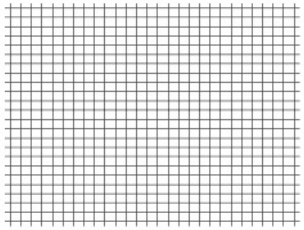
Does low intensity ultrasound have biological effects?

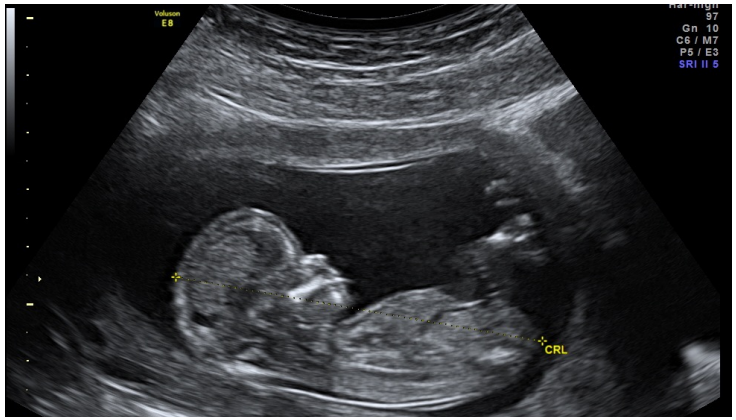
Ultrasound effects on cells and tissue



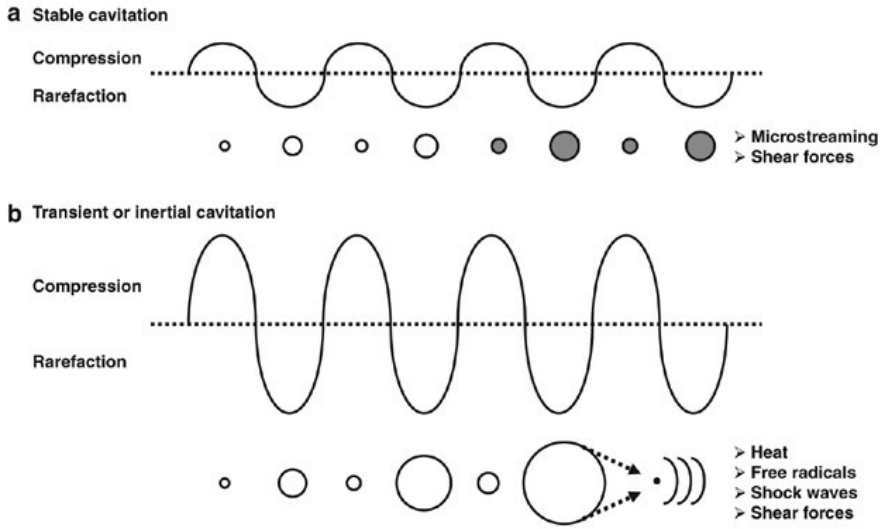
Pressure wave generates forces

$F = P/A$

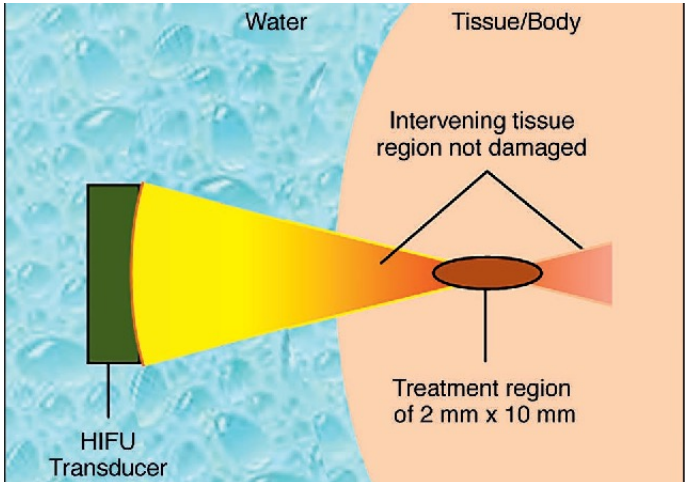
Imaging



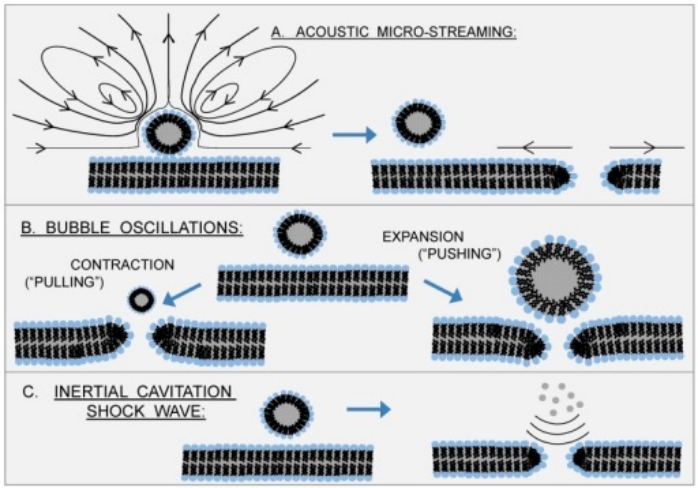
Cavitation



Heating



Microbubbles



Low intensity [pulsed] US (LI[P]US): No heating, no cavitation, no microbubbles

Medical low frequency US

Table 1
General characteristics of LF ultrasound for medical applications.

Intensity range	Application	Description	Low-frequency range	Temporal-average intensity[a]
Low intensity	Sonophoresis; Glucose extraction; Ultrasonic drug delivery to the brain	• Sonophoresis: Transdermal drug delivery • Glucose extraction: Extraction of glucose or other material from the skin in an opposite direction compared to sonophoresis	20–100 kHz (Polat et al., 2011a,b) Drug delivery to the brain: 80–180 kHz (Mitragotri, 2005)	0.05–3.5 W cm^{-2} (Polat et al., 2011a,b)
	Cosmetic applications	• Skin scrubbing, • Microderm-abrasion – a method for facial rejuvenation	20–500 kHz (Esenaliev, 2006)	Various intensities (Esenaliev, 2006)
High intensity	Dentistry	Two main applications are: • Dental descaling (tooth cleaning and calculus removal) • Root canal therapy	25–42 kHz (Arabaci et al., 2007; Plotino et al., 2007)	Equivalent of 0.58 W cm^{-2} (Walmsley, 1988a)[b]
	Lithotripsy	Extracorporeal shock wave lithotripsy (ESWL) -the non-invasive breaking of kidney stones using acoustic pulses	100–200 kHz (Miller, 2007)	In the range of 10 MPa (Miller, 2007)
	Sonothrombolysis	Sonothrombolysis – the break down (lysis) of blood clots using acoustic waves	20–25 kHz (Siegel and Luo, 2008)	0.5–50 W cm^{-2} (Nesser et al., 2002)
	Surgical	Tissue dissection and fragmentation	20–60 kHz (O'Daly et al., 2008)	10–850 W cm^{-2} (O'Daly et al., 2008)
	Lipoplasty	Ultrasonic lipoplasty – the use of ultrasound waves to loosen fat beneath the skin's surface before its removal by means of suction	20–50 kHz (Mitragotri, 2005; Cooter et al., 2001)	10–300 W cm^{-2} (Cooter et al., 2001)
	Phacoemulsification	Phacoemulsification cataract surgery - break up and removal of a cloudy lens, or cataract, from the eye to improve vision	25–62 kHz (Fine et al., 2002)	Up to 1000 W cm^{-2} continuous wave (Topaz et al., 2002)

Thermal effect of US

- Ultrasound is attenuated in tissue
- $\frac{dQ}{dt} = 2\alpha I_{TA} = \frac{\alpha p p^*}{\rho c}$
- $\alpha(\nu) = \frac{\alpha}{\nu_0}\nu = \alpha'\nu$ or
- $\alpha(\nu) = \alpha_0 \left(\frac{\nu}{\nu_0}\right)^n, n \in [1,2]$
- $P(x) = P_0 e^{-\alpha x}, \quad \ln\left(\frac{P}{P_0}\right) = -\alpha x$
- How do I relate «attenuation coefficient» in dB/cm to the alphas?

α'

Tissue/Medium	Attenuation Coefficient (dB/cm/MHz)	Acoustic Impedance (Mrayl)
Water	0.0022	1.5
Blood	0.15	1.6
Soft tissue	0.75	1.6
Air	7.50	0.00001
Bone	15.0	8.0
Fat	0.63	1.4
Kidney	1.0	1.6
Lens of eye	0.05	1.7

α_0

Body Tissue	Attenuation Coefficient (dB/cm at 1MHz)
Water	0.002
Blood	0.18
Fat	0.63
Liver	0.5-0.94
Kidney	1.0
Muscle	1.3-3.3
Bone	5

Table 4
Summary of the bio-effects of LF ultrasound contact exposure

Test Set	Body part	Exposure case	Coupling	Frequency/mode	Exposure duration	Intensity/power	Results	Author Comments	Author
Rats	Brain	In vivo	Water/Transducer at 5 mm above the skull	20 kHz/continuous wave	20 min	0, 0.2, 0.5, 1.1 and 2.6 W cm^{-2}	No changes detected for the group of rats with low power output sonication (0.2 W cm^{-2}) Intensities of 0.5 and 1.1 W cm^{-2}, caused cytotoxic edema 2.6 W cm^{-2} caused significant neuron loss.	Low-frequency ultrasound represents a potential hazard to healthy brain tissue in a dose dependent fashion. The observed bio-effect was considered non-thermal.	(Schneider and Gerriets, 2006)
Human	Blood cells	In vitro	PBS (water based)	20–100 kHz/ continuous wave	1 min	20 kPa	Total destruction of blood cells		(Nagel and Nagel, 1999)
Human	Blood vessels	In vivo	Gel based	29 kHz/Pulsed, duty cycle 30%, pulse repetition frequency 25 Hz.	1,2,3 min	I_{SATA} (spatial-average, temporal-average intensity) of about 0.12 W cm^{-2}.	Human brachial artery would be dilated when exposed to transdermal low-frequency ultrasound.	The mechanism through which the brachial artery is dilated by low-frequency ultrasound might be a result of local vibrations and stimulation of endothelial cells.	(Lida and Luo, 2006)
Pig	Lungs	In vitro	Water at 10 and 50 cm distance	22–36 kHz Pulsed (pulse length 4–12 ms with intervals of 0.5–1 s, duty cycle 0.5–1.5%)	2–20 h	SPL of 166–182 dB	No macroscopic lesions. Micro-bleeding was detected through microscopic examination.		(Shupak and Arieli, 1999)
Mice, rabbits and pigs	Lungs	In vivo	Water (distilled/ degassed)	30 kHz/continuous ultrasound	5, 10, 20 min	0, 100 and 145 kPa (equal to 134–197 dB)	At an acoustic pressure level of 145 kPa, for all three exposure durations, severe lung damage occurred for mice, with blood in the chest cavity. Extrapolating the 30 kHz ultrasound threshold findings of animal models to man suggested that the human lung hemorrhage damage acoustic pressure threshold might be in the range of 500 kPa for a 10 min continuous wave exposure duration.		(O'Brien and Zachary, 1994, 1996)
Rabbit	Skin	In vivo	NR	105 kHz Pulsed, pulse length 5 s at 5 s intervals	90 min	5000 Pa pressure amplitude	Examination of the skin by the naked eye/Histological findings by microscope showed no damage to the skin upon ultrasound application.		(Tachibana, 1992)

