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Oncotripsy and Ultrasound Neuromodulation: Targeting cells selectively by means of tuned ultrasound

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With: M. Gharib, D. Mittelstein, E.F. Schibber, A. Salahshoor, M. Shapiro (Caltech) and P. Lee, J. Ye (City of Hope) and M.A. Keip, L. Werneck (Universität Stuttgart) and M. Sitti, E. Yidiz (MPI-IS, Stuttgart)

Universidad de Zaragoza, November 17, 2022

A Zaragoza, *grato animo*

Instituto de Educación Secundaria Goya (1967-1971)

Facultad de Ciencias de la Universidad de Zaragoza (1971-1972)

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Lecture plan

- *Oncotripsy*: Targeting cancerous cells selectively with tuned low-intensity pulsed ultrasound (*LIPUS*)
  - *Does it work? Experimental study of cells in suspension subjected to LIPUS*
  - *How does it work? The mechanics of healthy vs. cancerous cells (band gaps and resonance), spectral gap* and *cell fatigue*
  - *Model validation: Can we predict cell life, dependence on frequency, amplitude duty cycle…?*
- *Neuromodulation:* Targeting neurons selectively with tuned low-intensity focused ultrasound (*LIFUS*)
  - *Does it work? Can US be focused on precise targets in skull?*
  - *How does it work? From mechanosensitive Ca++ channels to neuronal activation potential*
  - *Model validation: Can we dependence on frequency, amplitude?*
- *Harnessing the Data Revolution:* Towards *patient-specific*, *in situ, in vivo*, *Data-Driven* US neuromodulation *therapies*…

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Oncotripsy: Early exploratory work

S. Heyden

M. Ortiz

Computational Solid Mechanics Laboratory
Division of Engineering and Applied Science
California Institute of Technology

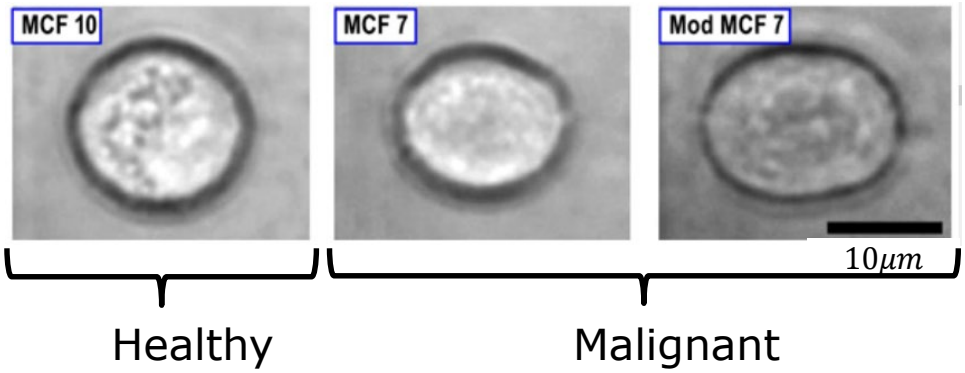
Heyden, S. and Ortiz, M., *JMPS*, **92**:164-175, 2016.
Heyden, S. and Ortiz, M., *CMAME*, **314**, 09 2016.

Oncotripsy: The key observation

- Studies suggest that aberrations in both *cellular morphology* and *mechanical properties* of different cell constituents are typical of cancerous tissues
- Criterion for malignancy: *Size difference* between normal nuclei (average diameter of 7 to 9 microns) and malignant nuclei (diameter of over 50 microns)

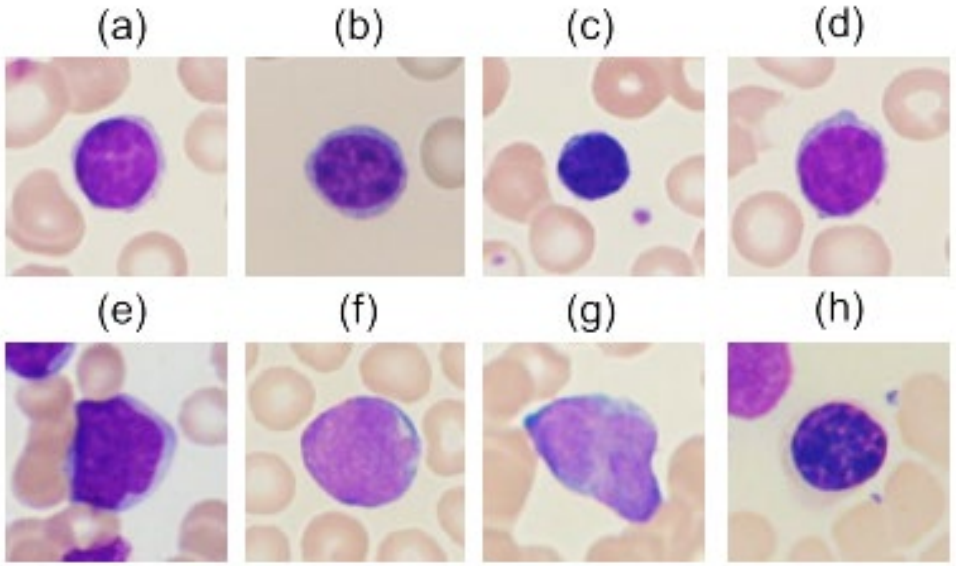
Oncotripsy: The key observation

Cells from MCF-7 breast cancer cell line. Morphological changes induced by malignancy[1]



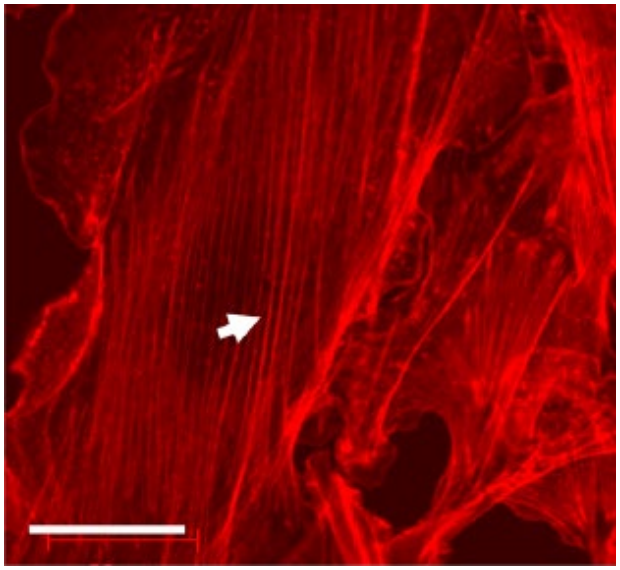
(a-d) Healthy lymphocyte cells.[2]

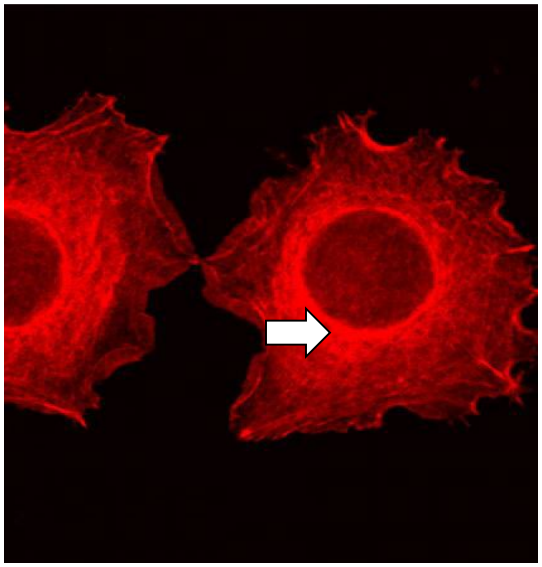
(e-h) Acute lymphoblastic leukemia (ALL) cells.[2]



[1]Suresh, S. (2007). *Acta Materialia*, *55*(12), 3989–4014.
[2]Labati, R. D., Piuri, V. & Scotti, F., *IEEE ICIP* (2011) 2045–2048.

Oncotripsy: The key observation





Low cancer potential

High cancer potential

Cytoskeletal organization in tumor cells.
Actin filaments well (randomly) organized
for the less (more) invasive tumor cells

Smolyakov, G. *et al*., *Amer. Chem. Soc.,* **8** (41) 2016, pp. 27426 - 27431.

Oncotripsy: The key observation

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- Criterion for malignancy: *Size difference* between normal nuclei (average diameter of 7 to 9 microns) and malignant nuclei (diameter of over 50 microns)
- *Mechanical stiffness* of various cell components are found to vary significantly in healthy and diseased tissues (cancerous cells are softer, ECM stiffer)

Oncotripsy: The key observation

Hepatocellular Carcinoma (HCC)

Malignant	κ [kPa]	μ_1 [kPa]	μ_2 [kPa]
Plasma membrane	39.7333	0.41	0.422
Cytoplasm	39.7333	0.41	0.422
Nuclear envelope	239.989	2.41	2.422
Nucleoplasm	239.989	2.41	2.422
Nucleolus	719.967	7.23	7.266
ECM	248.333	5.0	5.0

Healthy	κ [kPa]	μ_1 [kPa]	μ_2 [kPa]
Plasma membrane	71.5199	0.738	0.7596
Cytoplasm	71.5199	0.738	0.7596
Nuclear envelope	431.98	4.338	4.3596
Nucleoplasm	431.98	4.338	4.3596
Nucleolus	1295.94	13.014	13.0788
ECM	198.666	4.0	4.0

Heyden, S. and Ortiz, M., *JMPS*, **92**:164-175, 2016.
Heyden, S. and Ortiz, M., *CMAME*, **314**, 09 2016.

Oncotripsy: The key observation

- Studies suggest that aberrations in both *cellular morphology* and *mechanical properties* of different cell constituents are typical of cancerous tissues
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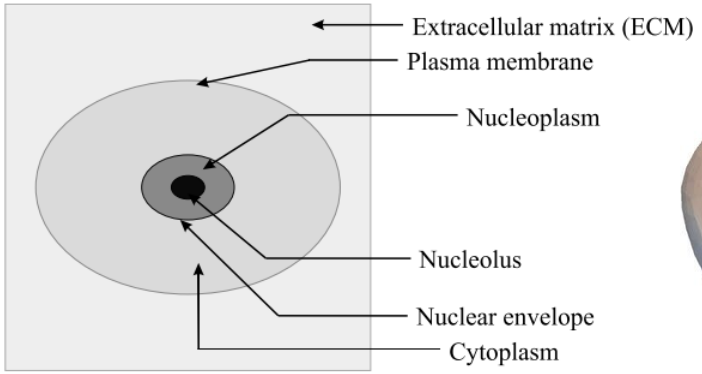
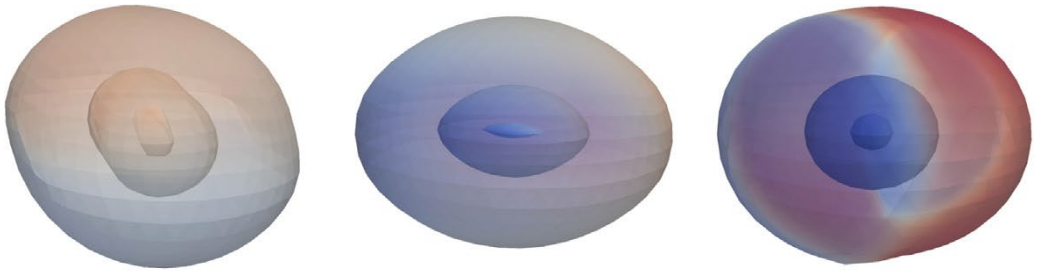
- *Mechanical stiffness* of various cell components are found to vary significantly in healthy and diseased tissues (cancerous cells are softer, ECM stiffer)
- *Question*: Can cancer cells be *selectively targeted* by harmonic excitation at their resonance frequency? (*oncotripsy*)
- What are the *therapeutic ranges* of frequency, duty cycle, intensity, exposure time?

