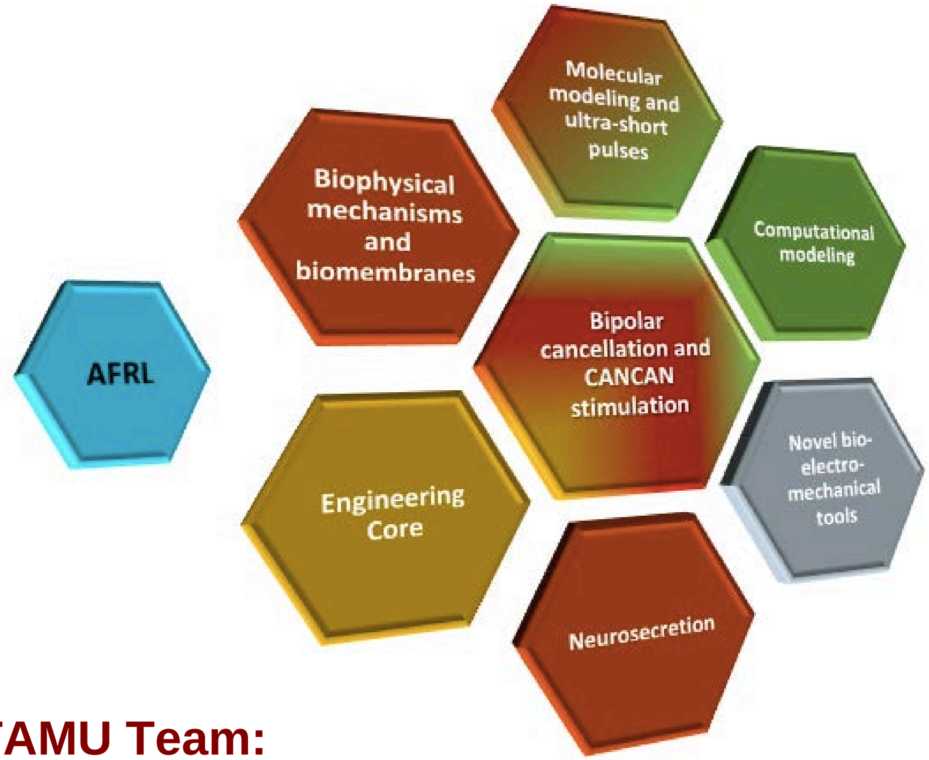
Task 4: Novel bio-electro-mechanical tools

Nascent biophysical tools to elucidate nanoelectropulse-induced electromechanical interactions

Vladislav Yakovlev

TEXAS A&M UNIVERSITY

Acknowledgement



TAMU Team:
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MURI Team
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Dr. Tom Vernier
Dr. Xiao Shu
Dr. Jim Weaver
Dr. Gale Craviso
Dr. Normand Leblanc

Dr. Warwick Bowen

The University of Queensland
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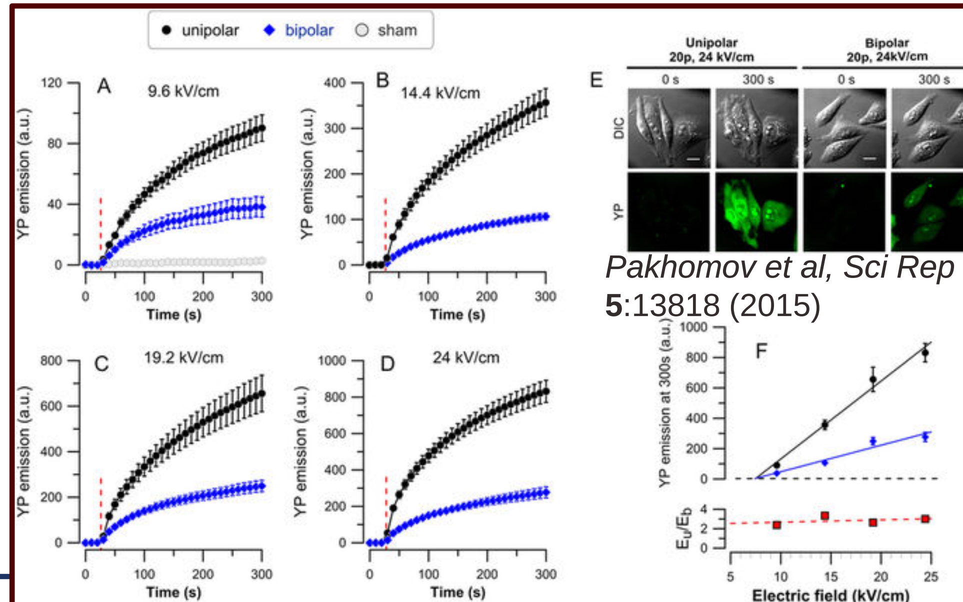
Motivation

Electric field measurements across membrane

Local chemistry assessment

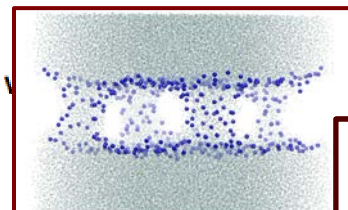
Local mechanical properties assessment



Pakhomov et al, Sci Rep **5**:13818 (2015)

Mechanism?

- **assisted membrane discharge** (?) - need more v … ments and models!
- calcium in and out
- low frequency content
- **a 2-step process:**
  - *- oxidation (or some chemistry), then long-lasting permeabilization* - need experiments
  - *- "foot-in-the-door" occlusion of transient pores* - need experiments (GUV?)

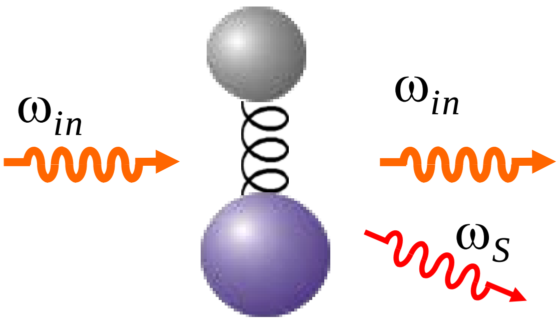


RS Son, KC Smith, TR Gowrishankar, PT Vernier, and JC Weaver. *J. Membr. Biol.* 247:1209-1228 (2014).

Motivation

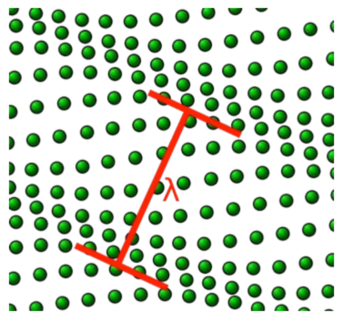
Develop **advanced** optical tools for **non-invasive** assessment of **electrical potentials** and mechanical properties (**elasticity and viscosity**).

Raman spectroscopy



Molecular vibrations carry information about **structure and chemical** composition

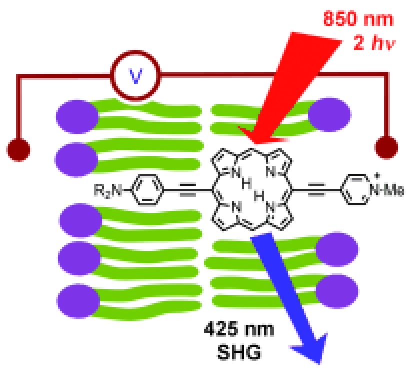
Brillouin spectroscopy



$$\Delta\nu \propto \sqrt{K}$$

Acoustic oscillations carry information about **longitudinal modulus and viscosity**

Other spectroscopies



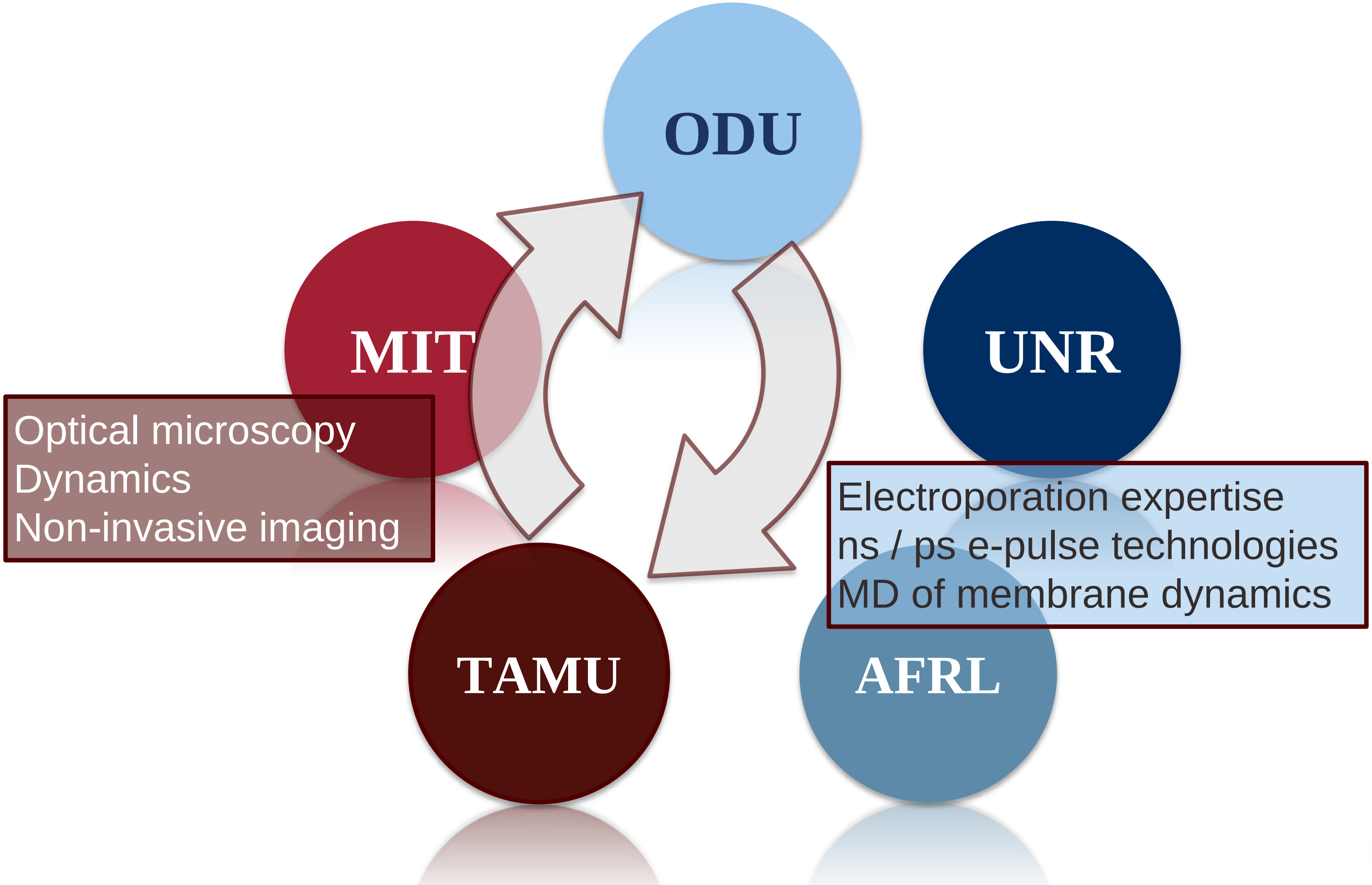
True electro-optic effect(s)

$$\vec{E}(2\omega) = \chi^{(3)}(2\omega;\omega,\omega,0)\vec{E}(\omega)\vec{E}(\omega)\vec{E}(0)$$