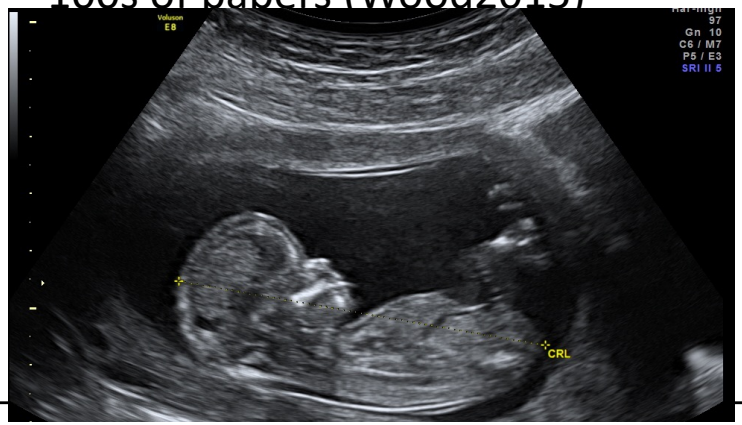
Table 4 (*continued*)

Test Set	Body part	Exposure case	Coupling	Frequency/mode	Exposure duration	Intensity/power	Results	Author Comments	Author
Mice and human	Skin	In vitro	Saline (water based)	48 kHz	5 min	0.5 W cm^{-2}	The effect on mouse skin was much more significant than on human skin. Cells of the stratum corneum of the mouse skin surface were almost completely removed. Electron Microscopy of the surface of the skin shows that Ultrasound-induced large craterlike openings of diameter of 100 μm and injury to the stratum corneum of hairless mouse (as a possible result of micro-jets produced by transient cavitation in the coupling medium).	This effect was attributed mostly to cavitation.	(Yamashita et al., 1997)
Pig	Skin	In vitro/3 mm from the skin	PBS (water based)	19.6–93.4 kHz (continuous wave)	2–14 min	0.2–2.7 W cm^{-2}	The data showed that for each frequency (in the range of 19.6–93.4 kHz), there exists a threshold intensity below which no detectable change (conductivity enhancement in the skin) was observed. The threshold intensity increased with frequency. The threshold intensity for porcine skin increased from about 0.11 W cm^{-2} at 19.6 kHz to more than 2 W cm^{-2} at 93.4 kHz. At a given intensity, the enhancement decreased with increasing ultrasound frequency.	The threshold intensities correspond to the transient cavitation in the aqueous coupling medium.	(Tezel et al., 2001)
Dog	Skin	In vivo	Saline (water based)	20 kHz, 6 s of every second (pulsed mode).	60 s	Maximal energy output of 400 W. intensities of 4%, 10%, 20%, 30% and 50% were applied using three probes, 1-cm cylindrical, 5-cm cylindrical, and 10-cm disc-shaped (diameter).	Low-frequency ultrasound at low intensities appears safe. Higher-intensity ultrasound produced significant thermal effects even second-degree burn.	Skin heating, particularly at the interface between epidermis and dermis appears to be the mechanism.	(Singer et al., 1998)
Human	Skin	In vivo/The transducer at a distance of 1 cm from the skin.	PBS (water based)	20 kHz, pulsed, (5 s on, 5 s off) duty cycle of 50%	2 min	10 W cm^{-2} (spatial-average temporal peak intensity).	Human subjects reported no pain during ultrasound application and no visible effects of the ultrasound were detected on the skin. Low-frequency ultrasound used, did not seem to induce damage to skin or underlying tissues.		(Kost et al., 2000)

Some reported biological effects of LIPUS

Killing cancer cells selectively:

- "Oncotripsy": 4 publ. 2016-2020 (J.Appl.Phys.++)
- US+hyperthermia ⇒ Apoptosis (Feril 2002)
- Mike Sheetz group: Piezo ⇒ Ca^{2+} ⇒ apoptosis
- Apoptosis while other cells increased proliferation (Schuster2013)
- Microbubbles, cavitation or thermal ⇒ 100s of papers (Wood2015)

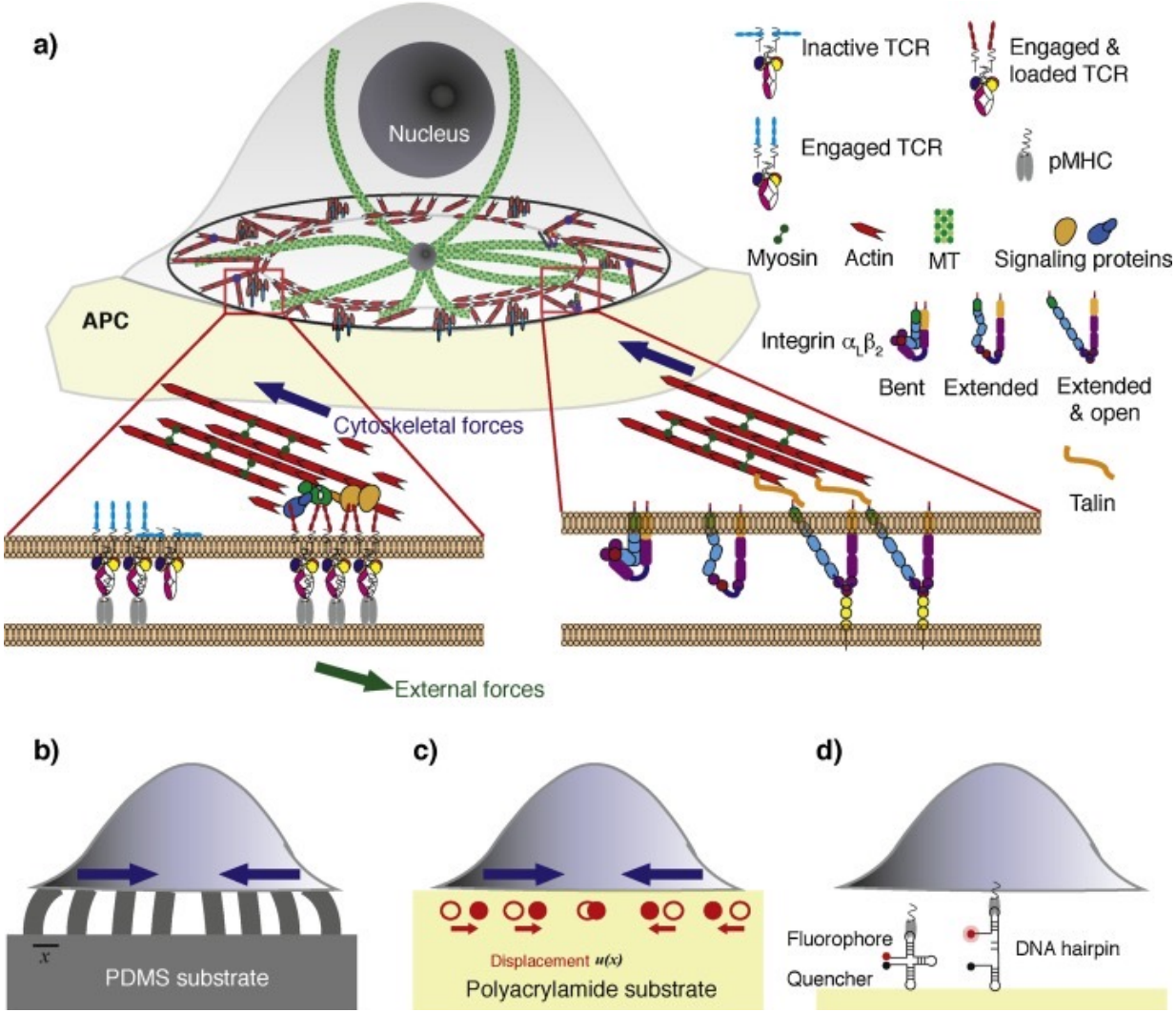


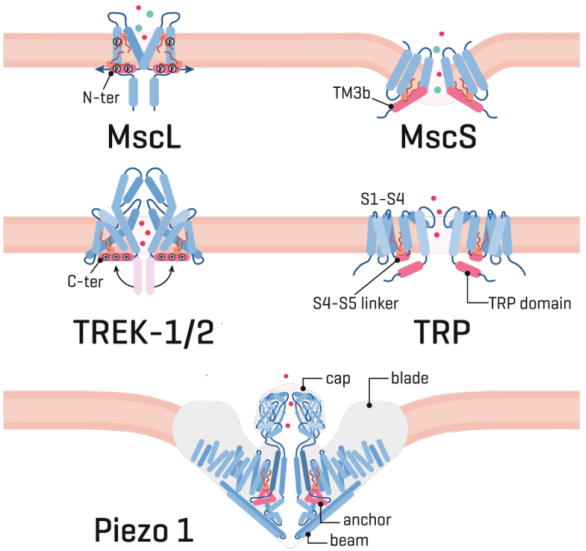
No effect: One single study (Lucas 2021)!

- Increased insulin release from pancreatic beta cells (Castellanos 2017)
- Enhanced diverse transcription factors, increased proliferation (Puts 2016,2018,2018b)
- Improved osteogenic comittment & differentiation (Costa 2018)
- Enhanced viability & proliferation of iPSC (Lv 2013)
- Increased expression of chondrogenic markers (Subramanian group 2012, 2013, 2017)
- Perturbs cytoskeleton dynamics (Misrahi 2012, incl. Dave Weitz!)
- Increased growth & proliferation of stem cells (Gao 2016)
- Modulates ion channel currents (Kubanek 2016)
- Interleaflet cavitation (Kimmel group, PNAS 2011)
- +++++

Mechanosensing

how can cells be affected by US pressure?