Oncotripsy: The spectral gap



Cell in extracellular matrix

Hepatocellular Carcinoma (HCC)



ω = 501576 rad/s ω = 502250 rad/s ω = 508795 rad/s

Lowest fundamental modes

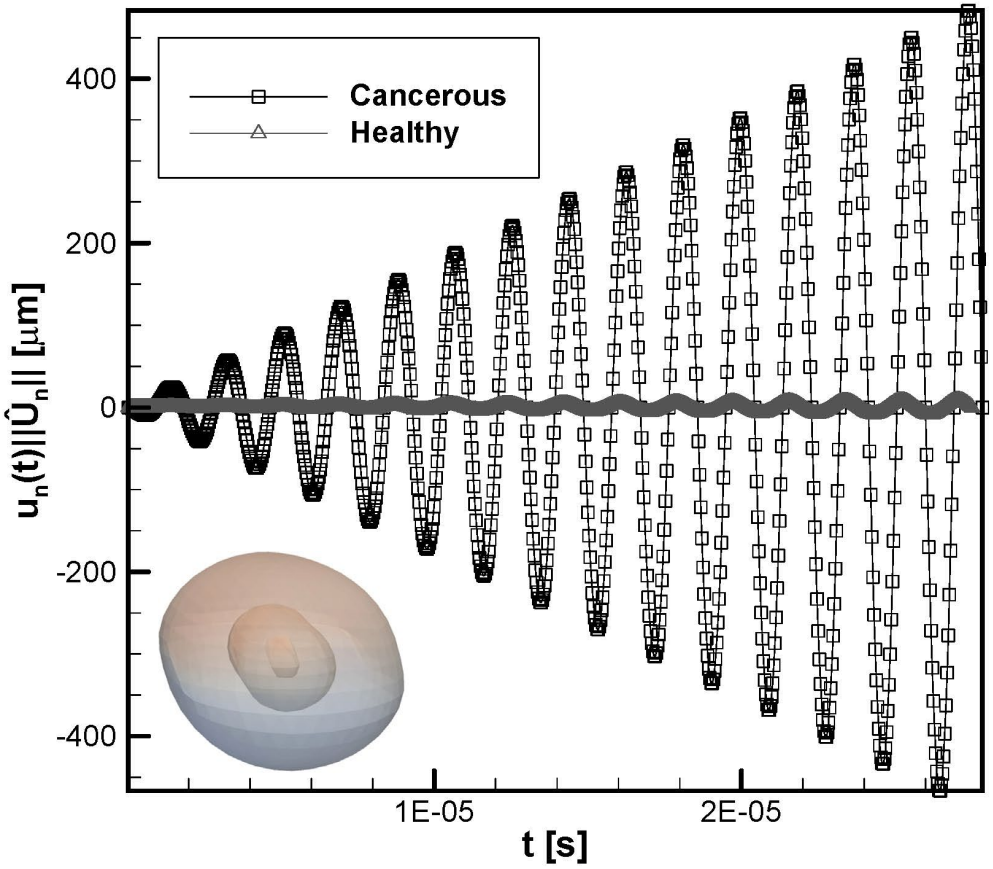
	ω_1 [rad/s]	ω_2 [rad/s]	ω_3 [rad/s]	ω_4 [rad/s]	ω_5 [rad/s]
Cancerous	501576	502250	508795	532132	537569
Healthy	271764	274141	364259	364482	367413
	ω_6 [rad/s]	ω_7 [rad/s]	ω_8 [rad/s]	ω_9 [rad/s]	ω_{10} [rad/s]
Cancerous	538512	557291	667107	678287	678771
Healthy	375570	376000	380063	424226	425327

Vibrational spectrum of HCC and healthy cells

Heyden, S. and Ortiz, M., *JMPS*, **92**:164-175, 2016.
Heyden, S. and Ortiz, M., *CMAME*, **314**, 09 2016.

Oncotripsy: The spectral gap

Hepatocellular Carcinoma (HCC)



- Modal *displacements* of HCC and healthy cells excited at HCC *resonant frequency*
- Distortions in HCC and healthy cells grow at *vastly different rates*!
- *Malignant cells come to lysis first!*

Heyden, S. and Ortiz, M., *JMPS*, **92**:164-175, 2016.
Heyden, S. and Ortiz, M., *CMAME*, **314**, 09 2016.

Oncotripsy: The spectral gap

- Computational studies of HCC give natural frequencies of ~80 kHz (malignant) and ~43 kHz (healthy): *Ultrasound!*
- *Spectral gap* of ~37 kHz: Window for *selective targeting* of malignant cells (*oncotripsy*)
- Energy deposition rates ~1 W/m^2: Low-intensity pulsed ultrasound (*LIPUS*)
- LIPUS is widely used in clinical applications, *new non-invasive cancer therapies*?
- *Is oncotripsy observed in the laboratory*? (*in vitro, in vivo*, models, humans…)
  - *First step: In vitro testing of cells in suspension*
  - *Second step: In vivo testing in animal models*
  - *Third step: In vivo testing in human subjects*

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Oncotripsy: Laboratory studies

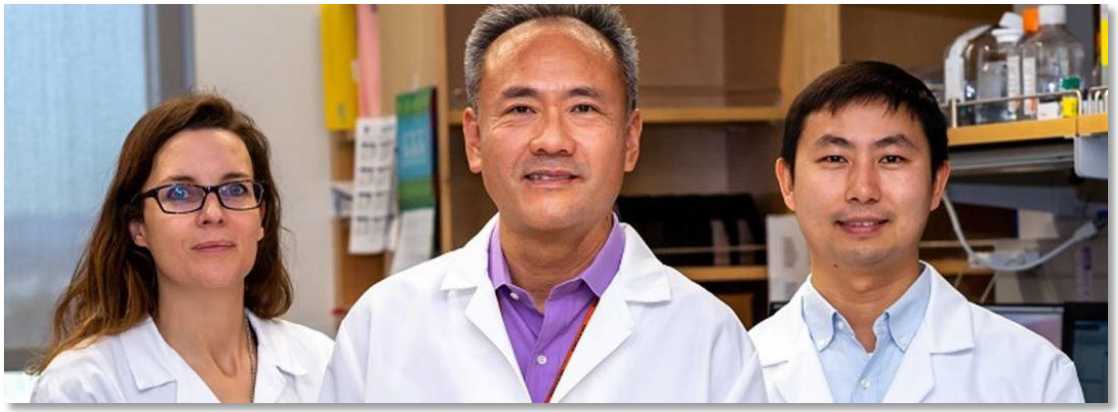




E.F. Schibber M. Ortiz M. Gharib D. Mittelstein M. Shapiro

Division of Engineering and Applied Science

Division of Chemistry and Chemical Engineering



Left to right:
C. Hoffman, P.P. Lee, J. Ye
Dept. Immuno-Oncology

City of Hope

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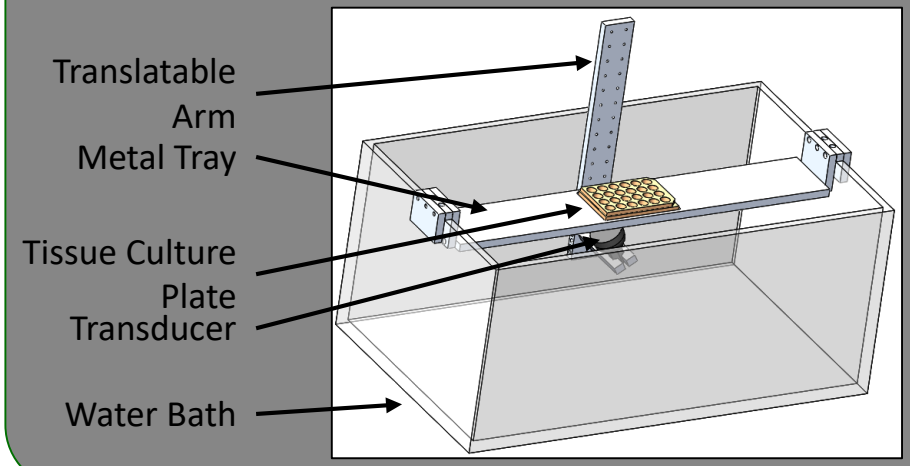
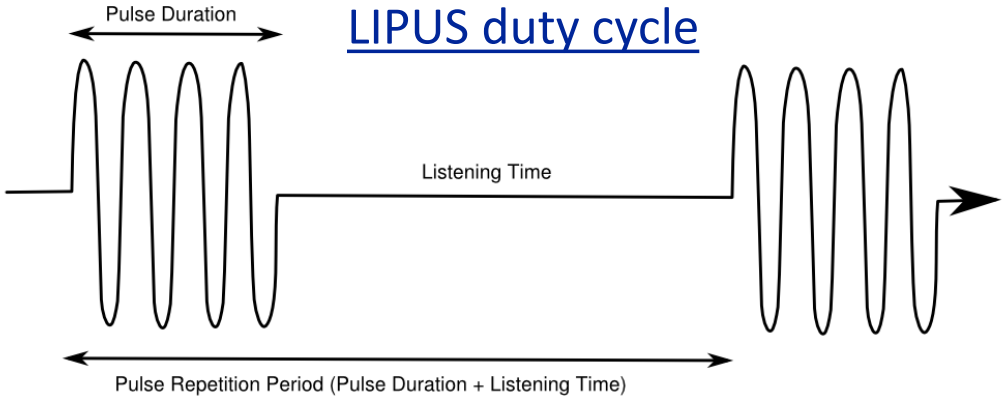
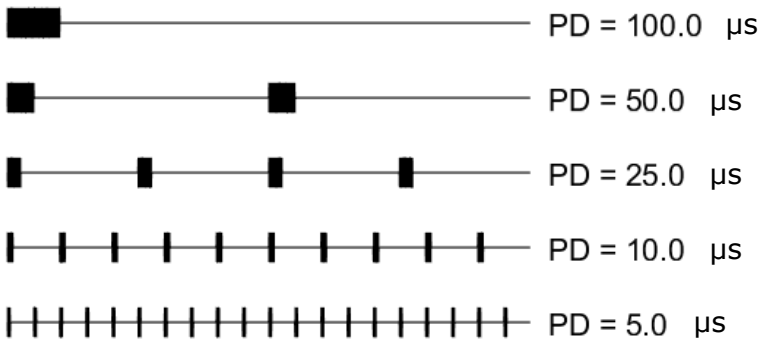
In vitro testing of cells in suspension

In-vitro experimental setup:



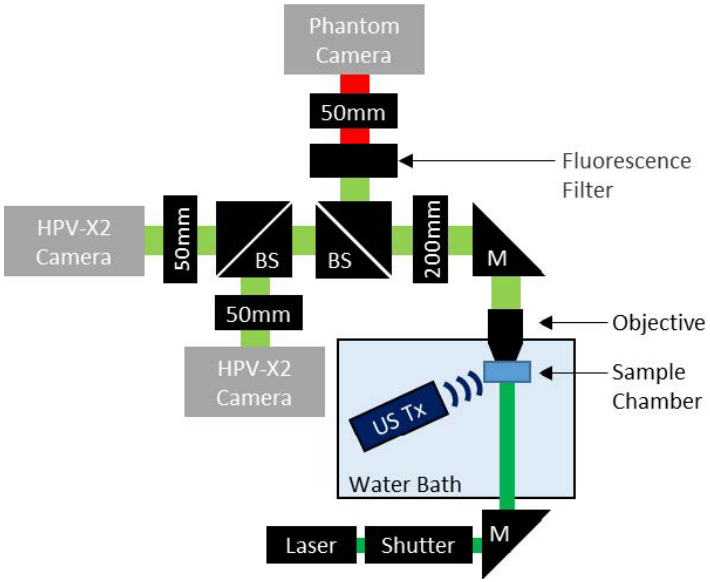
LIPUS duty cycle

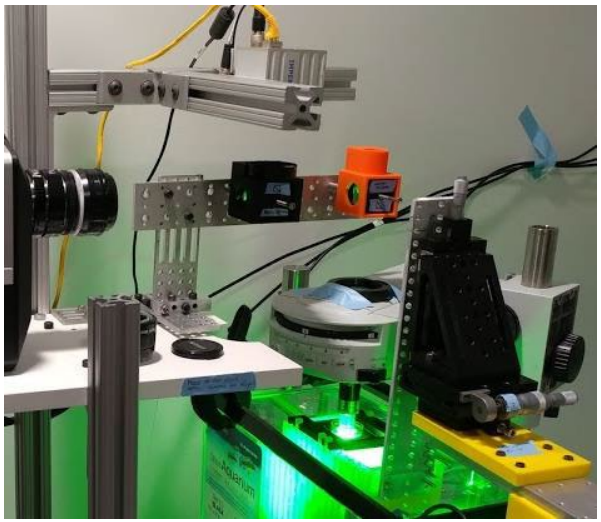




D.R. Mittelstein *et al.*, *Appl. Phys. Lett.*, **116**, 013701 (2020).

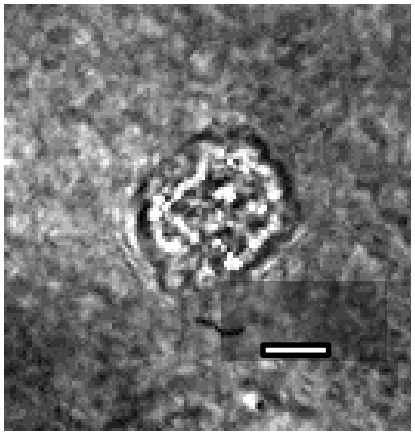
In vitro testing of cells in suspension

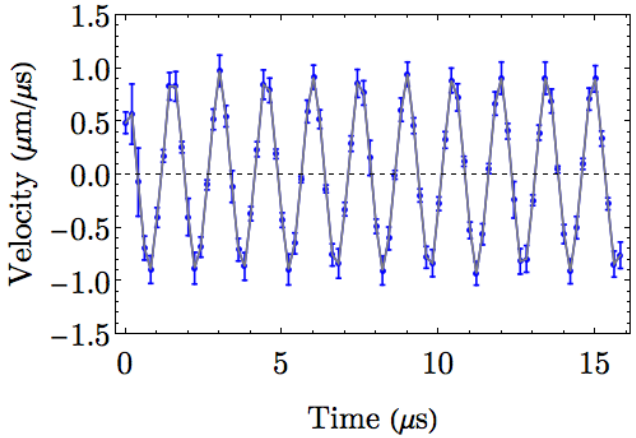




High-speed camera setup

High frame-rate camera recordings showing minimal K-562 cell distortion after 100 ms of 670 kHz ultrasound exposure (scale bar 20 microns)





Data reduction from video showing nearly harmonic rigid motion of the cell

D.R. Mittelstein *et al.*, *Appl. Phys. Lett.*, **116**, 013701 (2020).

