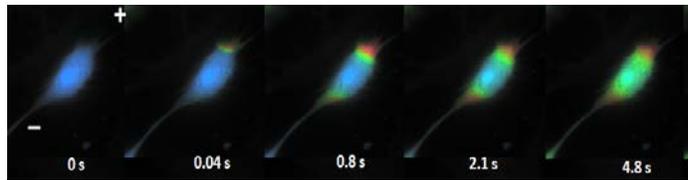


Nanoelectropulse-induced electromechanical signaling and control of biological systems

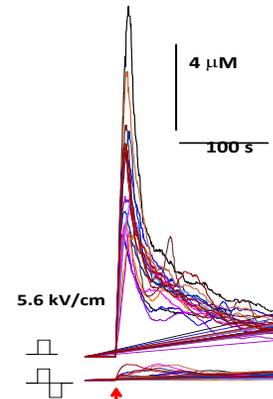
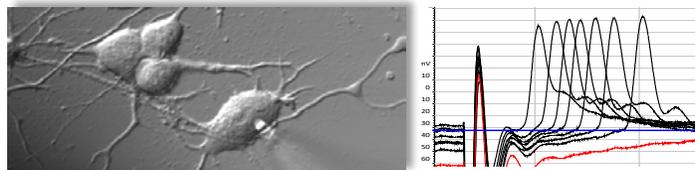


Objectives

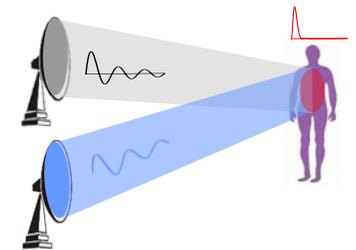
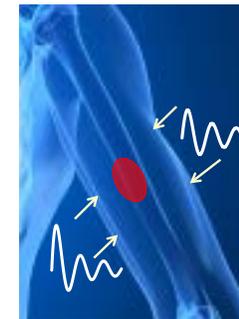
- Advanced understanding of stimulation and cell injury by nanosecond pulsed electric field (nsPEF)
- Comprehensive action spectra for nsPEF and bipolar cancellation at cellular and subcellular levels
- Uncovering mechanisms of bipolar cancellation and its optimization
- Achieving remote stimulation and control of biological systems using a “cancancellation of cancancellation” (CANCAN) paradigm



nsPEF → electropermeabilization
nerve and muscle excitation



bipolar cancellation

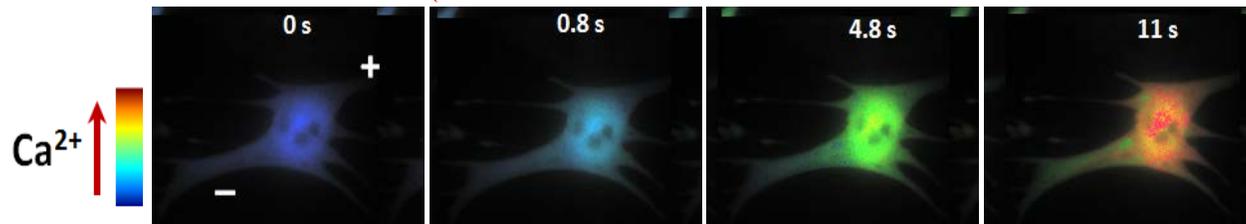
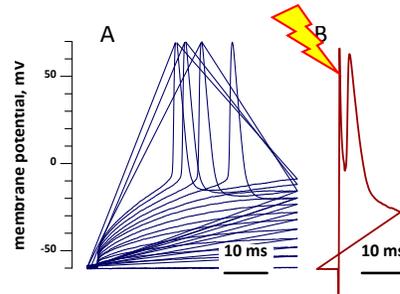
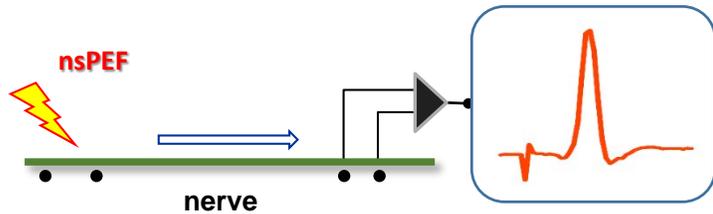
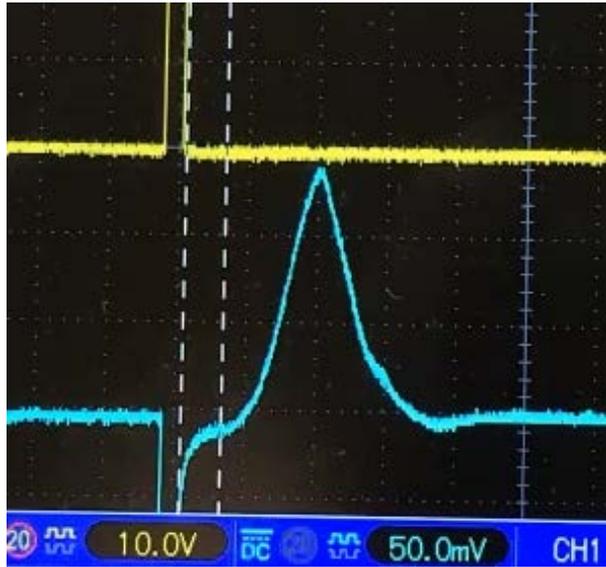
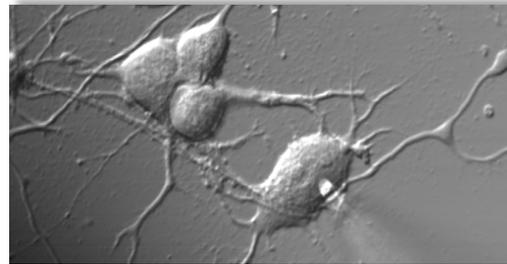


remote stimulation by
CANCAN paradigm

Primary bioeffects of nsPEF

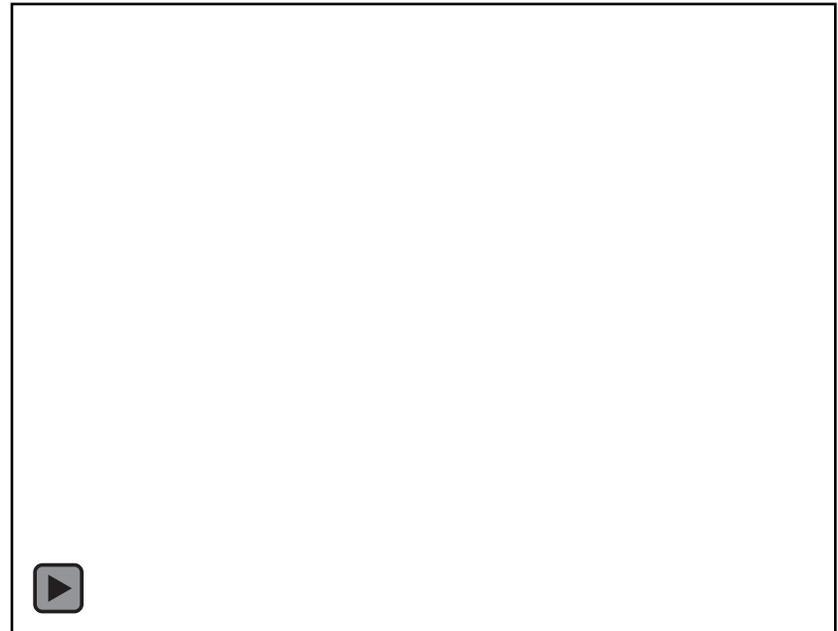
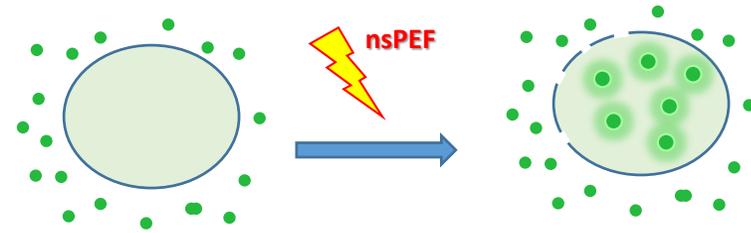
Excitation