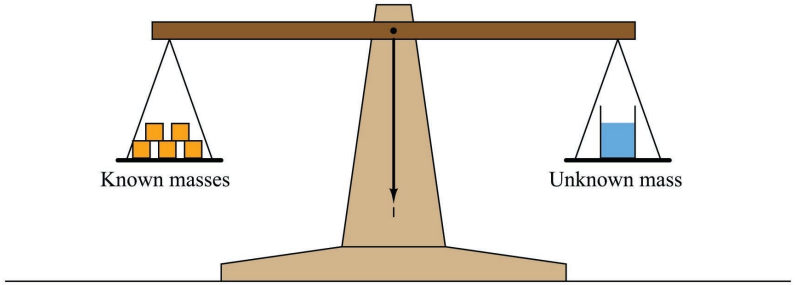




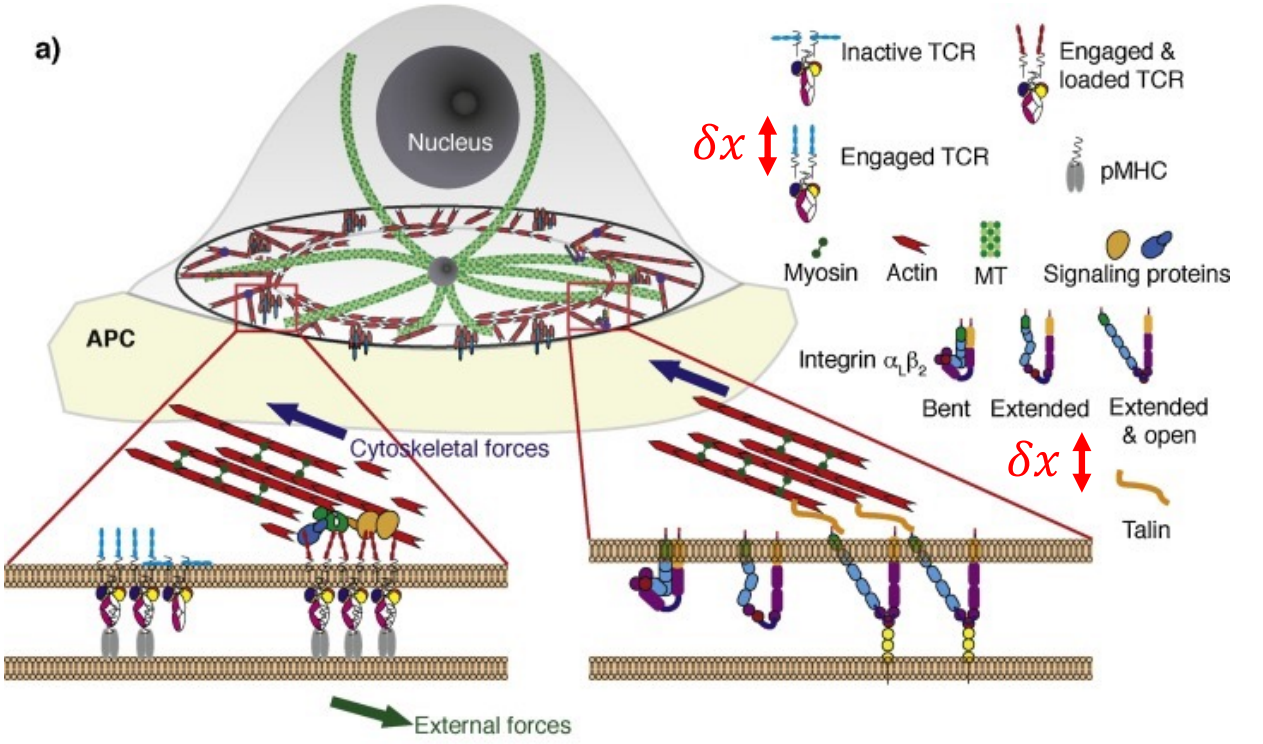
How do you measure a force?

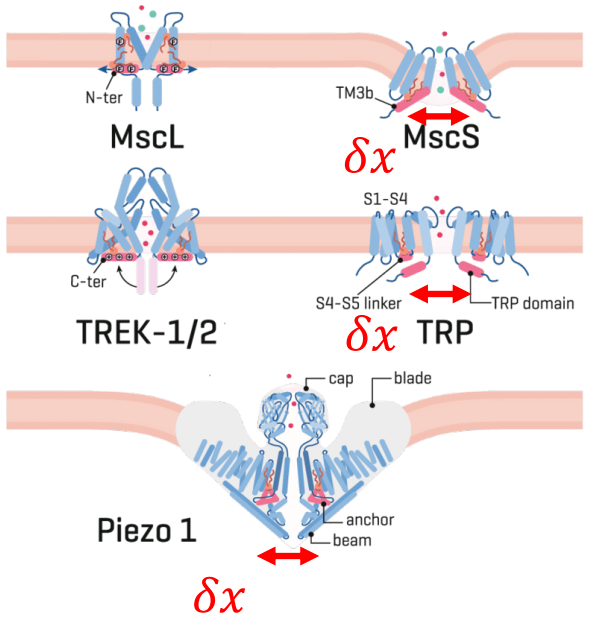
- Newtons law: $f = ma$
- Force balance: $f_1 = f_2$
- Elasticity: $f = \mathrm{k}\delta x$

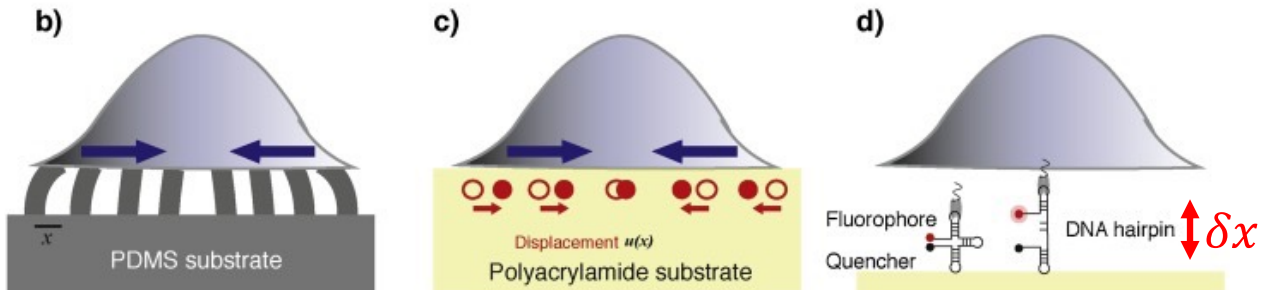
That's how cells do it



Mechanosensing







2-states: open/closed
open probability:

$$P(f) = \frac{1}{1 + e^{-(\Delta F - f\delta x)/kT}}$$

displacement δx is necessary!

Most discussion of mechanosensing molecules are about second - minute time scale. Effectively static

1 MHz	≙	1 μs	conformation changes?
1 GHz	=	1 ns	time to equilibrate ab vacuo cond. time correlation functions
1 THz	=	1 ps	~ 10^3 timesteps in MD

MHz vs × is too slow × to act as extra thermal bath.
× to nucleate phase changes in small volume

× is sufficient time to reach near equilibrium densities and conformations of relatively simple molecules & structures

Stress, strain and waves in homogeneous, isotropic solids

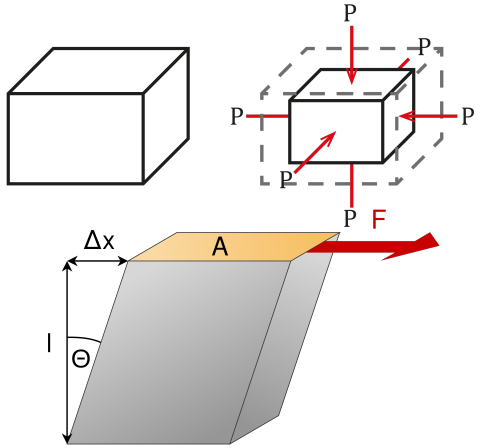
Bulk modulus: $K = -V\frac{dP}{dV} = \frac{\text{normal stress}}{\text{normal strain}}$

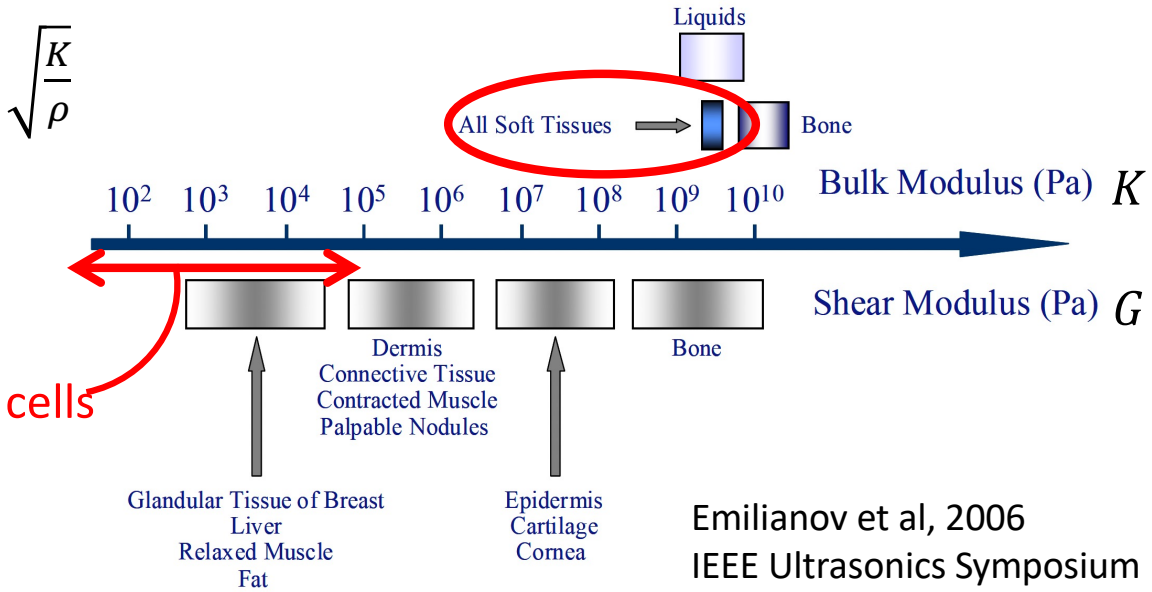
Shear modulus: $G = \frac{\sigma_{xy}}{\gamma_{xy}} = \frac{F/A}{\Delta x/l} = \frac{\text{shear stress}}{\text{shear strain}}$

In soft tissues $K \gg G$

Pressure waves: $v_p = \sqrt{\frac{K+{}^3/_4 G}{\rho}} \approx \sqrt{\frac{K}{\rho}}$