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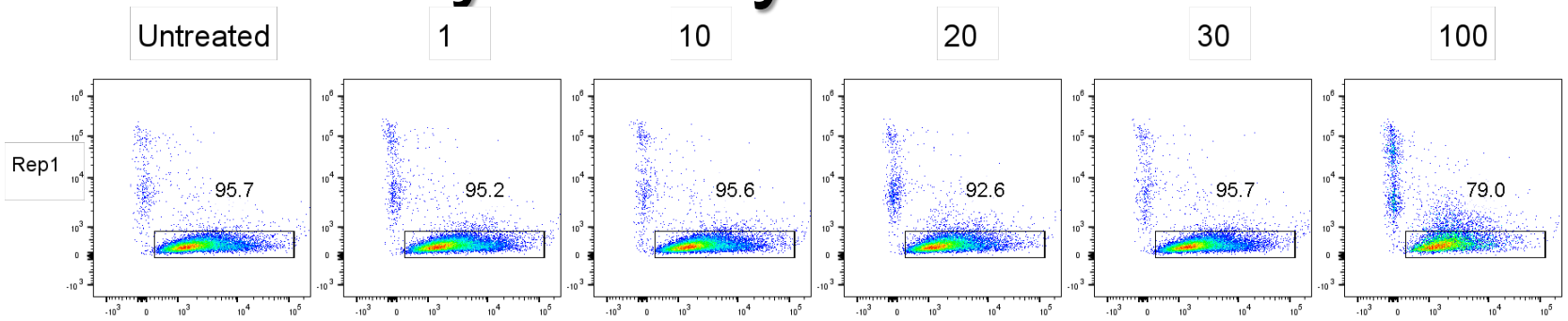
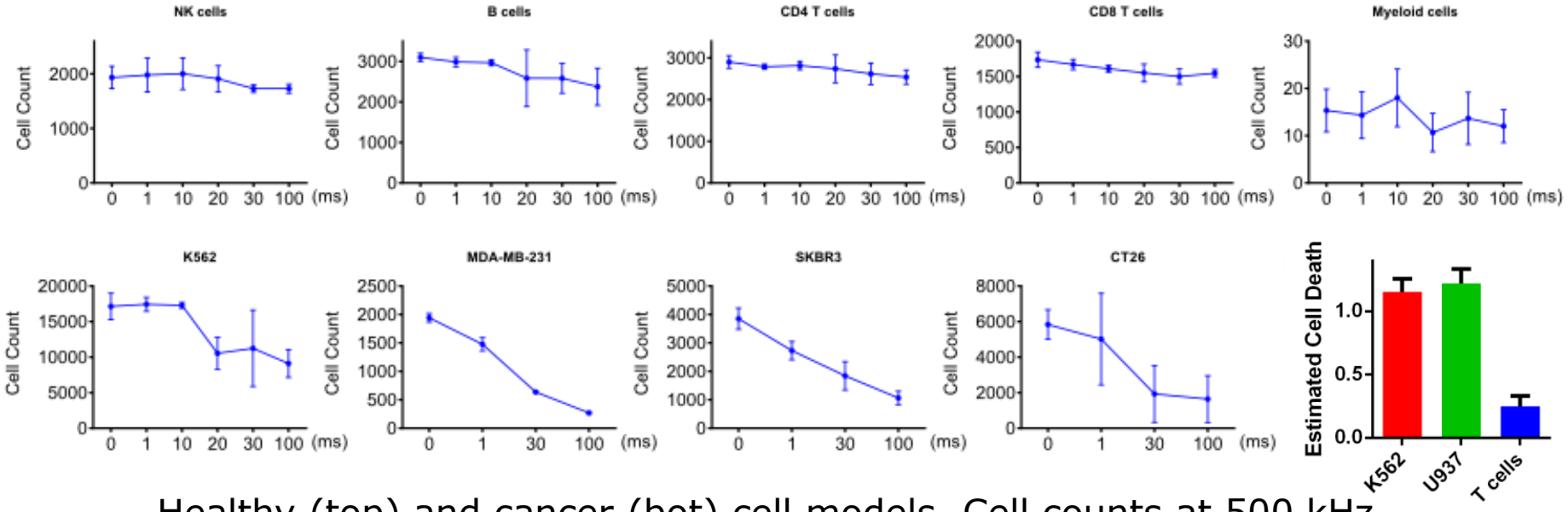
Flow cytometry measurements



Double fluorescence dot plots from cytometry analysis of K-562 line.
Dead-cell fractions as a function of exposure and duty cycle



Healthy (top) and cancer (bot) cell models. Cell counts at 500 kHz (20 µs PD) demonstrate ***therapeutic index*** after ms exposure

Source: Lee, P. and Ye, J., *City of Hope,* 2019.

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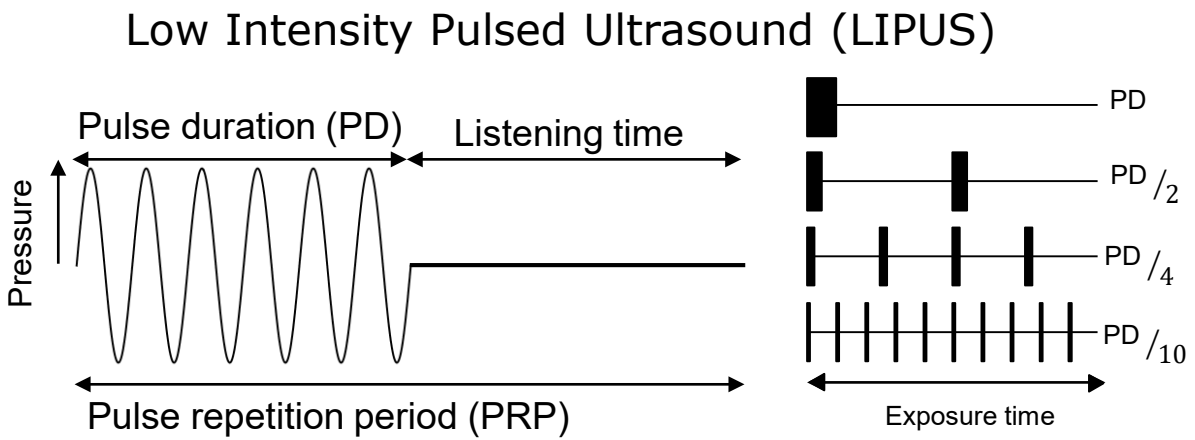
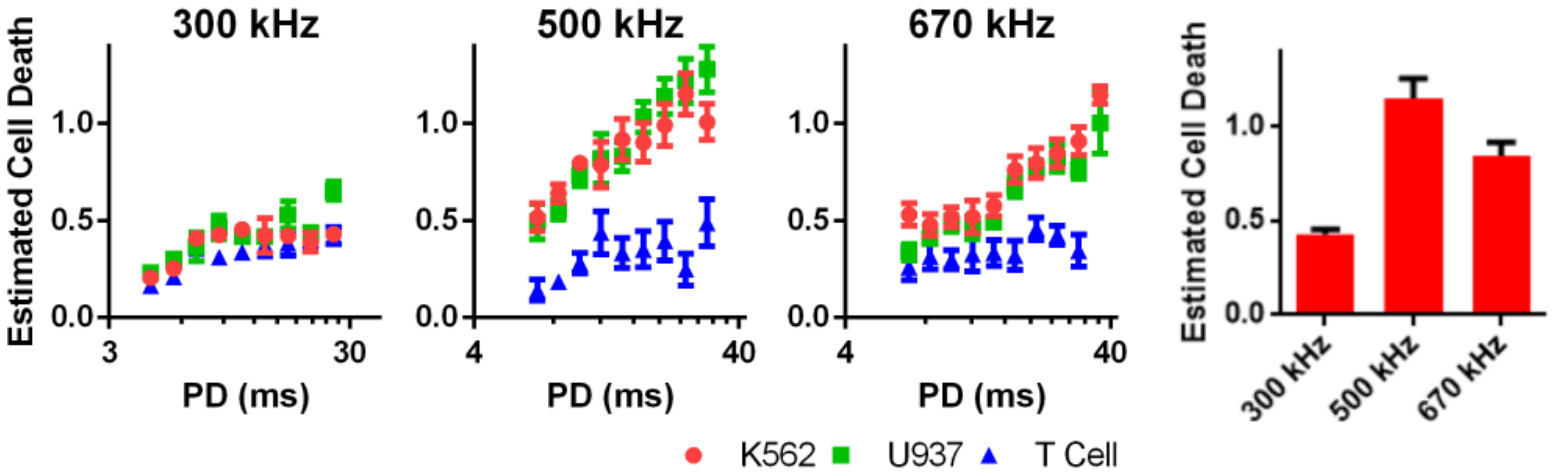
Flow cytometry measurements





NB: Cell death requires large (millions) number of cycles and depends on the pulse duration despite same acoustic energy deposited!

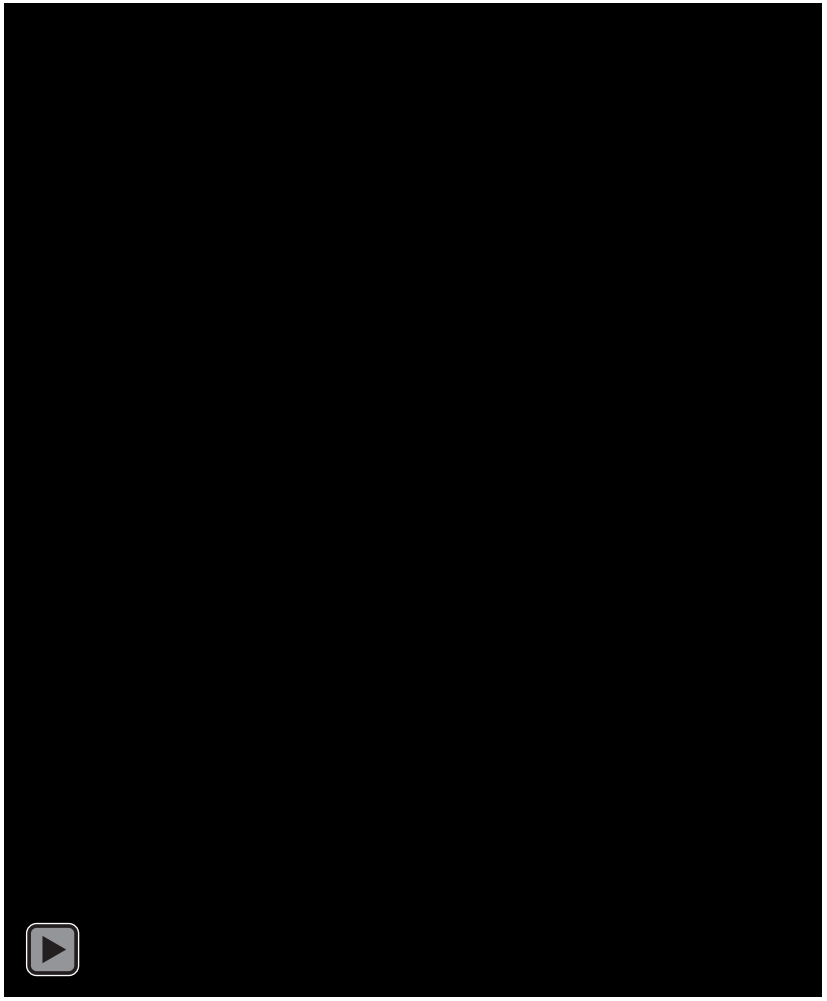
D.R. Mittelstein *et al.*, *Appl. Phys. Lett.*, **116**, 013701 (2020).

In vitro testing of cells in suspension

- Cell death in response to ultrasound exhibits *frequency-dependence,* peaks at *resonant frequency*
- Targeted US induces *highly selective cell death*, demonstrating significant *therapeutic index,* potential
- These observations bear out the *oncotripsy* concept
- *But*: Cell death requires *large number of pulses*
- Cell death dependent on *pulse duration*, despite constant energy deposited
- How can we understand, model, this behavior?
- *Hypothesis*: Cells in aqueous suspension behave as *internal resonators*, die by slow accumulation of *damage to the cytoskeleton* (cell fatigue)
- Levels of description: i) Discrete networks; ii) Continuum models; iii) Reduced models.

D.R. Mittelstein *et al*., *Appl. Phys. Lett*., **116**, 013701 (2020).
E.F. Schibber *et al*., *Proc. R. Soc. London A*, **476**: 20190692 (2020).