- neurostimulation
- muscle stimulation
- activation of endocrine and immune cells

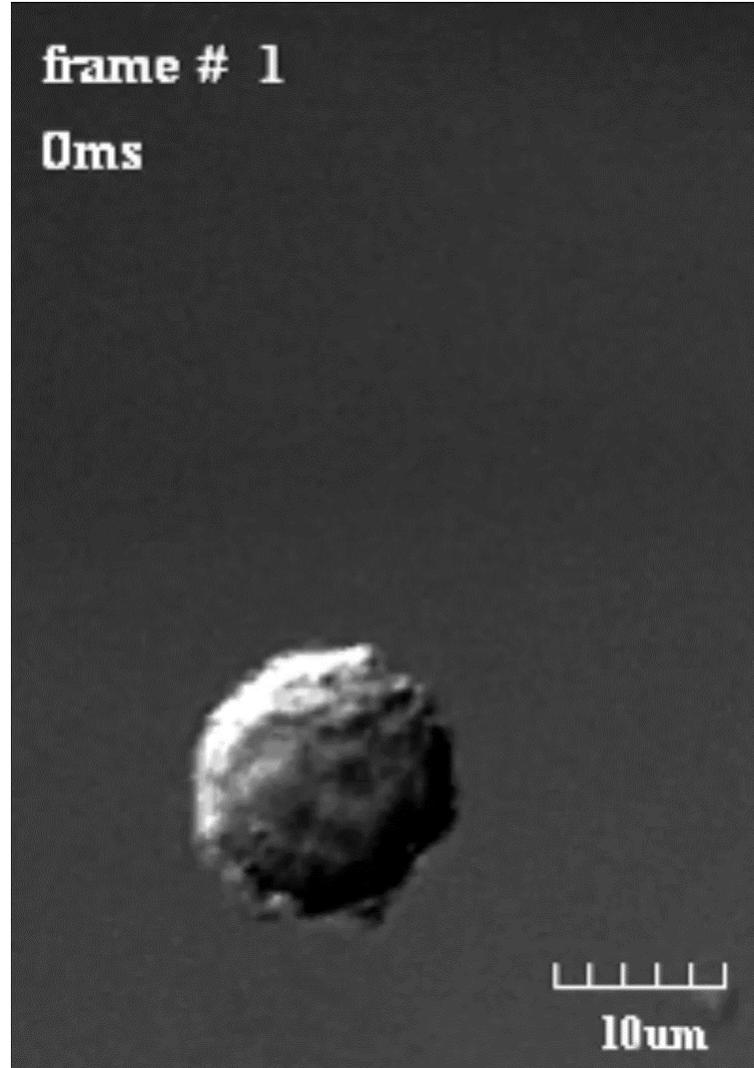
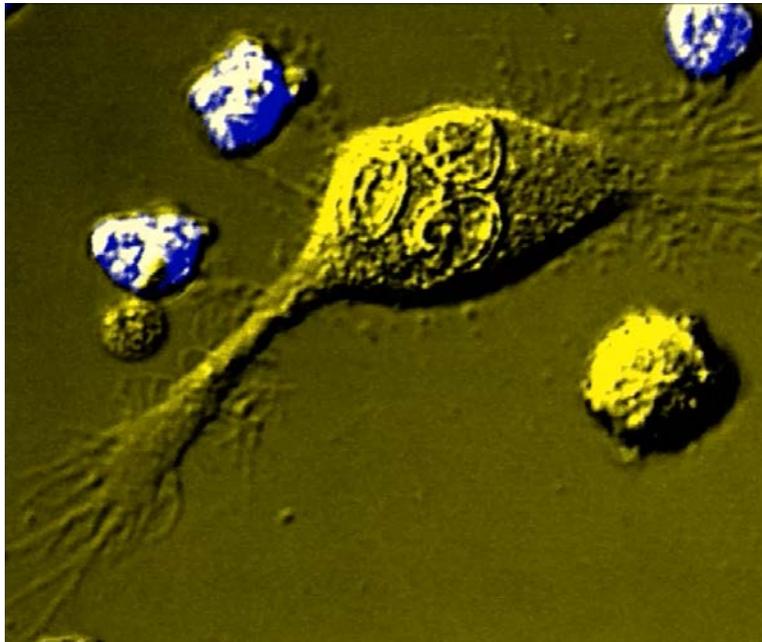


NanoElectroporation

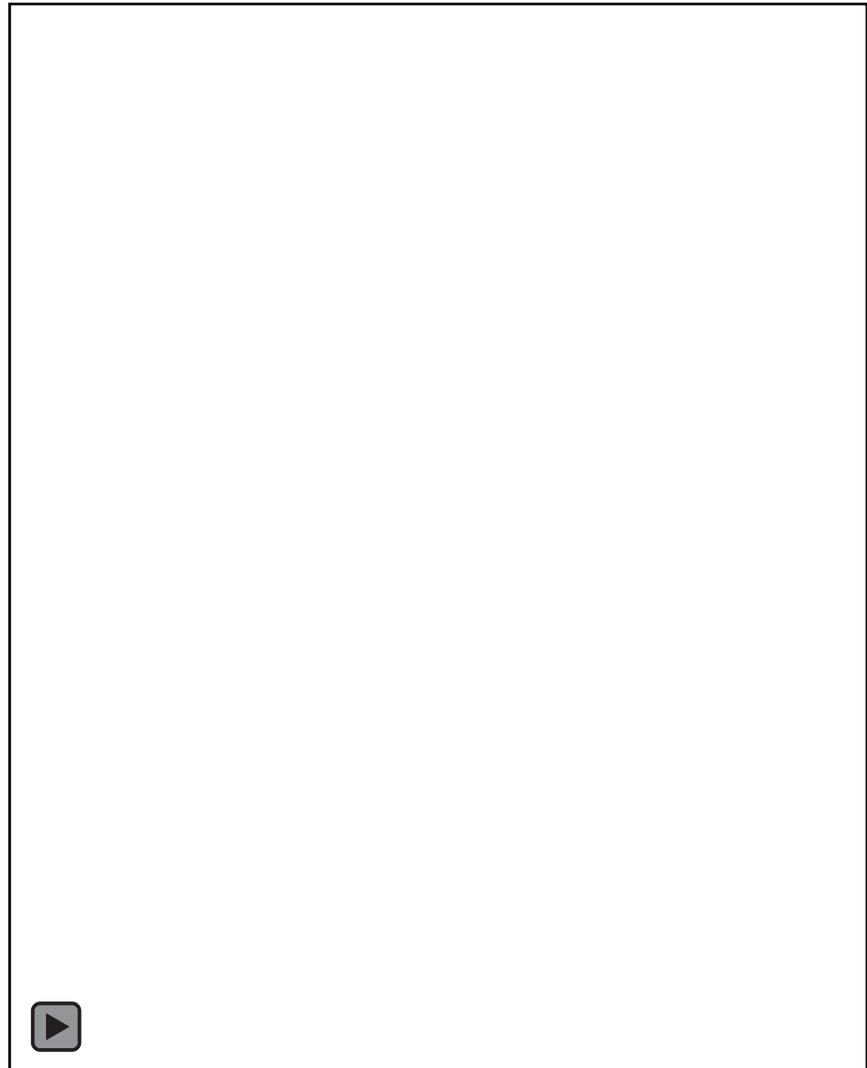
- formation of cell membrane nanopores due to dielectric breakdown