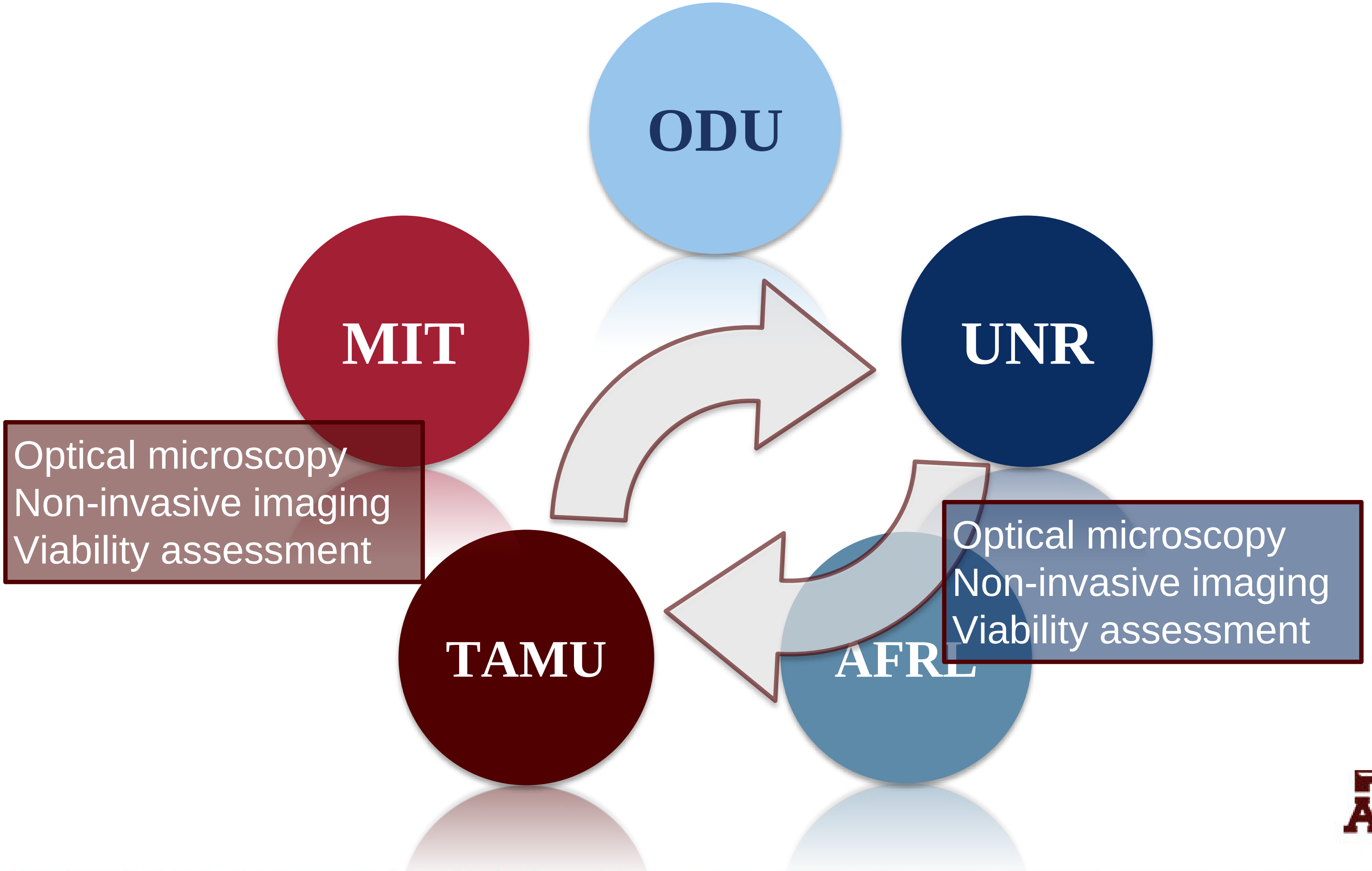
Long-tem goals

- Gain fundamental understanding of structure / property relationship on a nano/micro-scale and dynamical processes involved in nsPEF interaction with lipid membranes
- Develop new biophysical tools and instruments, which push sensitivity, molecular and structural specificity and spatial/temporal resolution
- Educate and train students and research personnel in a broad area of biophysics and bioengineering for DoD mission