Response of actin network to LIPUS



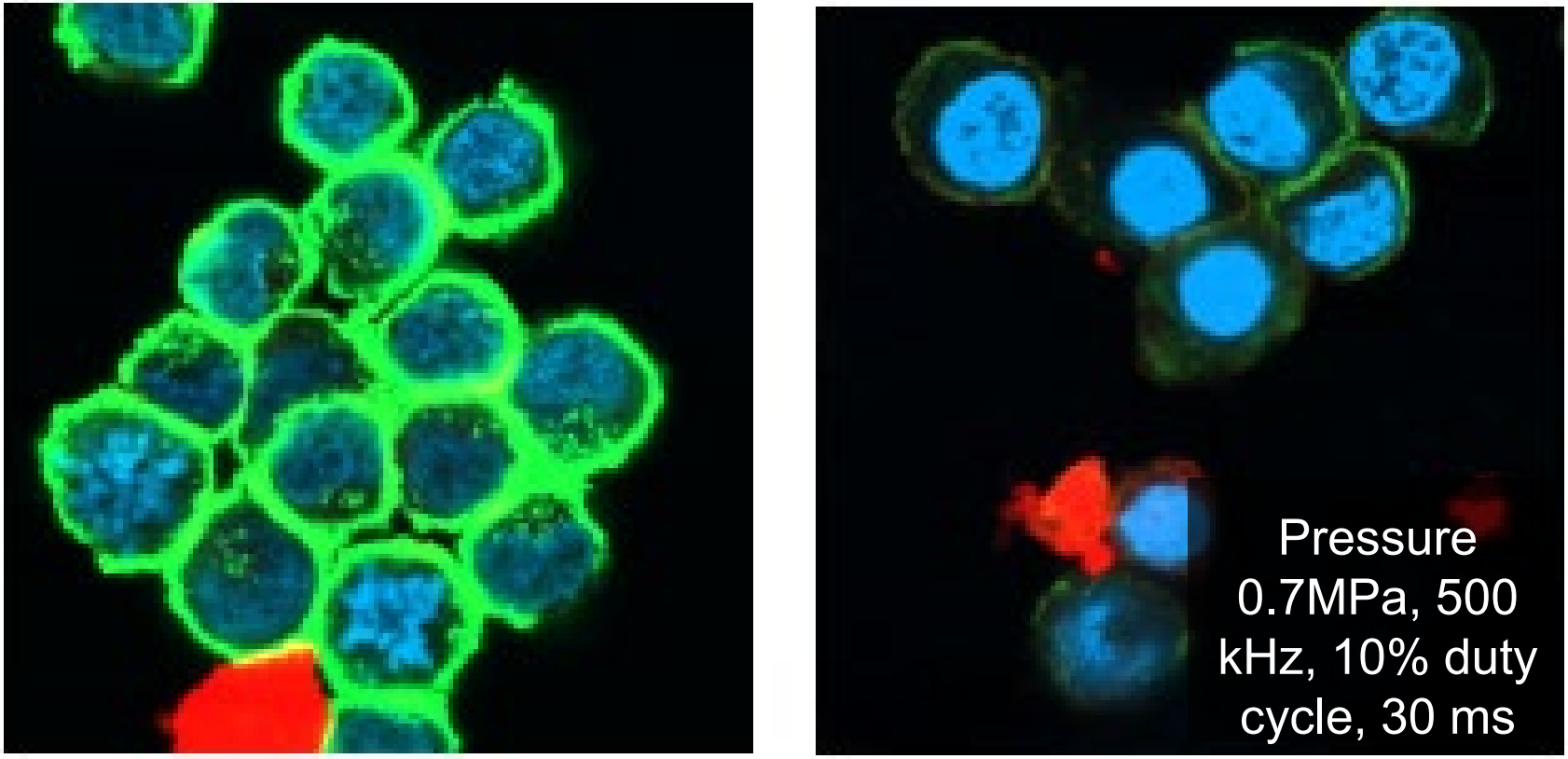
Cell actin network subjected to high-intensity LIPUS, progressively disassembles within 3 min exposure (Mizrahi et al., 2012)

Mizrahi, N., et al (2012). Soft Matter, 8(8), 2438–2443.

Response of actin network to LIPUS

Control: no ultrasound

After ultrasound

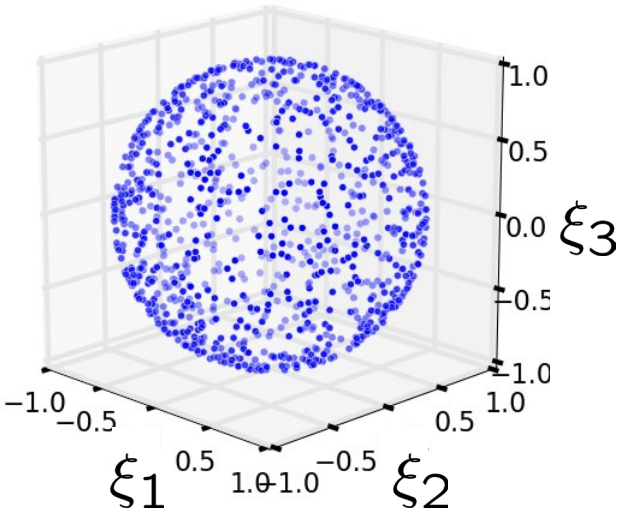


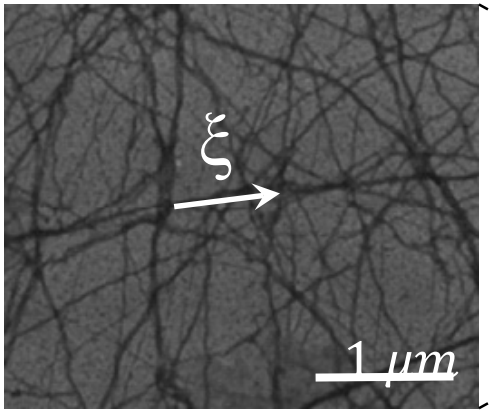
Dead Cell Actin Nucleus

Cell actin network subjected to low-intensity LIPUS, progressively disassembles within 60 seconds

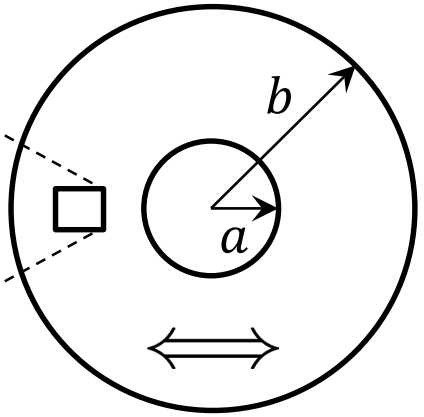
D.R. Mittelstein *et al*., *Appl. Phys. Lett*., **116**, 013701 (2020).

High-cycle cell fatigue model





(Gardel *et al.*, 2008)
(Ingber, 1997)



$$v(t) = V \sin \omega t$$

- *Network theory* of elasticity: $A(F,T,q) =$

$$\int_{S^2} p(\xi)\left(\frac{\mu(T)}{2}\underbrace{(1-q(\xi))^2}_{\text{damage}}\left(\lambda^2(\xi)+\lambda^{-2}(\xi)\right)\underbrace{+\frac{\beta}{2}q^2(\xi)}_{\text{healing}}\right) d\Omega$$

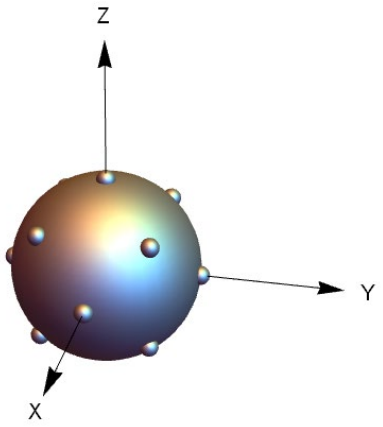
- *Linear damage kinetics*: $\alpha\dot{q}(\xi) + \dfrac{\partial A}{\partial q(\xi)} = 0$

E.F. Schibber *et al.*, *Proc. R. Soc. London A*, **476**: 20190692 (2020).

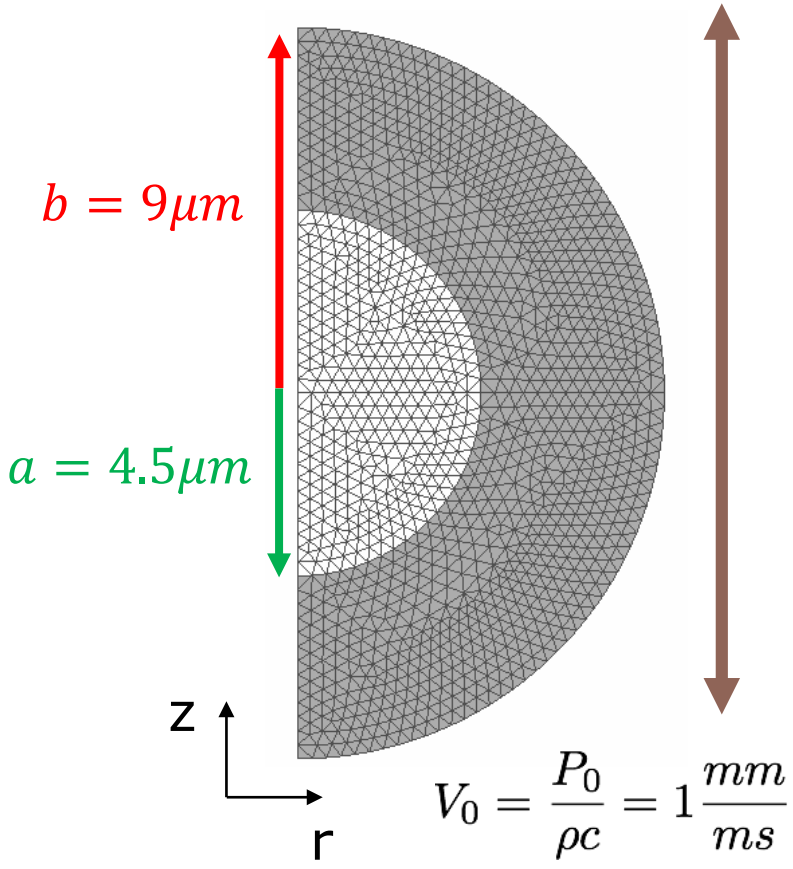
Continuum FE calculations

	Healthy	Cancerous
Geometry	Equal size	
Viscosity	~10 times higher than water	
Kinetics	$\alpha = 0.1$kPa ms	$\beta = 0.5$kPa
Shear modulus	33kPa	66kPa
Resonance	500kHz	700kHz