Complex behavioral and physiological responses in response to nsPEF

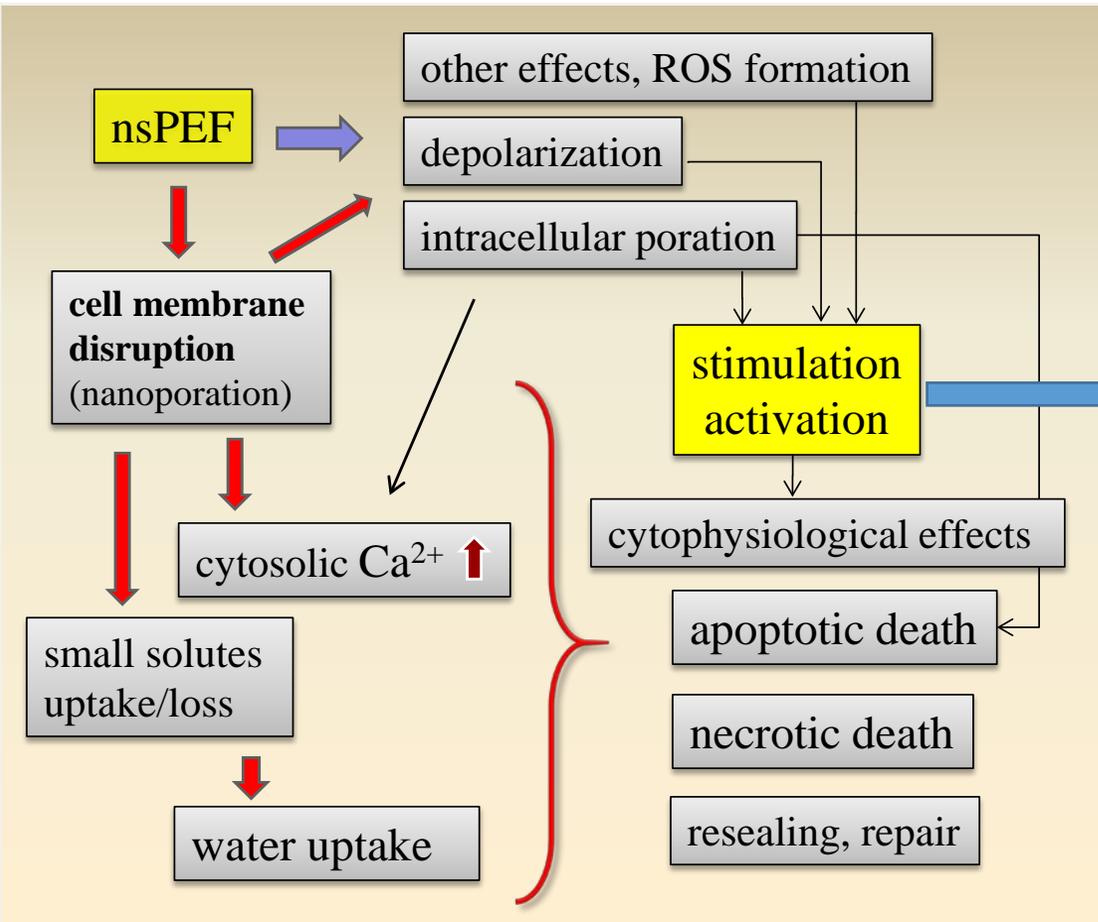


anode



cathode

Downstream bioeffects of nsPEF: Multi-level control of biological systems



Complex behavioral and physiological responses

Cellular level

- Activation of diverse signaling cascades
- Changes in gene expression
- Changes in excitability
- Induced endo- and exocytosis
- Programmed cell death
- Cell motility
- Cell fusion
- ...

