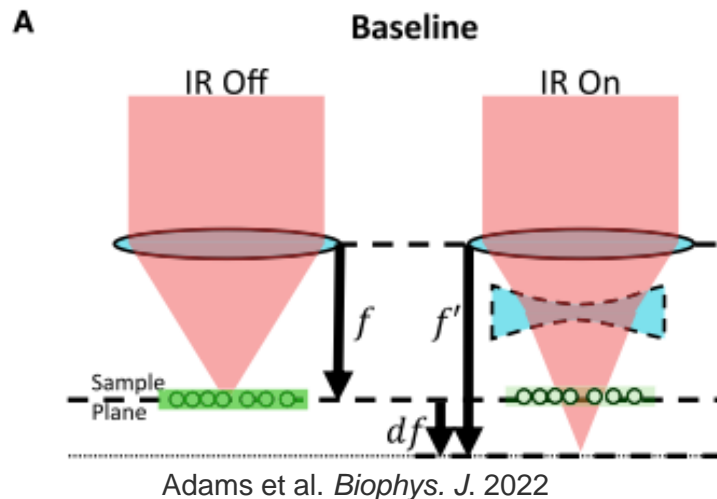
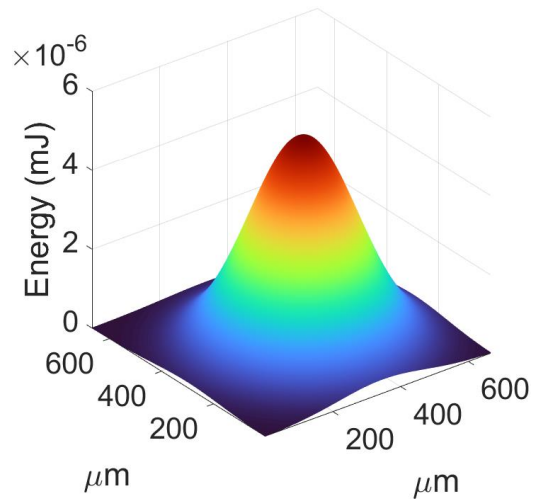
2 ms, 8 mJ, 4.44 J/cm<sup>2</sup>



[1] Wells et al. *J. Neuro. Methods* 2007, [2] Albert et al. *J. Neurophys.* 2012, [3] Izzo et al. *Biophys. J.* 2008, [4] Bec et al. *Las. Sur. Med.* 2012, [5] Throckmorton et al. *Neurophotonics* 2021

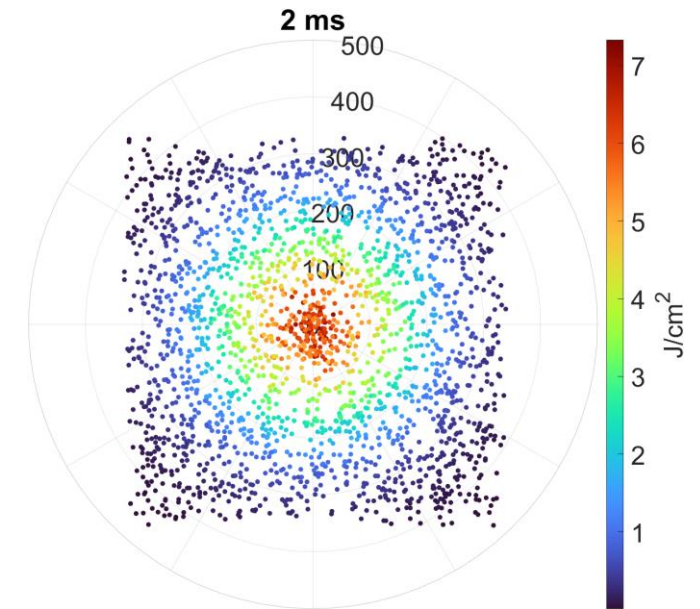
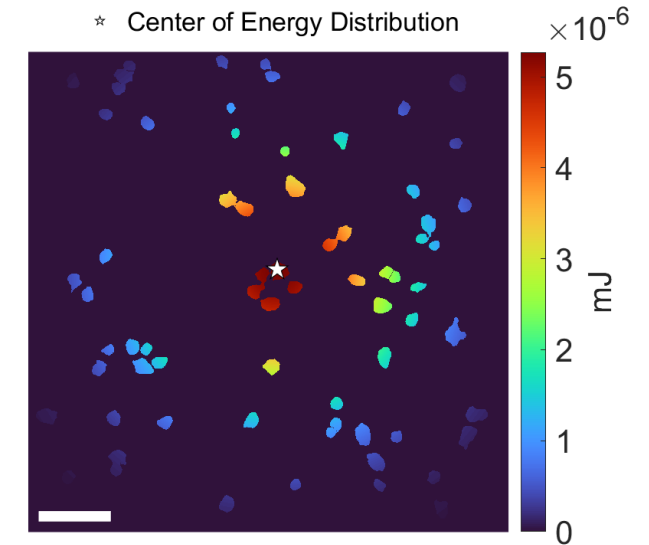
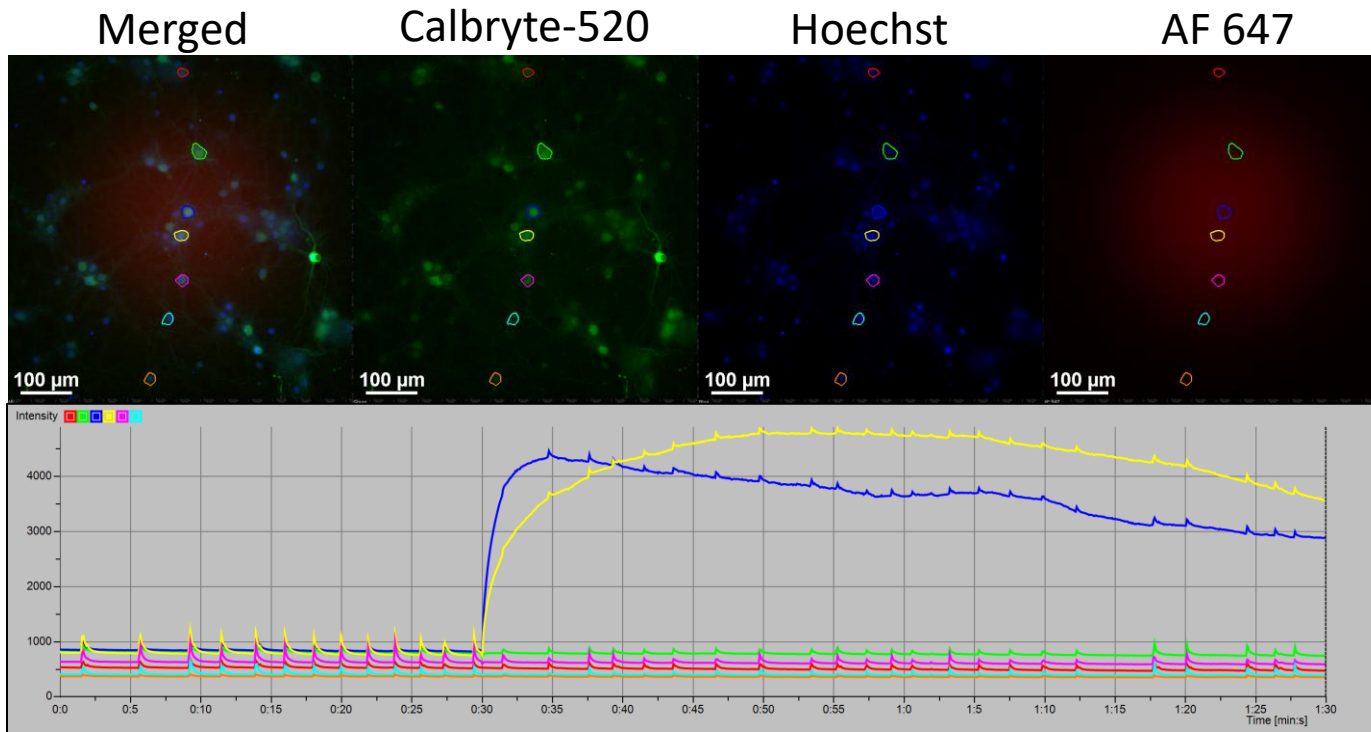
# Thermal Lensing Artifact as Fiducial for FTG exposure

- Thermal lens phenomenon
  - Index of refraction dependent on temperature
  - Change in focus because of heating
  - Lensing proportional to gaussian distribution of beam
- Visualize artifact by imaging diffuse Alexafluor 647 and irradiating sample
- Can develop model of energy distribution from artifact



# Position Dependent Stimulation

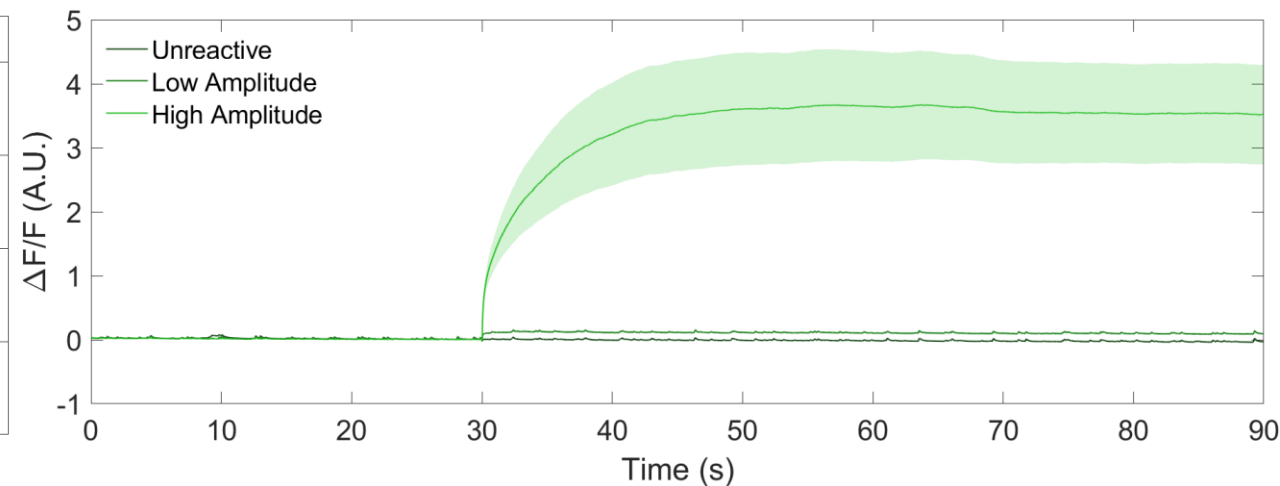
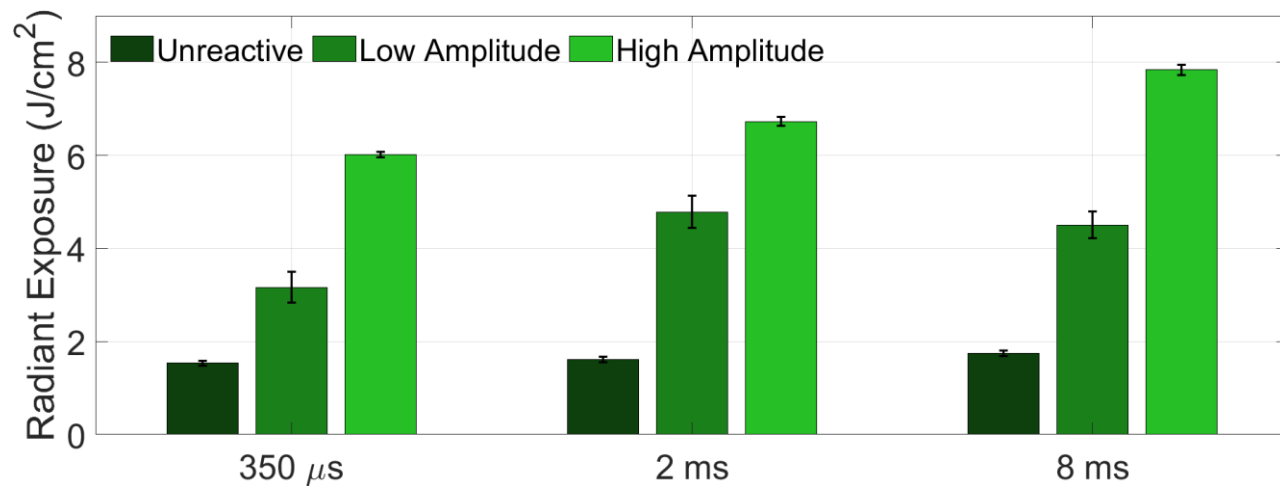
- Responses vary by position in FOV
- Cell reaction correlated with position on Gaussian energy distribution
- Reactive cells cluster near center of optical axis of laser





# Soma Reactions Exhibit 3 Phenotypes

- Phenotypes
  - Unreactive: Same response as unstimulated control group
  - Low Amplitude: Between 0 – 100% increase in fluorescence
  - High Amplitude: Above 100% increase in fluorescence
- Responses can be predicted based on local radiant exposure dosage