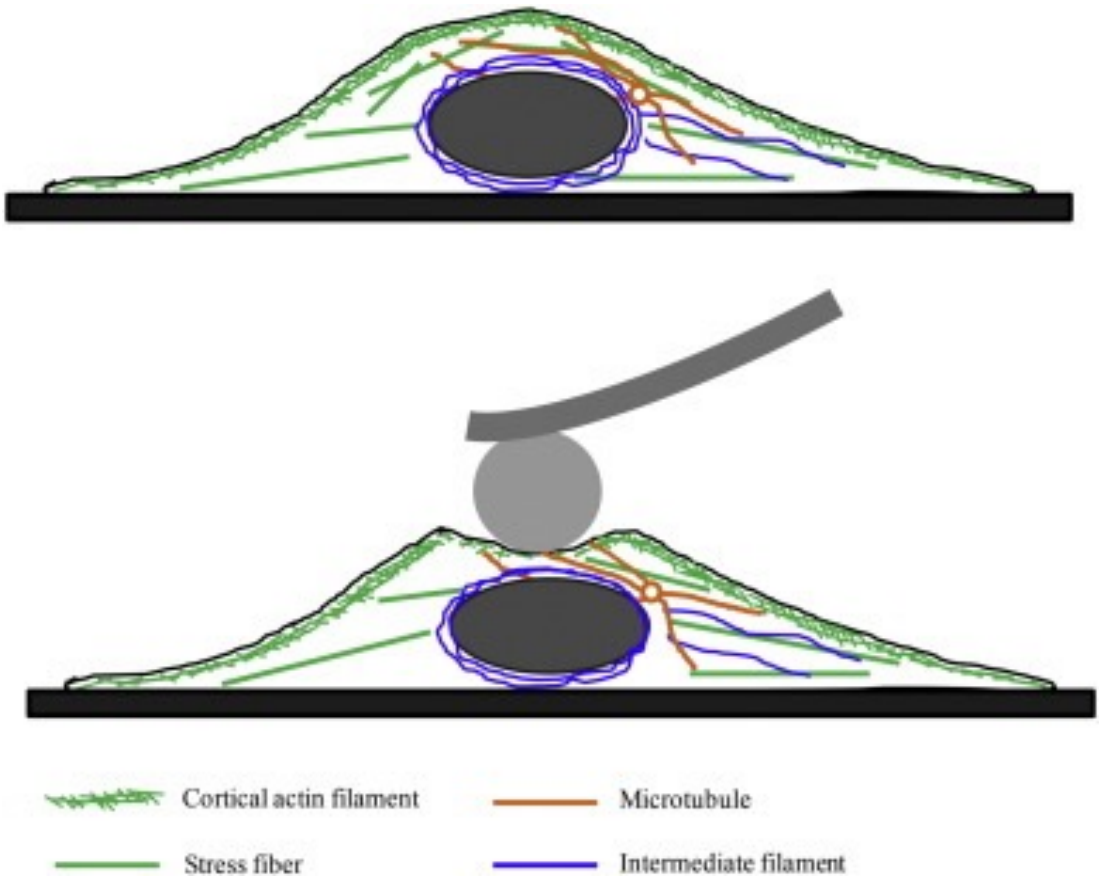
Shear waves: $v_s = \sqrt{{}^G/_\rho}$





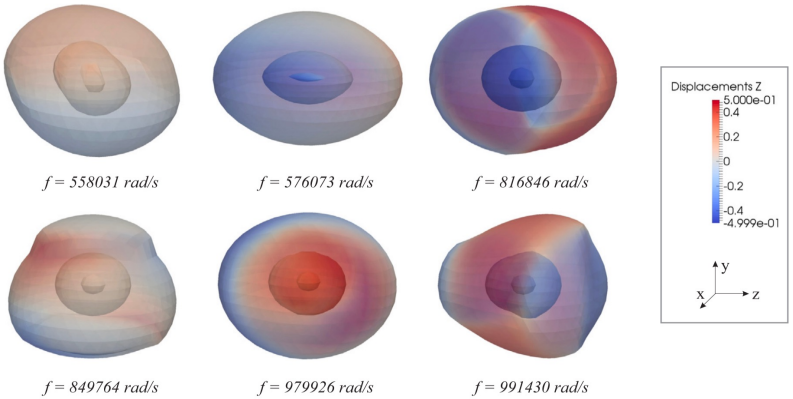
Emilianov et al, 2006
IEEE Ultrasonics Symposium

Soft tissue, soft cells



- "stiffness"
- $= G$: shear modulus
- $\approx \frac{E}{3}$: Youngs modulus
- $\approx 1 - 100$ kPa

Oncotripsy



- Heyden & Ortiz 2016, 2017: theoretical
- Mittelstein et al 2020, J. Appl. Phys: ”Moreover, our experiments revealed that the formation of standing waves and the emergence of cavitation were necessary to disrupt cancer cells.”

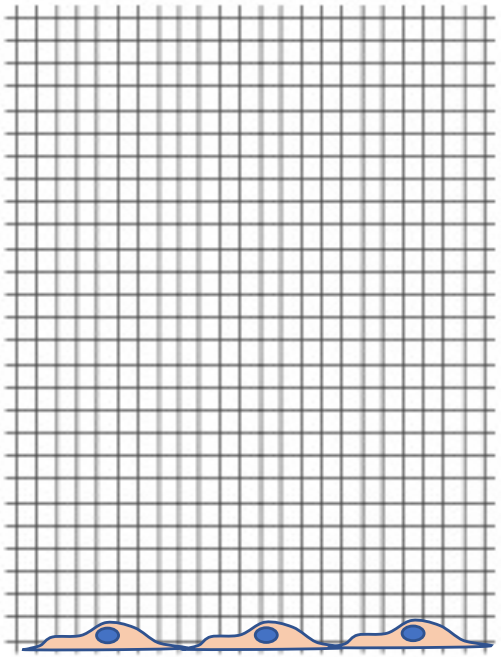
Table 1
Set of constitutive parameters (bulk modulus κ and shear moduli μ_1 and μ_2) used in the eigenfrequency analyses.

	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

water: 2 GPa

- Data based on data fitting of AFM indentation by Kim et al Med Biol Eng Comput (2011) 49:453–462 assuming Poisson ratio ν= 0.499, Heyden & Ortiz assume ν= 0.49!

Sound wave



water, cells: $K \approx 2$ GPa

$$v_p \approx \sqrt{\frac{K}{\rho}} \approx 1500 \text{ m/s}$$

$$\lambda = {}^{v_p}/_{\nu}$$

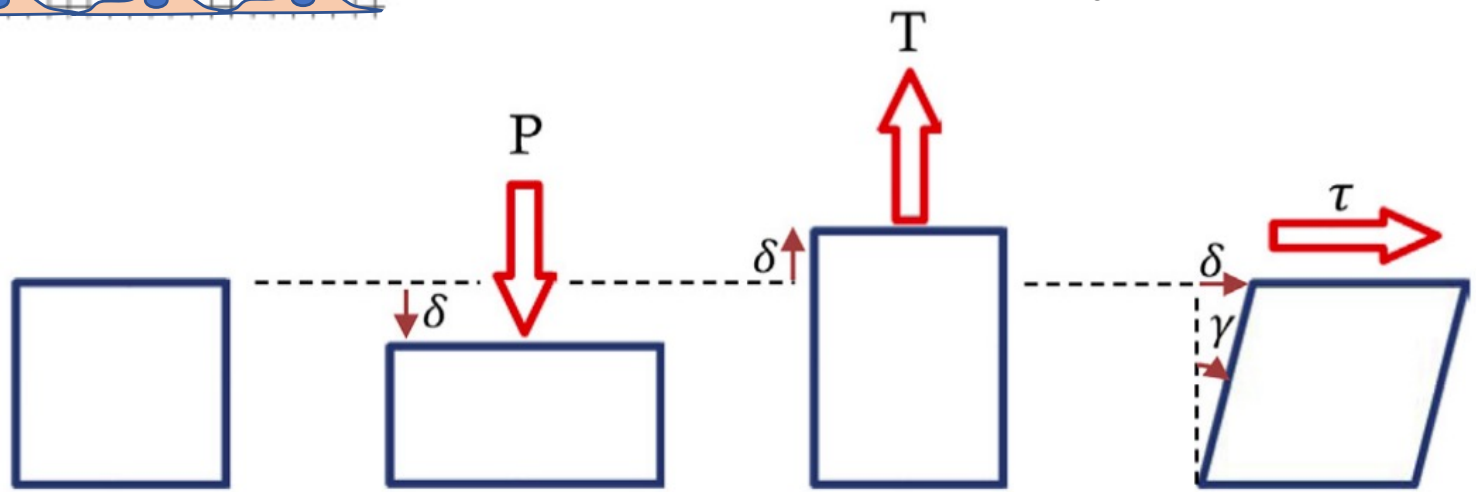
Tissue or Material	Density (g/cm^3)	Speed of Sound (m/sec)
Water	1	1480
Blood	1.055	1575
Fat	0.95	1450
Liver	1.06	1590
Kidney	1.05	1570
Brain	1.03	1550
Heart	1.045	1570
Muscle (along the fibers)	1.065	1575
Muscle (across the fibers)	1.065	1590
Skin	1.15	1730
Eye (lens)	1.04	1650
Eye (vitreous humor)	1.01	1525

20k	100k	1M	10M	1G
7.5cm	1.5cm	1.5mm	150µm	15µm

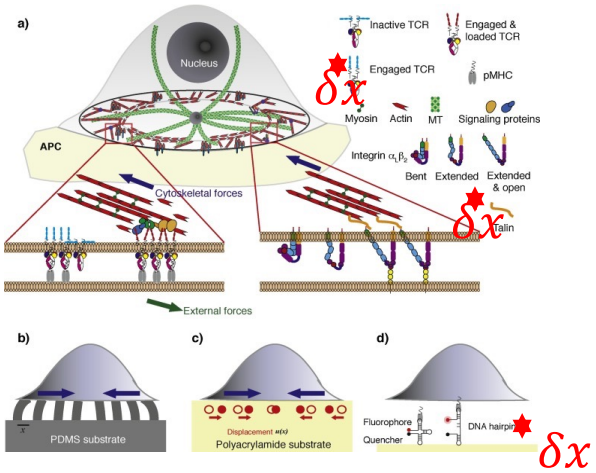
low intensity amplitude: $p_{max} < 10^5$ Pa

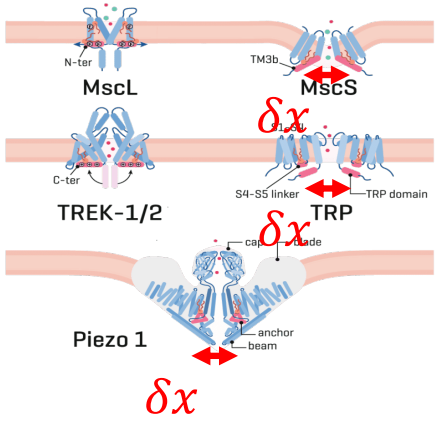
For a cell $l \sim 10\ \mu m$:

- hydrostatic pressure variation
- $\delta = \varepsilon l = \frac{pl}{K} < \frac{10^5 10^{-5}}{2 \cdot 10^9} = \mathbf{5\ Å}, \quad (\varepsilon < 5 \cdot 10^{-5})$



I will not believe it unless I see it!