Organism level

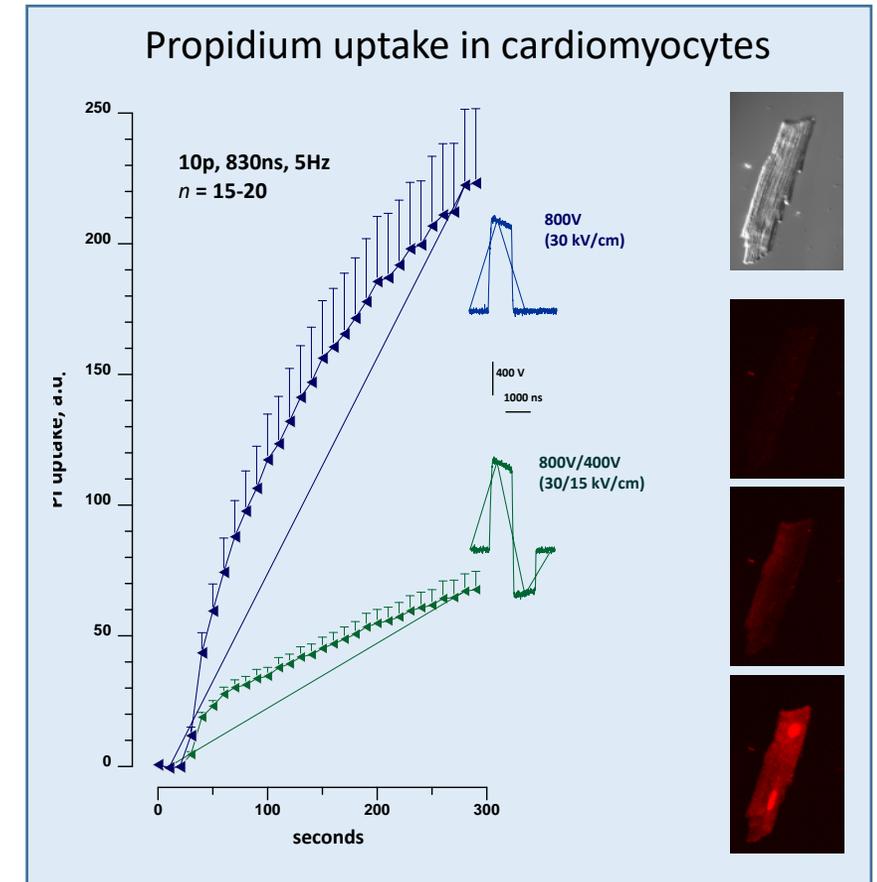
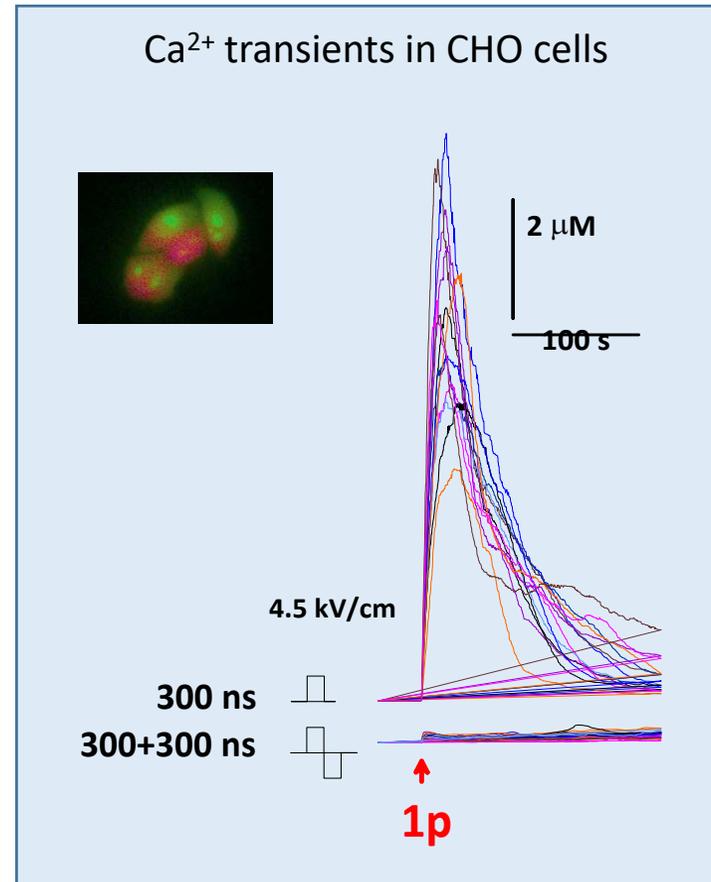
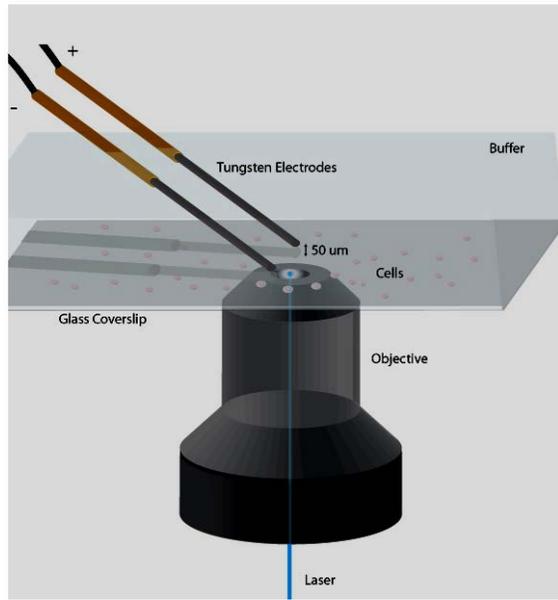
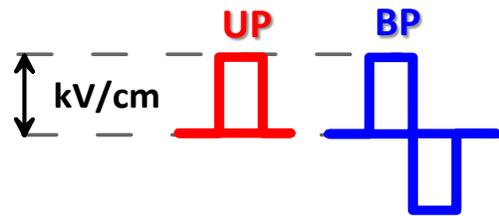
- Brain stimulation
 - enhancing cognition, memory, response time
- Motor and sensory nerve and muscle stimulation
 - pain elimination, man-machine interfaces
- Endocrine stimulation
 - counteracting fatigue
- Immune stimulation
 - infections, autoimmune conditions, cancer...
- ... – probably “Yes”!

Can we do it remotely? – probably “Yes”!

Bipolar Cancellation: A unique feature of nsPEF

A completely unexpected but (almost) universal phenomenon

- Two pulses of the opposite polarity are *less efficient than any one of these pulses*
- Adding an opposite polarity phase to a unipolar nanosecond pulse reduces its bioeffects

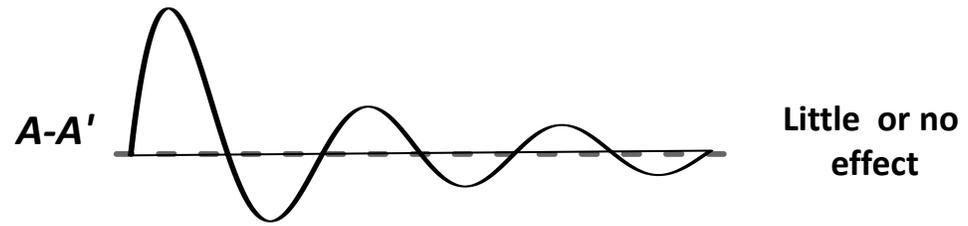
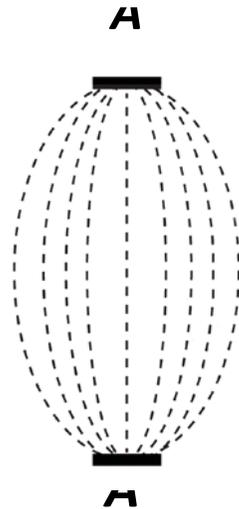


OK, we can suppress nsPEF bioeffects by adding the 2nd phase... now what?

- A novel and unique biophysical phenomenon, new insight ultra-short pulse interaction with living matter

- *Can it enable targeted nsPEF stimulation at a distance?* - Maybe it can. By means of **CANCAN!**

In bipolar nsEP, the 2nd phase cancels the stimulatory effect of the preceding phase