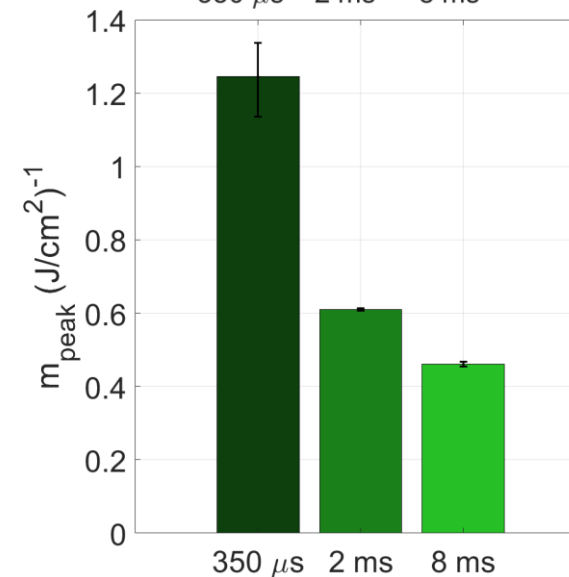
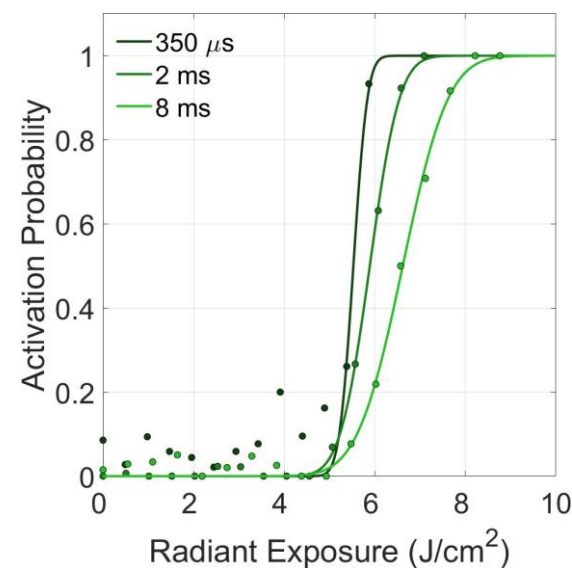
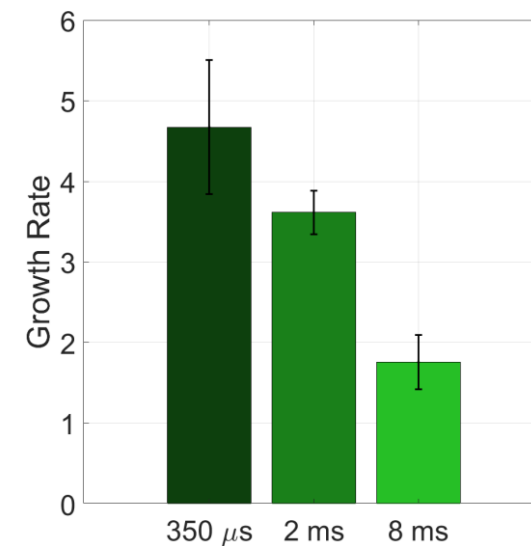
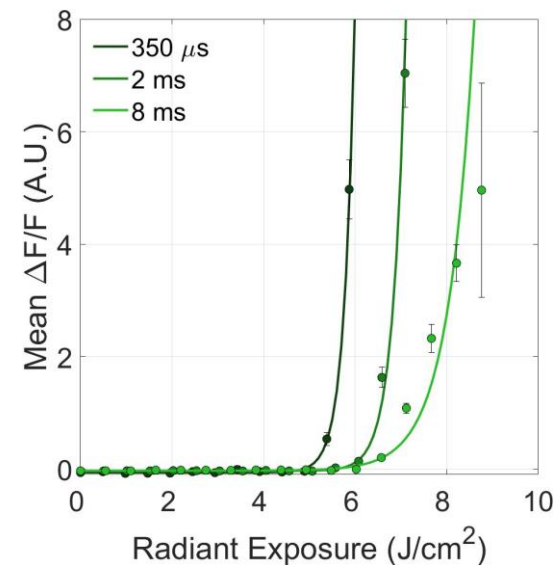
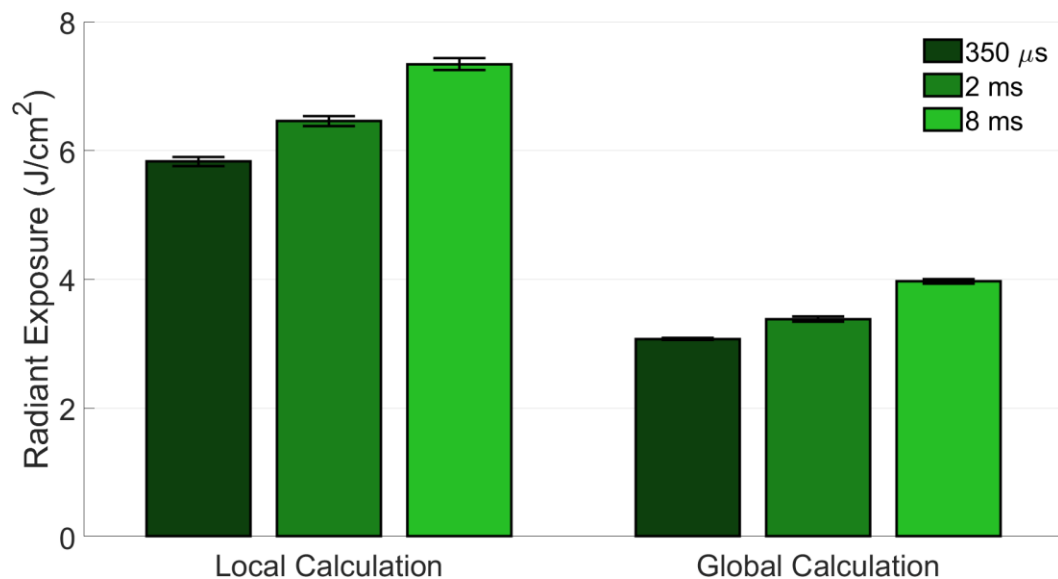






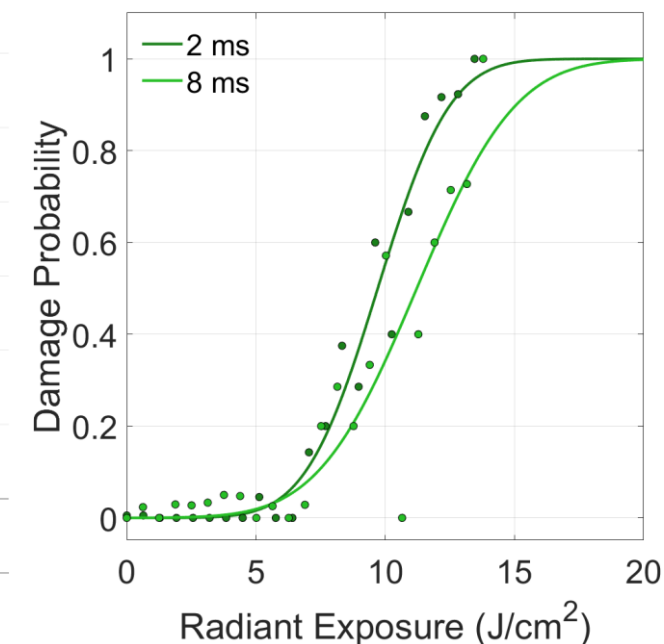
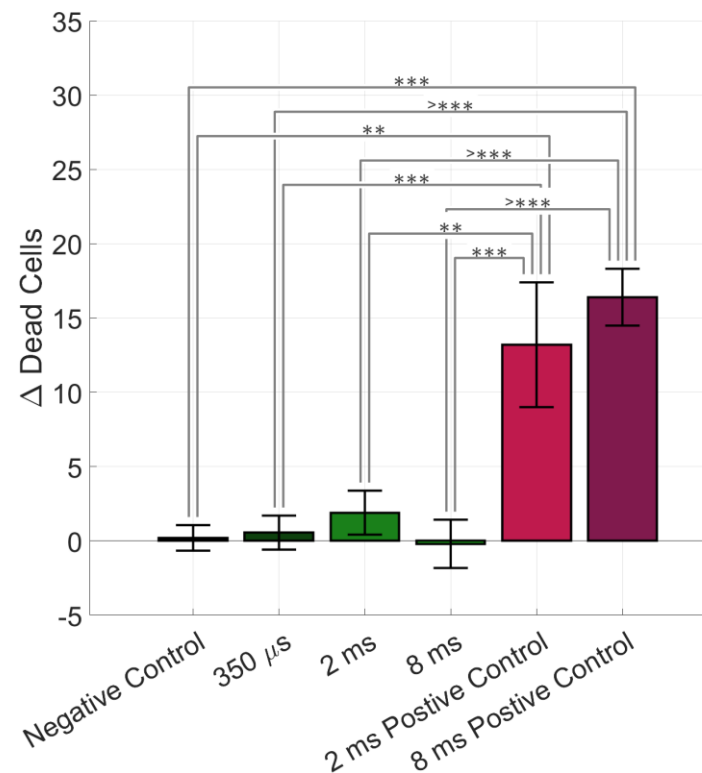
# Shorter Pulse Width Elicits More Efficient Stimulation

- The radiant exposure of reacting cells is ~2x the radiant exposure calculated using the spot size of the laser
- Strength-response curve shows exponential increase in fluorescence.
  - Growth rate of exponential increases with decreasing pulse width
- Probit responses indicate there is a faster transition in the probability that a cell will be stimulate using shorter pulse widths

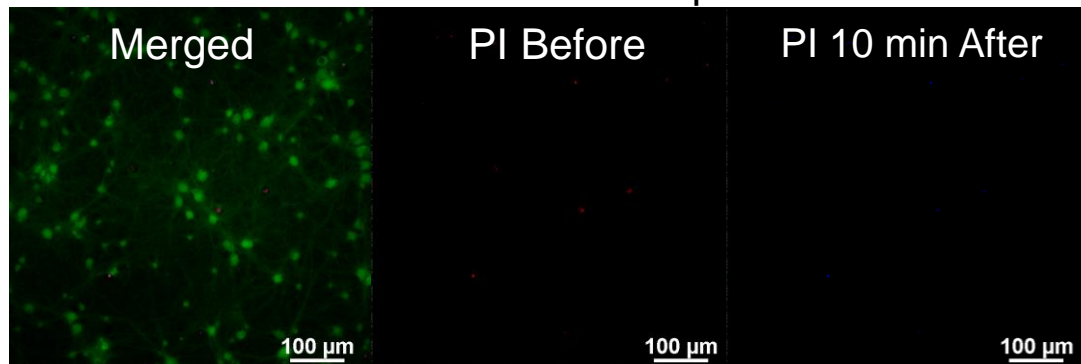


# Damage Assay

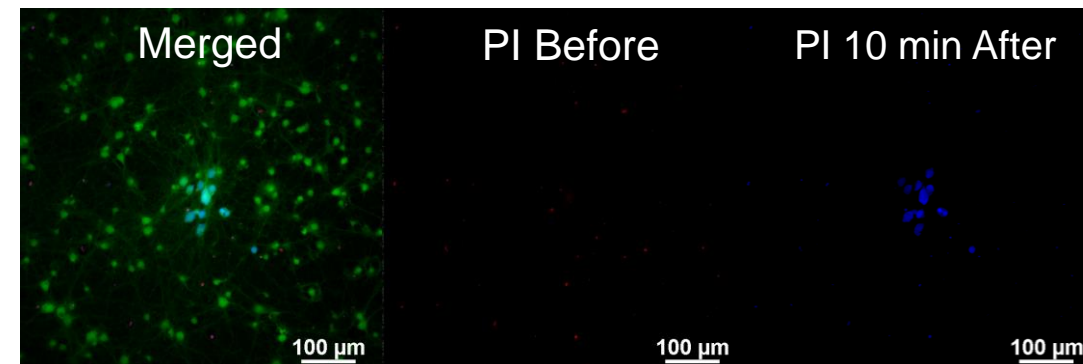
- Markers
  - Live Cell Marker: **Calcein-AM** - 5  $\mu\text{M}$
  - Damage Marker: **Propidium Iodide (PI)** - 1  $\mu\text{M}$
  - Stimulation fiducial: **Alexafluor 647** - 100 nM
- No statistically significant changes in cell death after exposing cells to pulse energies known to induce calcium responses.
- Mean radiant exposure for 2 ms and 8 ms pulse width correlates to ~10 % chance in cell death.
  - No damage was observed using the maximal laser power (20 W) for the 350  $\mu\text{s}$  pulse width