2-states: open/closed
open probability:

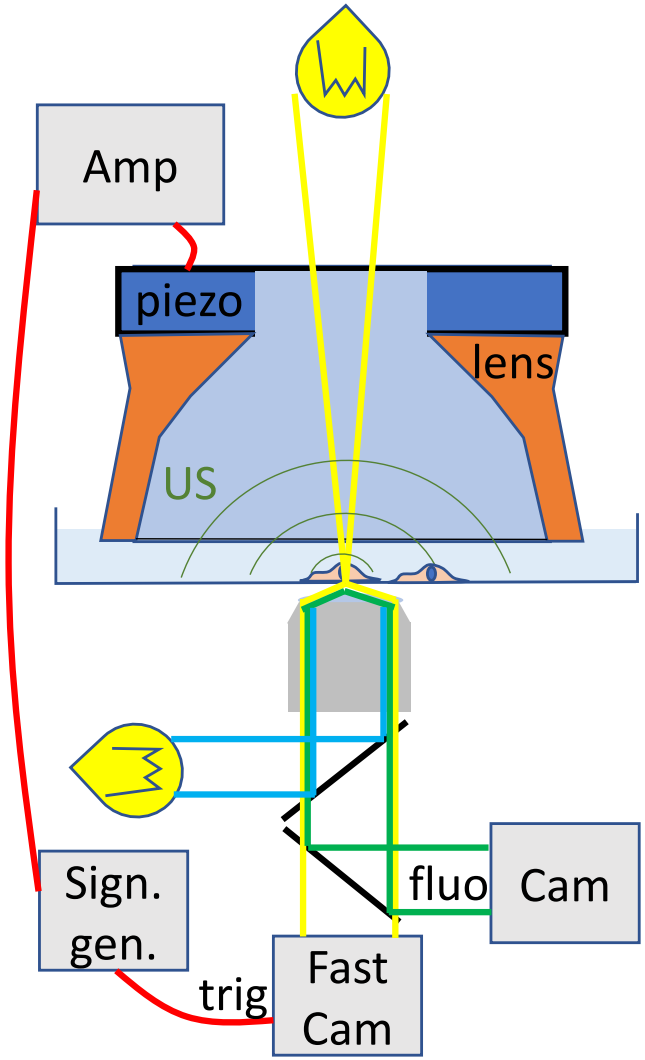
$$P(f) = \frac{1}{1 + e^{-(\Delta F - f\,\delta x)/kT}}$$

$\delta x < 5$ Å

⇒ **Attempt direct measurement of**

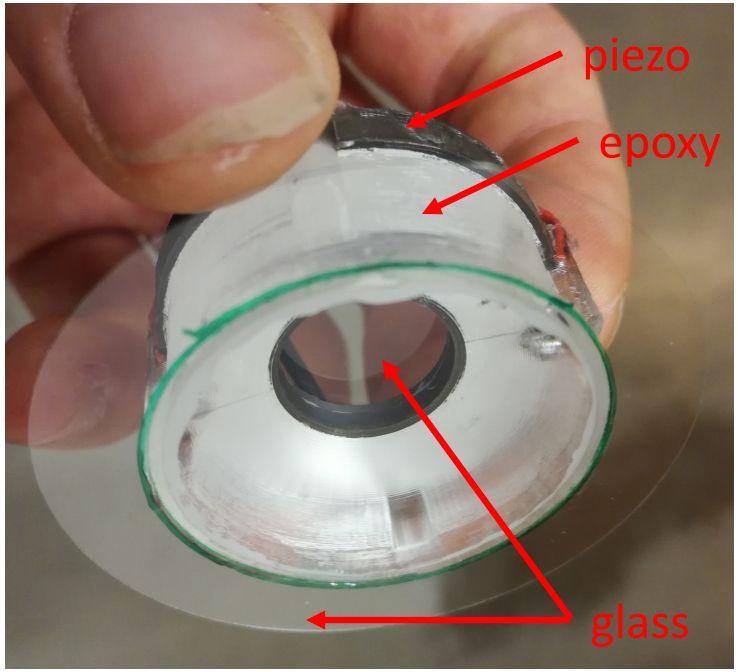
- pressure field
  - calibrate transducer
  - high speed (interferometric) imaging of substrate displacements
  - simulate wave propagation
- displacement field
  - high speed imaging of cells & tracers

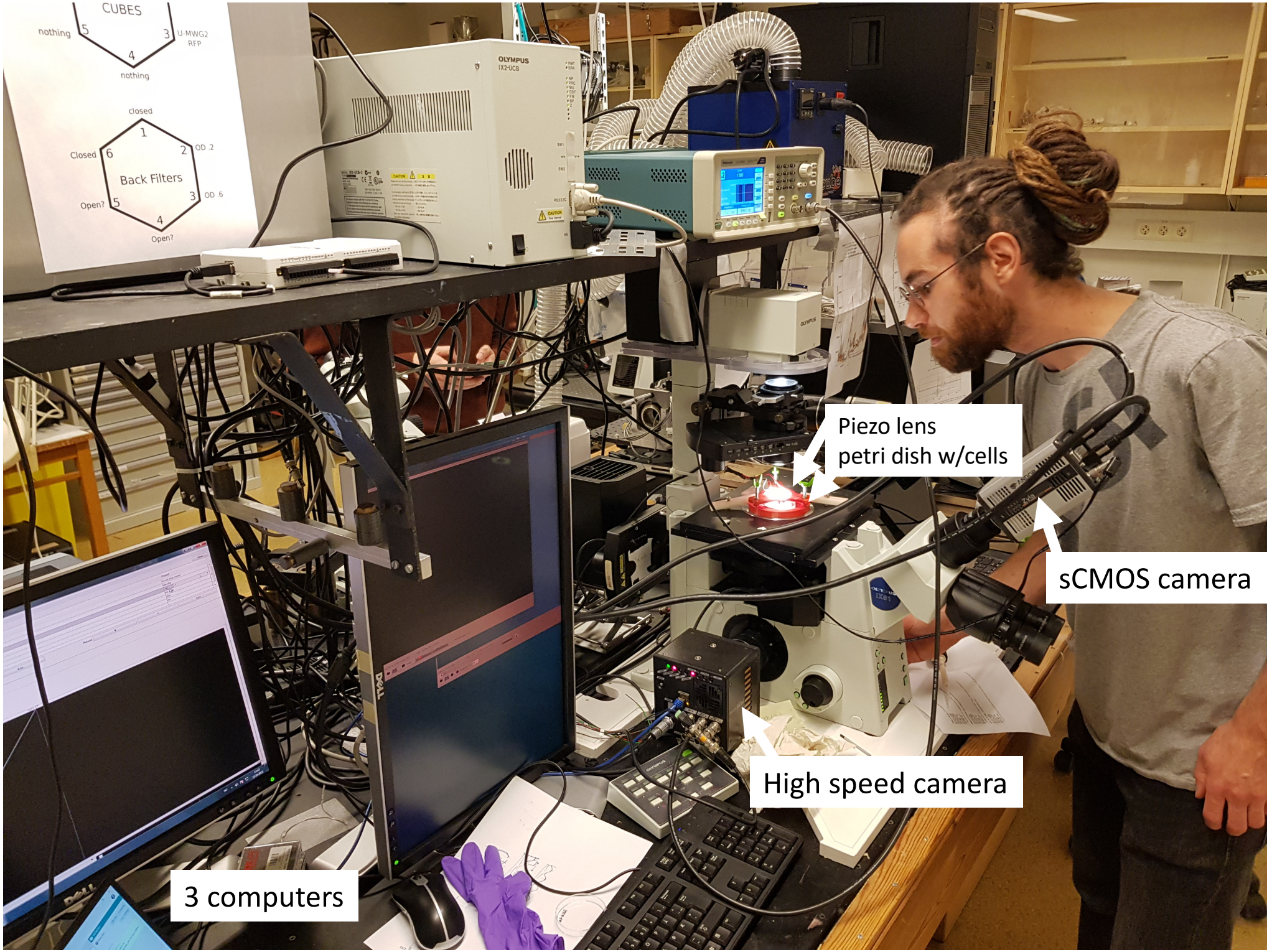
Current instrument



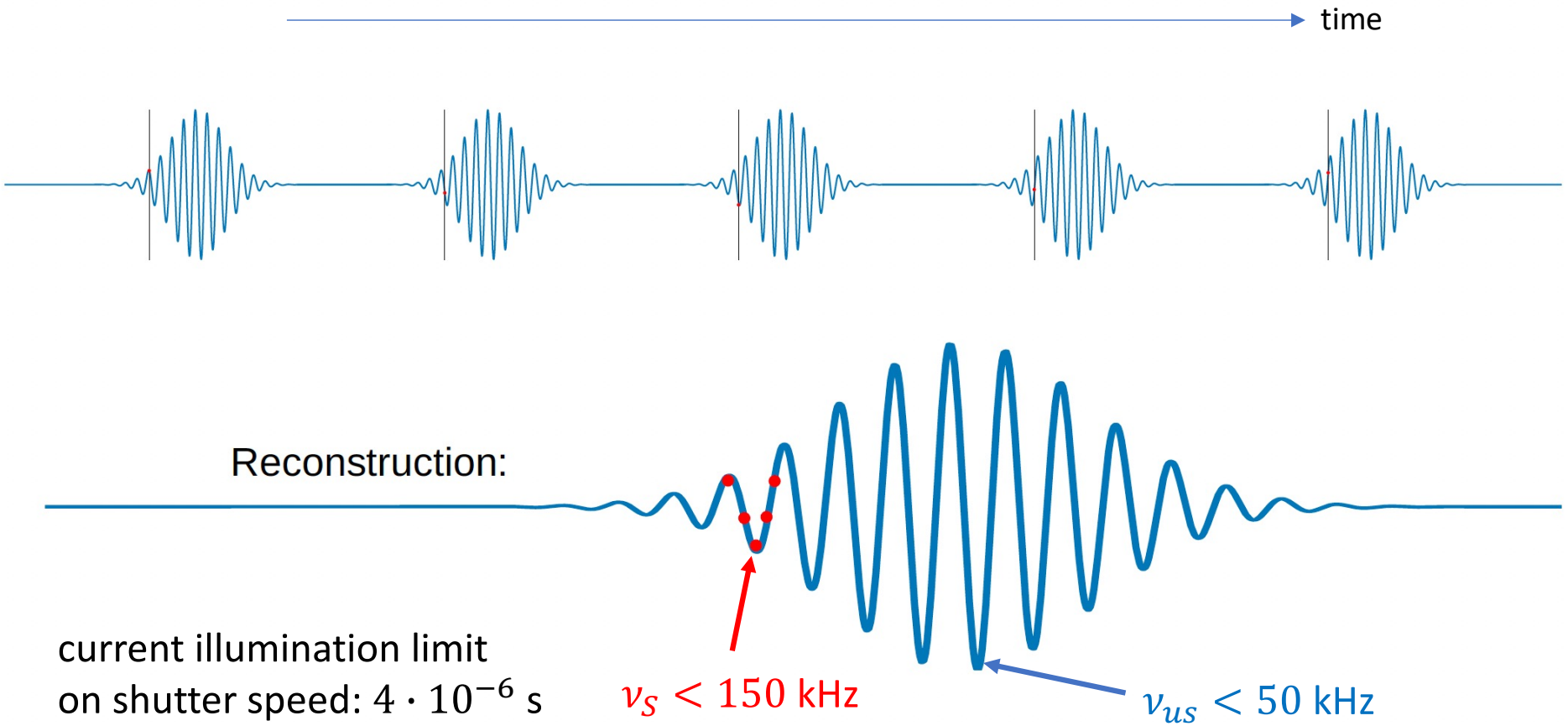
US transducer

- annular for illumination
- lens focuses US
- water filled for impedance matching
- submersible in petri dish