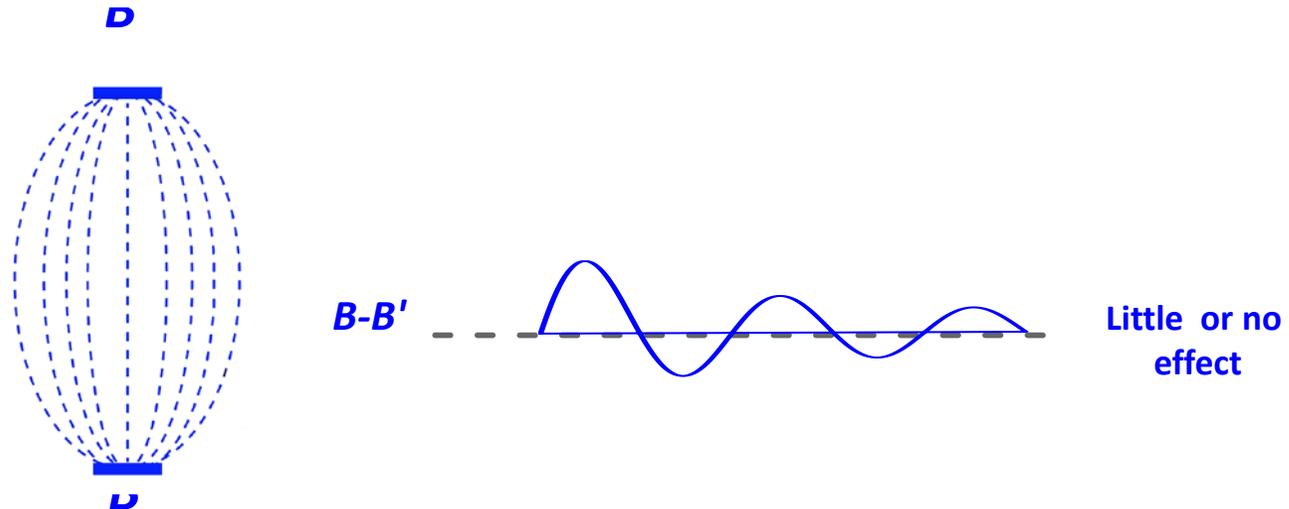


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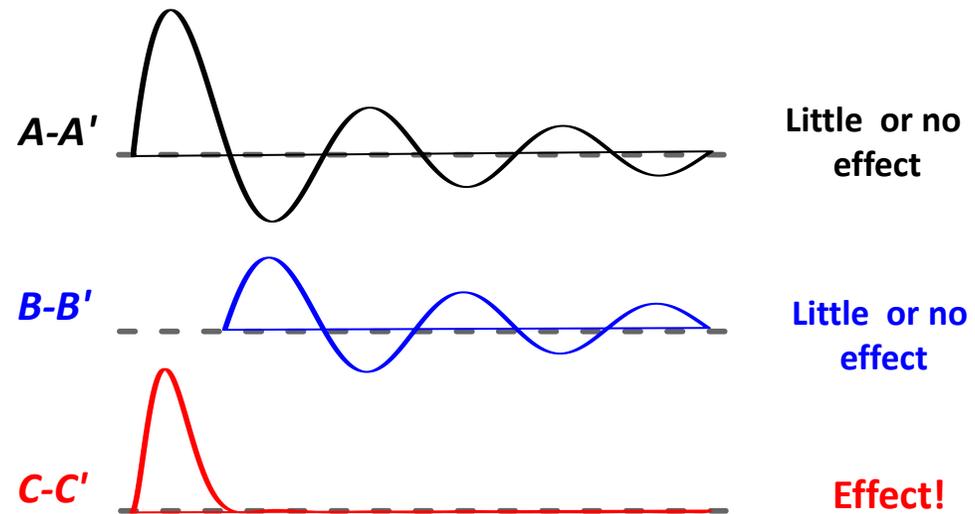
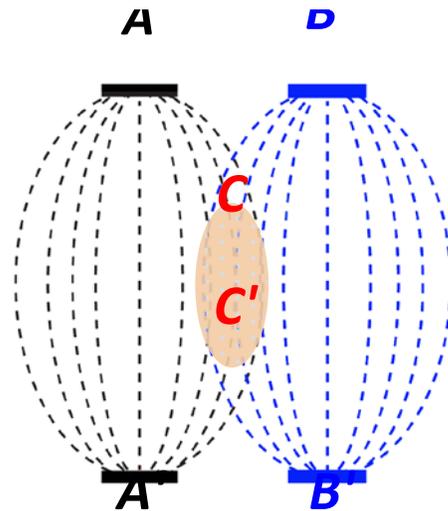
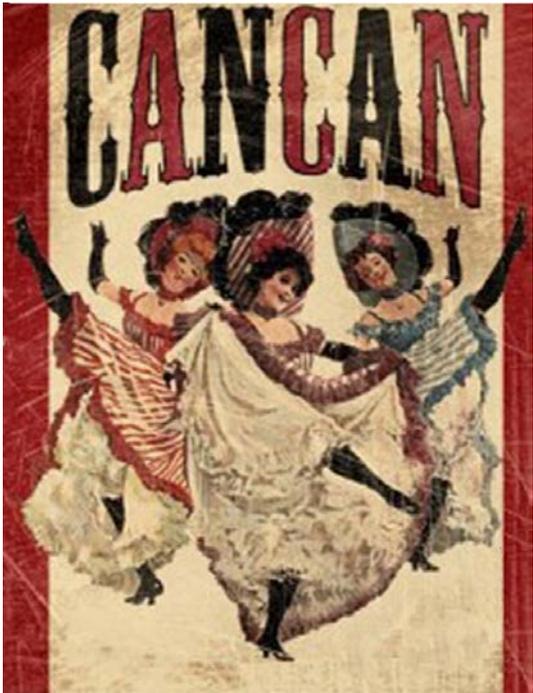


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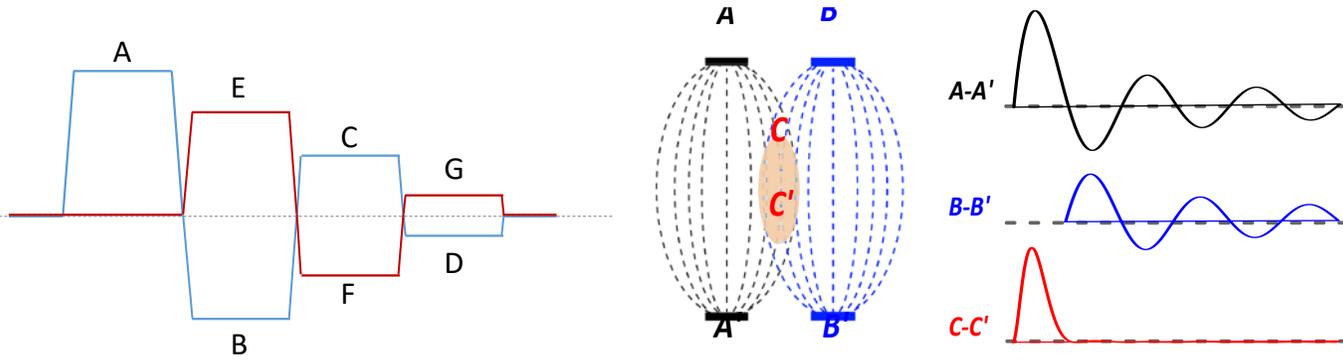
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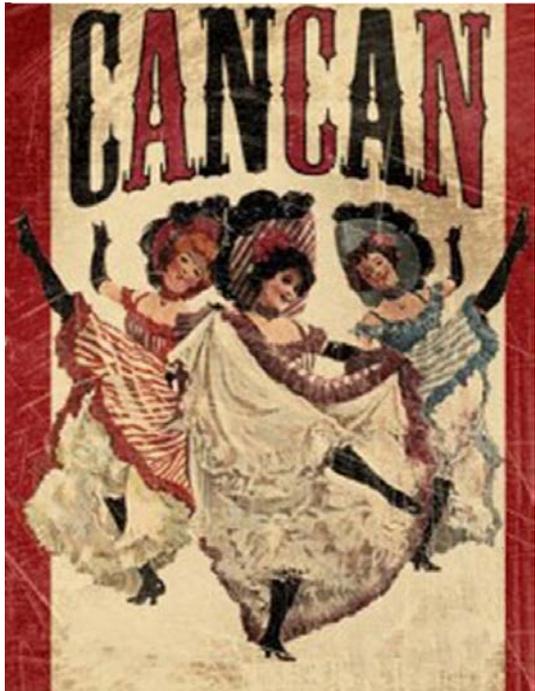
CANCAN: canceling of cancellation by the superposition of two bipolar stimuli back into a unipolar one



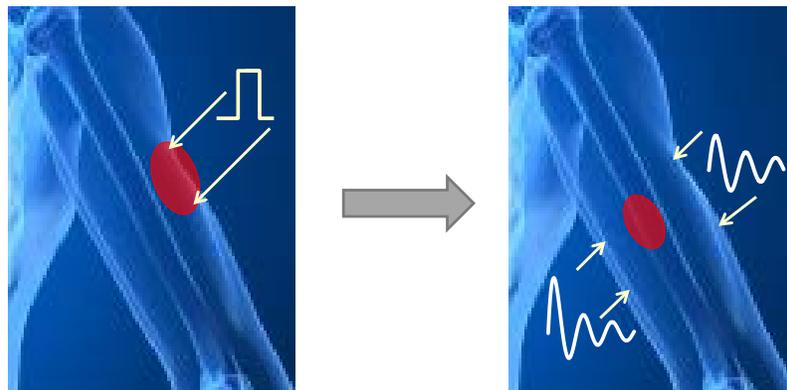
CANCAN-ES: electrostimulation by cancellation of bipolar cancellation