2 ms 7.2mJ Test Exposure

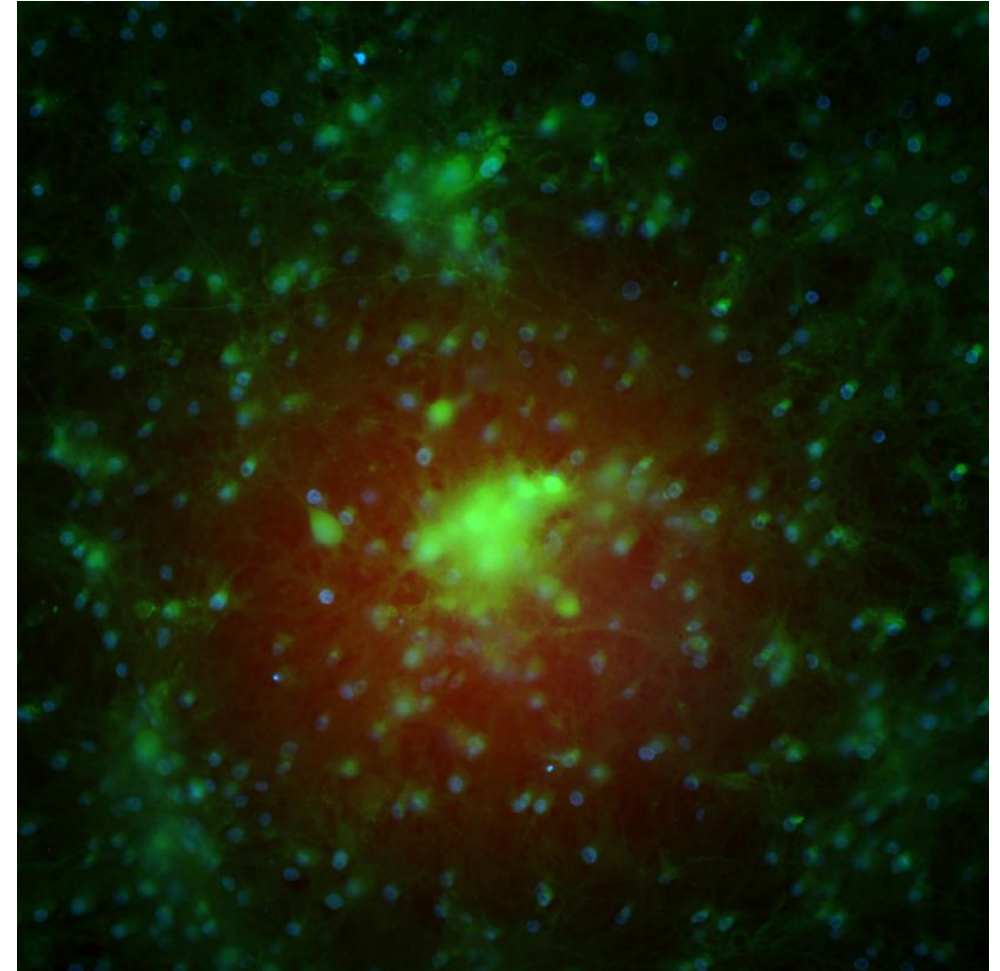


2 ms 14 mJ Positive Control



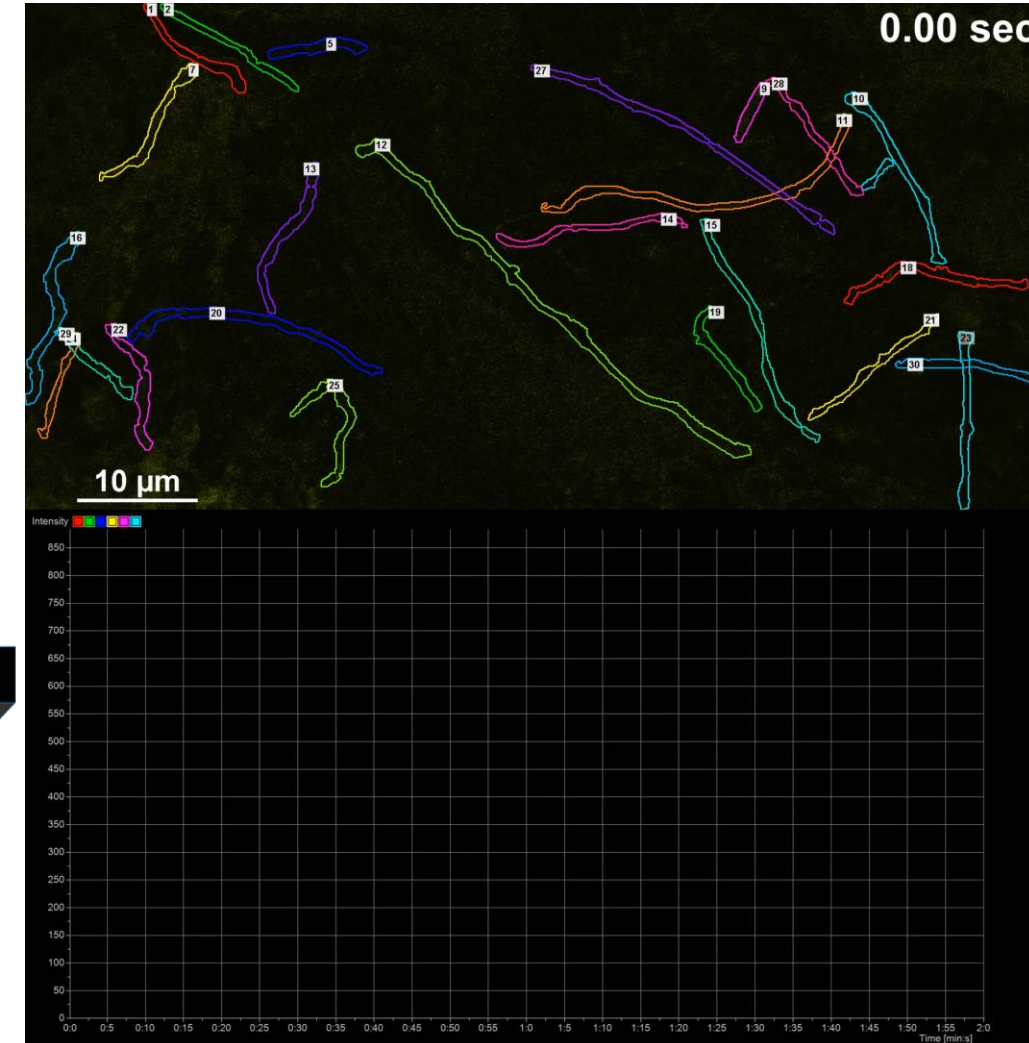
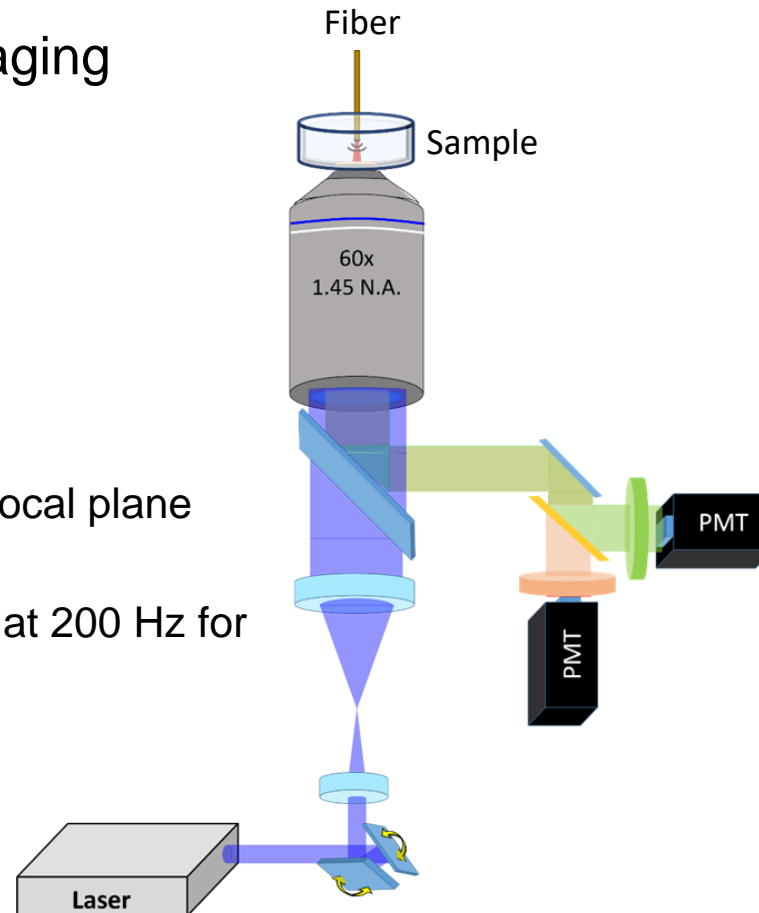
# Parameter Optimization Summary

- Neuronal reactions to stimulus have a spatial dependence.
- Critical to measure radiant exposure dosage at level of cells.
- Shorter pulse widths are more efficient for inducing stimulation in cortical neurons.
- Parameters tested are non-damaging



# Neurite Imaging During FTG Exposure

- Widefield imaging does not provide sufficient resolution
- Confocal Fluorescence Imaging
  - Nikon AX confocal
  - **Calbryte-520** - 1.5  $\mu\text{M}$
  - Resolution: 0.082  $\mu\text{m}/\text{pixel}$
  - FPS: 7.5 Hz
- INS Parameters
  - Fiber placed 200  $\mu\text{m}$  above focal plane
  - $\lambda = 1875\text{nm}$
  - Pulse train: 250  $\mu\text{s}$  repeated at 200 Hz for 500 ms, 100 pulses
  - Pulse Energy:
    - 1.2 mJ/pulse
    - 94.6 J/cm<sup>2</sup> @ fiber face

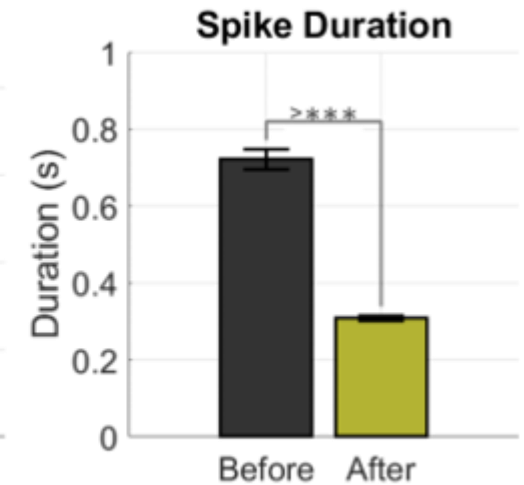
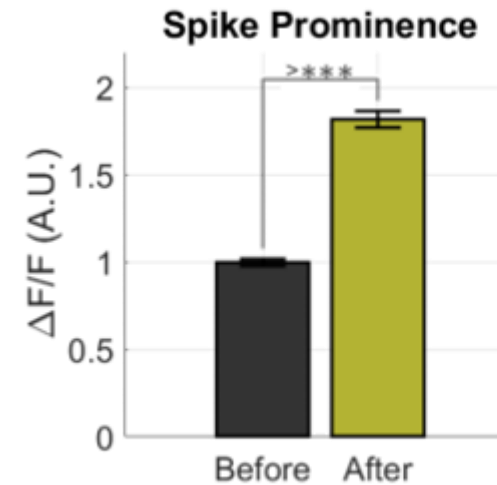
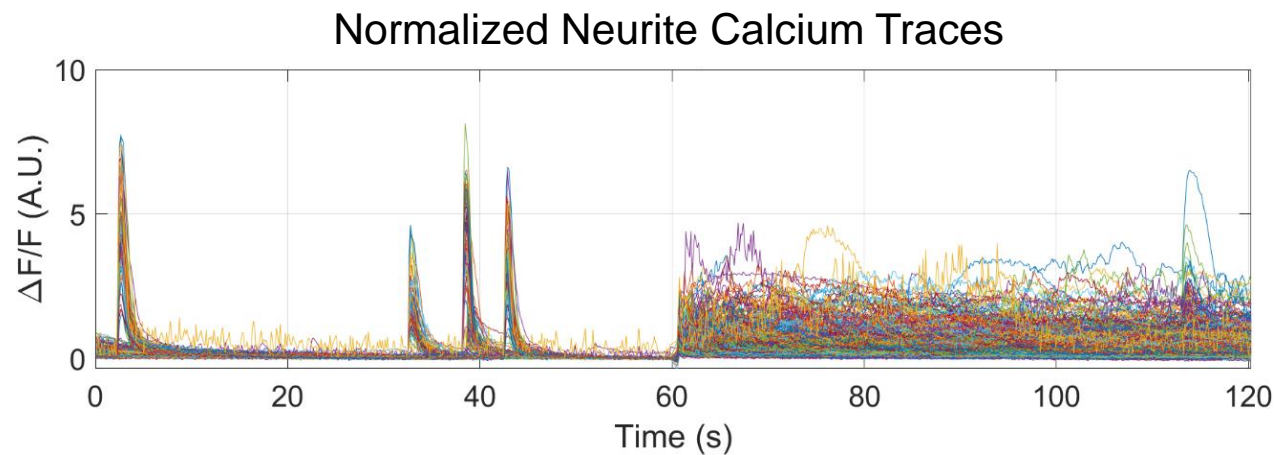
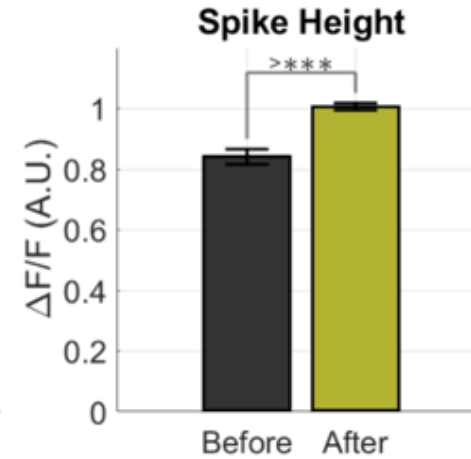
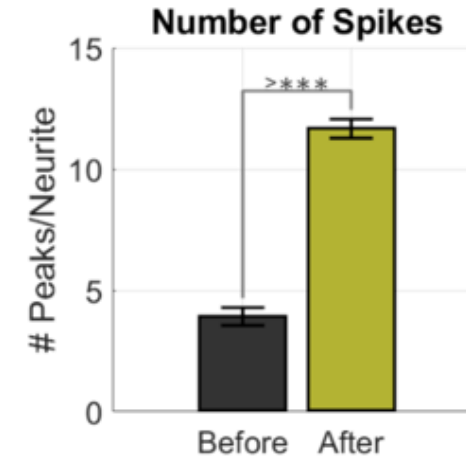
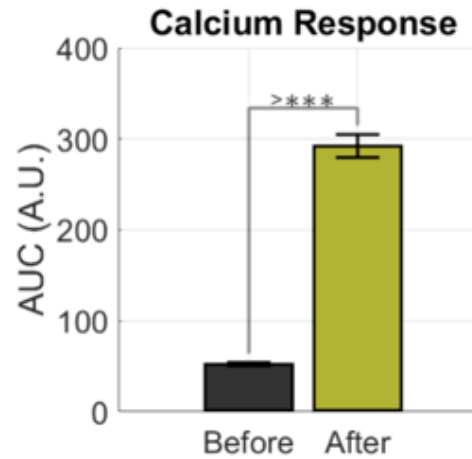






# INS Neurite Response

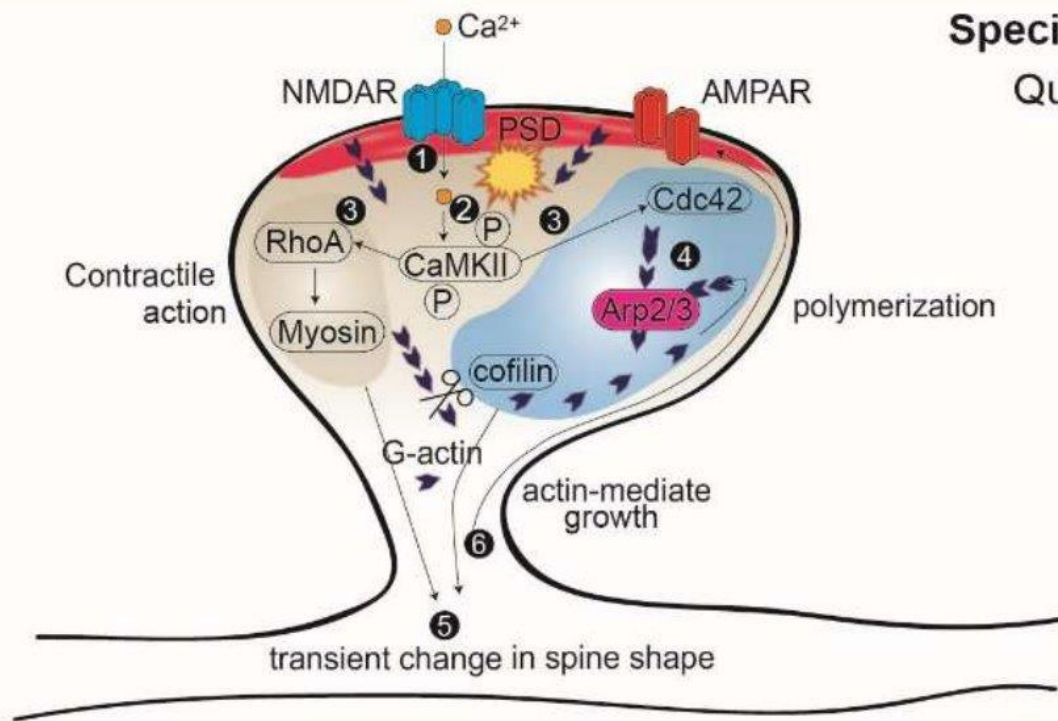
- Neurite responses to FTG exposure are stochastic
- Different temporal characteristics than spontaneous spiking
  - Increased DC signal
  - Increased AC spiking
  - Lower spike duration