Isotropic fibers
Gaussian quadrature
on unit sphere



White blood cell

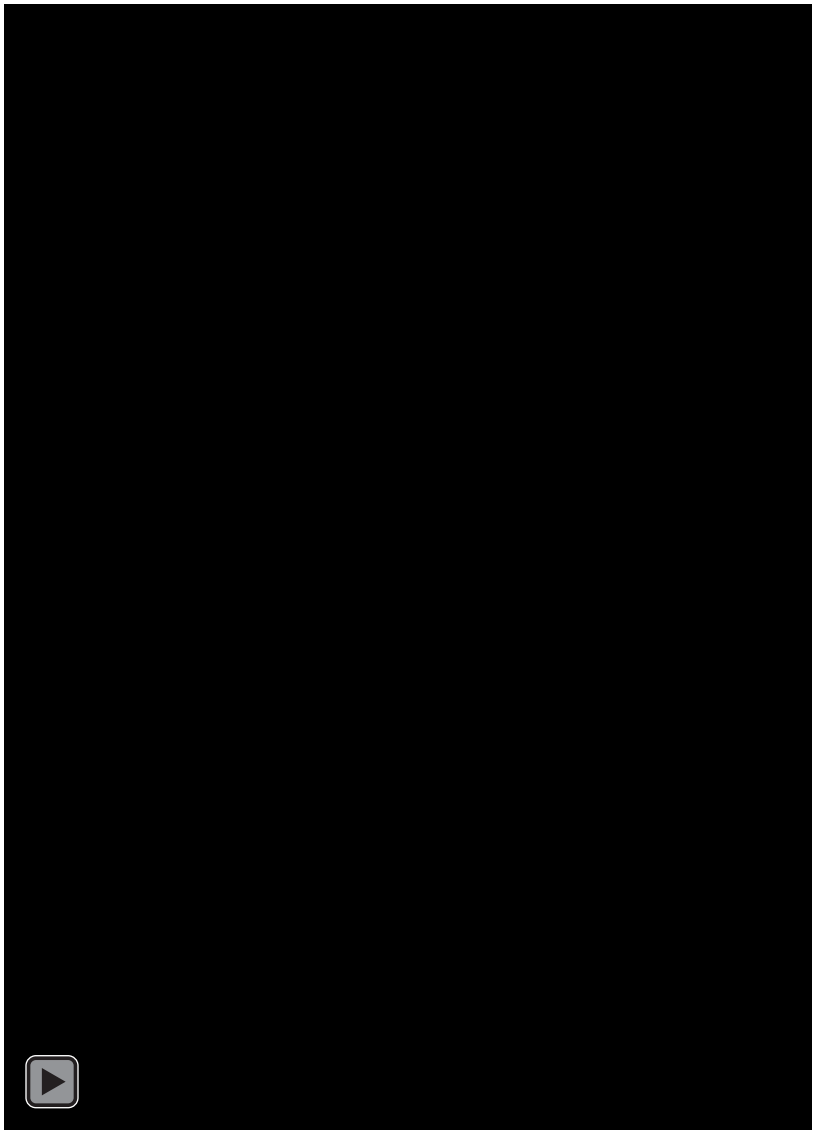


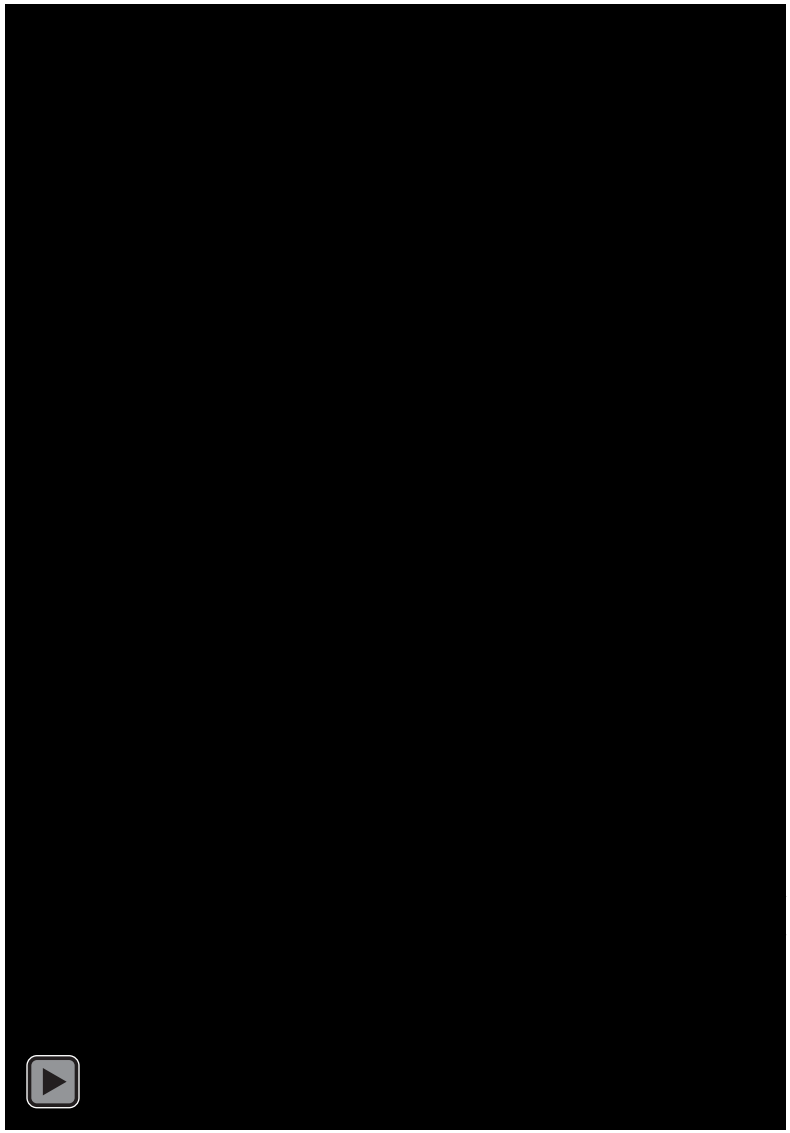
K Luby-Phelps et al. (1987) *PNAS*, **84** (14) 4910-4913

Li, M. *et al.* (2012). *Science China Life Sciences*, **55**(11), 968–973.

E.F. Schibber *et al.*, *Proc. R. Soc. London A*, **476**: 20190692 (2020).

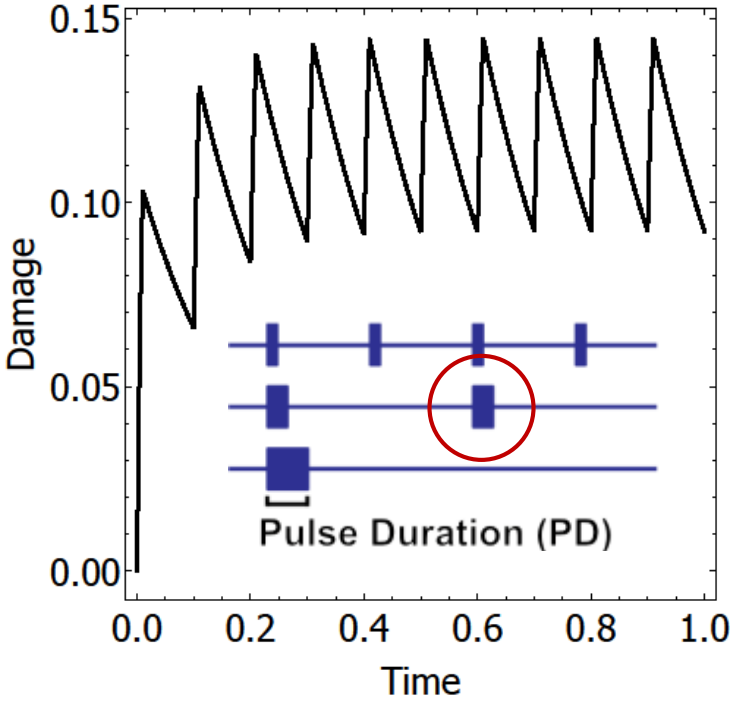
Continuum FE calculations

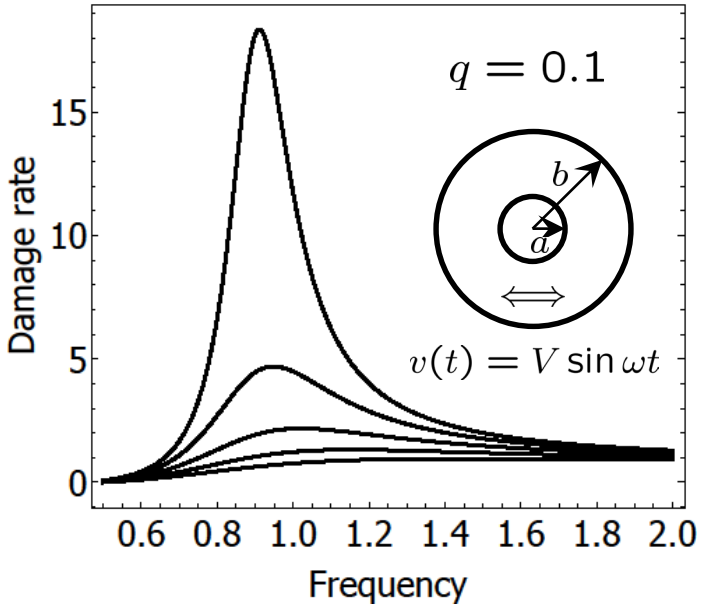


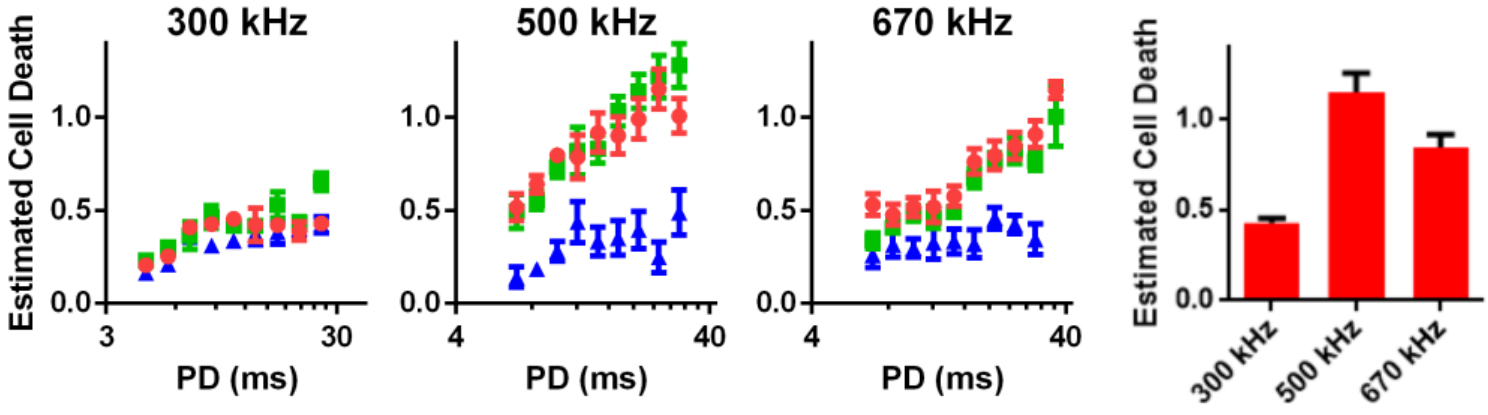


(Units: mm, ms)

Cell life *vs* frequency and pulse duration







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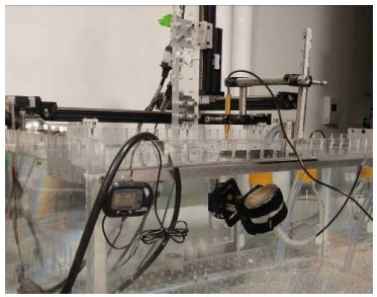
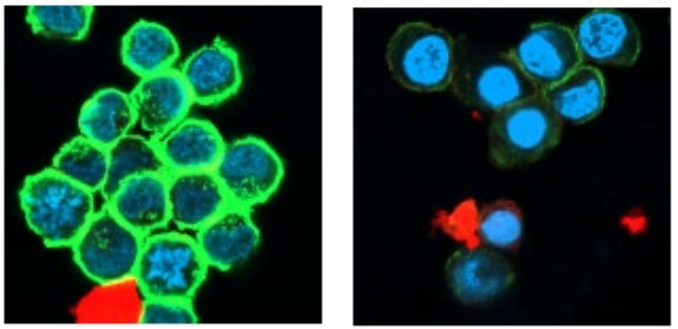
Dead-cell fraction *vs* time



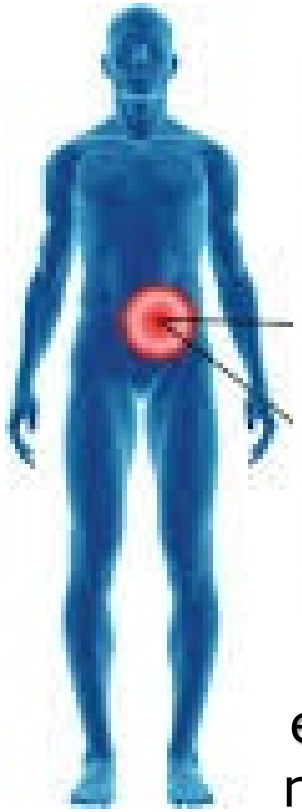
- Assume: Death when damage attains value q_c
- Assume: Log-normal variability of cells
- *Cell fatigue explains observations on oncotripsy for cells in aqueous suspension!*

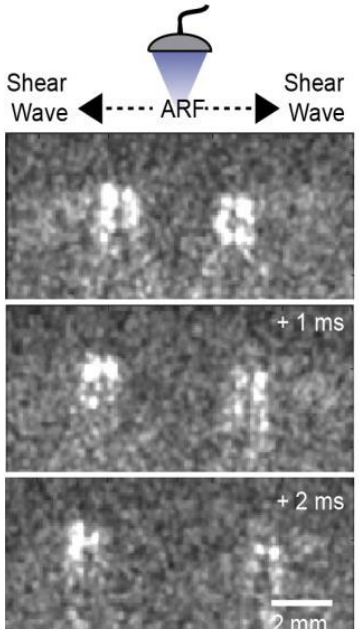


E.F. Schibber *et al.*, *Proc. R. Soc. London A*, **476**: 20190692 (2020).