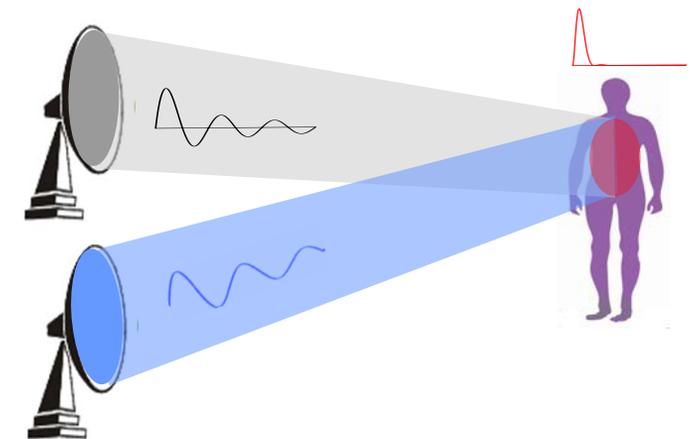
Superposition of two biologically-**ineffective bipolar** signals produces, **at a distance**, a biologically-**effective unipolar** signal



Targeted deep stimulation or electroporation from surface electrodes
- relies on the change in pulse shape, not the amplitude increase



Remote stimulation utilizing pulsed RF transmitters (*CANCAN-RF*)



CANCAN-ES: electrostimulation by cancellation of bipolar cancellation

- *A multifaceted challenge in uncharted territory*

- *Pushing the state of the art of pulsed power engineering, biophysical modeling and detection*

Unknown:

- biophysics of nsPEF stimulation
- mechanisms of bipolar cancellation
- action spectra for bipolar cancellation

Nonexistent:

- high-power bipolar nsPEF generators
- CANCAN generators
- CANCAN dosimetry or simulations

Undeveloped:

- non-disruptive analytical tools

CANCAN-ES: electrostimulation by cancellation of bipolar cancellation

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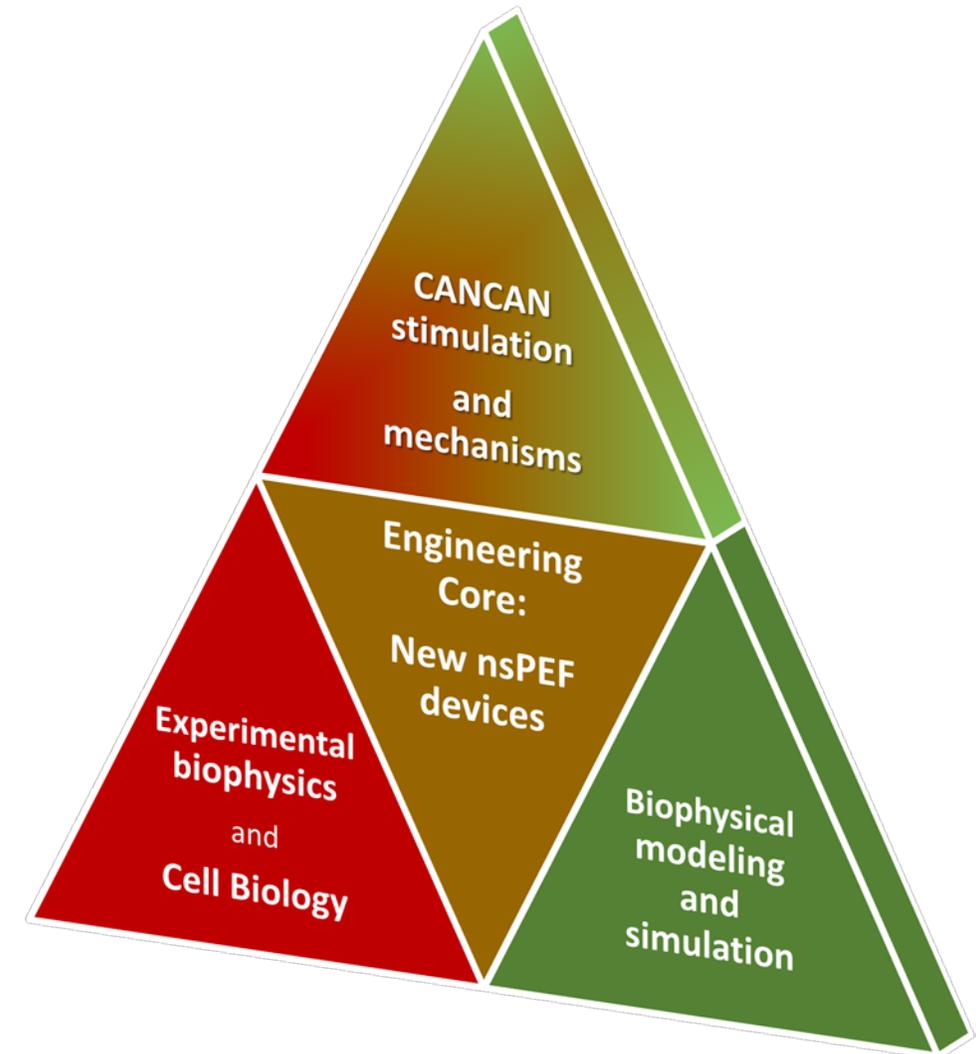
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MURI Approach:

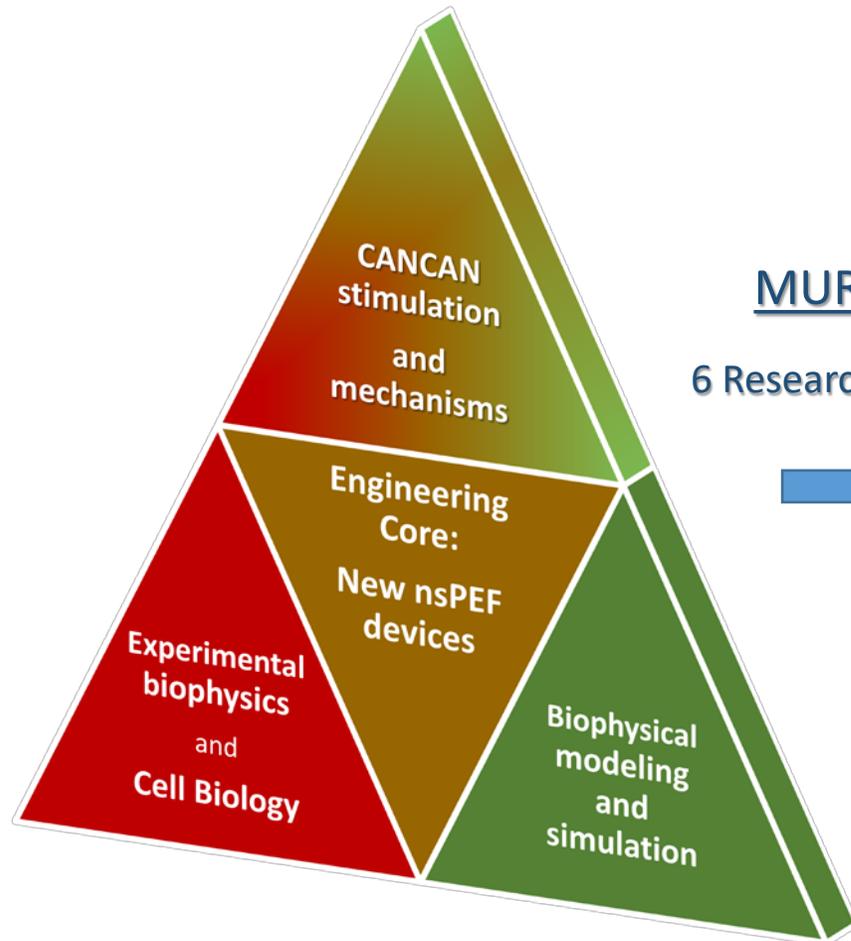
A highly interdisciplinary project which combines advanced engineering and physical modeling with electrophysiology and cell biology