# Specific Aim 1

## Specific Aim 1

Quantify how FTG exposure modifies key players at the dendritic spine



1. Dendritic spine morphology



2.  $Ca^{2+}$  tranients



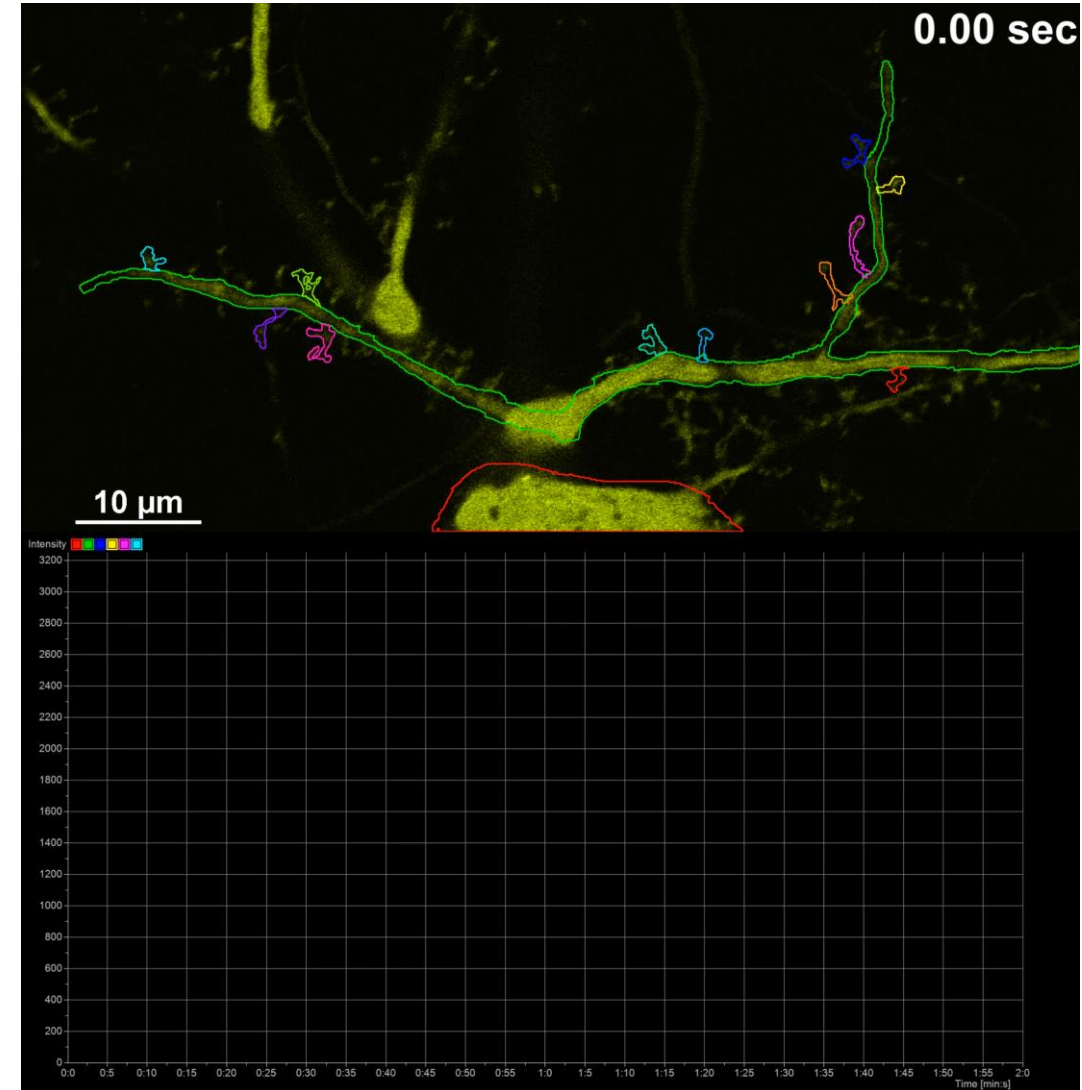
3. PSD dynamics



4. F-actin dynamics

# Dendritic Spine Imaging During INS

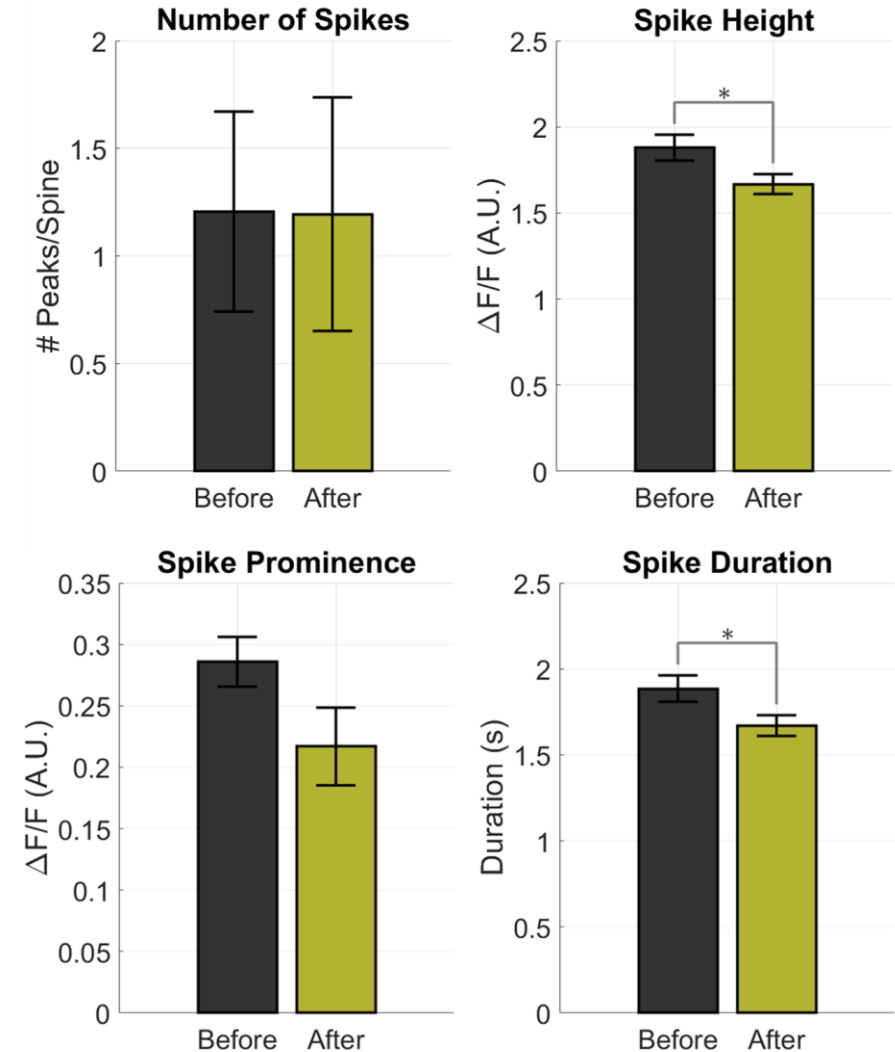
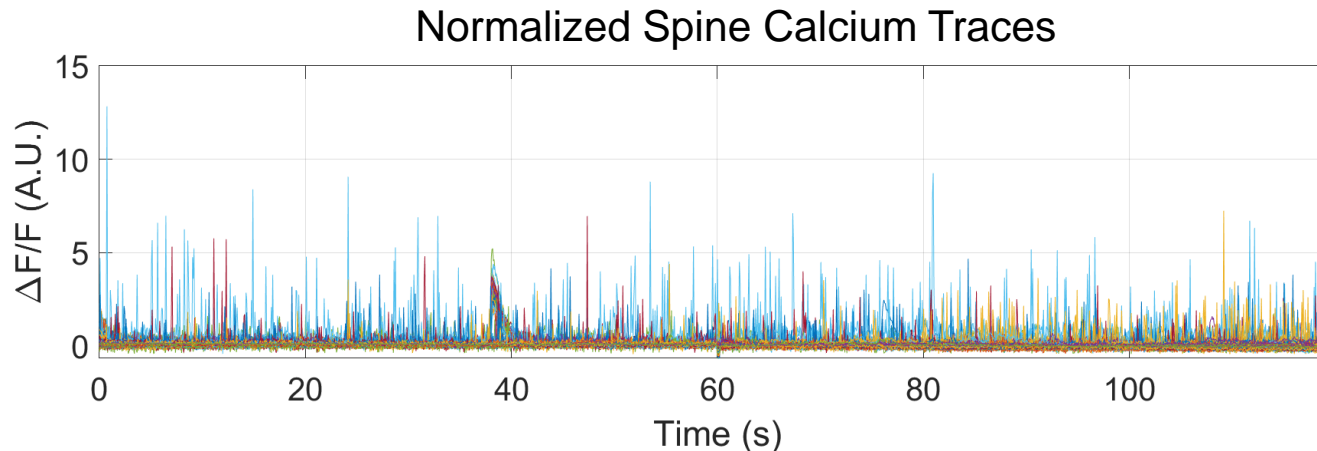
- Calbryte good for recording reaction from multiple neurons
- GCaMP better to study effects in single neurons
  - Can use to study functional effects of INS on dendritic spines
  - Transfected with GCaMPf8 plasmid using lipofectamine at DIV 7 imaged on DIV 20
- Calcium activity in spines
- Confocal fluorescence imaging
  - Nikon AX confocal
  - **GCaMP** – transgenic calcium indicator
  - Resolution: 0.082  $\mu\text{m}/\text{pixel}$
  - FPS: 7.5 Hz





# Dendritic Spine Reaction to Single FTG Exposure

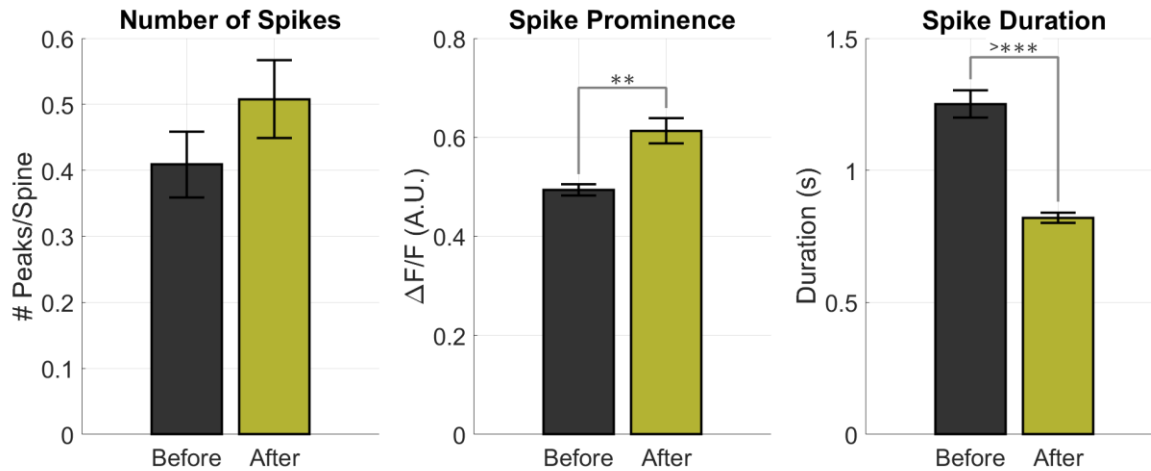
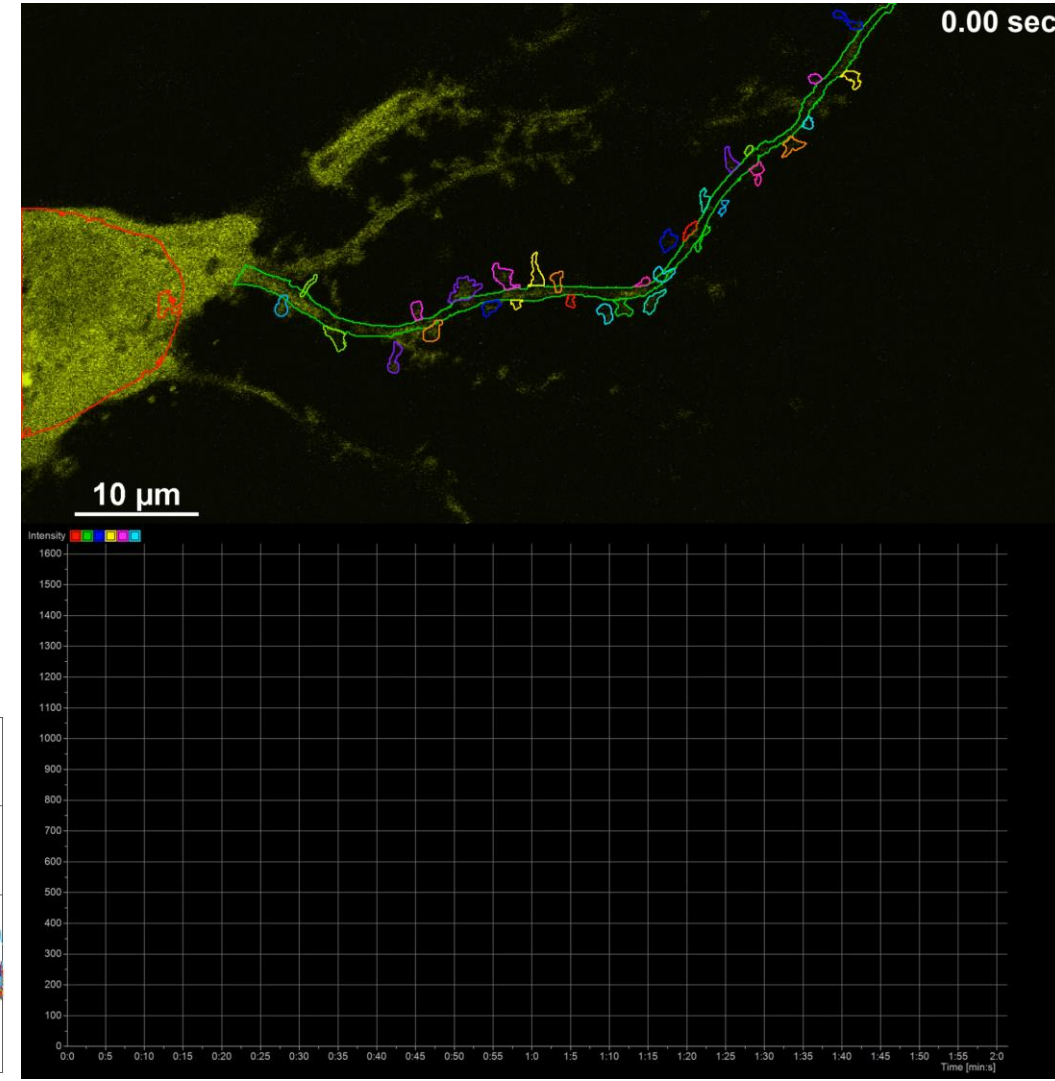
- Single exposure triggers neurite activity
- Dendritic spines largely unaffected
  - Some spines do respond
- Photobleaching could be responsible for statistically significant differences in spike height