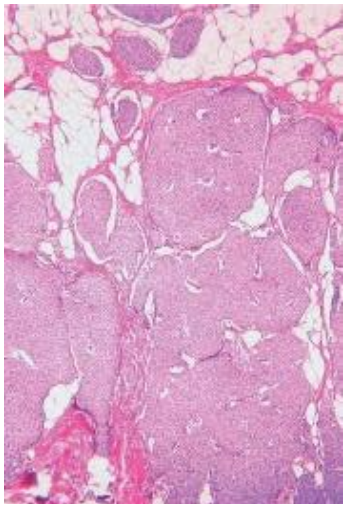
Oncotripsy - Outlook

- *Ultimate goal: Personalized oncotripsy/immunotherapy!*

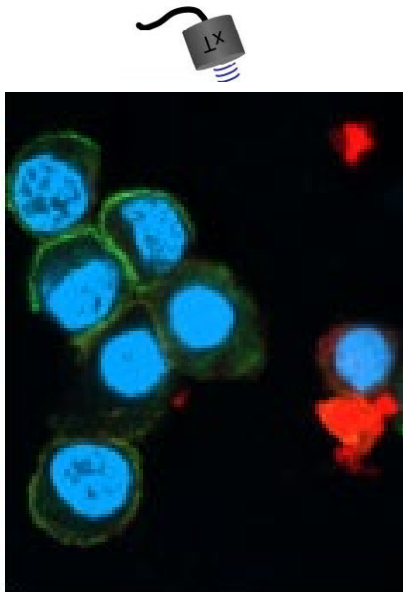




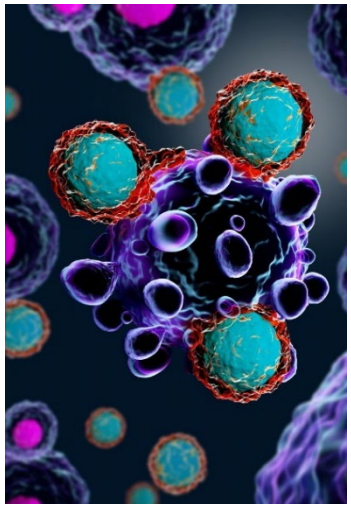
In situ elastographic measurement of dispersion relation of tissue



Inverse scattering identification of histological data, cell parameters



Patient-specific oncotripsy to disrupt target cell population



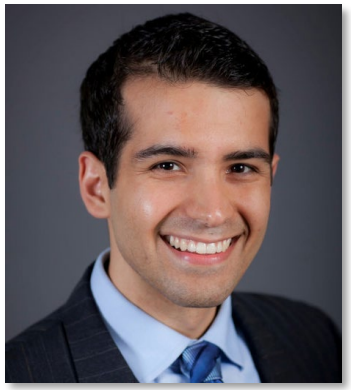
Efficacy increased through immunotherapy

Lecture plan

- *Oncotripsy*: Targeting cancerous cells selectively with tuned low-intensity pulsed ultrasound (*LIPUS*)
  - *Does it work? Experimental study of cells in suspension subjected to LIPUS*
  - *How does it work? The mechanics of healthy vs. cancerous cells (band gaps and resonance), spectral gap* and *cell fatigue*
  - *Model validation: Can we predict cell life, dependence on frequency, amplitude duty cycle…?*
- ***Neuromodulation:*** Targeting neurons selectively with tuned low-intensity focused ultrasound (***LIFUS***)
  - ***Does it work?*** *Can US be focused on precise targets in skull?*
  - ***How does it work?*** *From mechanosensitive Ca++ channels to neuronal activation potential*
  - ***Model validation****: Can we dependence on frequency, amplitude?*
- ***Harnessing the Data Revolution:*** Towards ***patient-specific***, *in situ, in vivo*, ***Data-Driven*** US neuromodulation ***therapies***…

LIFUS across scales: Axon to skull



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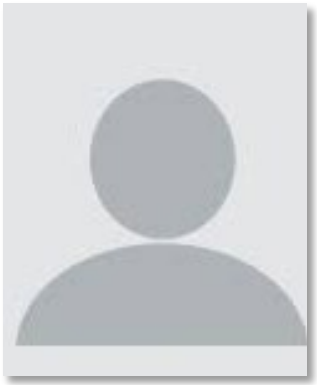


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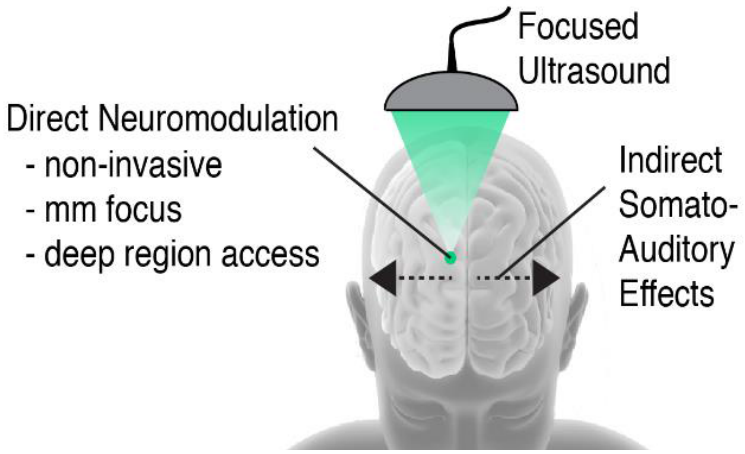
Ultrasound Neuromodulation (UNM)

- Novel non-invasive technique that uses *low intensity focused ultra-sound* (LIFUS) to stimulate the brain.
- Proposed by A. Bystritsky in 2002 as having therapeutic benefits.
- W. Tyler et al. discovered that UNM is stimulates neuron activity.
- UNM is currently used clinically to treat neurological disorders and improving cognitive function.



- UNM has the potential to address deep-brain structures non-invasively with *mm precision*, but also elicits indirect *somato-auditory* effects that need to be eliminated and controlled for widespread clinical use.
- Deployment of UNM therapies in a clinical setting can benefit from *advanced patient-specific data-acquisition and simulation capability*.

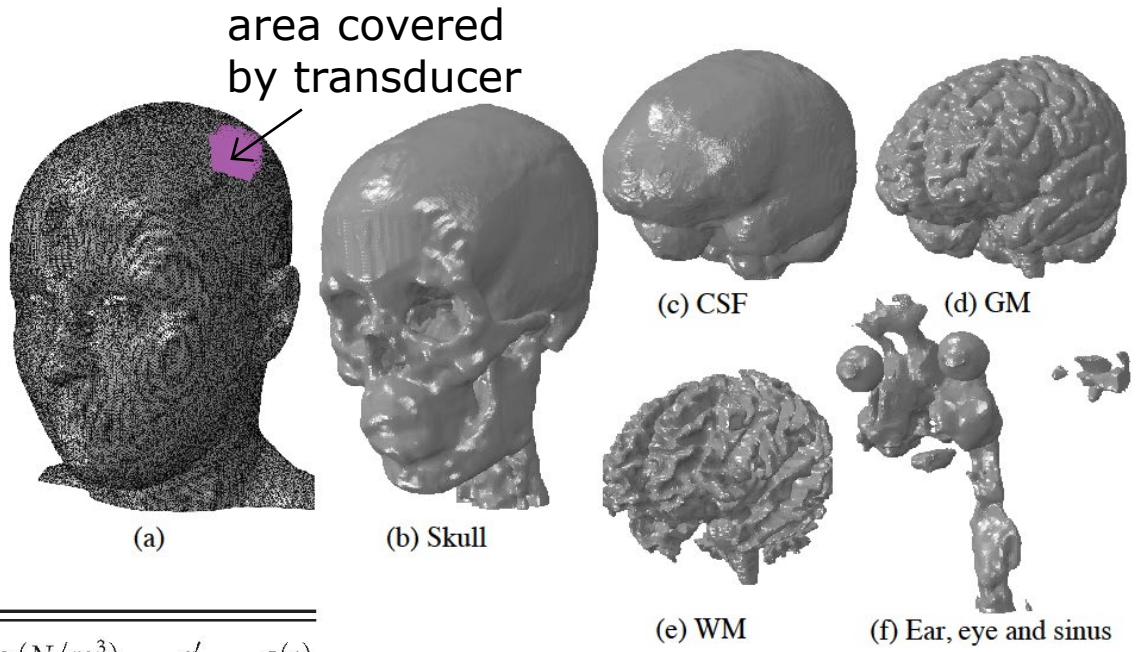
Bystritsky A., USPTO patent 7,283,861, 2002.
Tyler, W.J., Tufail, Y., Finsterwald, M., Tauchmann, M.L., Olson, E.J., Majestic, C., PLoS One. 2008;3(10):e3511.

FEA of US focusing – Forward problem

High-resolution solid model of the human cranium[1]:

a) Complete model:
   i. 8.5 million nodes
   ii. 48.5 million tet FE elements.

b) skull; c) cerebrospinal fluid; d) gray matter e) white matter; f) ear, eye and sinus.



	κ (Pa)	G (Pa)	$\rho\,(N/m^3)$	g'	$\tau(s)$
Skull	4.76×10^9	3.28×10^9	1721	…	…
Scalp	3.36×10^9	6.7×10^5	1100	0.6	3e-5
GM	1.2×10^9	1.2×10^3	1060	0.8	80
WM	1.5×10^9	1.5×10^3	1060	0.8	80
CSF	1.33×10^9	20	1040	…	…
Ear/Sinus	8.33×10^5	3.85×10^5	1000	…	…
Eye	1.13×10^7	2.28×10^3	1078	…	…

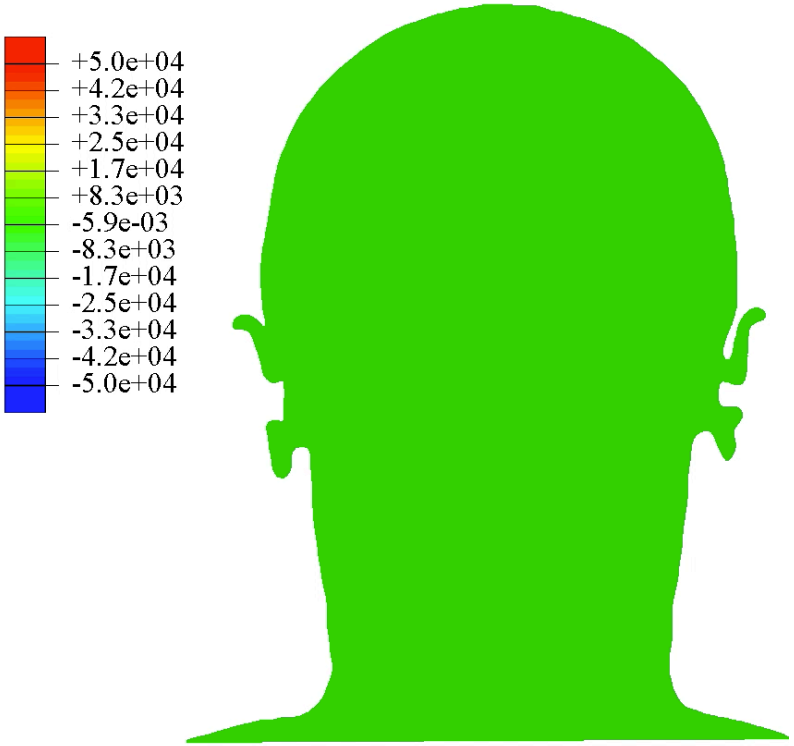
Mechanical properties of tissues[2]:

i) Bulk modulus
ii) Shear modulus
iii) Mass density
iv) Viscoelastic constants

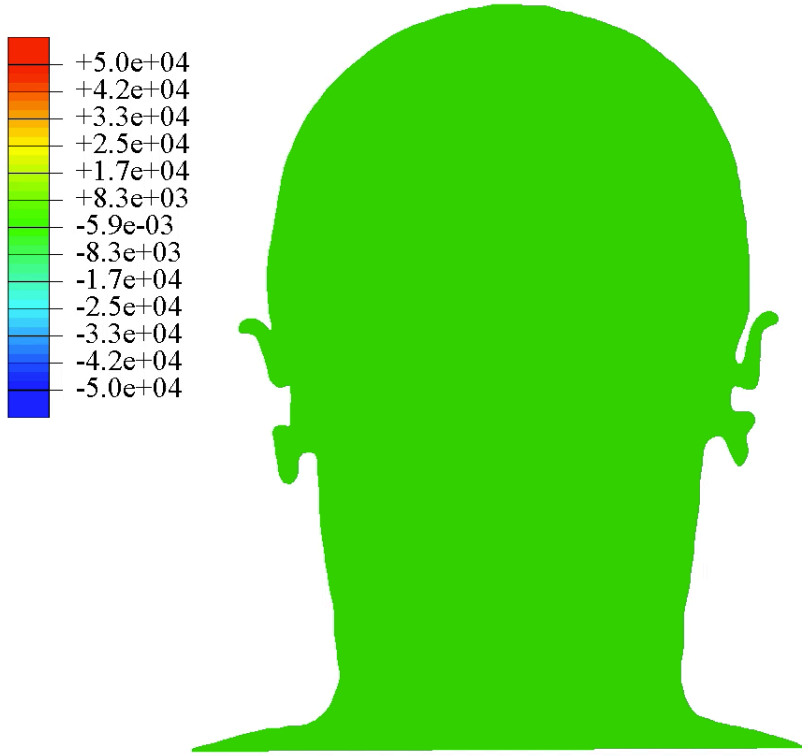
[1]Warner, A., Tate, J., Burton, B., and Johnson, C.R., *bioRxiv*, 552190 (2019).
[2]Salahshoor, S., Shapiro, M. and Ortiz, M. *Appl. Phys. Lett.* **117**, 033702 (2020).

FEA of US focusing – Forward problem

Pressure



100 kHz



Shear

Coronal cross sections of *complex pressure and shear waves* due to the application of continuous ultrasound of amplitude 0.6 MPa and frequency of 200 kHz to a region proximal to the intersection of parietal and temporal regions of the cranium.