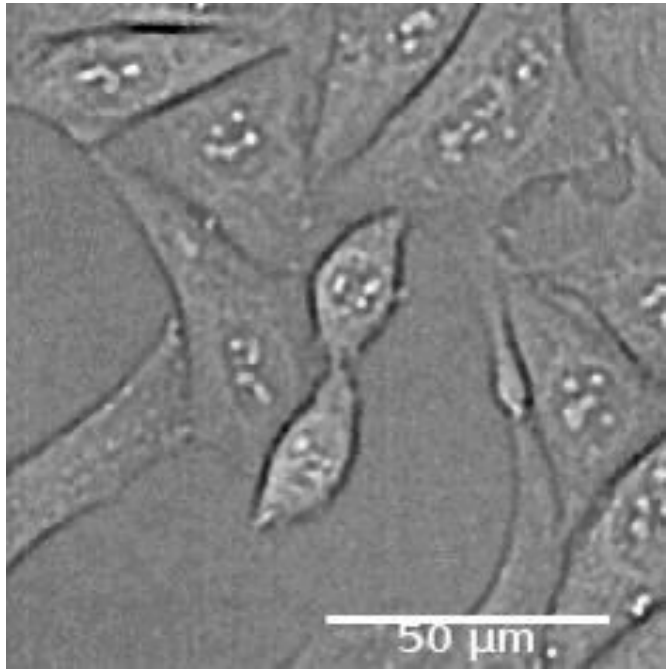


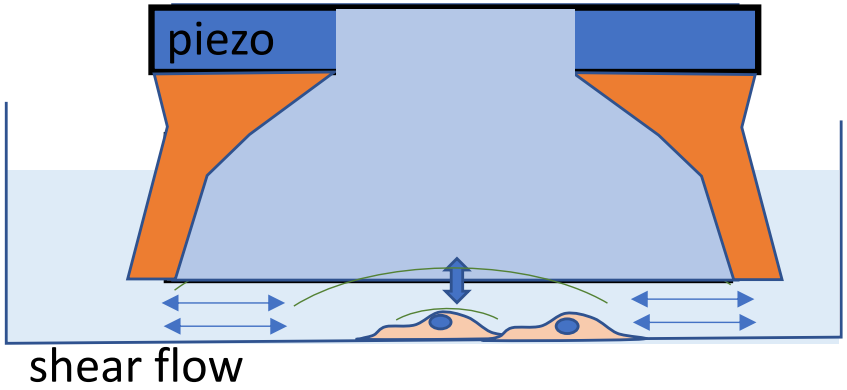

CUBES
nothing
5
3
U-MWG2
RFP
4
nothing
closed
1
Closed
6
2
OD .2
Back Filters
Open?
5
3
OD .6
4
Open?
Piezo lens
petri dish w/cells
sCMOS camera
High speed camera
3 computers

STROBOSCOPIC IMAGING - PRINCIPLE

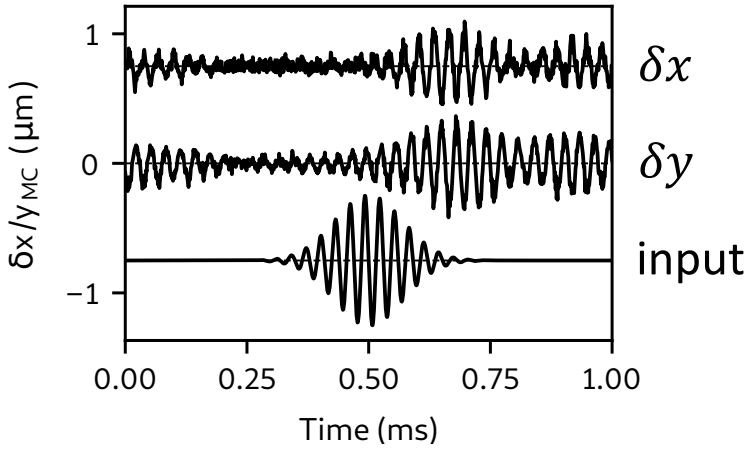


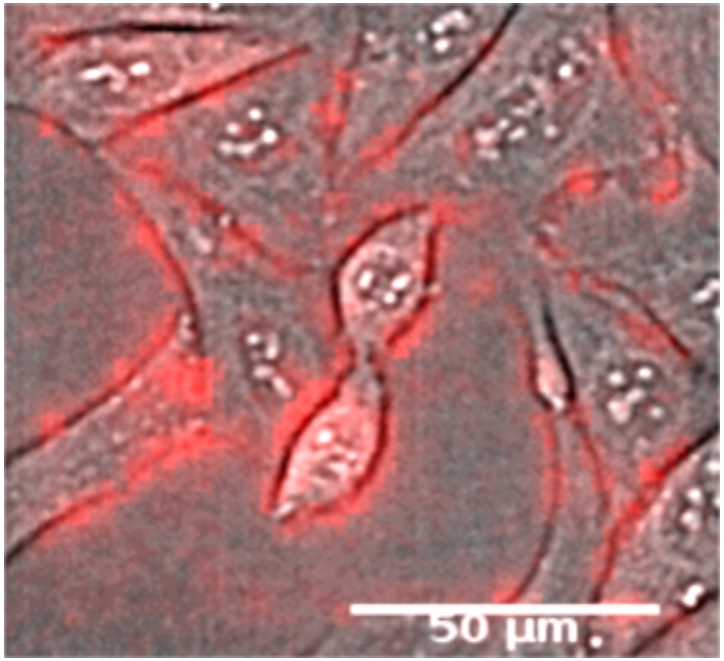
High speed imaging reveals shear



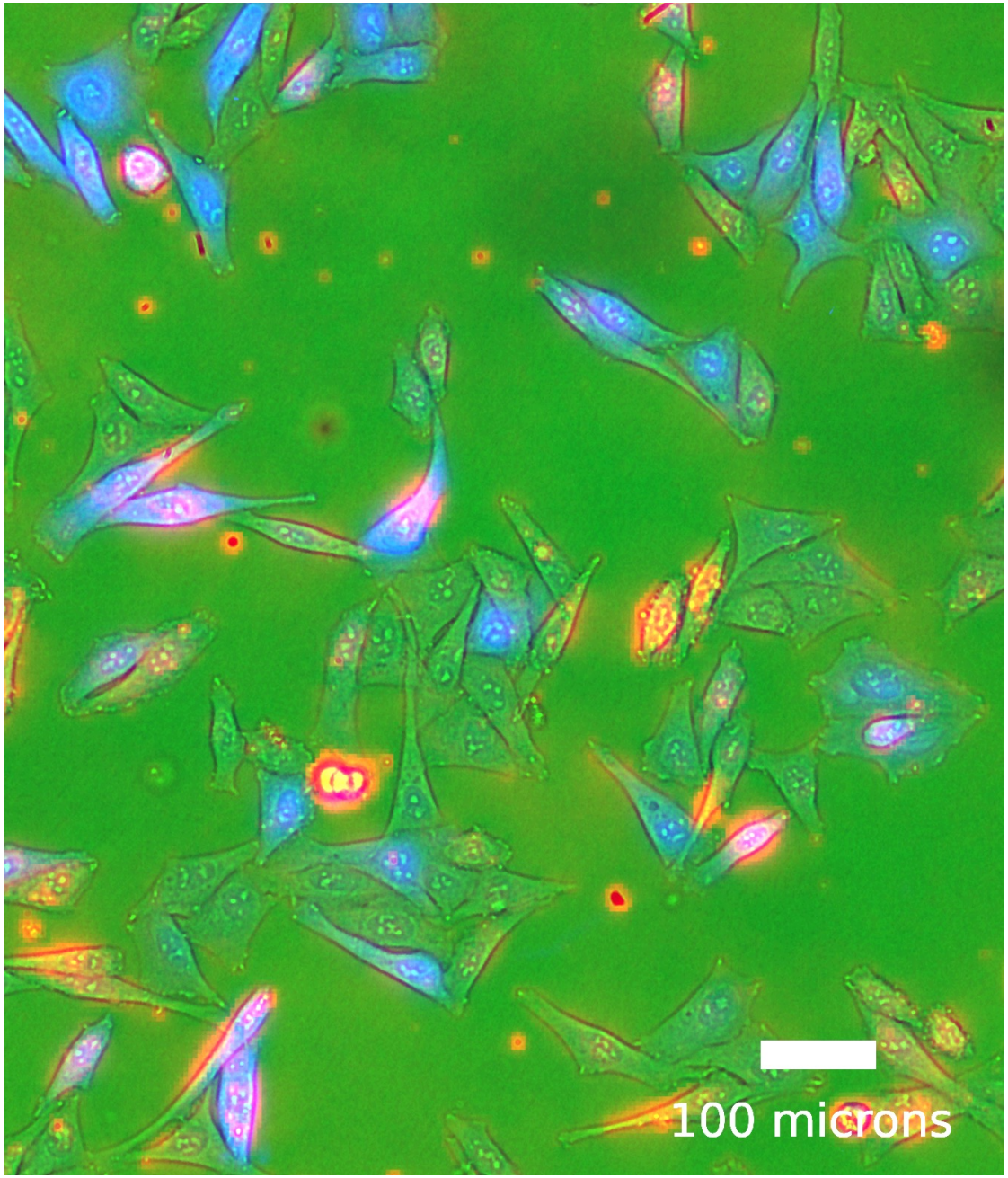


Digital image correlation (DIC)





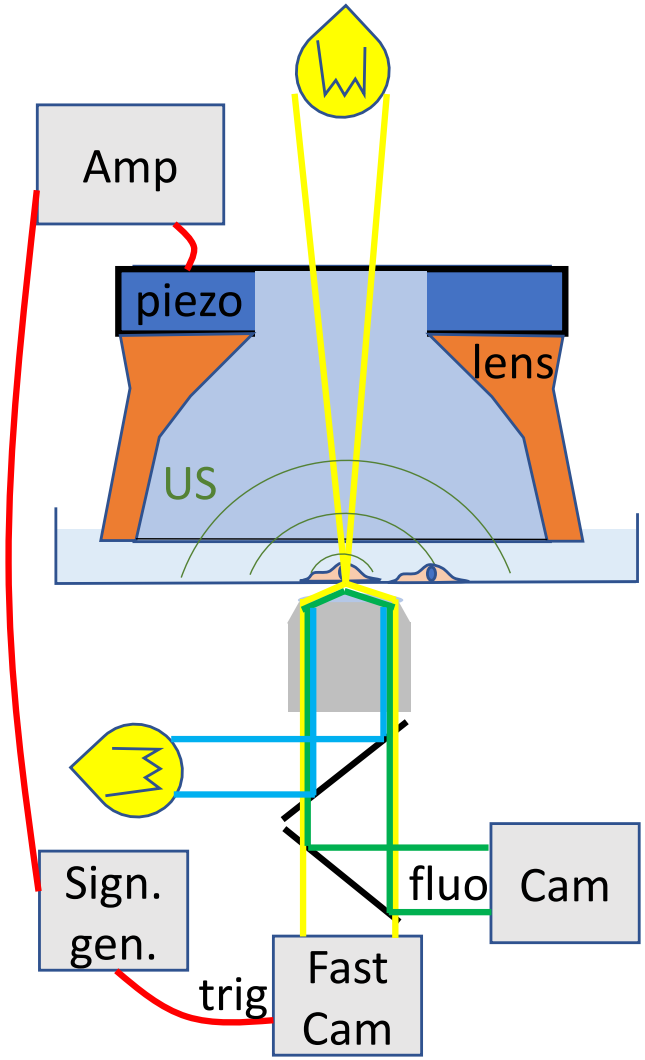
Correlative imaging

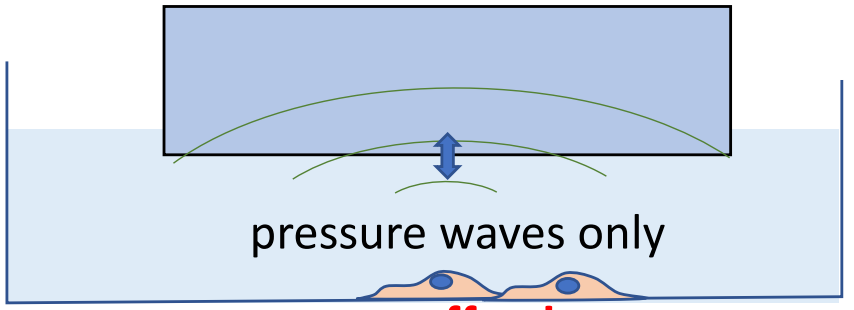


- phase contrast
- **mechanical stimulus:**
  mean displacement
  33 kHz
- **biological response:**
  Ca^{2+} fluorescence
  2s - 2h