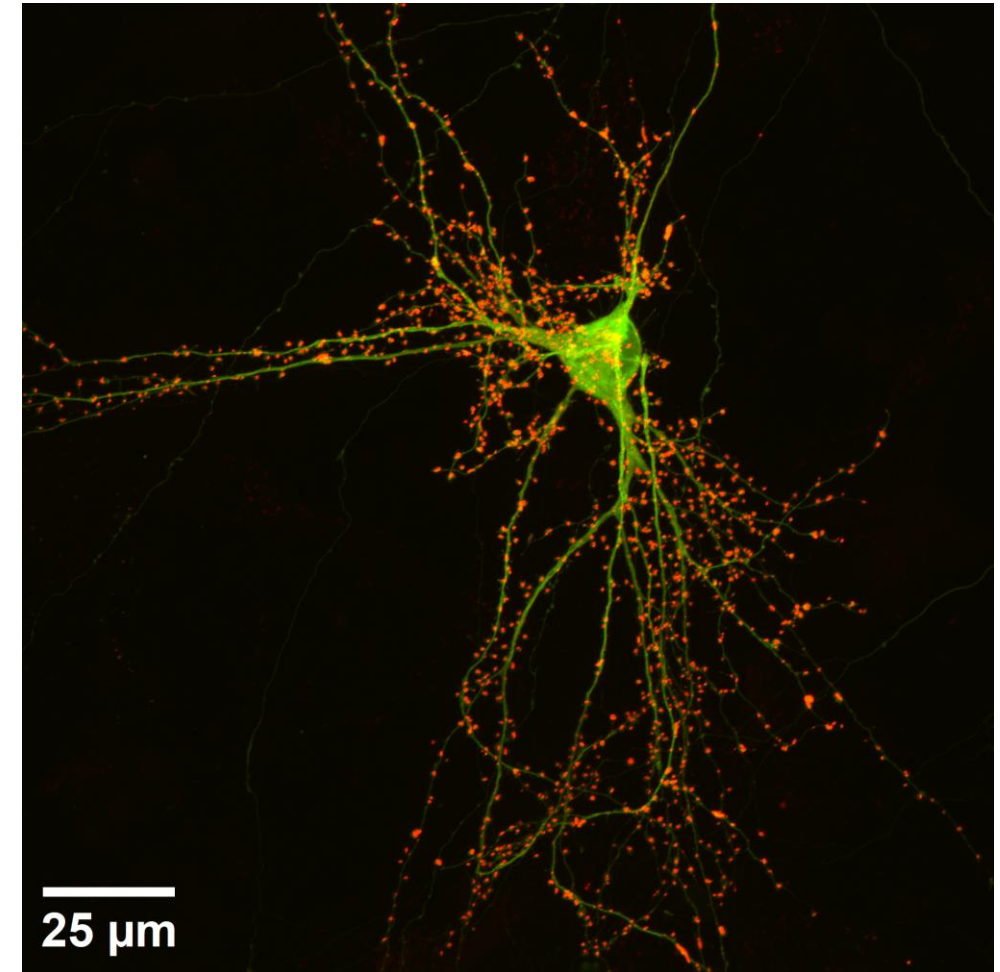
# Dendritic Spine Response to Multiple Exposures

- INS applied 30 seconds into imaging
  - 5 exposures with 5 seconds between subsequent exposures
- Dendritic spines exhibit increased activity
- Statistically significant in 2 categories



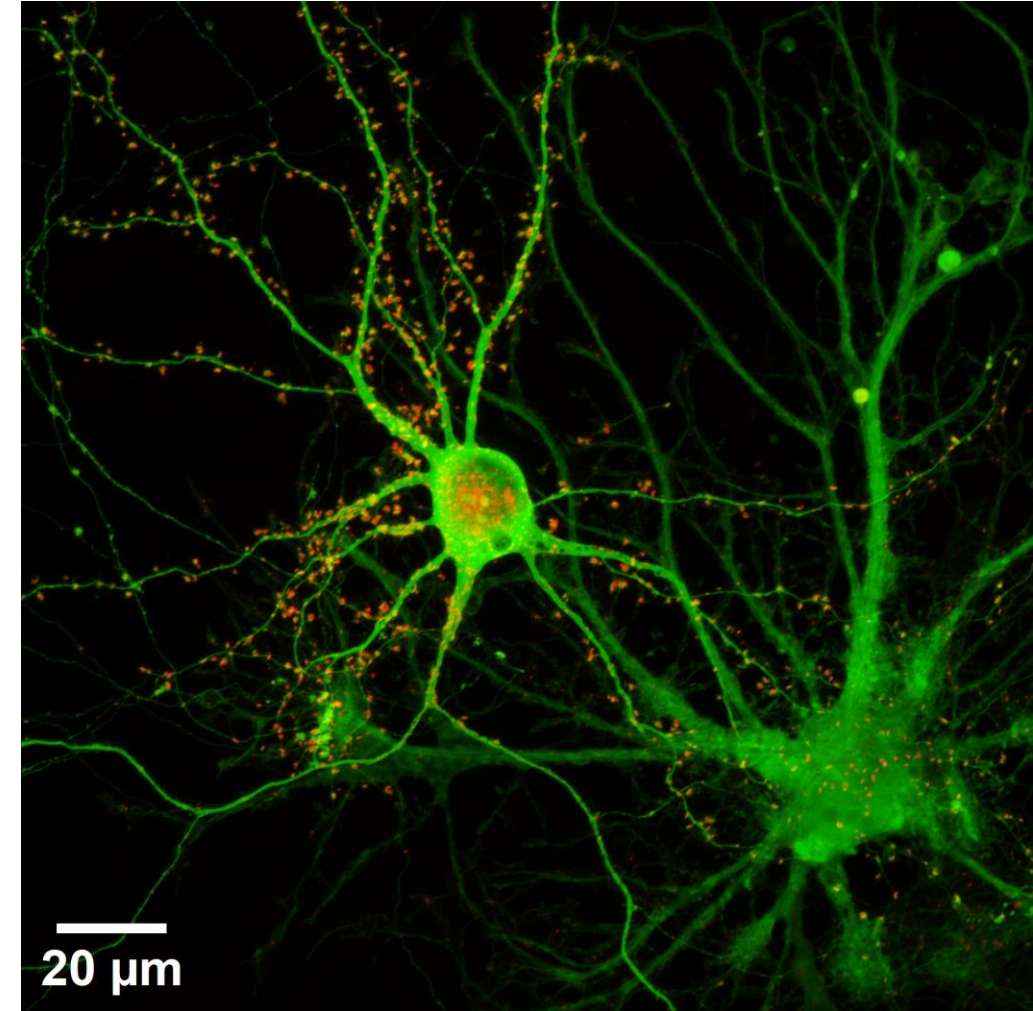
# Conclusions

- Shorter pulse widths are more efficient for inducing stimulation in cortical neurons
  - Likelihood for damage also increases with decreasing pulse width
- FTG induce multiple effects in cortical neurons.
  - Calcium reactions in soma exhibit a stepwise increase in calcium fluorescence unlike transient activity
  - Calcium signaling in neurites and spines is stochastic and differs from action potential related activity
  - Likelihood of spine response is correlated with number of exposures



# Ongoing Experiments and Future Directions

- Calcium
  - Q-switched Ho:YAG laser for evaluating pressure effects on neurons
  - Pharmacology to locate source of Calcium
- Plasticity Effects in Dendritic Spines
  - Spine Morphology
    - Longer imaging periods for dendritic spines
    - Light sheet volumetric imaging
  - Effects on post synaptic density
  - Actin dynamics
- Glia and neuronal interactions in response to FTG

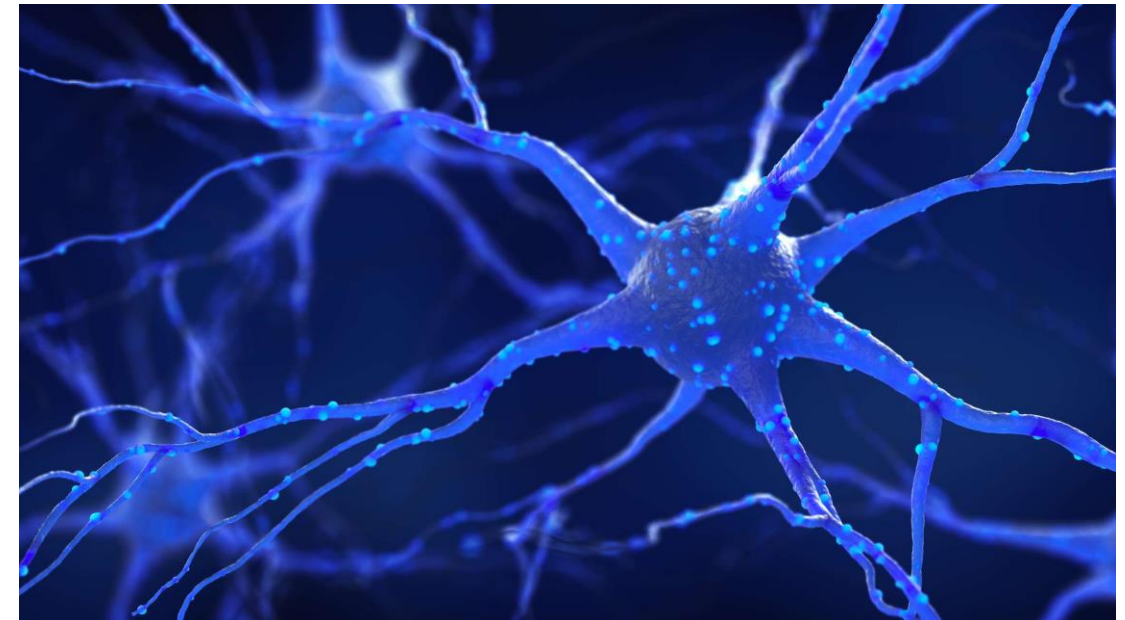


# Modulation of Neurons through IR Induced FTG

## Goal:

Determine specific IR exposure parameters (i.e. frequency, pulse duration, amplitude, energy) that enhance or reduce neuronal synaptic activity without damaging the dendritic spine

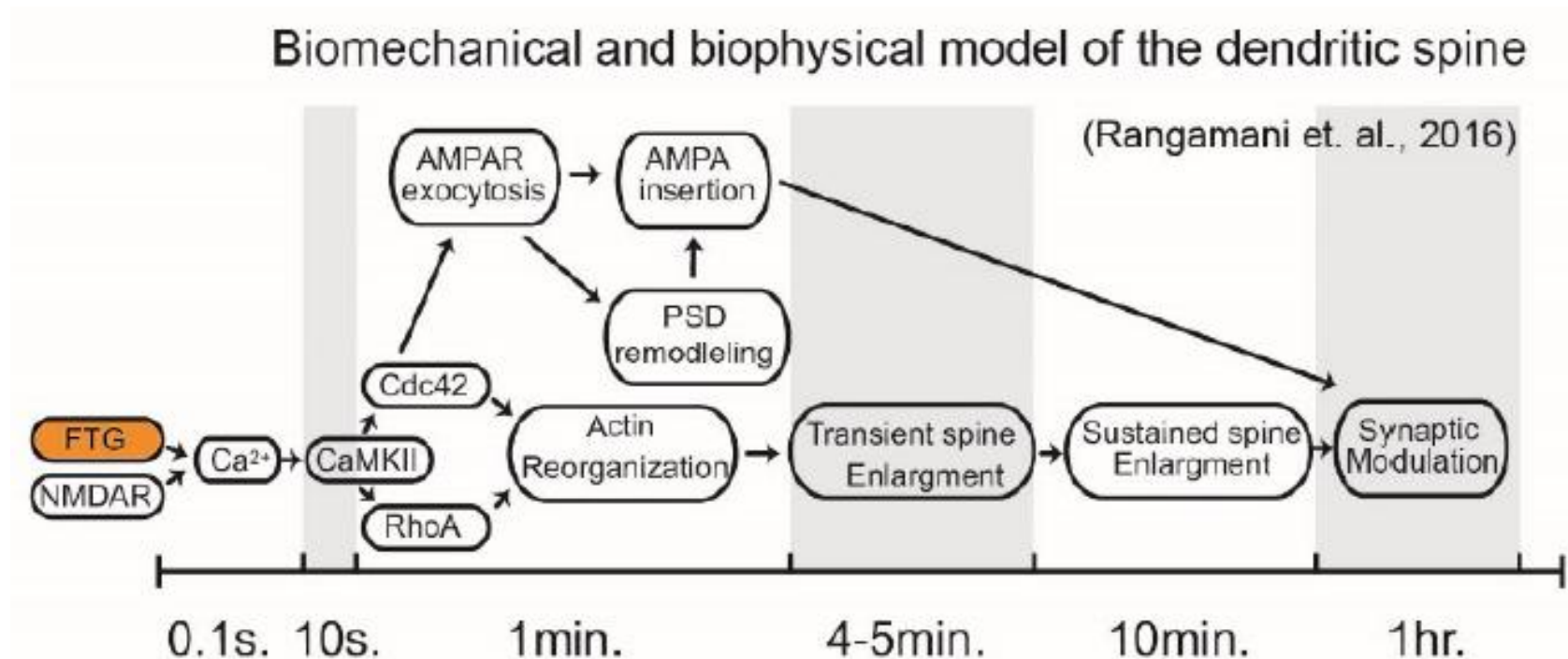
- Specific Aim 1
  - Determine the structural and functional effects of IR-induced FTG exposure on dendritic spines
- Specific Aim 2
  - Develop multiscale biophysical models of FTG exposure on dendritic spines







## Specific Aim 2





# Phenomenological Approach to Temperature Dependence

- Wide-held assumption:
  - The reaction rate depends on
    - Energy barrier
    - Entropy