CANCAN-ES: electrostimulation by cancellation of bipolar cancellation

- A multifaceted challenge in an uncharted territory

- pushing the state of the art of pulsed power engineering, instrumentation, and biophysical detection

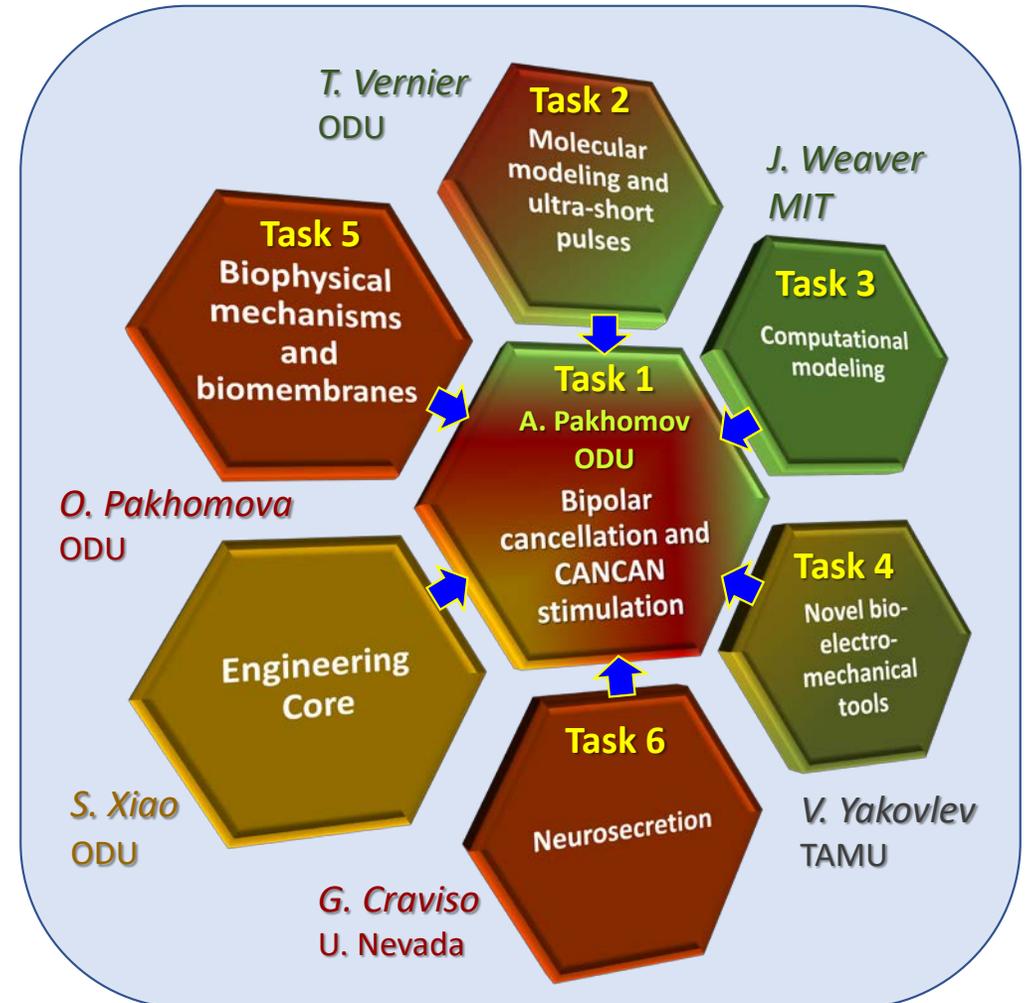


MURI Team Structure:

6 Research Tasks + Engineering Core



Experiments
Modeling
Engineering



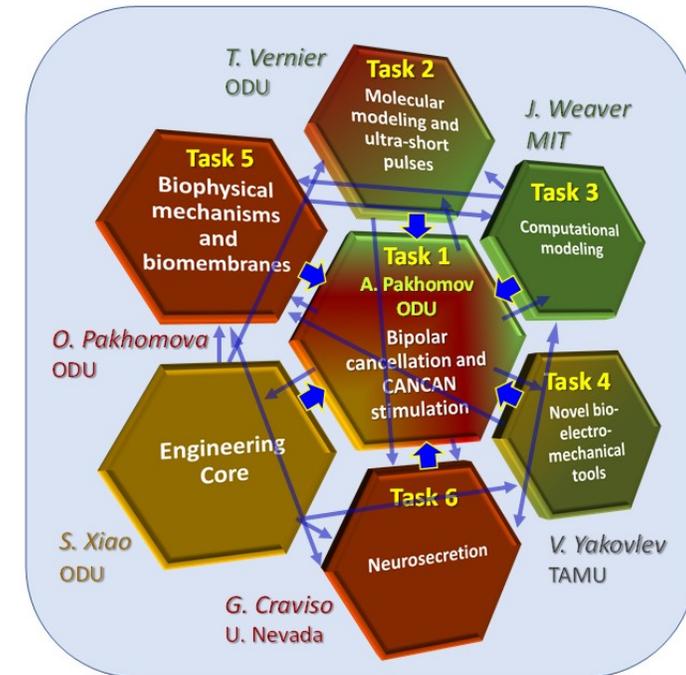
CANCAN-ES: electrostimulation by cancellation of bipolar cancellation

- *A multifaceted challenge in an uncharted territory*

- *pushing the state of the art of pulsed power engineering, instrumentation, and biophysical detection*

Team Work

- Bi-monthly Skype teleconferences of all teams +AFRL
 - reports of each group, discussions, plans for the future and coordinated research
- Weekly biophysics research seminars / journal club meetings
- Site visits between the teams (including seminars and data discussions)
- MURI Workshop at the 2nd World Congress: Lots of team work put together
- MURI Synergy Development Workshop, Jan. 15 2018, Norfolk, VA
- Students and postdocs are trained in different labs, being helped by a different PI
- Equipment sharing and exchange between the teams
- Engineering Core manufactures new devices for ALL teams doing experimental work



Results would not be possible without MULTIDISCIPLINARY TEAM WORK

None of the team leaders have expertise needed to perform dosimetry, make and use pulse generators, set-up biology experiments, analyze the data, put them in logical models, and come up with new concepts, devices and applications...

Together we learned a lot about fundamentals of nsPEF interaction with biological systems, underlying mechanisms, came up with new equipment and new directions of research.