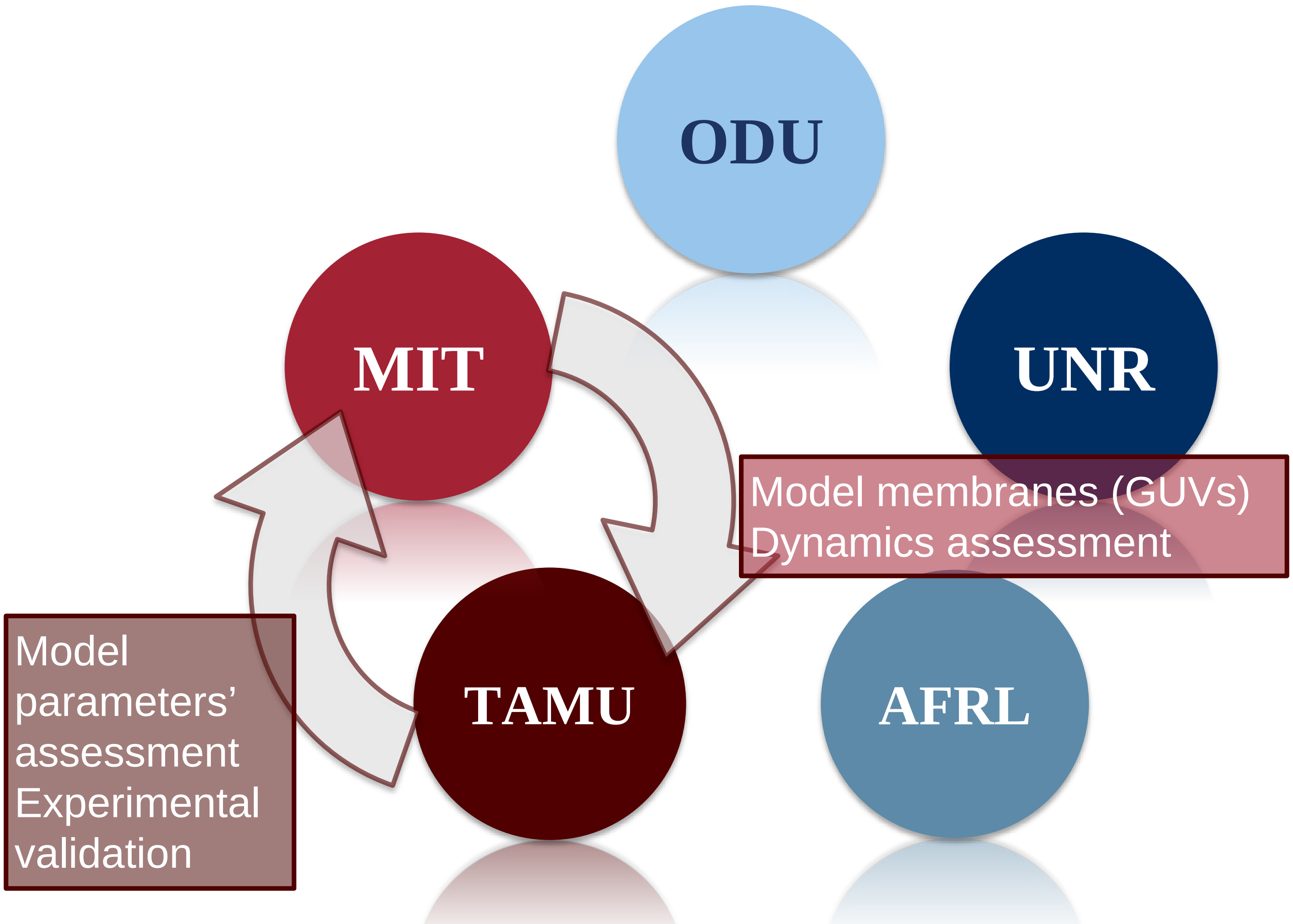
Relationship to other tasks



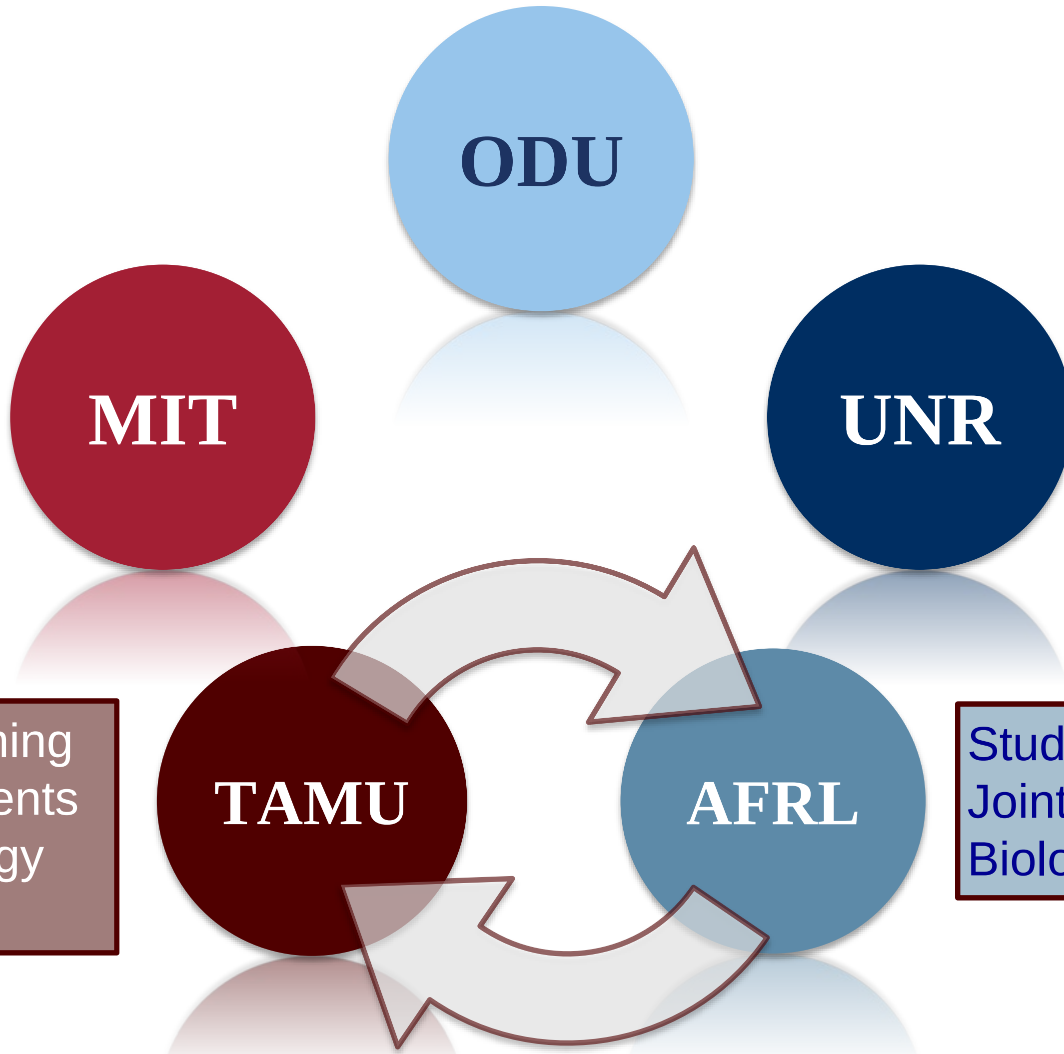
Relationship to other tasks



Relationship to other tasks



Relationship to other tasks

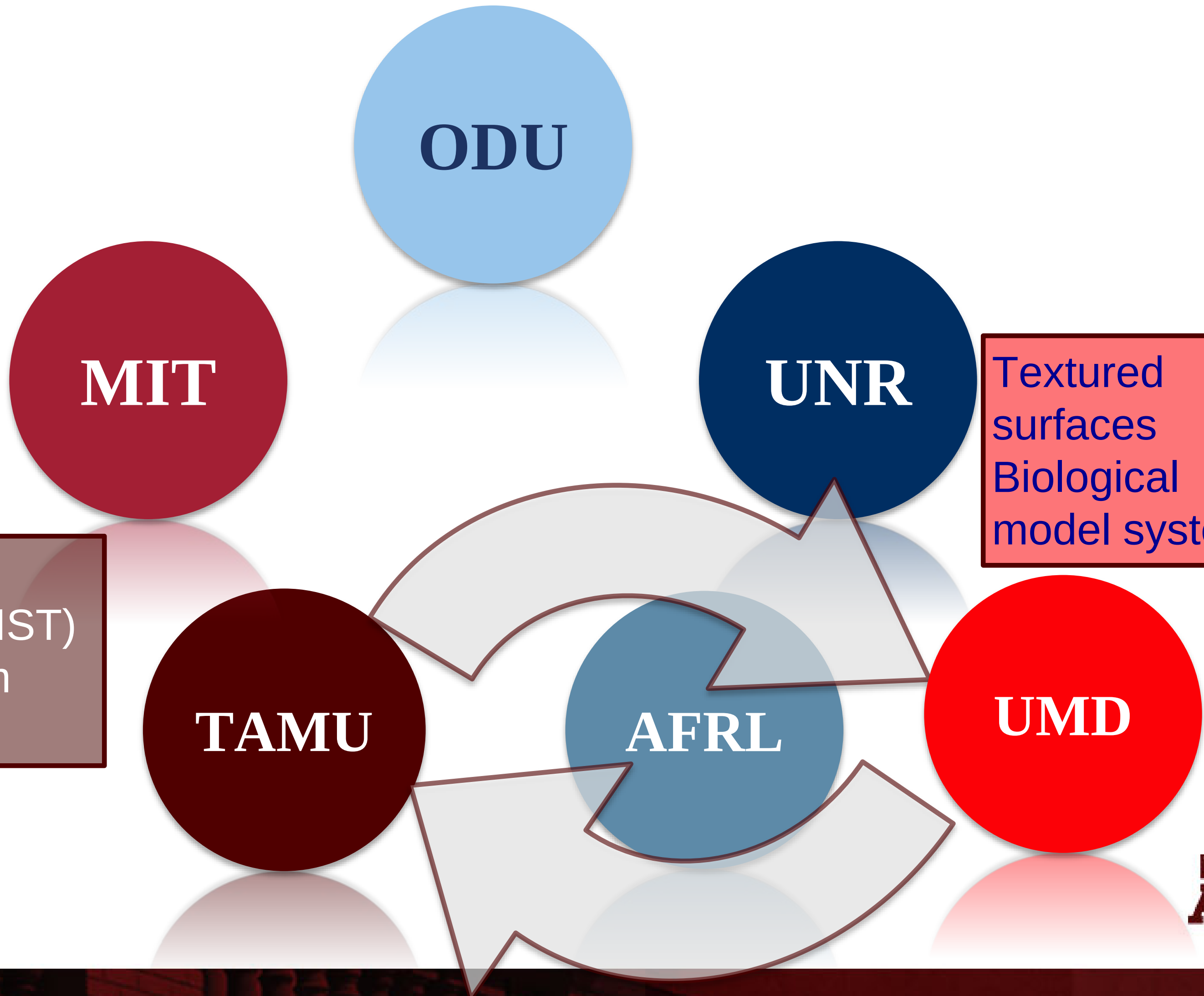


Students’ training
Joint experiments
New technology developments

Students’ support
Joint experiments
Biology expertise

Relationship to other MURI

Postdoctoral researcher (NIST)
Laser / system development



Textured surfaces
Biological model systems

Impact

Major scientific impact:

(1) Developed instrument which allows microscopic mechanical assessment of living cells and tissues
(2) Developed instrument which allows non-invasive measurements of electric potential across the membrane
(3) Developed instrument which allows 1000x more accurate instrument to assess local chemical / structural composition

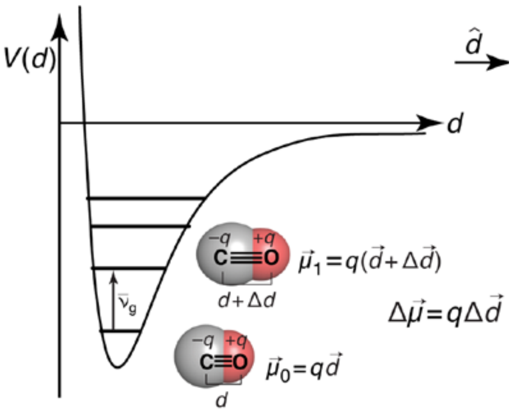
25 journal publications (+2 under review)
65 conference proceedings
34 conference presentations. including 1 Keynote, 1 Plenary, and 6 Invited

3 PhD students have graduated and are working for DoD
7 graduate students were involved in different aspects of the work
10 undergraduate students were involved
8 students worked as interns at AFRL

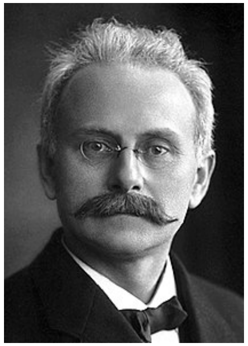
Advancements and Challenges

Successes and advancements	Challenges and setbacks
State of the art instrument for broadband coherent Raman imaging: unprecedented sensitivity to structure / chemical changes	Imaging electrical fields potentials was found to be more challenging than it was thought at the time of proposal submission
State of the art instrument Brillouin imaging: unprecedented sensitivity to mechanical properties	Unexpected laser malfunction (now fixed) forced to explore some alternative strategies and techniques
Achieved the desired sensitivity of electric field imaging	Initial hypothesis of signal significant enhancement via overtone resonant excitation only partially worked out: 20x signal enhancement, but the line-shapes were significantly distorted
Strong collaborative effort with the Air Force Research Laboratory	

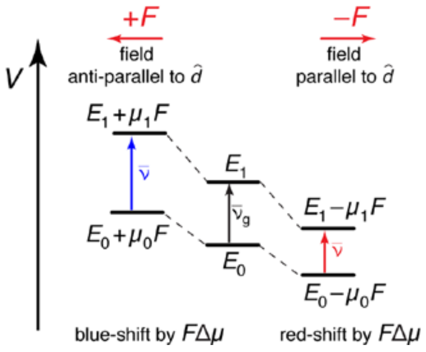
Electrical potential measurements via vibrational Stark effect

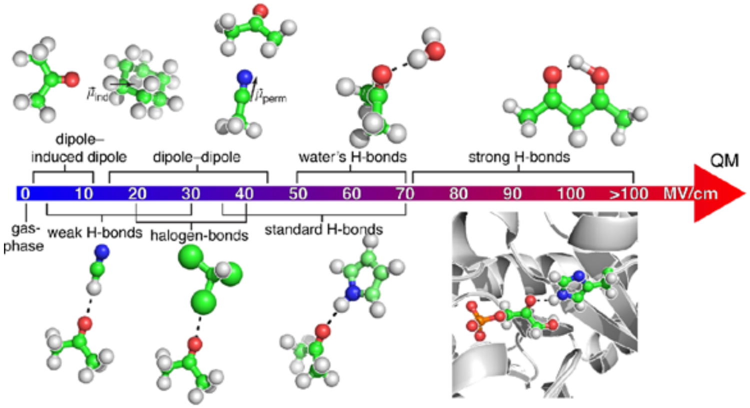


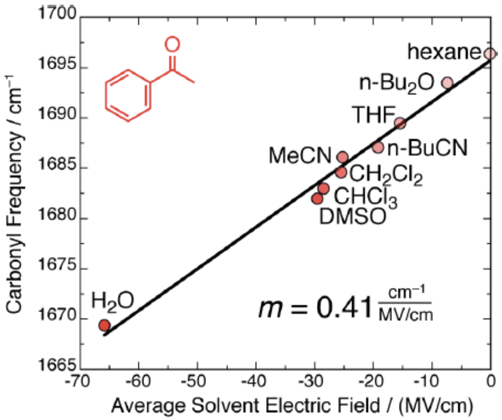
Johanes Stark (Nobel Prize 1913)
Shifting and splitting emission lines in the presence of external electrical field.
Considered one of the first verifications of quantum mechanics.