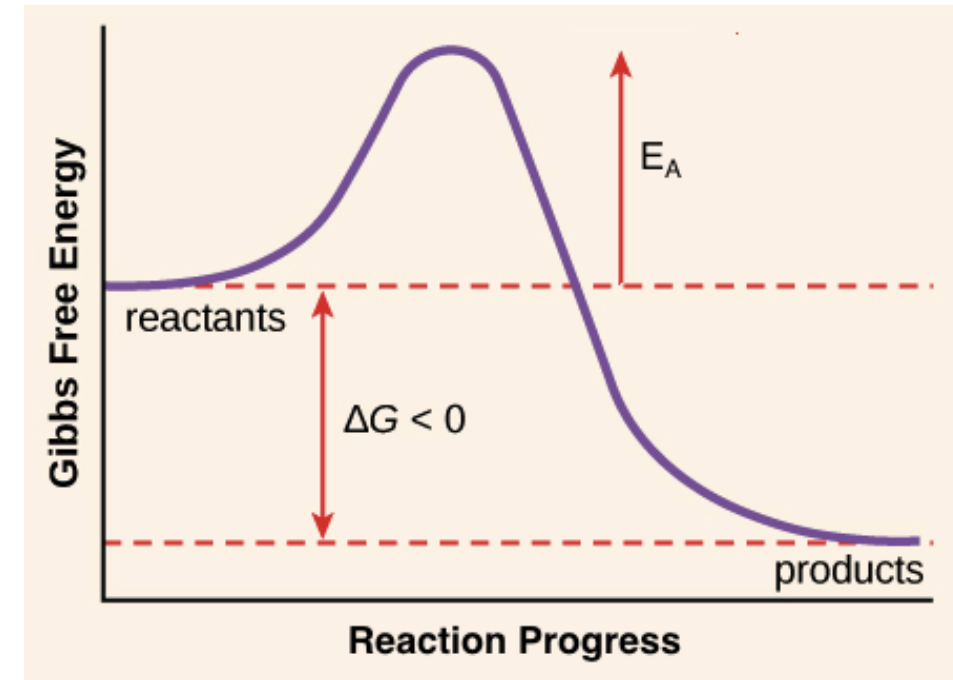
In 1889 Arrhenius proposed a phenomenological function

$$k^{Arr}(T) = Ae^{-E_a/RT}$$

In this case k is the **rate coefficient**

From Transition State Theory (1935) we move to Gibbs's/Free energy:

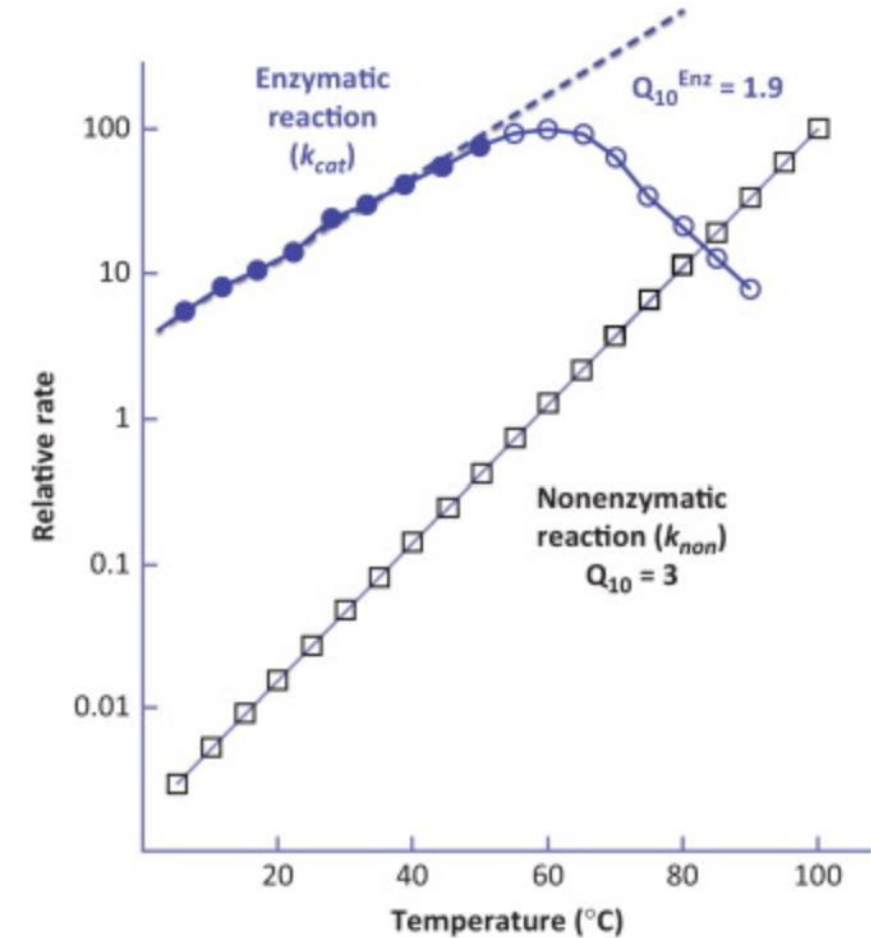
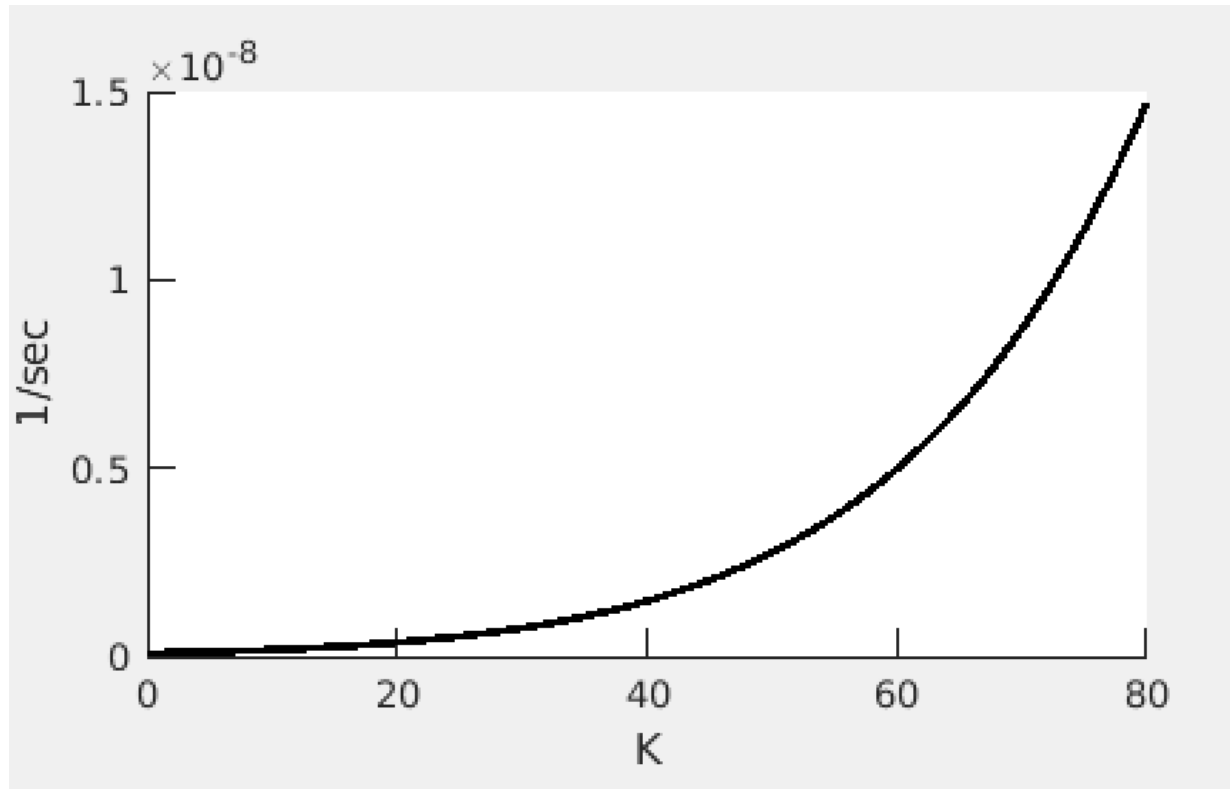
$$k^{tst}(T) = (k_B T/h) e^{-\Delta G^\ddagger/RT}$$



From any Intro Biology book. OpenStax



# While Arrhenius Predicts Infinite Increase in Reaction Rates, the Reality is Different



Elias et al. Trends in Biochem Sci. **The universality of enzymatic rate–temperature dependency** 2014.



# Free Energy, Enthalpy, and Entropy

- The reaction rate coefficient is temperature dependent

$$k^{tst}(T) = (k_B T/h) e^{-\Delta G^\ddagger/RT}$$

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$$

$$k^{tst}(T) = \left( (k_B T/h) e^{\Delta S^\ddagger/R} \right) e^{-\Delta H^\ddagger/RT}$$





# Macro-molecular Rate Theory (originally developed for enzymatic reactions)

- **Macro-molecular rate theory** includes the effect of heat capacity  $\Delta C_p^\ddagger$

At constant pressure, which are our experimental conditions

From the definition of enthalpy

$$\Delta C_p^\ddagger dT = d\Delta Q = d\Delta H$$

$$\Delta C_p^\ddagger = \frac{\partial \Delta H^\ddagger}{\partial T}$$

From the definition of entropy

$$\Delta C_p^\ddagger dT = d\Delta Q = T d\Delta S$$

$$\Delta C_p^\ddagger = T \frac{\partial \Delta S^\ddagger}{\partial T}$$

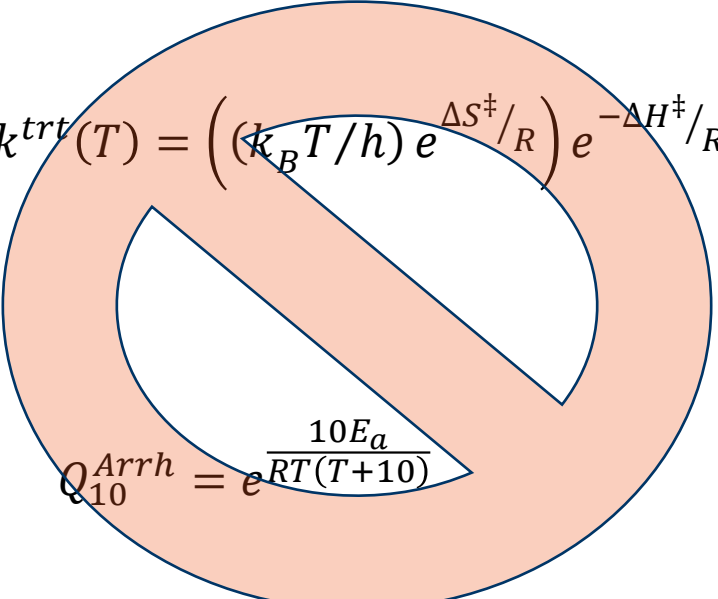
Hobbs, et al. ACS Chem Biol 8(11):2388-93 (2013)

# What Does a Change in $\Delta C_p^\ddagger$ Imply?

- Proteins are not monoliths
- The movement of a macro-protein to the transition state could change the conformational state and affect non-covalent bonds
  - Hydrogen, van der Waals, salt bonds, hydration shells

## How Does Including $\Delta C_p^\ddagger$ Affect $k$ and $Q_{10}$ ?

- Macro-molecular rate theory** includes the effect of heat capacity  $\Delta C_p^\ddagger$



$$k^{trt}(T) = \left( \left( \frac{k_B T}{h} \right) e^{\Delta S^\ddagger / R} \right) e^{-\Delta H^\ddagger / RT}$$

$$Q_{10}^{Arrh} = e^{\frac{10 E_a}{RT(T+10)}}$$

$$k^{MMRT} = \frac{k_B T}{h} e^{\frac{-\left( \Delta H^\ddagger + \Delta C_p^\ddagger (T - T_0) \right)}{RT} + \frac{\left( \Delta S + \Delta C_p^\ddagger \left( \log \frac{T}{T_0} \right) \right)}{R}}$$

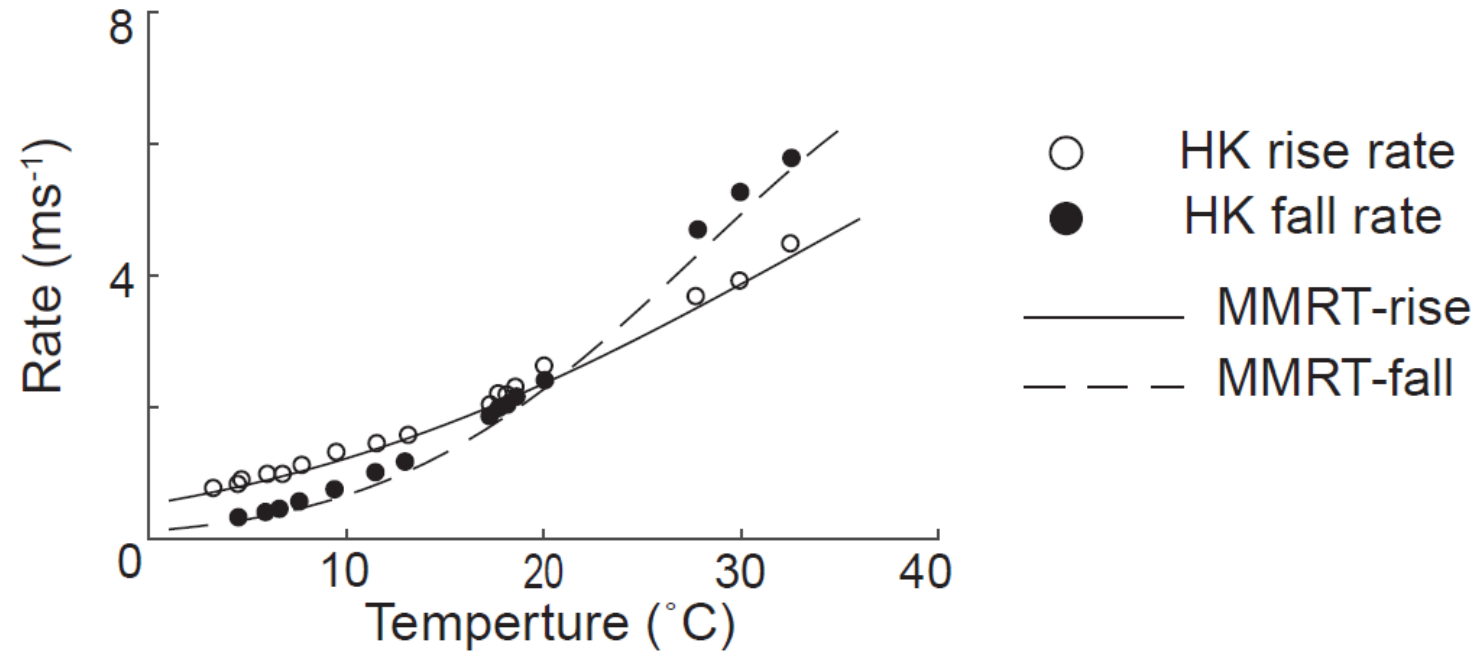
$$Q_{10}^{MMRT} = e^{\frac{10 T \left( \Delta H^\ddagger + \Delta C_p^\ddagger (T - T_0) \right) + 50 T \Delta C_p^\ddagger - 500 \Delta C_p^\ddagger}{RT^2 (T + 10)}}$$