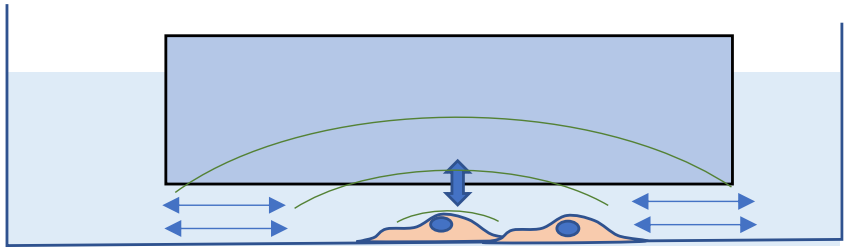
frequencies, amplitudes and fluorescent reporters to be varied

Summary

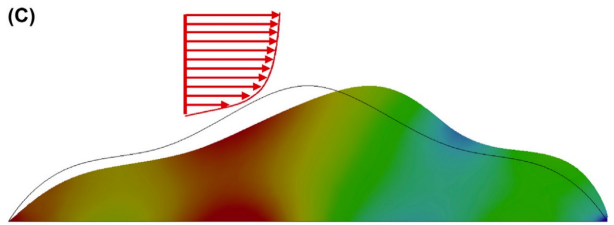


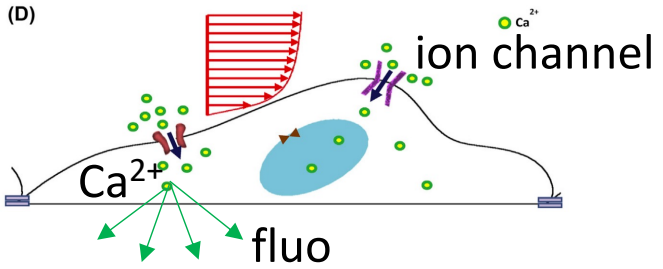


no effect!



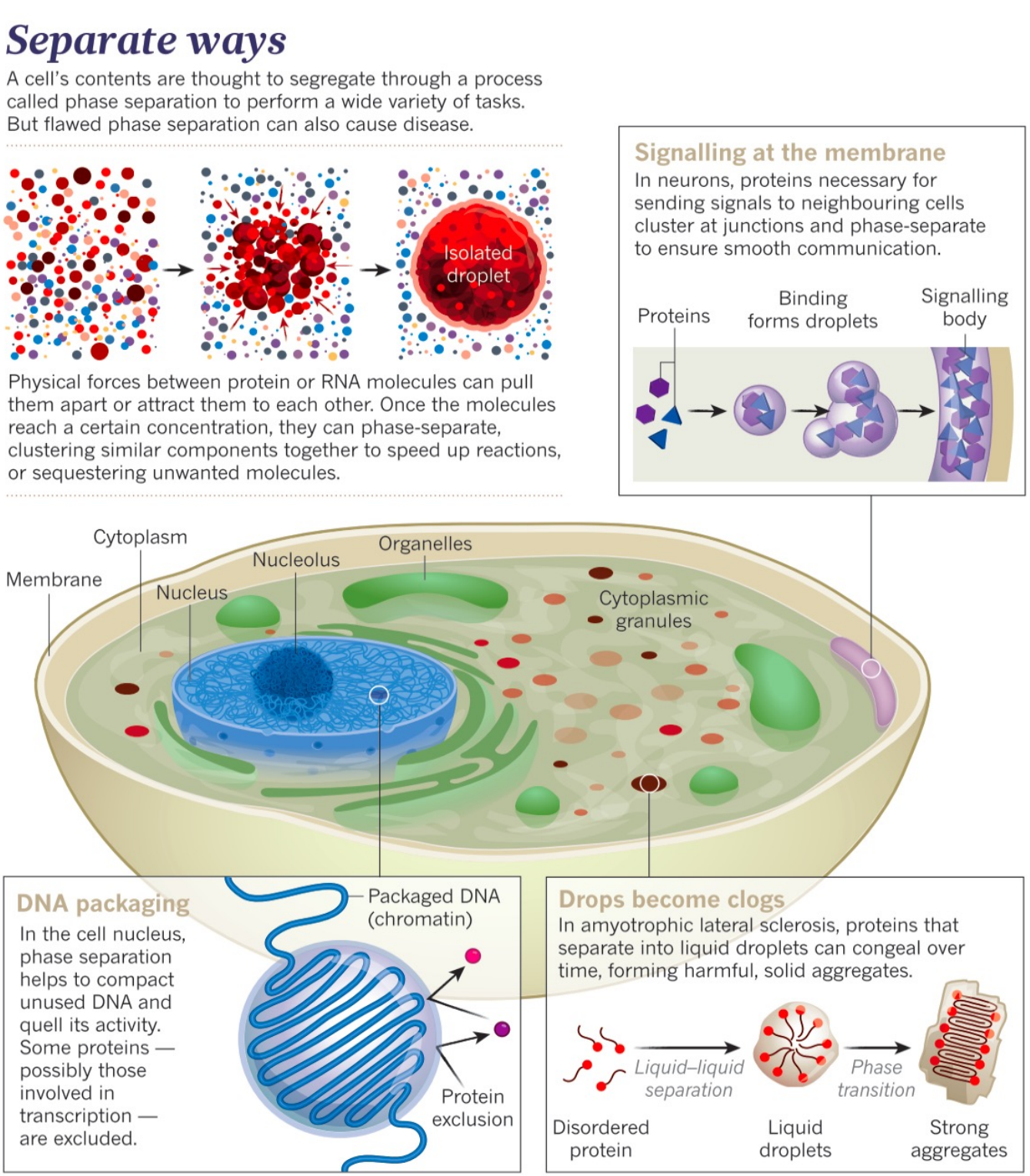
shear flow



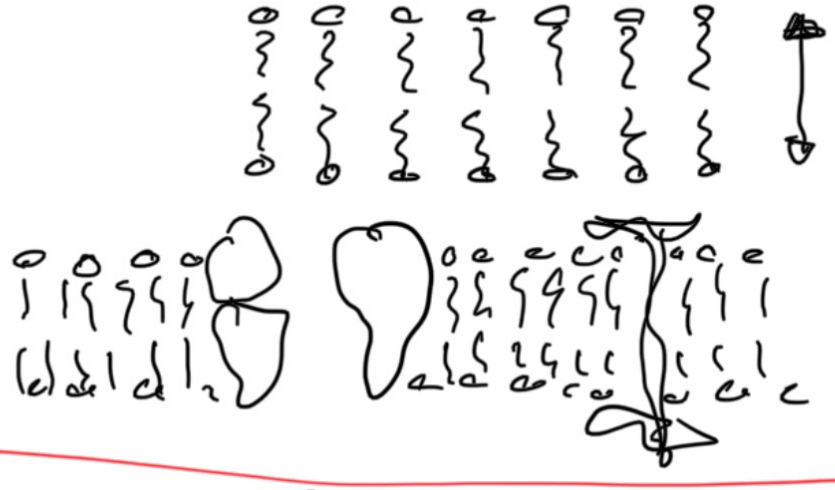


Other mechanisms are possible

Pressure waves trigger phase separation?



Dolgin, *Nature* 2018



how stable is this distance
how compressible?

Is there enough time to flow around more static structures?

Stress – changes activation energy of a transition

Boschadsley 2006

Bunny Geiger!

Intramembrane cavitation as a unifying mechanism for ultrasound-induced bioeffects

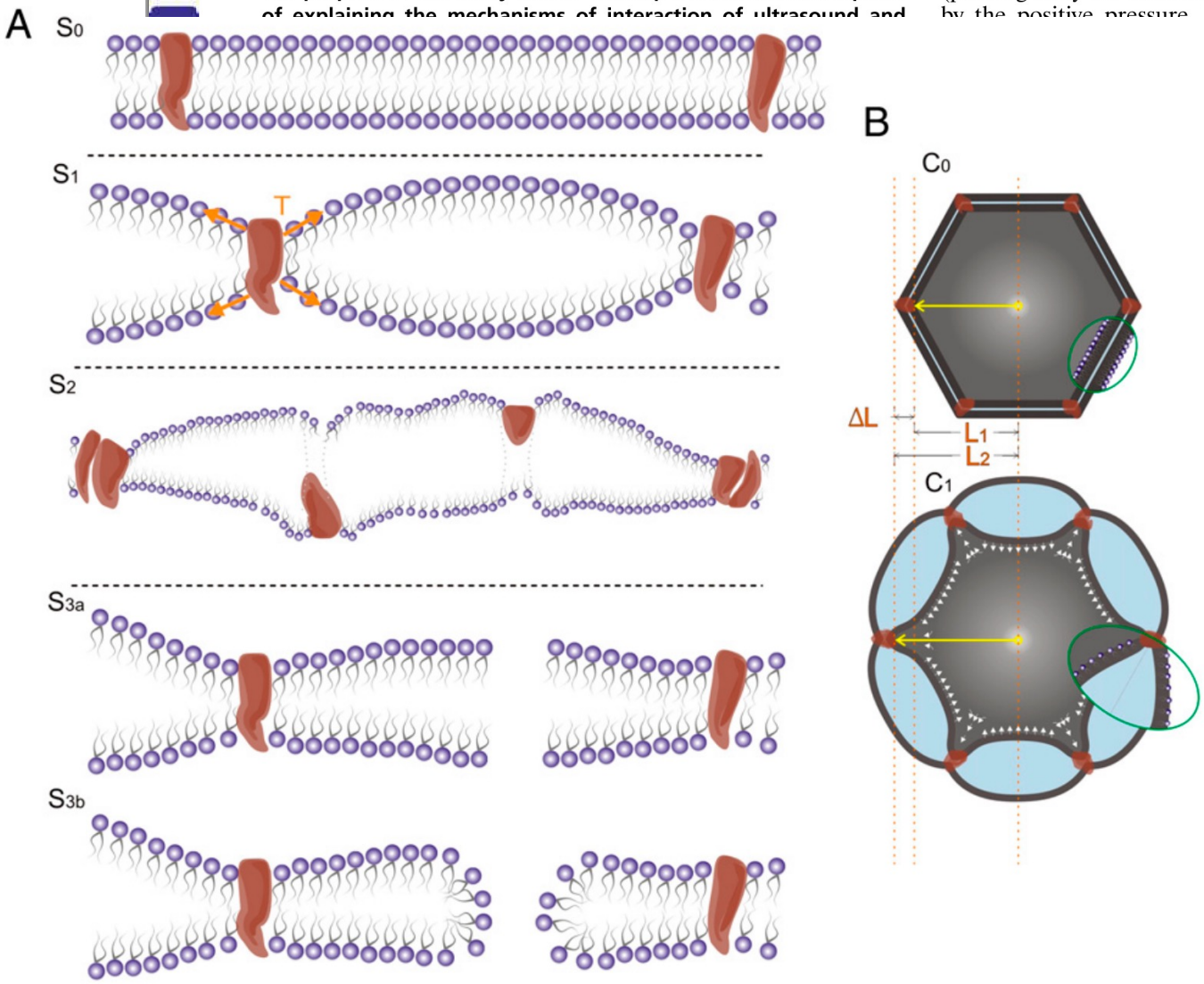
Boris Krasovitski[a], Victor Frenkel[b], Shy Shoham[a], and Eitan Kimmel[a,1]