Vibrational Stark effect (quantum effect):
1 cm^{-1} shift per **MV/cm** electrical field



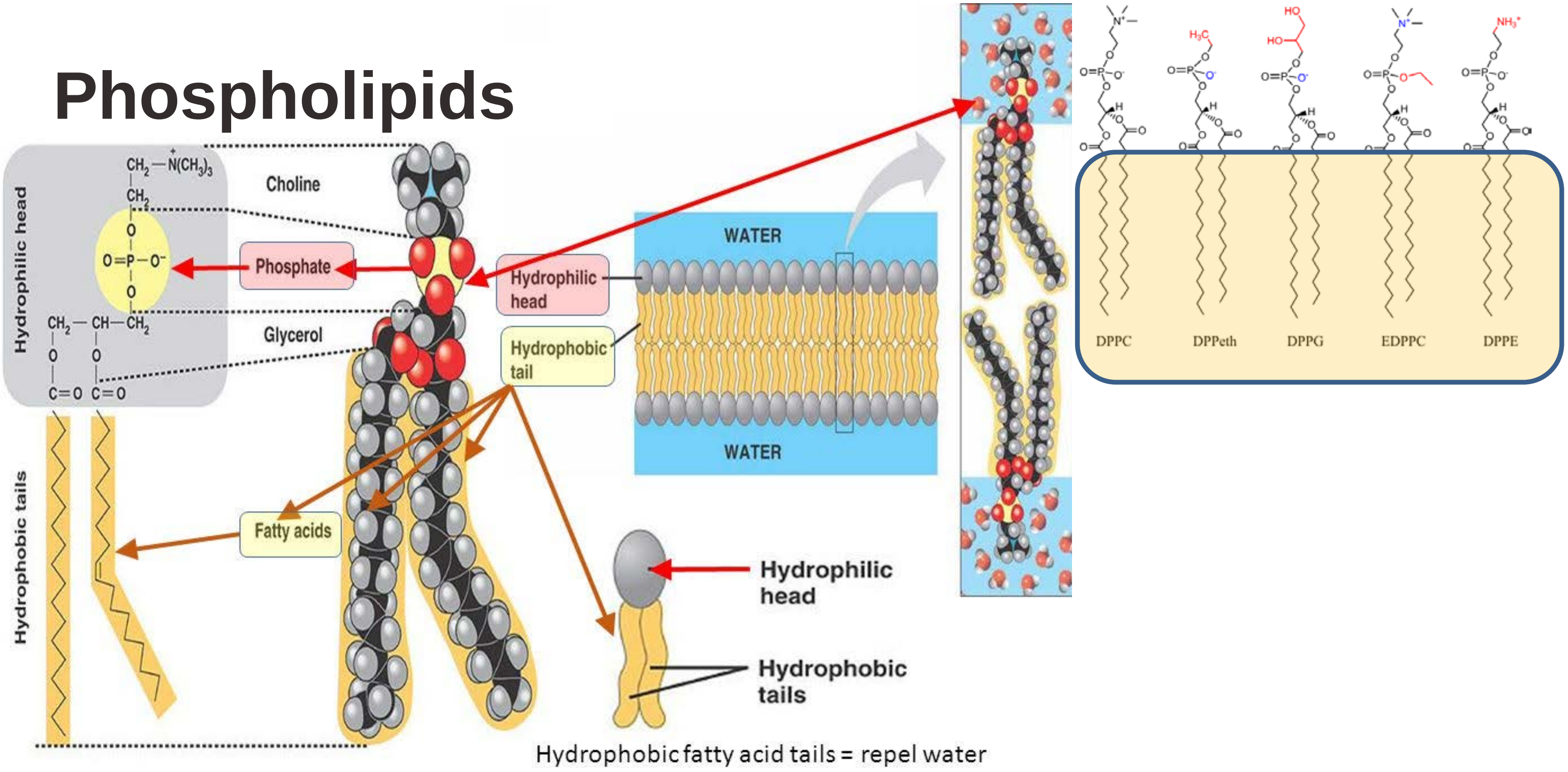




S. D. Fried, S. G. Boxer, "Measuring electric fields and noncovalent interactions using the vibrational stark effect," Acc. Chem. Res. 48(4): 998-1006 (2015).

Structure of biological membrane

Phospholipids



Model system: giant unilamellar vesicle (GUV)

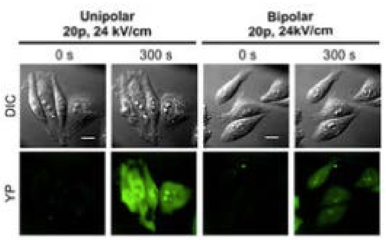
Biophysical Journal Volume 88 February 2005 1143–1155 1143

Electro-Deformation and Poration of Giant Vesicles Viewed with High Temporal Resolution

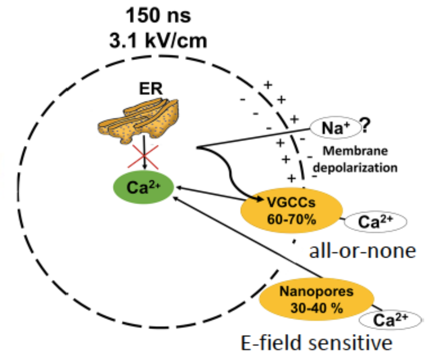
Karin A. Riske and Rumiana Dimova
Max Planck Institute of Colloids and Interfaces, 14476 Golm, Germany

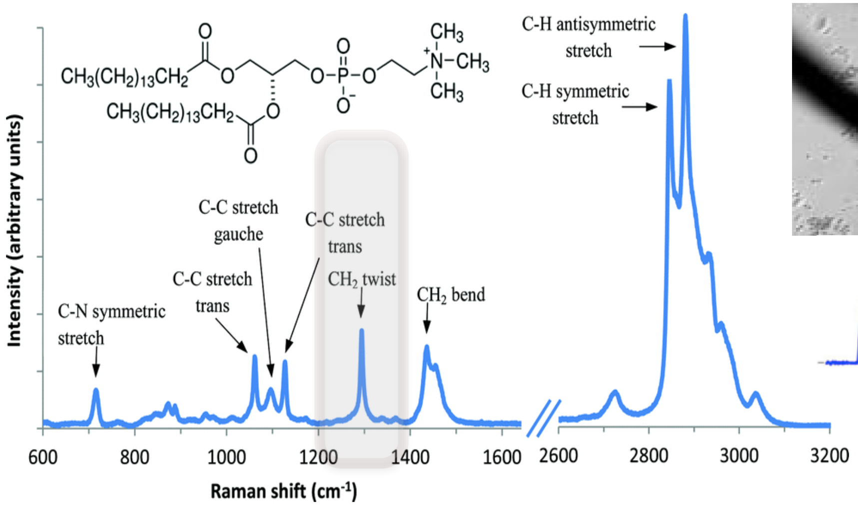
GUV provides a common motive for cellular membrane; it is simpler to model and is easier for interpretation, there are less unknown parameters; controllable shape, size and environment. It is also provides stable model for instrumentation validation.