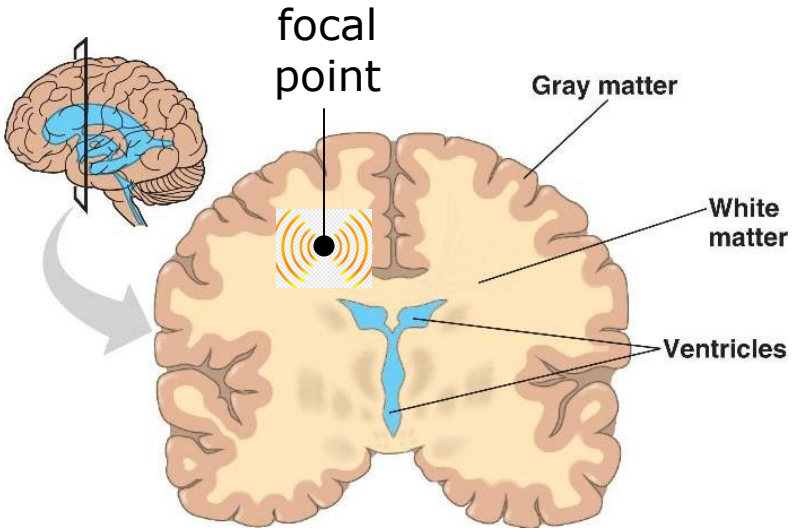
Salahshoor, S., Shapiro, M. and Ortiz, M. *Appl. Phys. Lett.* **117**, 033702 (2020)

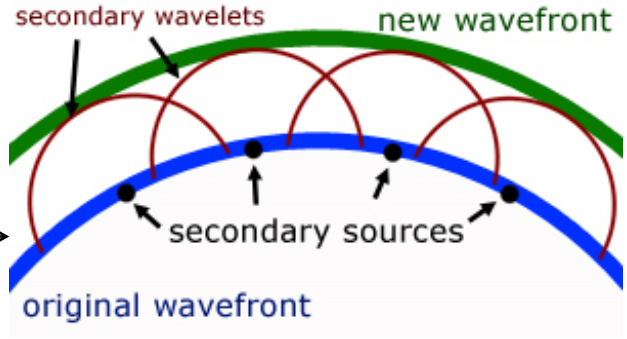
FEA of US focusing – Inverse problem

- Can US from transducers *really be focused* on deep brain structures with mm accuracy and control?
- What is the *optimal arrangement of transducers* required to focus US on a desired point of the brain?
- *Inverse problem!*
- *Near-field* solution at focal point: Bessel functions of first kind,

$$p(r) = B\,\frac{\sin(kr)}{kr}, \qquad k = \frac{\omega}{c}.$$

- Propagate to the boundary by *Huygen's construction*:
- *Optimal distribution and modulation of trans-ducers over the skull!*
- *But: Need patient-specific imaging/mechanical data*





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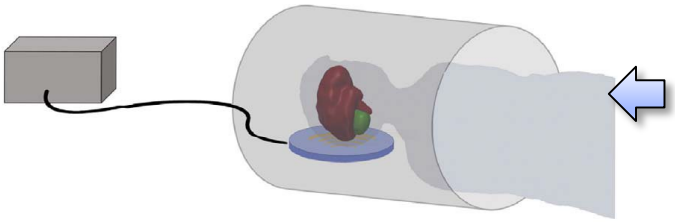
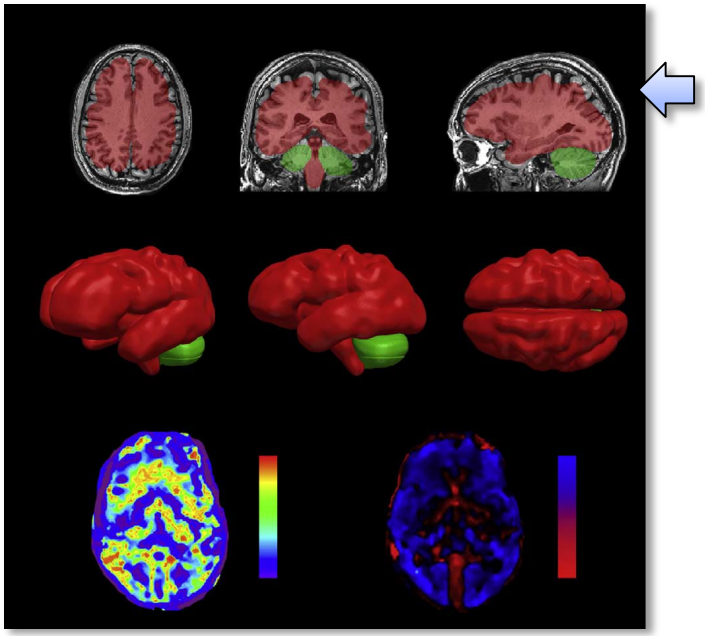
Towards optimized patient-specific UNM

- Data can be acquired *in vivo* through Magnetic Resonance Elastography (EMR).
- MRE is based on the magnetic resonance imaging of shear wave propagation.

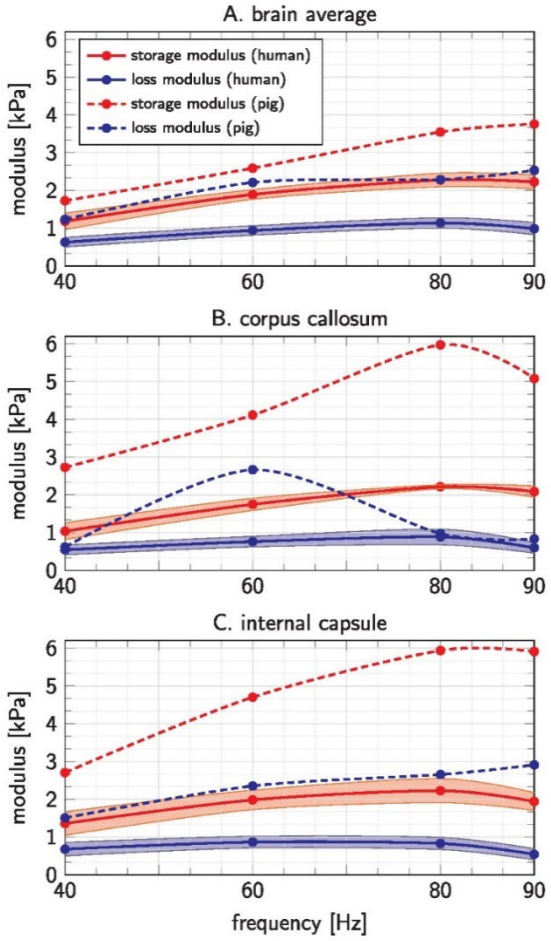


Human subjects scanned in the supine position



Structural scan, reconstruction map of storage and loss moduli

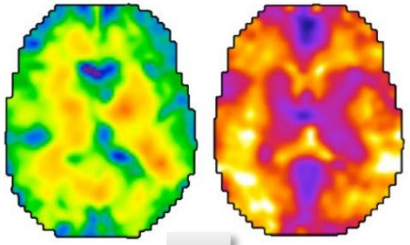
Region-specific storage and loss moduli for human and porcine brains as functions of driving frequency



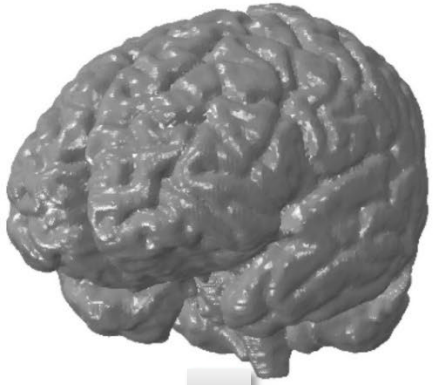
J. Weickenmeier, M. Kurt, E. Ozkaya, M. Wintermark, K.B. Pauly, E. Kuhl, *J. Mech. Behav. Biomed. Mater.*, **77** (2018) 702-710.

Towards optimized patient-specific UNM

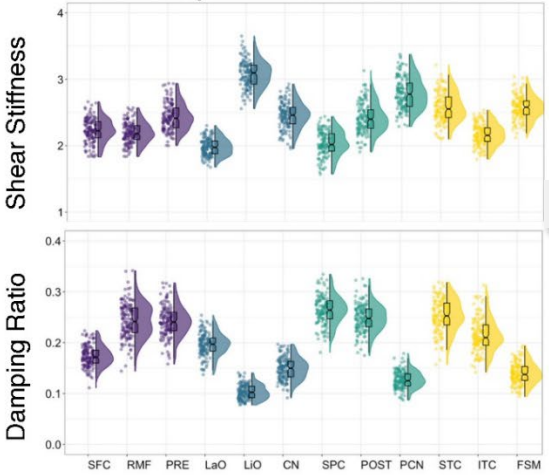
Experiment:
In-situ data acquisition (MRI & MRE)



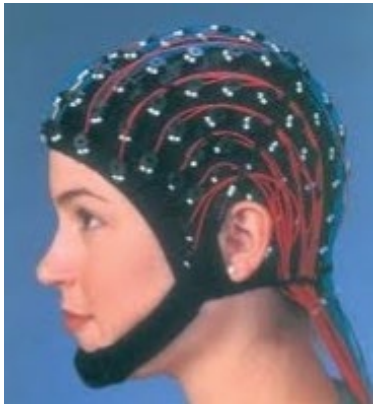
Anatomical Data



Brain Matter Data Repository:
Patient-specific viscoelastic atlas



Forward/inverse analysis:
Optimization with feedback control



L.V. Hiscox *et al.*, *Hum Brain Mapp.*, 2020;**41**:5282–5300.
H. Salahshoor and M. Ortiz, bioRxiv 2022.09.01.506248, Sept 1, 2022.

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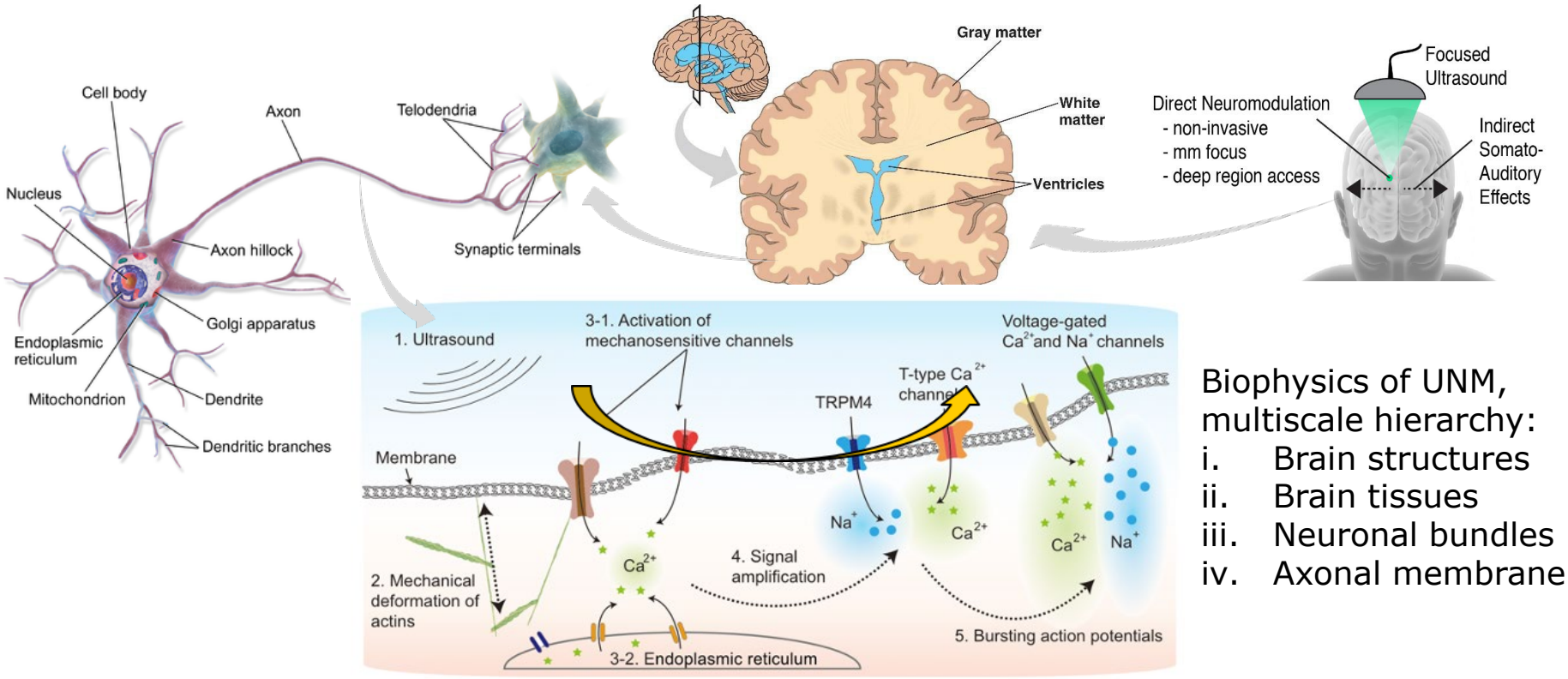
Multiscale biophysics of UNM