# Implementing Thermodynamic Equations in MMRT



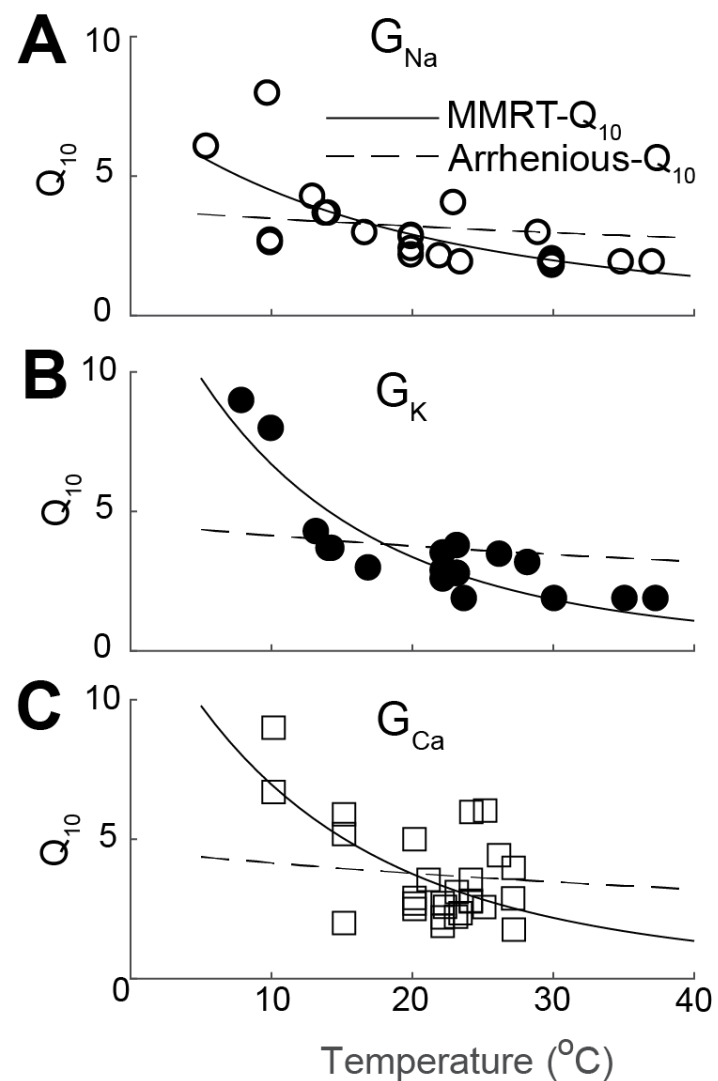
Dr. Bahram Pahlavan



Pahlavan, Buitrago, Santamaria  
Biophys J 2022



# Temperature Dependence of $Q_{10}$ on Neuronal Membrane Conductances



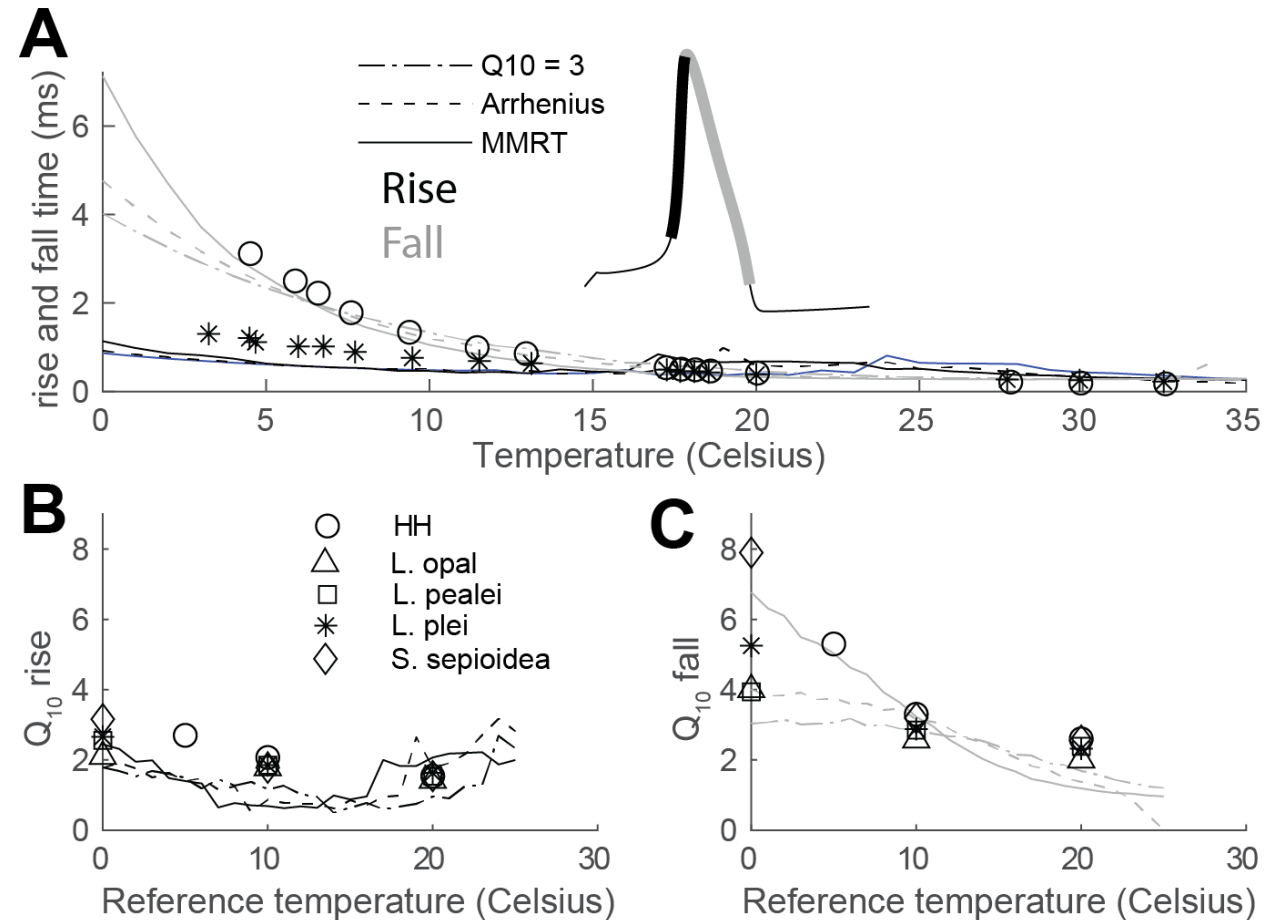
Pahlavan, Buitrago, Santamaria  
Biophys J 2022





# Explanation: Differences in Excitability Among Squid Species that Live in Different Temperatures

- Calculate the rise/fall of action potential.
  - For different squid species
  - MMRT is the only one that shows a parsimonious explanation



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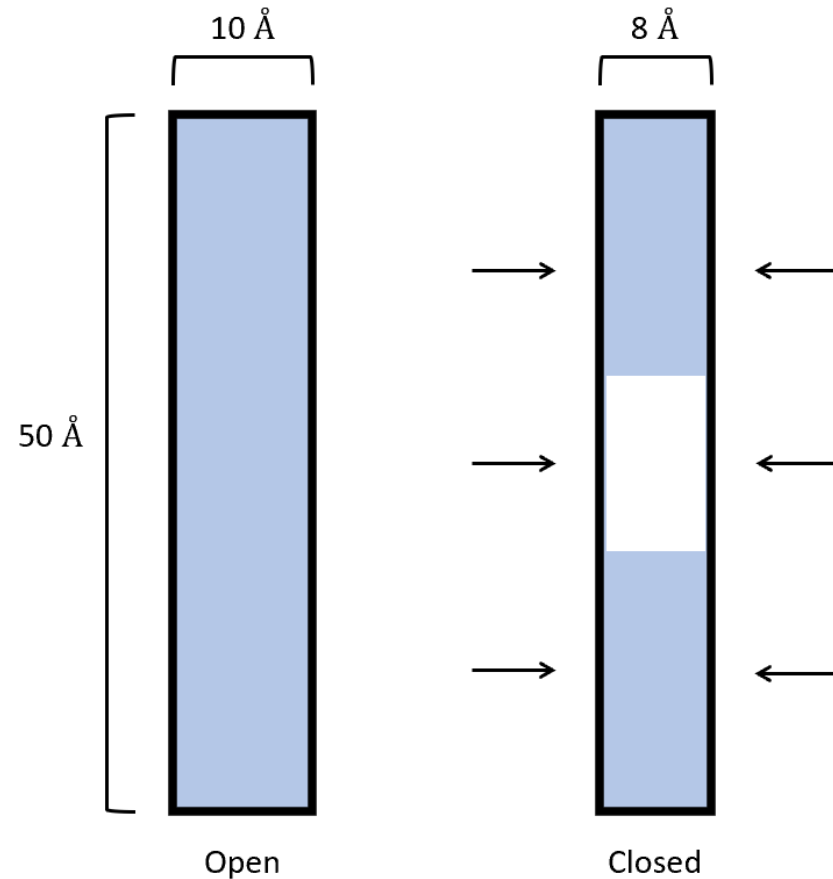


# Pressure Effect on Channel Pore

- $\Delta V^\ddagger$  : change in the activation volume during conformation change from closed to open states
- Hydration and dewetting



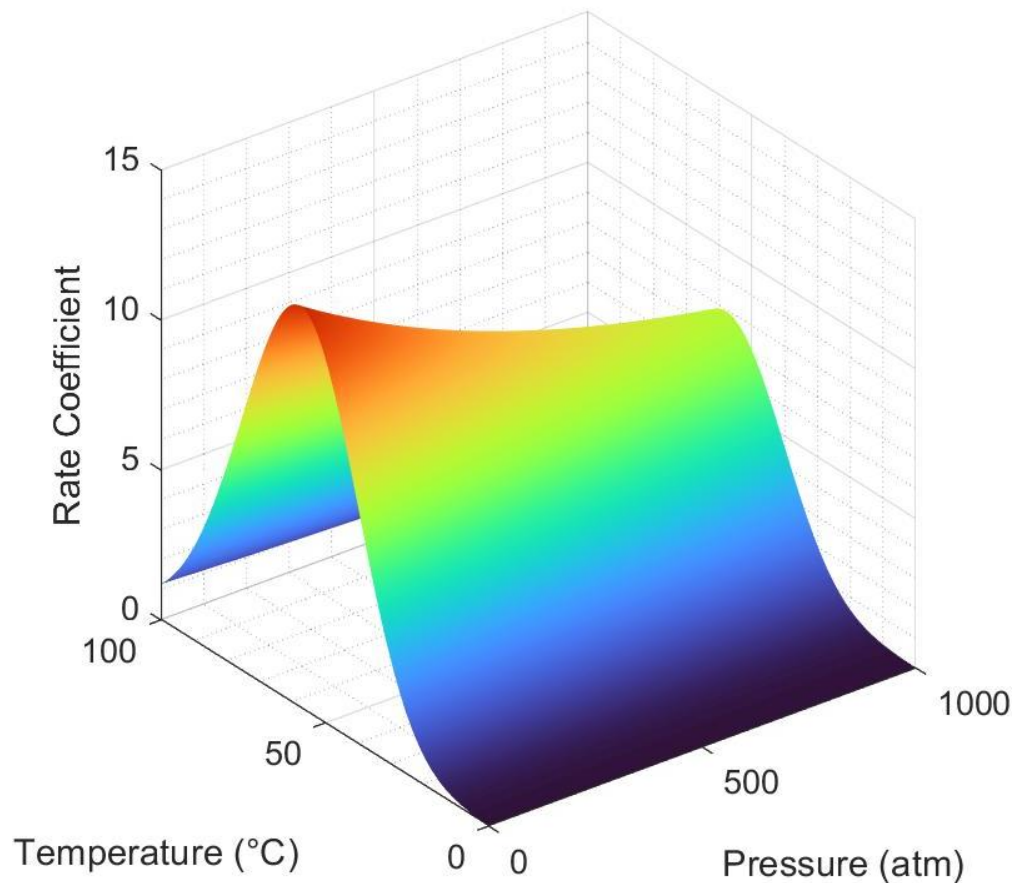
Mr. Jacob Miller





# How Does Pressure Changes Affect Neuronal Computation?

$$\Delta G^\ddagger = \Delta C_p^\ddagger \left[ (T - T_0) - T_0 \ln \frac{T}{T_0} \right] - T_0 \Delta S^\ddagger + \Delta \hat{\alpha}^\ddagger (T - T_0)(P - P_0) + \Delta V_0^\ddagger (P - P_0) - \frac{\Delta \hat{\kappa}^\ddagger}{2} (P - P_0)^2 + \Delta H_0^\ddagger$$



$$\Delta \hat{\alpha}^\ddagger = \Delta(\alpha V)^\ddagger \approx \alpha(\Delta V)^\ddagger + V(\Delta \alpha)^\ddagger$$

Isobaric expansivity  $\hat{\alpha}$

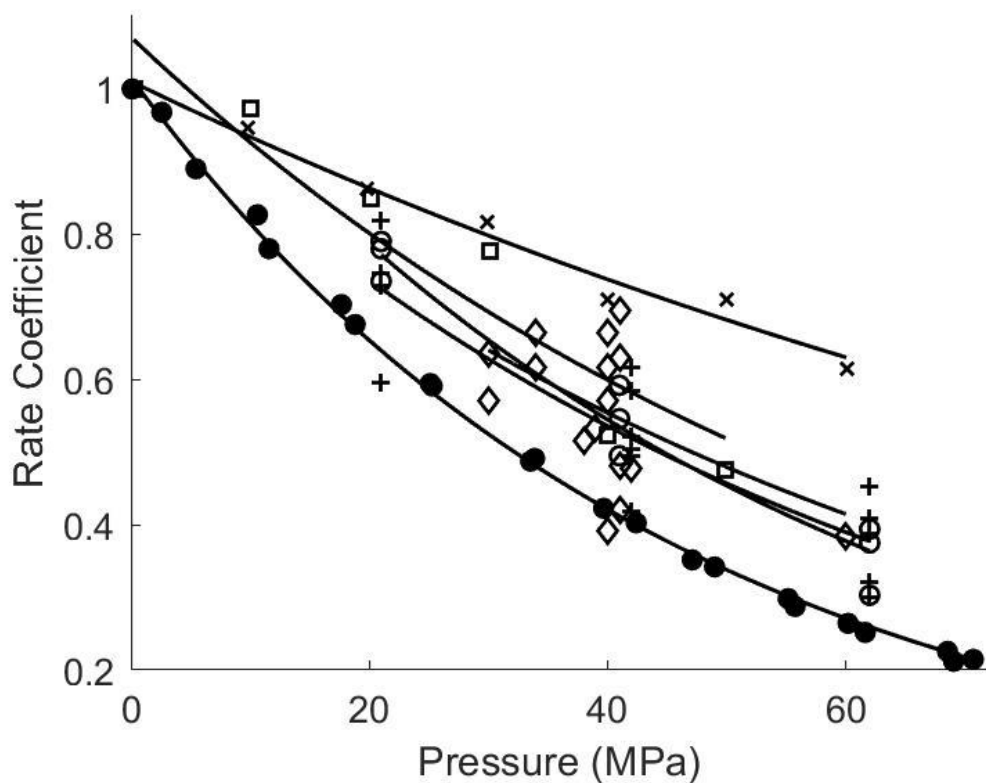
$$\alpha = \frac{1}{V} \left( \frac{\partial V}{\partial T} \right) \bigg|_P$$

isothermal compressibility  $\hat{\kappa}$

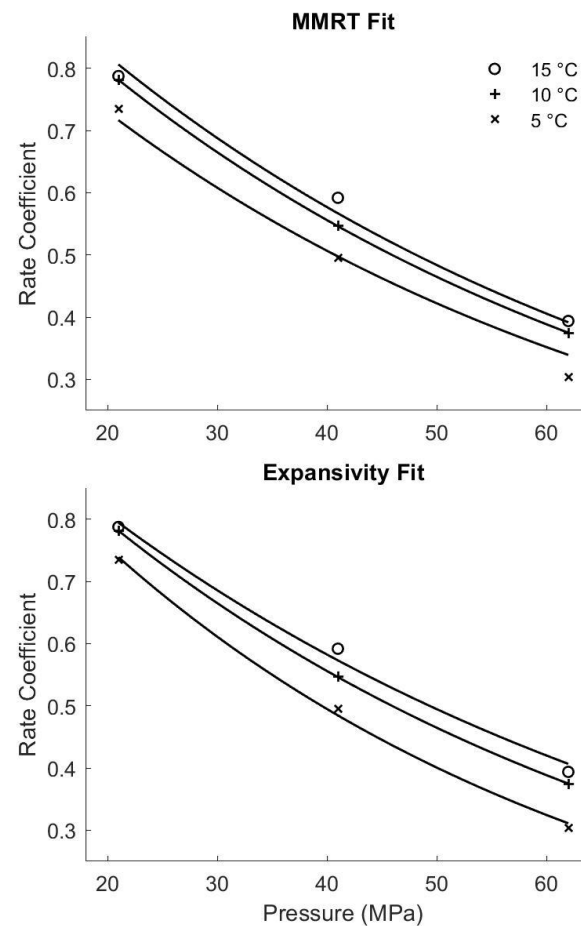
$$\kappa = -\frac{1}{V} \left( \frac{\partial V}{\partial P} \right) \bigg|_T$$