Pakhomov et al

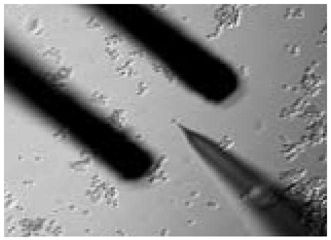


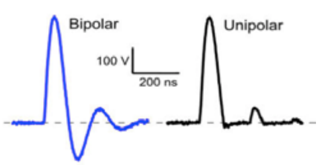
Craviso et al



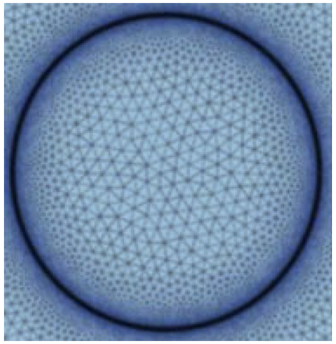


Technical core

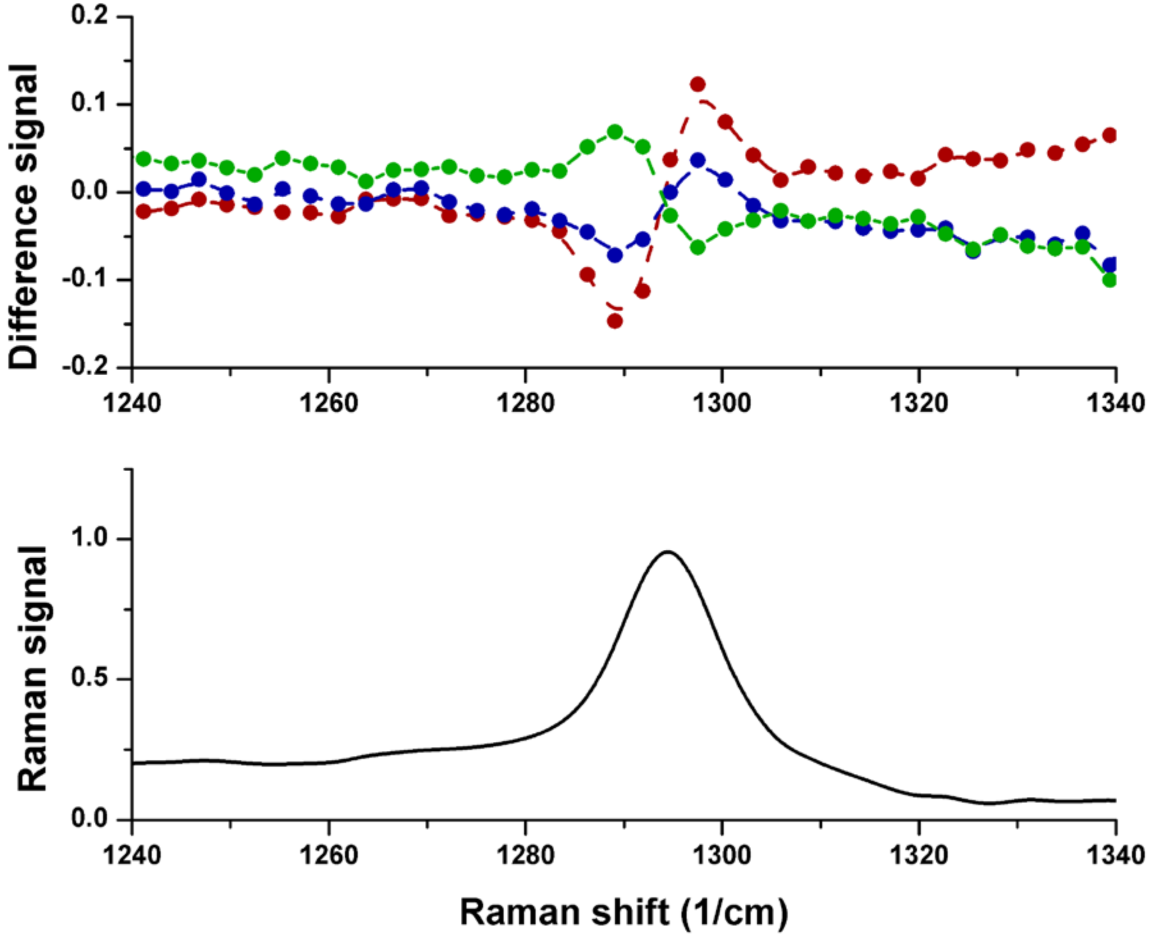


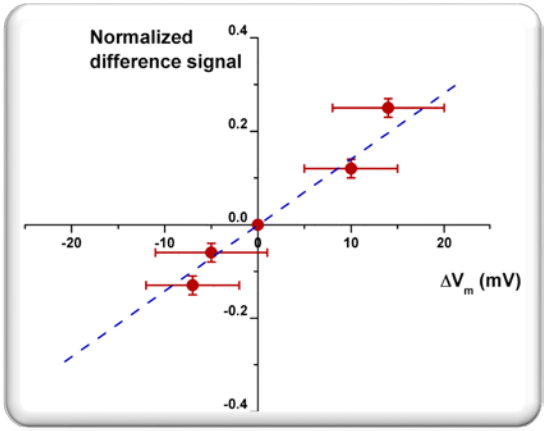


Theory group



Raman spectroscopy of GUV's

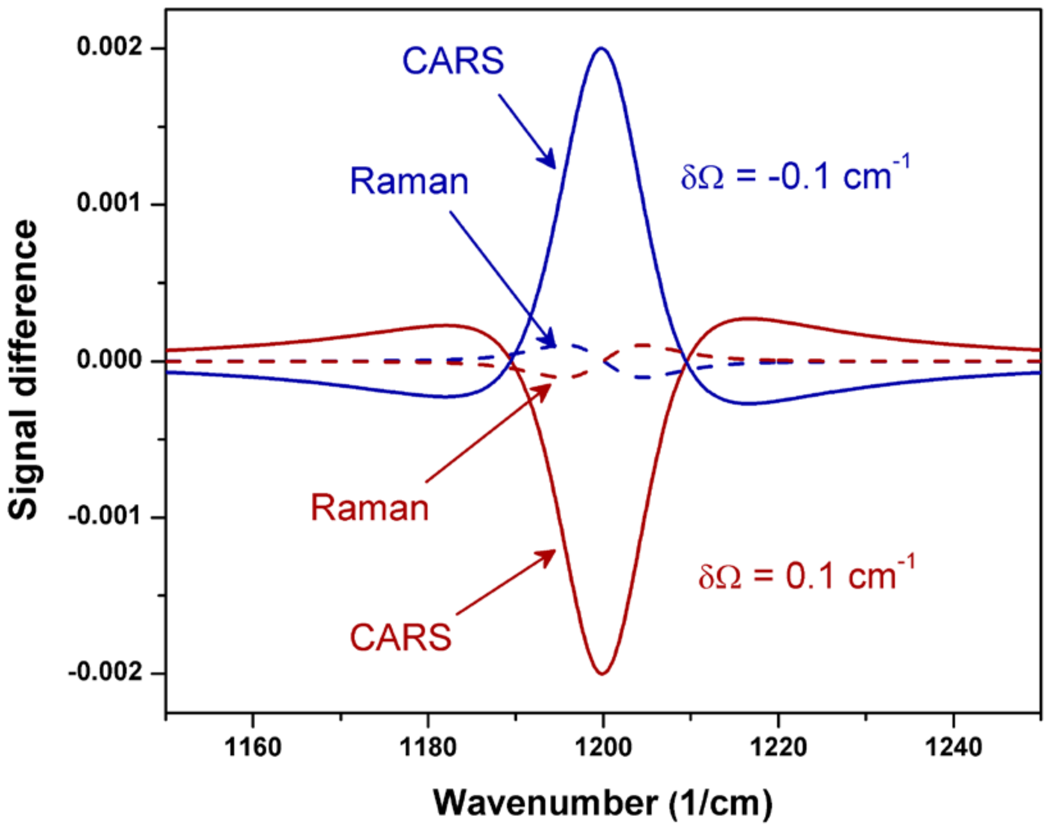




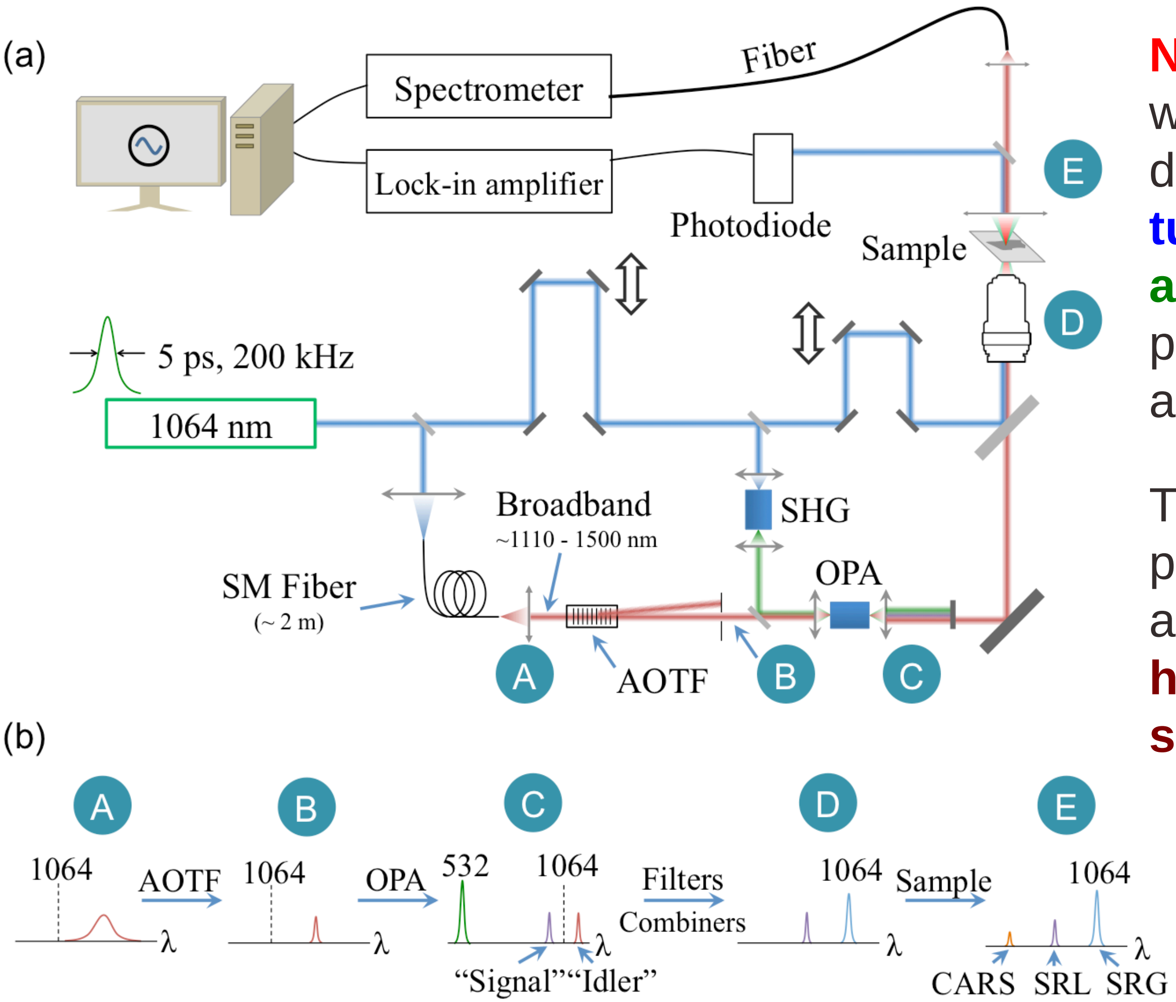
532-nm excitation, ~10 mW, 30-s acquisition time

Raman vs CARS: Stark spectroscopy

Nor only CARS spectroscopy produces **stronger signal**, but it is also **stronger affected** by the frequency shift of vibrational transition