[a]Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel; and [b]Department of Biomedical Engineering, Catholic University of America, Washington, DC 20064

Edited by Robert Langer, Massachusetts Institute of Technology, Cambridge, MA, and approved January 12, 2011 (received for review October 21, 2010)

The purpose of this study was to develop a unified model capable of explaining the mechanisms of interaction of ultrasound and

(pushing away the surrounding tissue) and pushed back together by the positive pressure. We propose the term "bilayer sono-



What pressures are needed to open an inter-leaflet cavity?
Should the bilayer be considered a "bubble"?
Is there a nucleation barrier?

Fig. 4. Different stages in the interaction of a BLS and an ultrasound field can induce different bioeffects on the cell membrane and the cytoskeleton. (*A*) As tension increases gradually in the leaflets around a pulsating BLS, from the reference stage (S_0), the slightly stretched leaflets might at first activate mechanosensitive proteins (S_1); growing tension in the leaflets might damage membrane proteins (S_2) and then might induce pore formation (S_{3a}, S_{3b}) or cause membrane rupture at high levels of stretching. (*B*) Pulsations of the BLSs that surround a cell initially (at C_0) might induce from reversible mild stretching of cytoskeleton fibers to irreversible rupture (C_1).

Questions for (your) further research

- Many experiments find that LIUS has an effect on biological cells and tissue. Can we find good arguments for not trusting them?
- What are considered safe itensities/pressures/frequencies for US imaging?
- Which itensities/pressures/frequencies can be considered to give no effect of US / safe, small effects / potentially harmful effects?
- What is the best / clearest definition of LI[P]US?
- Which hypothetical mechanisms on the molecular/ cellular level that transform LIUS into chemical/ biological signals can you explain?
- In our experiments shear-waves, not pressure-waves trigger Ca^{2+} release. How can these experiments be used to generalize the result? (range of parameters...)
- If it is true that all biological effects of LIUS are due to shear- and not pressure-waves, what are the consequences for future research on LIUS bio-effects on tissue and cells?
- Can we construct and simulate relevant examples of shear wave generation from pressure waves?

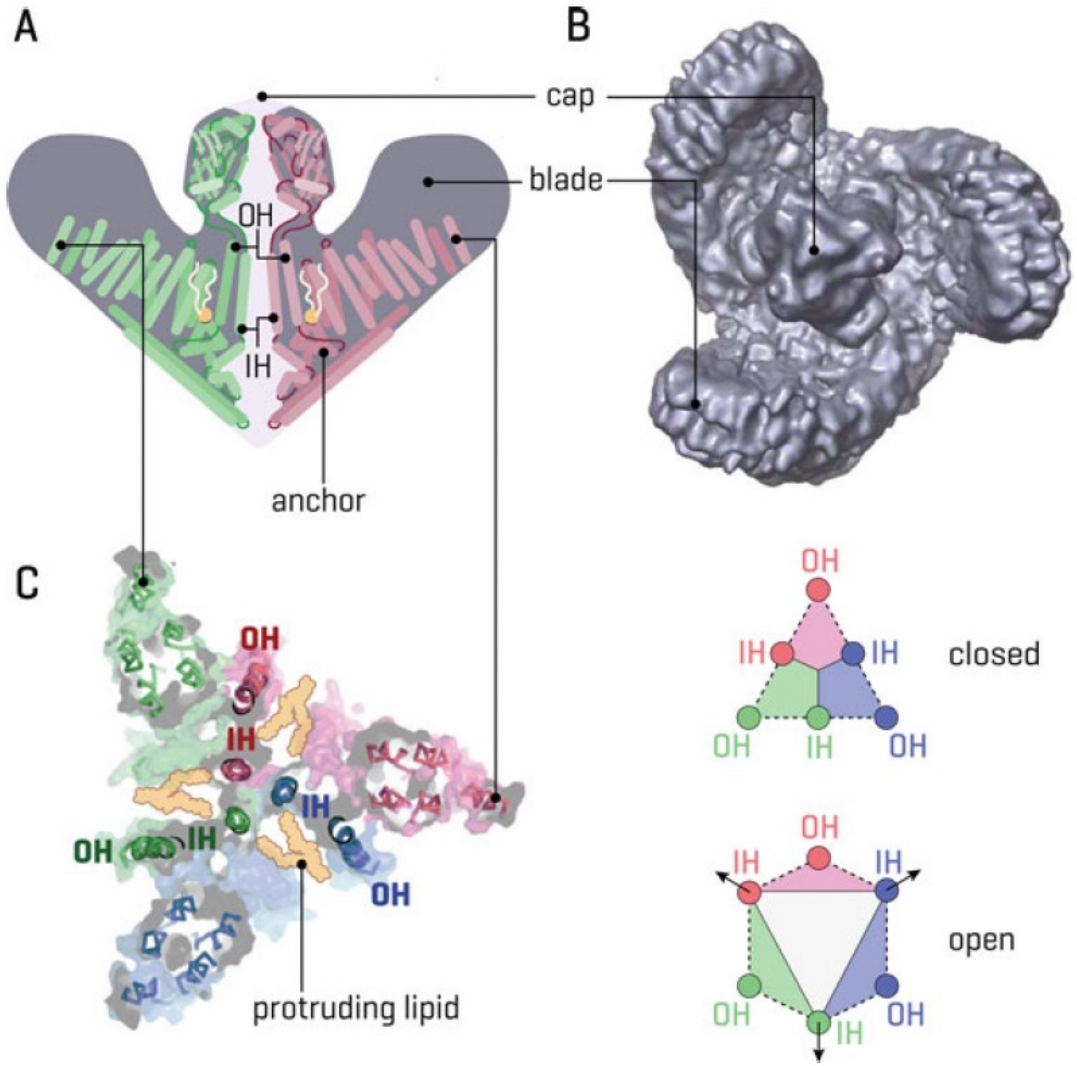
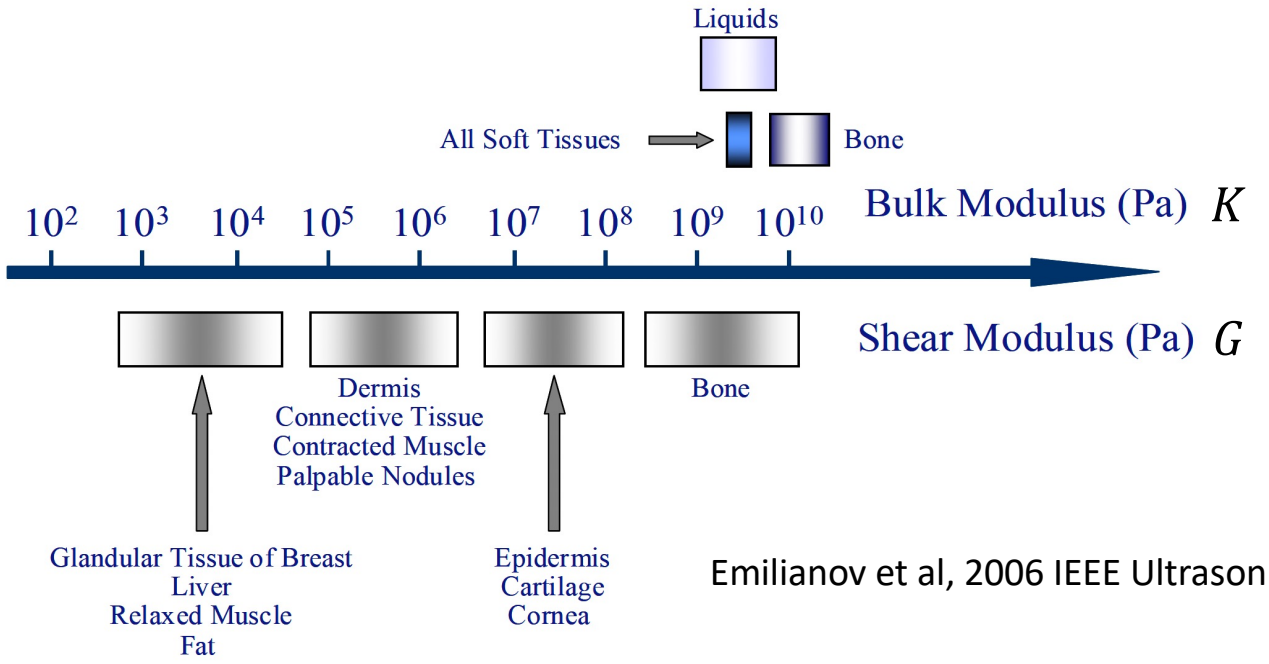


Fig. 4.5 Mammalian mechanosensitive Piezo1 architecture and a putative membrane-mediated gating mechanism. (**a**) Schematic of the side view of Piezo1 structure. (**b**) *Top view* of Cryo-EM structure of mouse Piezo1 as shown in *shaded grey* surface (PDB: 3JAC) [72]. (**c**) View from the *top* of the human Piezo1 (homology model based on mouse Piezo1) shows the interlocked arrangement of its 3 subunits at the level of the hydrophobic core of the lipid bilayer. An increase in lateral bilayer tension is thought to result in a clockwise or counter-clockwise deflection of the 'Blade' domains around the 'Anchor' and outer helix (OH) domains. This movement ultimately results in the displacement of the inner helices (IH) away from the center of the pore to allow ion conduction, as shown in the diagram. This hypothesis aims to explain the intrinsic mechanosensitivity of the channel different from the Blade-deflection model proposed by Ge et al. (2015) [72]

Bulk and shear modulus of tissues



Emilianov et al, 2006 IEEE Ultrasonics Symposium

$\Rightarrow$ soft tissue and cells can be considered incompressible and Poisson's ratio $\nu = 0.5$

$$\varepsilon_{ij} = \frac{1}{E}\left[\sigma_{ij}(1+\nu) - \nu\delta_{ij}\sum_{kk}\sigma_{kk}\right]$$

Young's modulus, E, shear modulus, G, and bulk modulus, K:

$$E = 2G(1+\nu) \approx 3G$$
$$= 3K(1-2\nu)$$