## Calculating Isobaric Expansivity from Data



## Calculating Isothermal Compressibility

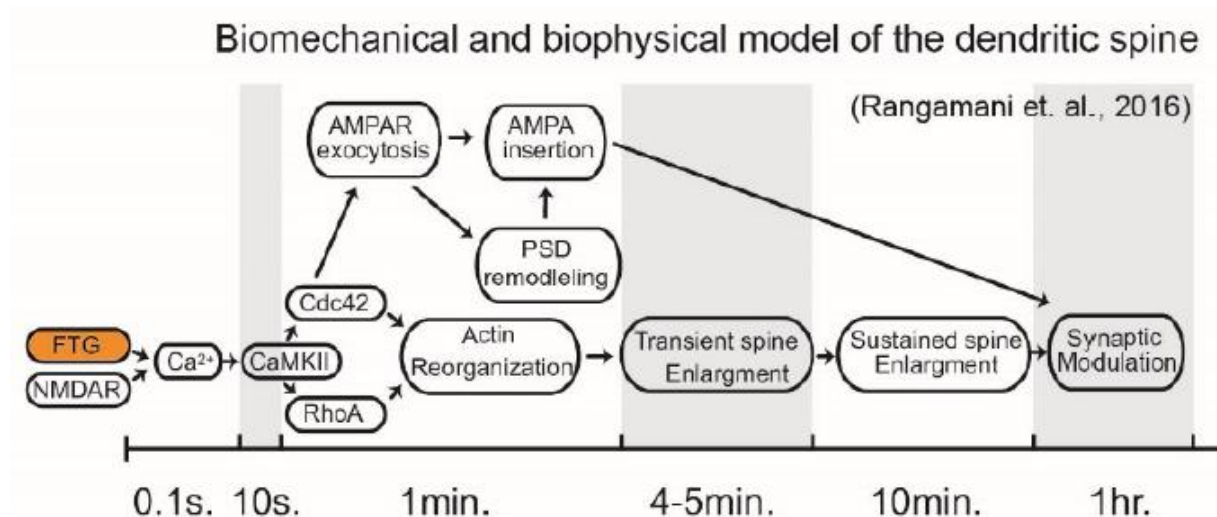






# Summary

- Temperature dependence is explained by MMRT
- Biophysical models of spiking neurons show realistic responses to temperature and pressure changes
- Thermodynamics based theory explains temperature and pressure dependence of neuronal membrane conductance kinetics
- The ability to calculate and implement isobaric expansivity and isothermal compressibility will allow for more accurate results from the biomechanical models developed
- Temperature and pressure compensation is a critical parameter and can be implemented in all biophysical models of neuronal excitability





- Technical: Provide MMRT modified conductances to the community
- Theoretical: Have a generalized thermodynamic theory of voltage gated channel activation
- Computational:
  - How do T and P affect single cell computation?
  - How do T and P affect network activity?

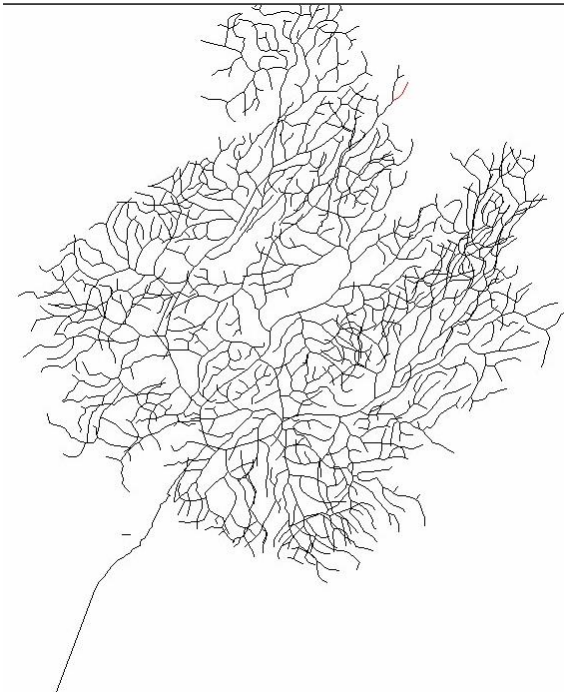
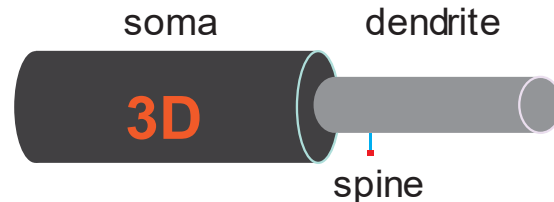
- Build a generic computational platform to be used by all AFRL efforts
  - Synaptic plasticity in Purkinje cells is the best model
    - Mostly post-synaptic
    - Well characterized biochemical reaction and easy model to test experimentally





# Benefits and Opportunities

- Implemented all the known biochemical reaction in long term synaptic depression (LTD) in cerebellar Purkinje cells
- Extended the model to use the latest computational algorithms that include 3D diffusion of all reactants.
- NEURON reaction-diffusion module
  - It allows us to model all intracellular biochemical reactions including
    - Calcium buffering reactions with calbindin and parvalbumin
    - Signal transduction mechanisms involved in cerebellar long-term depression of granule cells synapses onto Purkinje cells
  - It allows us to more realistically model the diffusion of ions and proteins
    - Calcium ions
    - Enzymes
- Platform could implement other synaptic mechanism processes such as LTP in cortical or hippocampal pyramidal cells





## Questions Remaining?

- Calcium Source
- Evaluate pressure effects on neurons
- Plasticity Effects in Dendritic Spines
  - Spine Morphology
  - Effects on post synaptic density
  - Actin dynamics
- Glia and neuronal interactions in response to FTG
- Develop model that incorporates FTG
  - Does the model accurately predict neural response



# Value of Research

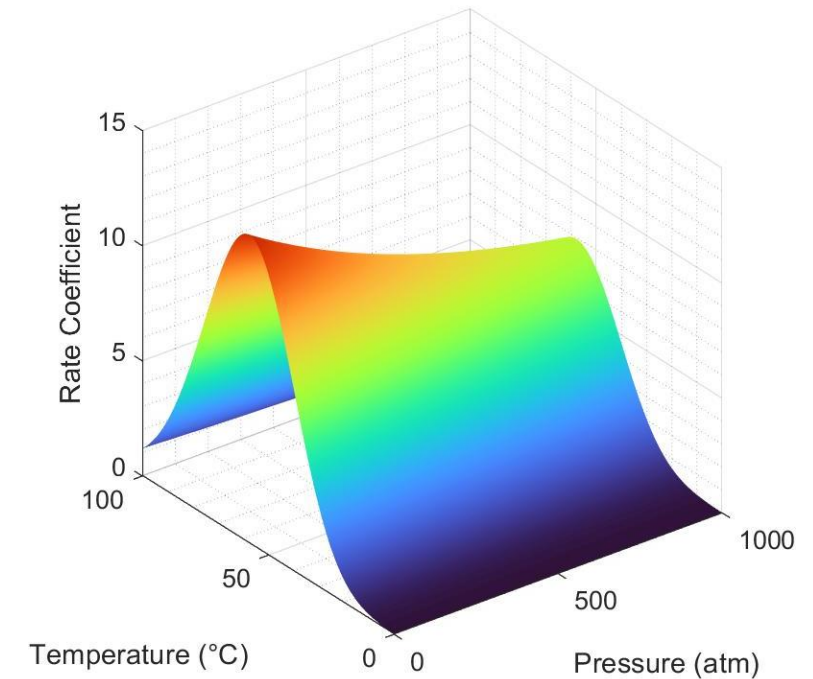
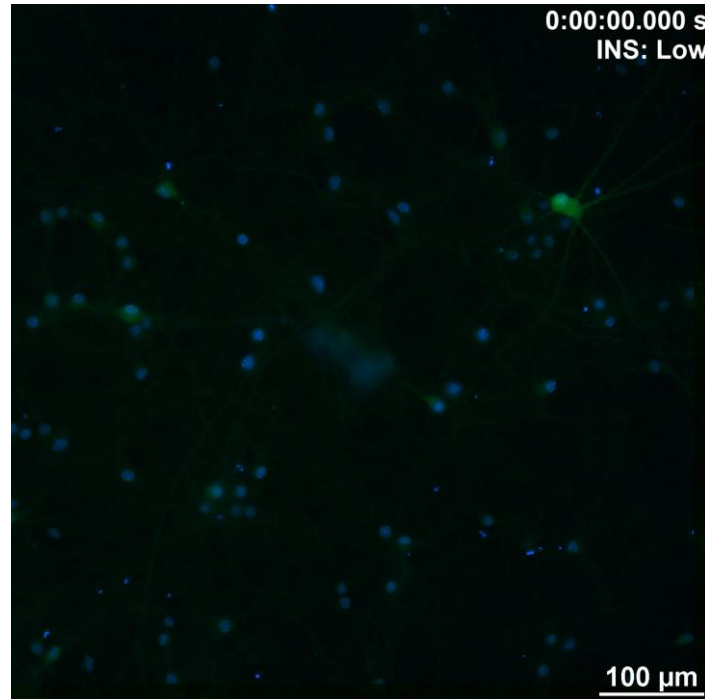
- Develop an integrative modeling result to predict the spatiotemporal interplay between thermodynamic phenomena and subcellular effectors at the dendritic spine
- Overall, this proposal will provide the foundational data for us to progress to neural slice preparations and in-vivo studies
- Generate testable predictions on FTG exposure parameters that selectively modulate synaptic plasticity
- Our future effort resides in unraveling the cohesive interplay between multiscale biophysical modeling, and execution of its experimentally testable predictions



<https://www.nih.gov/news-events/nih-research-matters/controlling-brain-circuits-mice>

# Summary

- One paper published
- One paper under review
- Three papers in preparation
- One student employed
- One doctorate acquired
- Four undergraduate students trained
- Two graduate students trained
- One postdoc trained





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- Dr. Joel Bixler



Ms. Anna Sedelnikova



Dr. Joel Bixler



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Dr. Fidel Santamaria



Ms. Mona Gerges



Mr. Jacob Hardenburger





# Defining How an Infrared-Evoked Fast Thermal Gradient Impacts Synaptic Mechanisms at the Dendritic Spine (FY 24) – AFRL, 711HPW/RHDR

## Objectives:

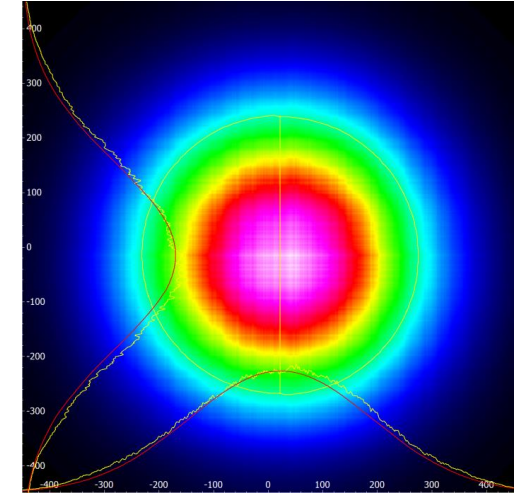
- [1] Understand FTG induced effects on calcium signaling in neurites and action potential related activity
- [2] Expand modeling outputs with biomechanical and biophysical model of the reaction rate dependence on temperature and pressure
- [3] Determine how isothermal compressibility and expansivity (dependence of  $Q_{10}$ ) affect the energy barrier of all voltage activated membrane conductances



Bryan Gamboa

## Technical Approach:

- Determine IR beam profile and observe effects on neural response
- Understand thermal lensing artifact and characterize effect on imaging
- Modeling and dosimetry
- Evaluate isothermal compressibility and expansivity in the governing equations for thermodynamics



## Accomplishments:

- Established the effect of heat and pressure simultaneously on neuronal membrane conductance kinetics and the free energy of biochemical reactions.
- Improved biophysical realistic models of specific neurons to study temperature and pressure effects on membrane conductances.
- Characterized the affect that the gaussian IR beam and thermal lensing artifact had on the imaging profile and the neuronal response
- Determined empirically that calcium signaling in neurites and spines is stochastic and differs from action potential related activity

## DoD Benefit:

- Understanding the effect of temperature and pressure on neuronal activity and optimizing reaction rates, activation energy and biochemical interactions would potentially enhance cognitive function and increase memory retention
- Recognizing techniques for manipulation and modulation of neuronal responses enables biological and cognitive technologies to be optimized to protect and enable the Airman's capabilities
- Experimentally validating the model would allow the US to predict exposure parameters for their specific need as modeling vastly increases results of interest



QUESTIONS?