Improved CARS/ SRS spectrometer



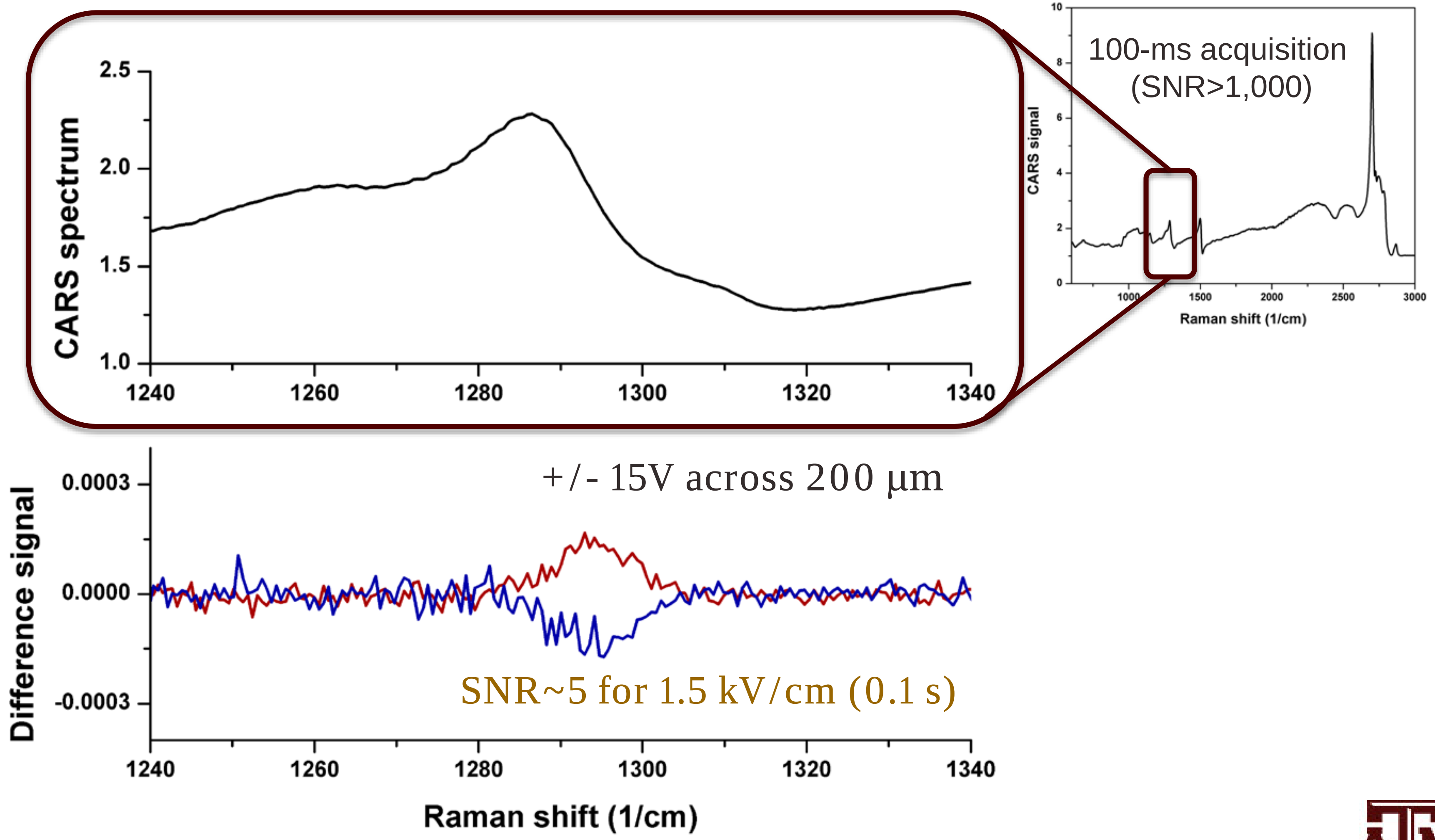
No moving parts: wavelength selection is done through **continuously tunable AOTF**, and light is **amplified using OPA** to provide sufficient intensity and stability at the sample.

The wavelengths of both pump and Stokes beams are **derived from from highly stabilized laser sources**

CW Ballmann, GI Petrov, VV Yakovlev, *Opt Lett* 42(1), 89-92 (2017); AJ Traverso et al *Light Sci Appl* 6(5), e16262 (2017).

Z Meng, GI Petrov, VV Yakovlev, *Sci Rep* 6, 20017 (2016)
GI Petrov, Z Meng, VV Yakovlev, *Opt Express* 23(19), 24669-24674 (2015)

CARS Stark spectroscopy



Mechanical assessment

Vol. 90, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 27, 1979 Pages 656-662

Electrical potential affects rigidity (stiffness) of membranes

POTENTIAL DEPENDENT RIGIDITY CHANGES IN LIPID MEMBRANE VESICLES

Peter I. Lelkes

Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

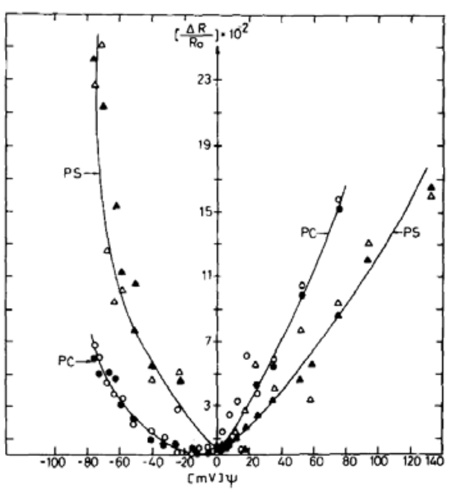
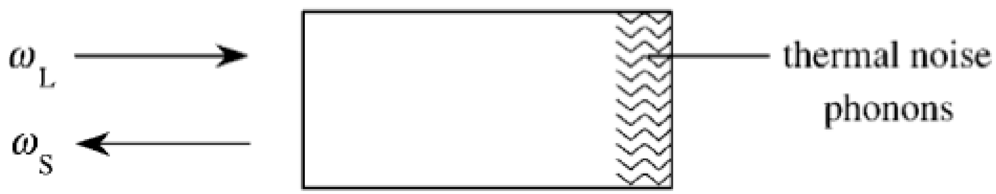
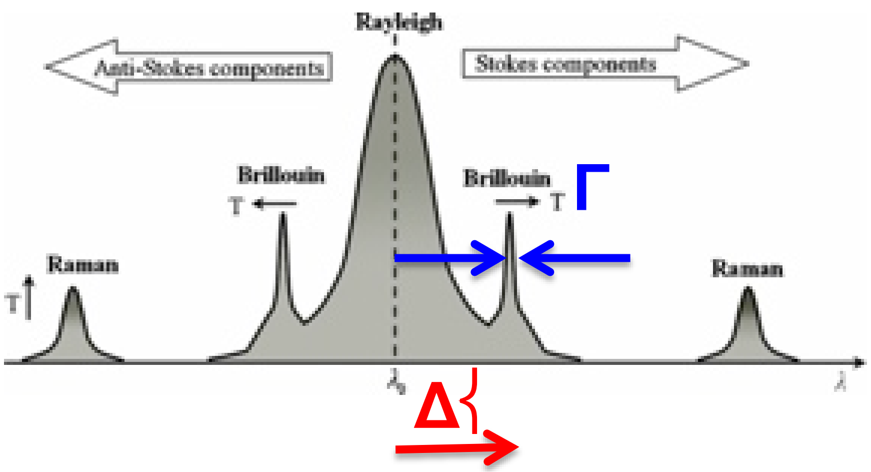


Figure 1 : The relative change in membrane rigidity $\Delta R/R_o$ in dependence of the transmembrane potential ψ, measured in phosphatidylcholine (PC) and phosphatidylserine (PS) vesicles. The different symbols represent independent experiments. For details, see text.

In this communication we present experimental evidence for a straightforward relationship between the transmembrane electrical potential and the membrane structure, expressed here as its rigidity. The biological implications of our results are significant and of general interest. A number of events on the cellular level are known to be initiated and/or accompanied by changes in the membrane potential, like ion-transport, hormonal action, enzyme-regulation, cell differentiation and proliferation, as well as cell growth and in some cases independently concomitant modifications in the membrane structure have been observed (25,26). Vertical translocations of proteins, and availability of receptor sites in biological membranes, which can be modulated by changes in the membrane fluidity (27), as well as by electrical fields across the membrane (28), underline the interrelation between membrane structure and transmembrane potential and its general occurrence in biological systems.

Brillouin microscopy: viscoelastic assessment





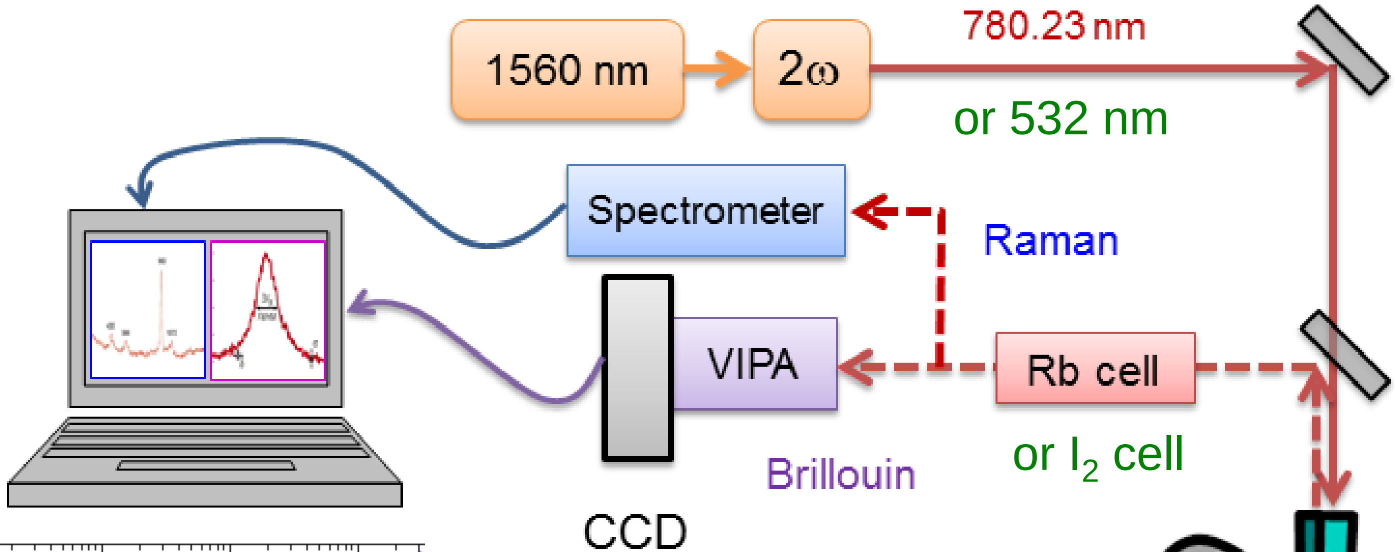
$G^{*}=G' + i\,G'' =$
$= \rho v^2 + i\,(2\rho v^3 \alpha),$

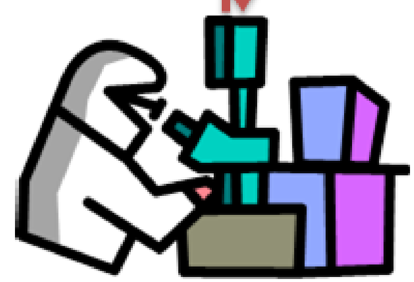
Velocity which is proportional to the **frequency shift**

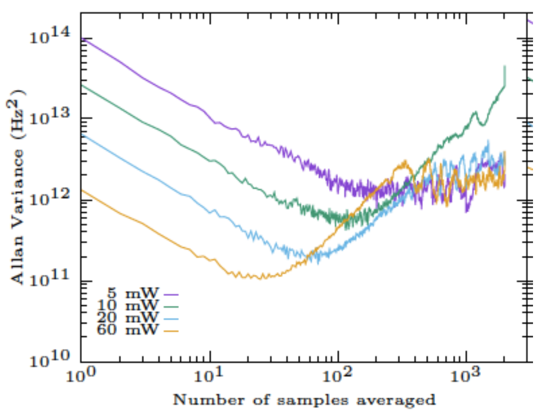
Attenuation which is proportional to the **line-width**)

Viscoelastic modulus is the function of frequency of acoustic wave, and, in Brillouin spectroscopy, we are assessing GHz waves. For nsPEF, those are the important ones.