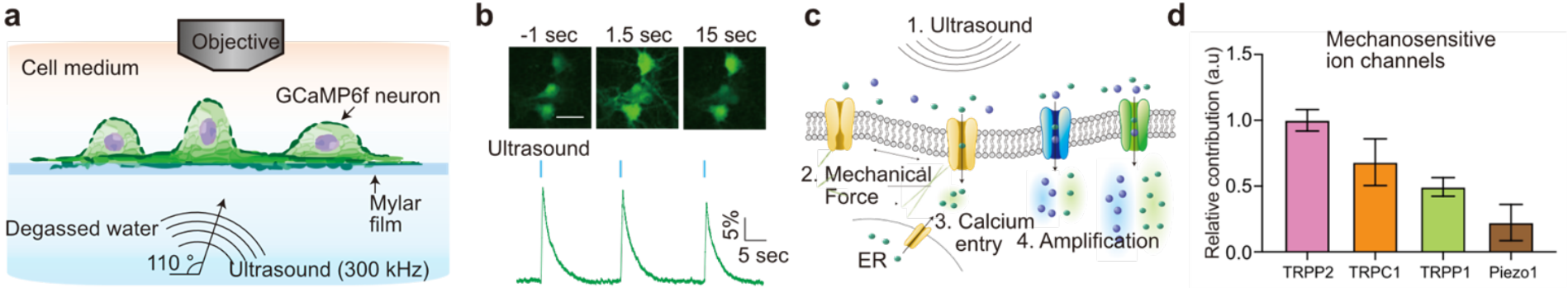
Biophysics of UNM, multiscale hierarchy:

i. Brain structures
ii. Brain tissues
iii. Neuronal bundles
iv. Axonal membrane

- *Aims and challenges:*
  - *Characterize, model, and validate the neurobiological, cellular, and circuit responses of neuronal cells to US stimulation.*
  - *Understand the biological and bio-informatic content of signals recorded from neuronal cells and circuits.*

Multiscale biophysics of UNM

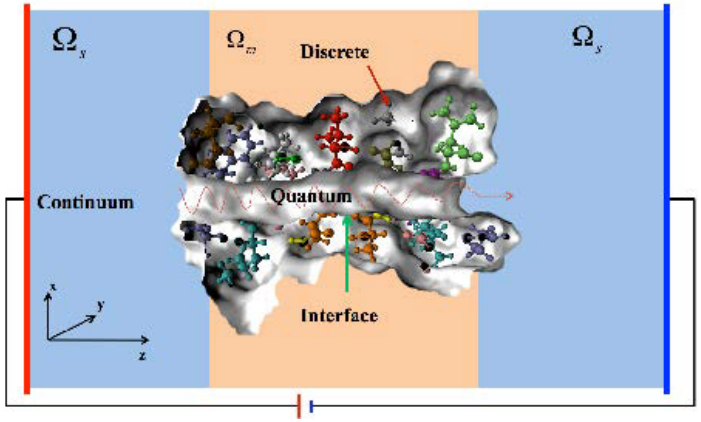
- The underlying *cellular and molecular mechanisms* of ultrasonic neuromodulation remain the subject of conjecture and debate…
- *Hypothesized mechanisms:* Temperature elevation; acoustic radiation force; acoustic streaming; cavitation; intramembrane cavitation; large-scale deformation; flexoelectricity; activation of mechanosensitive channels...



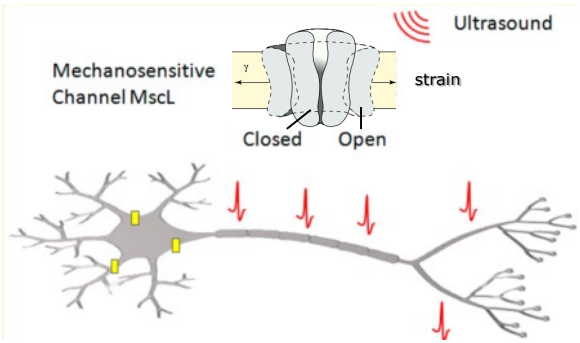
a) Yoo et al[1]: Optical setup for visualizing effects of FUS on cultured neurons.
b) Images and traces of GCaMP6f fluorescence changes in response to US.
c) Molecular pathway: Mechanosensitive Ca++ channels, signal amplification.
d) Contribution of specific mechanosensitive ion channels to UNM.

[1]Yoo, S., Mittelstein, D.R., Hurt, R.C., Lacroix, J., and Shapiro, M.G. (2022). *Nat. Commun.* **13**, 493.

Multiscale biophysics of UNM

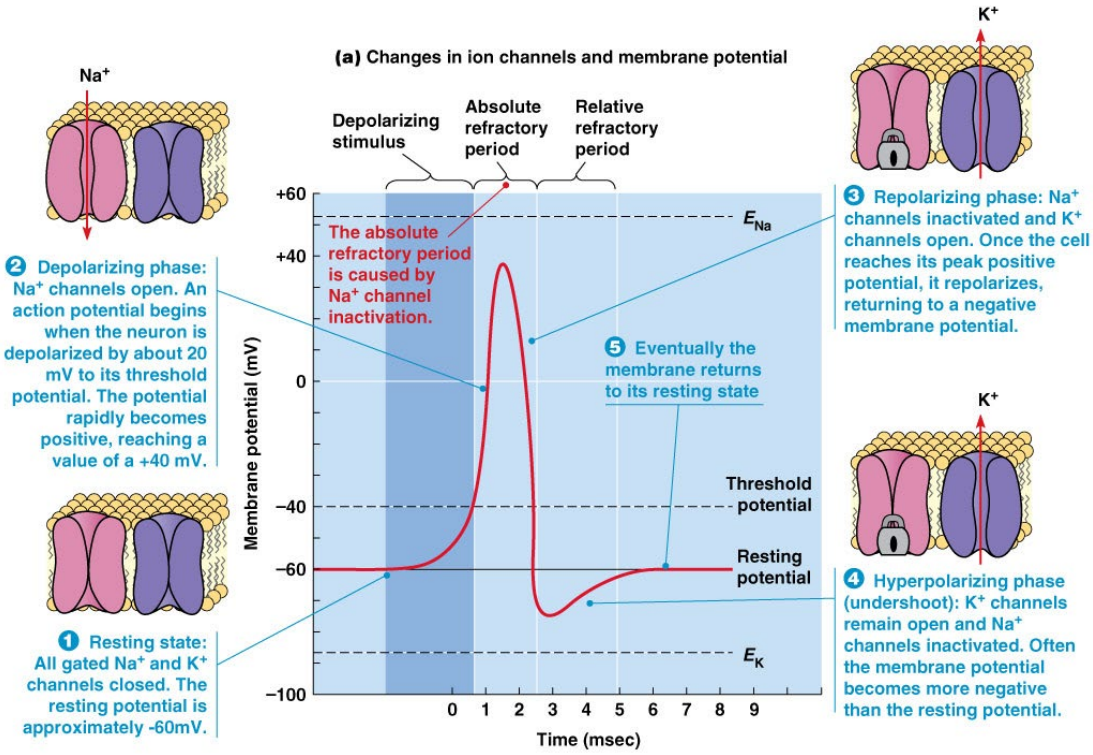


a) *Ca⁺⁺ ion channel conductance* is computed from Poisson-Nernst-Planck (PNP) transport model as a function of channel aperture.



b) Ca⁺⁺ channels are modelled as *two-state systems* (open/closed) coupled to the applied US strain

- *Our multiscale model of UNM:*



c) *Hodgin-Huxley (HH) model* of the axonal action potential is *augmented* to account for Ca⁺⁺ channel activation using time-dependent effective conductance from (a) and (b) to predict *signal amplification*

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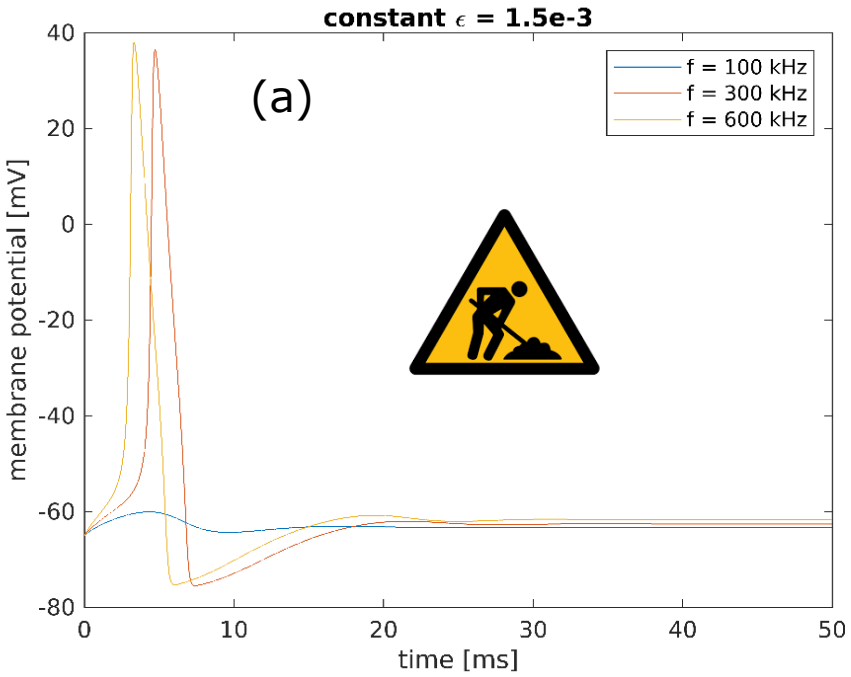
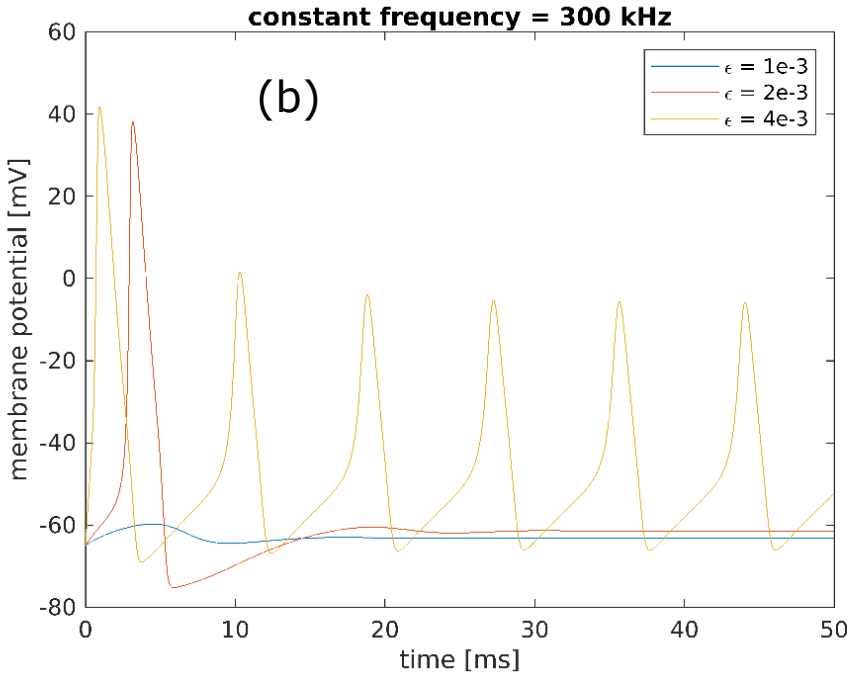
Multiscale biophysics of UNM





Preliminary calculations (courtesy L. Werneck) of axonal activation potential *vs*. time using ***multiscale model*** (two-state Ca^{++} channels coupled to elastic strain field of the axon coupled to HH model of the action potential through effective conductance) a) Effect of ***insonation frequency***, showing ***threshold*** for activation in the US range; b) Effect of ***strain amplitude***, implying ***threshold*** US amplitude for activation

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Lecture plan

- *Oncotripsy*: Targeting cancerous cells selectively with tuned low-intensity pulsed ultrasound (*LIPUS*)
  - *Does it work? Experimental study of cells in suspension subjected to LIPUS*
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  - *Model validation: Can we dependence on frequency, amplitude?*
- *Harnessing the Data Revolution:* Towards *patient-specific*, *in situ, in vivo*, *Data-Driven* US neuromodulation *therapies*…

Concluding remarks

- Despite (or perhaps because) epochal advances in non-invasive imaging, microscopy, molecular biochemistry … the fields of biology and medicine remain mostly *empirical* (trial and error) and *descriptive* (taxonomy)
- *Medical engineering* requires *quantitive models* for purposes of device *design, optimization and control* of therapeutic/clinical procedures
- Simple *physically-inspired models* supply a first level of quantitative understanding of complex phenomena such as *oncotripsy* and *neuromodulation*
- *Data-Driven computing* (machine learning, unsupervised learning…) introduces a novel and powerful approach to quantitative prediction and a pathway to the utilization of *in situ*, *in vivo*, data acquisition techniques in support of *patient-specific medicine*

Concluding remarks

Thank you!