Together we made a big impact in the field and now it is well recognized by the scientific community

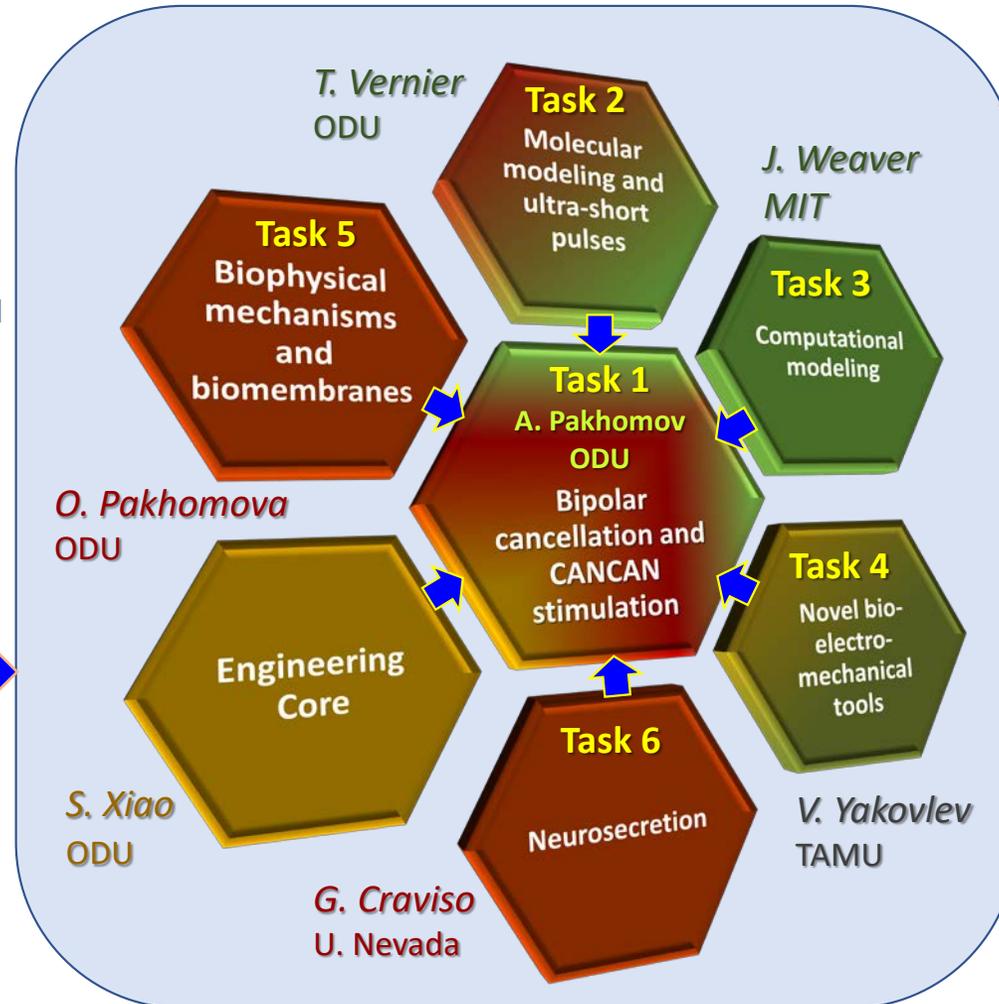
CANCAN-ES: electrostimulation by cancellation of bipolar cancellation

- A multifaceted challenge in an uncharted territory



Pulse Biosciences Inc.,
Hayward, CA

University of Ljubljana, Ljubljana, **Slovenia**
Universitat Pompeu Fabra, Barcelona, **Spain**
Institute for Electromagnetic Sensing of the Environment (IREA), Naples, **Italy**
La Sapienza University of Rome, Rome, **Italy**
Institute of Pulsed Power Science, Kumamoto University, **Japan**
XLIM Research Institute, Limoges, **France**
National Italian Agency for Energy, New Technologies and Sustainable Economic Development (ENEA), Rome, **Italy**
Vytautas Magnus University, Kaunas, **Lithuania**
Vilnius Gediminas Technical University, **Lithuania**



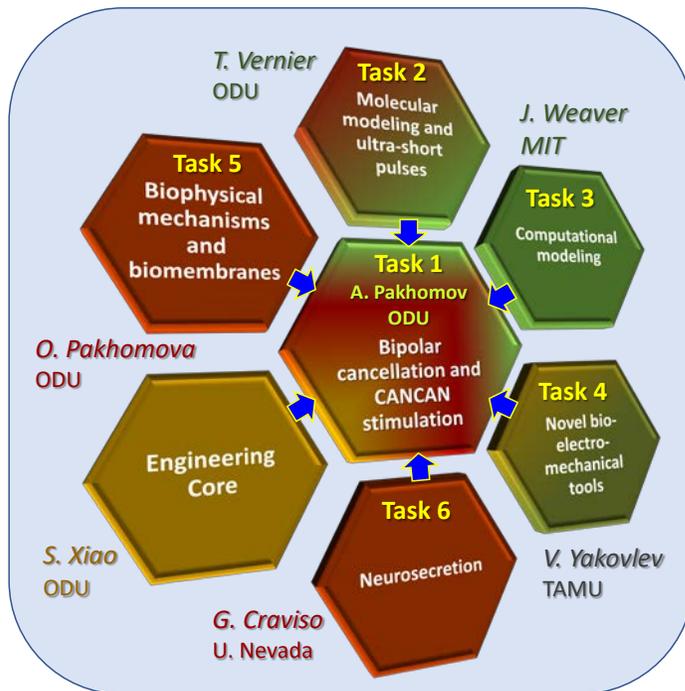
 AFOSR PM
S. Bin-Salamon

AFRL
B. Ibey
H. T. Beier


CANCAN-ES: electrostimulation by cancellation of bipolar cancellation

- A multifaceted challenge in an uncharted territory

- pushing the state of the art of pulsed power engineering, instrumentation, and biophysical detection



0850-0905	Multi-Disciplinary University Research Initiative: Nanoelectropulse-Induced Electromechanical Signaling and Control of Biological Systems	Prof. Andrei Pakhomov Old Dominion University	
0905-0920	Universality of Bipolar Cancellation for Nanoporation and Nanoelectropulse Stimulation	Prof. Andrei Pakhomov Old Dominion University	Task 1
0920-0940	Instrumentation for Studying Cancellation Effects Caused by Nanosecond Pulses	Prof. Shu Xiao Old Dominion University	Eng. Core
0940-1000	Membrane Biophysics of Biphasic Electrostimulated Molecular Transport	Prof. Thomas Vernier Old Dominion University	Task 2
1000-1020	A New Biophysical Model Can Explain Bipolar Cancellation of Molecule Transport	Prof. James Weaver Massachusetts Institute of Technology	Task 3
1020-1040	Nascent Biophysical Tools to Elucidate Nanoelectropulse-Induced Electromechanical Interactions	Prof. Vladislav Yakovlev Texas A&M University	Task 4
1040-1055	BREAK		
1100-1120	Nanoelectropulse and Excitable Membranes: Uncovering Mechanisms of Activation of Voltage-Gated Ca ²⁺ Channels	Prof. Olga Pakhomova Old Dominion University	Task 5
1120-1140	Toward the Application of CAN-CAN Technology – Attenuation of Ca ²⁺ Signaling by Bipolar nsPEFs in a Neurosecretory Cell Type Involved in the “Flight or Fight” Response	Prof. Gale Craviso University of Reno Nevada	Task 6
1140-1200	Summary of the Project Status: Principal Accomplishments, Scientific Impact, and Future Developments	Prof. Andrei Pakhomov Old Dominion University	