Mechanical assessment





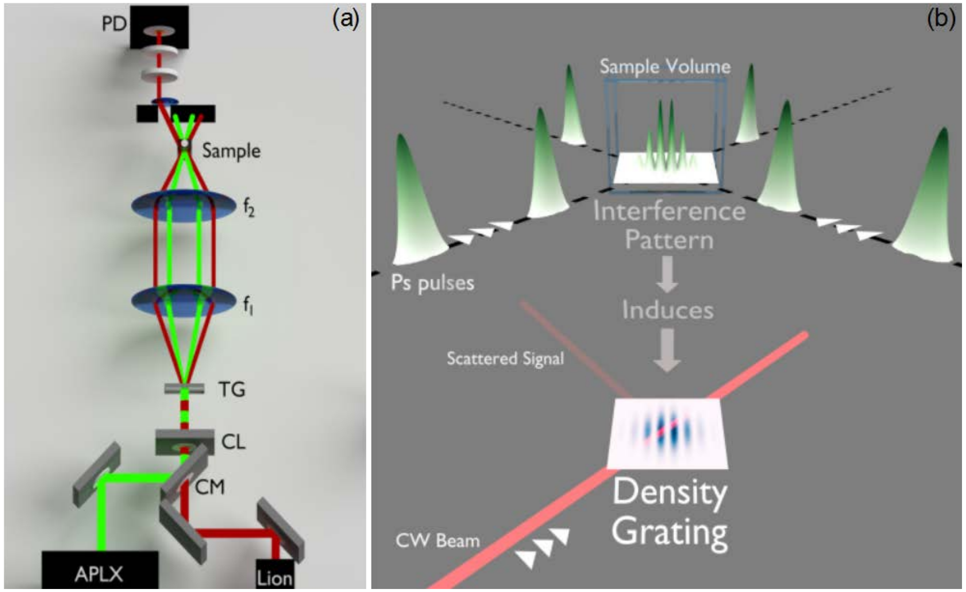


Z. Coker et al, *Opt. Express* 26(3), 2400-2409 (2018)

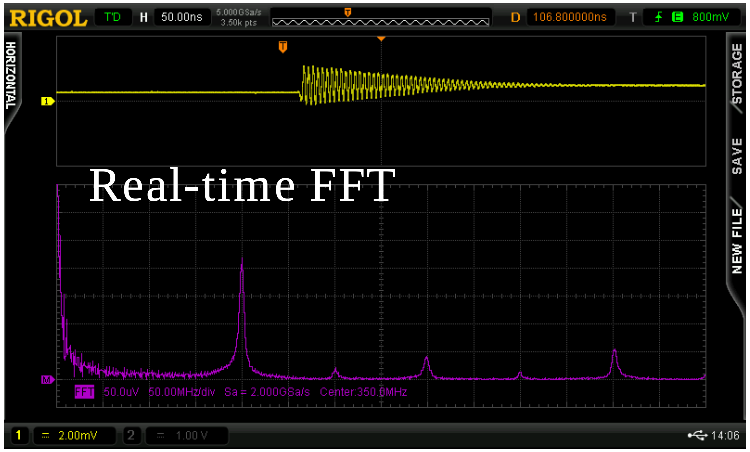
A. Traverso et al, *Anal Chem* 87 (15), 7519-7523 (2015)

Zh. Meng, et al, *Adv. Opt. Phot.* **8**(2), 300- 327 (2016).

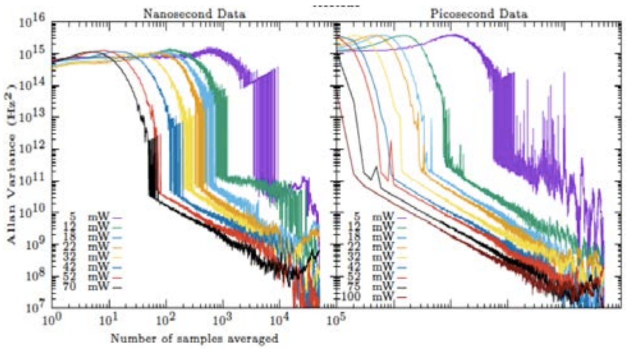
Mechanical assessment: BISTRO measurements



CW Ballmann, et al *Optica* 4(1), 124-128 (2017)

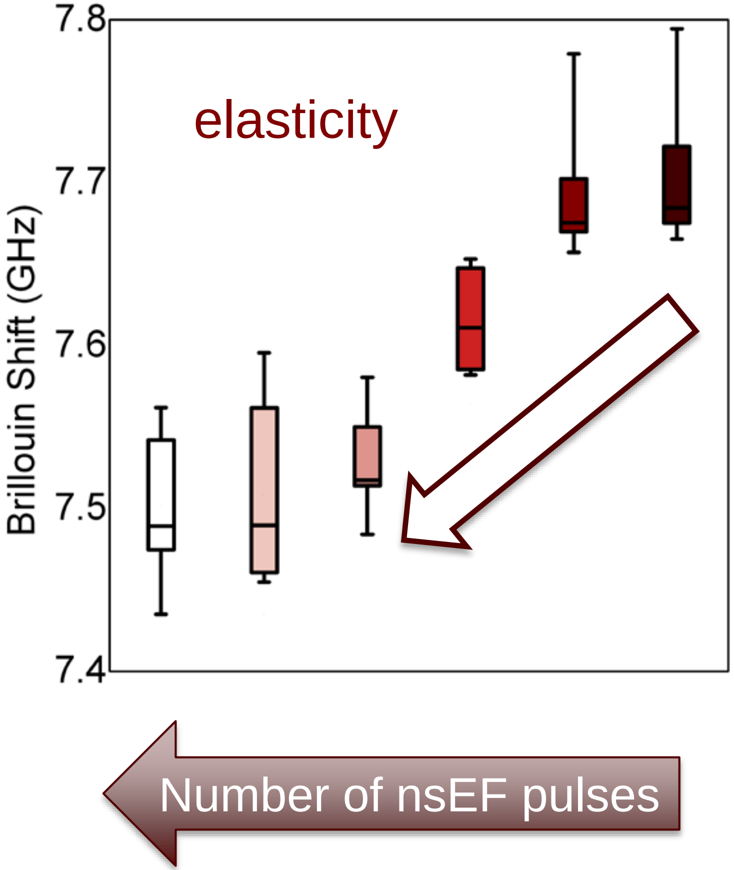


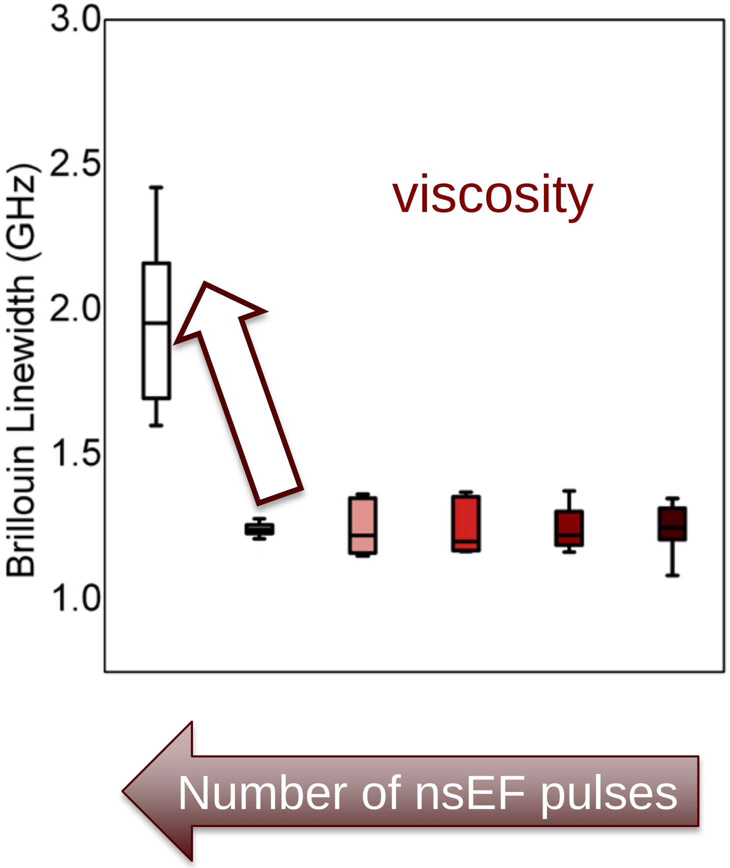
Brillouin **I**maging/**S**ensing via **T**ime-**R**esolved **O**ptical (**BISTRO**) Measurements



Shorter acquisition times (down to 1μs)
More accurate measurements

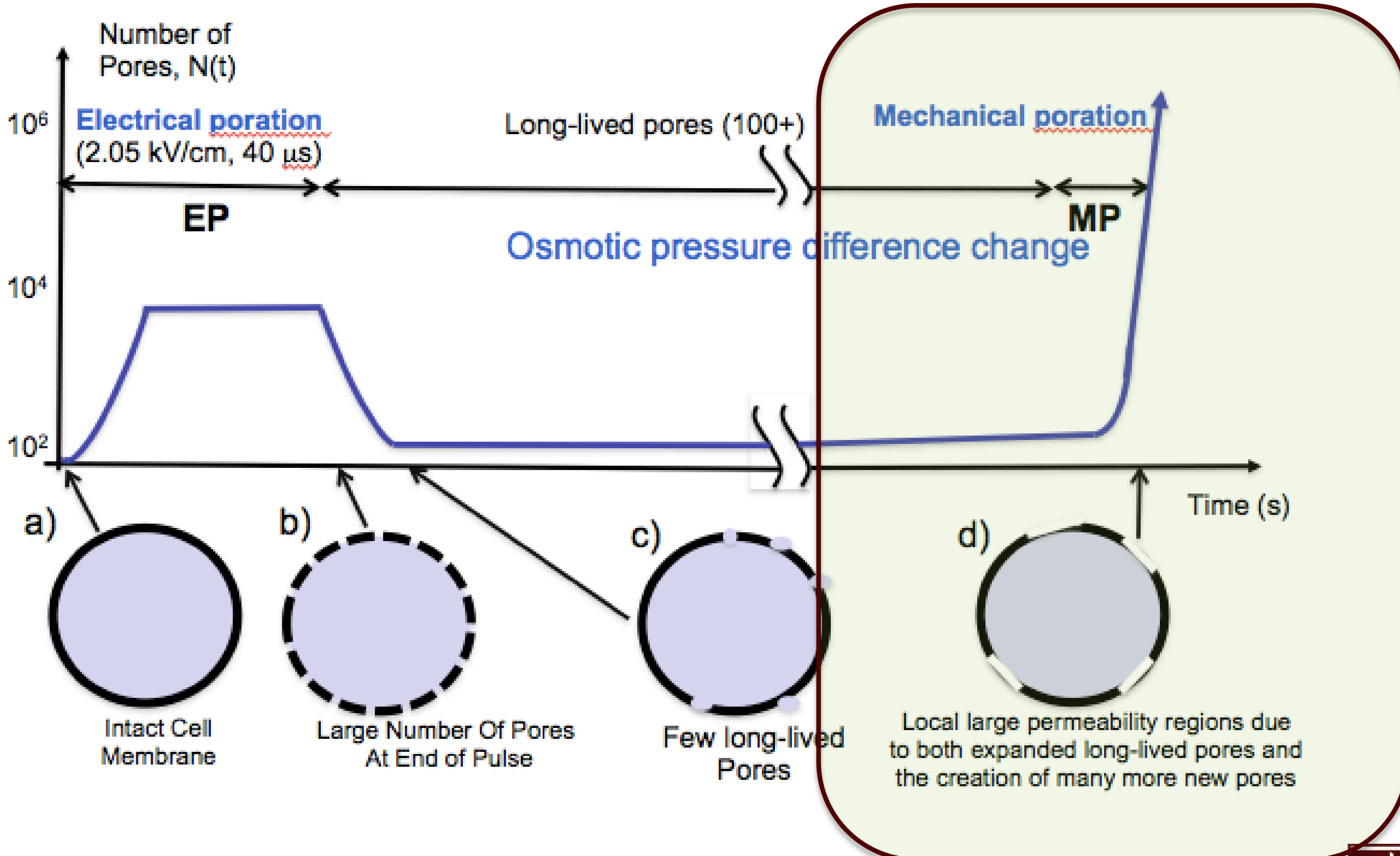
Mechanical assessment of nsPEF effects



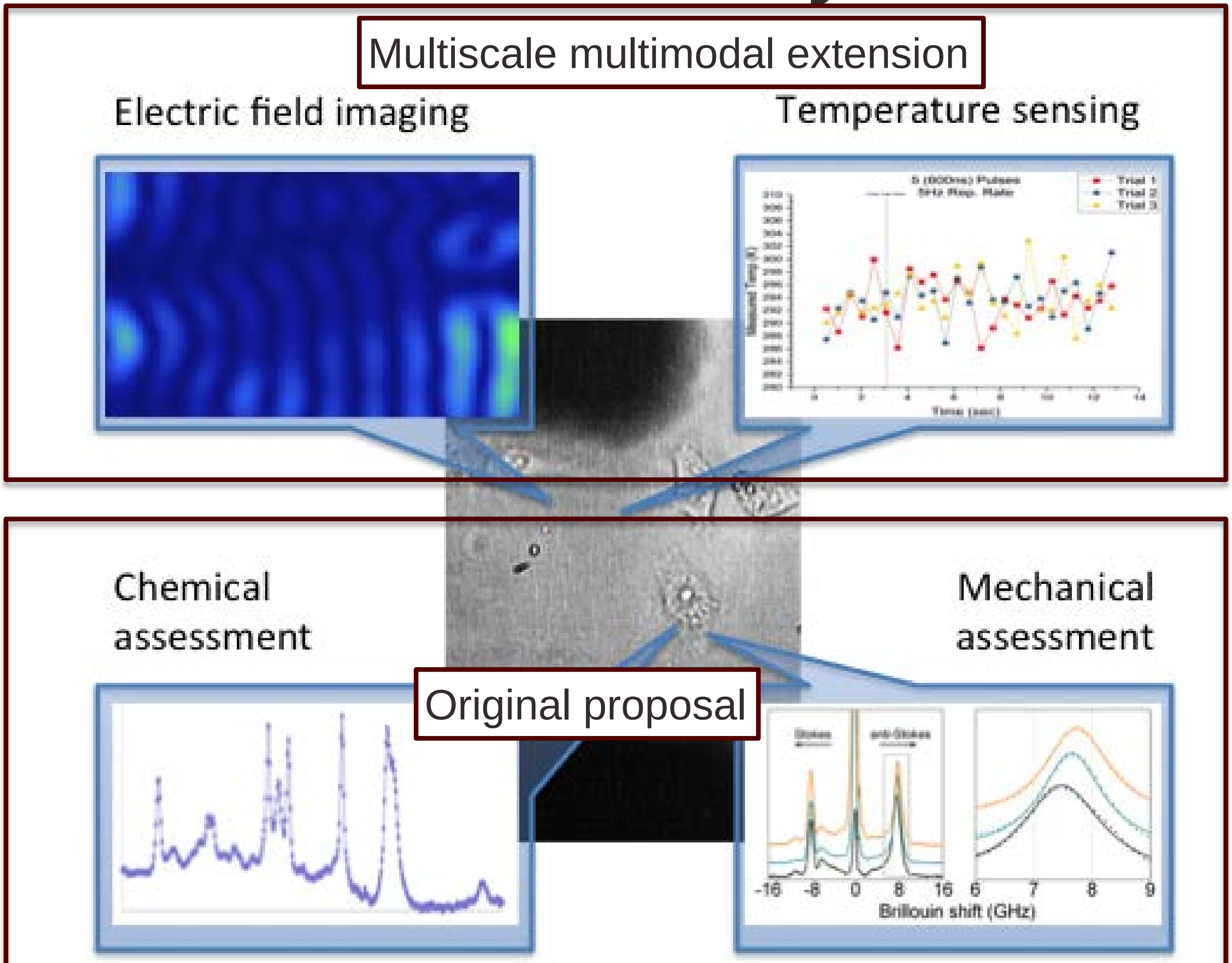


CHO (Chinese hamster ovarian) cells; 1.5 kV/cm, 600 ns pulse

Comparison with theory



Summary

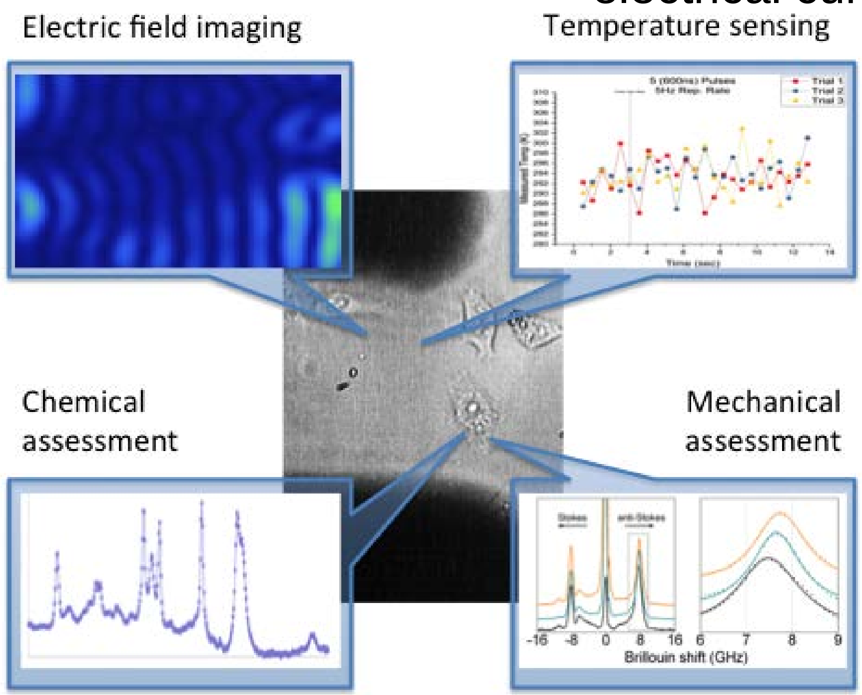

Multiscale multimodal extension
Electric field imaging
Temperature sensing
Chemical assessment
Mechanical assessment
Original proposal
Brillouin shift (GHz)

Sensitive **O**ptical **F**ields’ **I**maging

Pockels effect: possible at short time scales
Provides with EF distribution
Tomographic imaging makes E(x,y,z) possible

NP: “smart" substrate allows real-time temperature and magnetic field (due to electrical currents) sensing

CARS: first experimental demonstration of Stark effect in model system of GUVs



Diamonds NV: imaging electrical currents and magnetic field (with Phil Hemmer)

CARS: system is optimized for high SNR measurements, so it is possible to start looking for small variations due to oxidation, electroporation, etc.

Brillouin: sensitivity and signal strength are sufficient to look for dynamic changes immediately after nsPEF

Future work

Year	1				2				3				4				5			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Task 4: Novel bio-electro-mechanical tools																				
Develop broadband Raman imaging system																				
Develop coherent Brillouin imaging system																				
nsPEF experiments																				

Nascent set of tools has been developed: non-invasive microscopic assessment of electro-mechanical interactions in biological systems is now possible. State-of-the-art of sensitivity and accuracy measurements.

Fast kinetics imaging: pressing needs of nsPEF effects assessment.

- ✓ How fast is membrane discharge?
- ✓ What are the structure/chemistry changes associated with nsPEF?
- ✓ Are those changes permanent of transient?
- ✓ Are mechanical effects important?
- ✓ Theory vs Experiment. Can we connect those on molecular level?
- ✓ Any new physics?