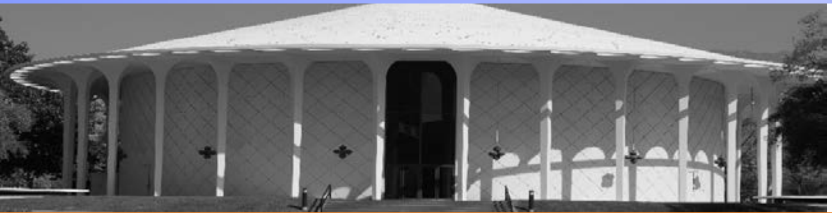


Oncotripsy: Targeting cancer cells selectively by means of tuned ultrasound

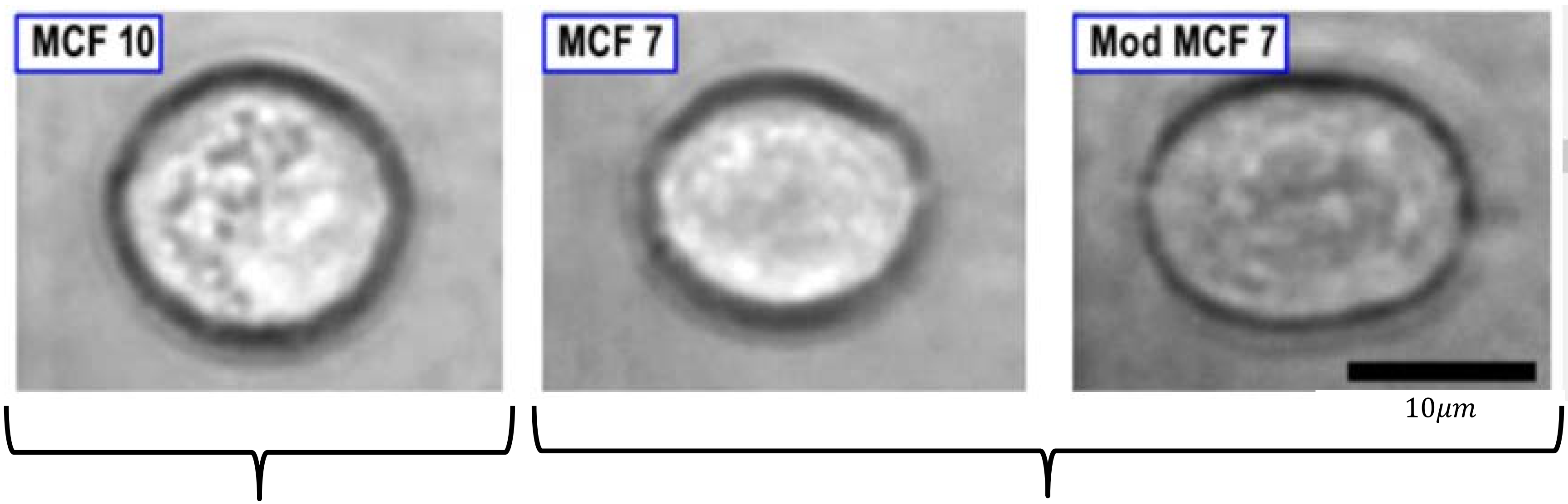
Michael Ortiz
California Institute of Technology

With: M. Gharib, D. Mittelstein, E.F. Schibber, M. Shapiro (Caltech) and P. Lee, J. Ye (City of Hope)

Inaugural SEMTA Colloquium
Madrid, Spain, March 28, 2019

Oncotripsy: The key observation

- Cancer cells have markedly different geometry, mechanical properties from healthy cells



Cells from MCF-7 breast cancer cell line
Morphological changes induced by malignancy

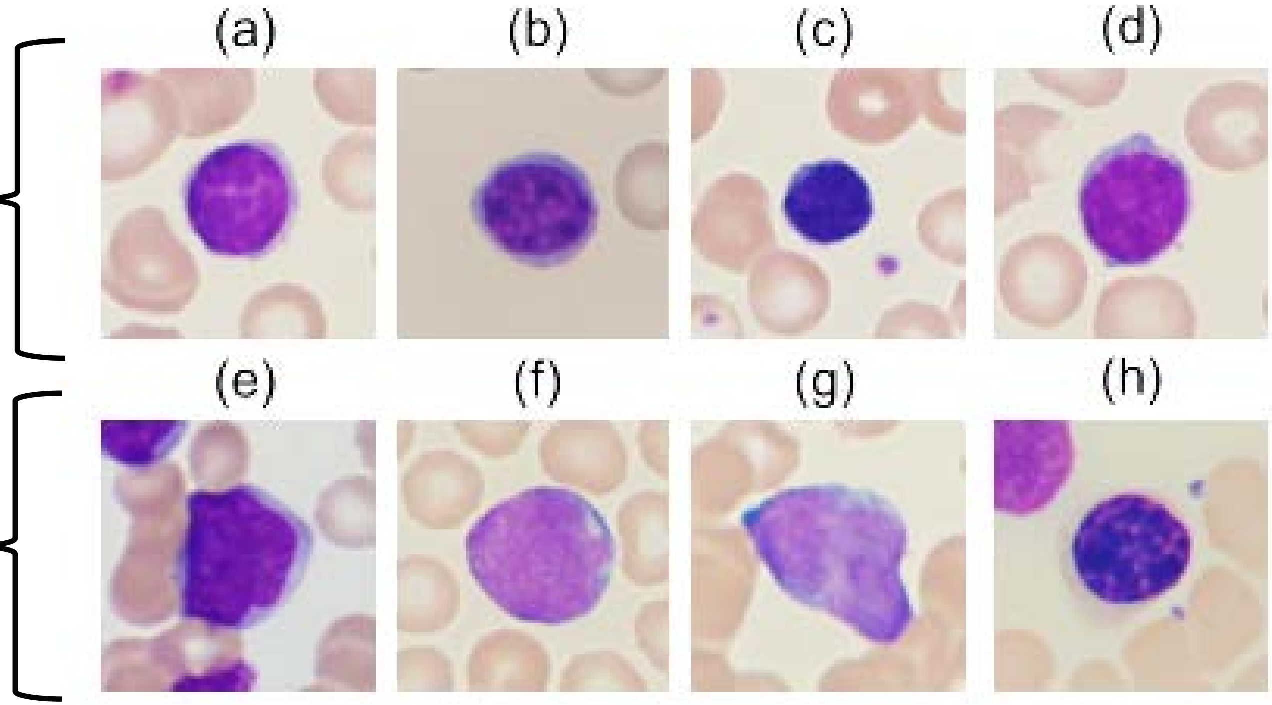
Suresh, S. (2007). *Acta Materialia*, *55*(12), 3989–4014.

Oncotripsy: The key observation

- Cancer cells have markedly different geometry, mechanical properties from healthy cells

Healthy

Malignant



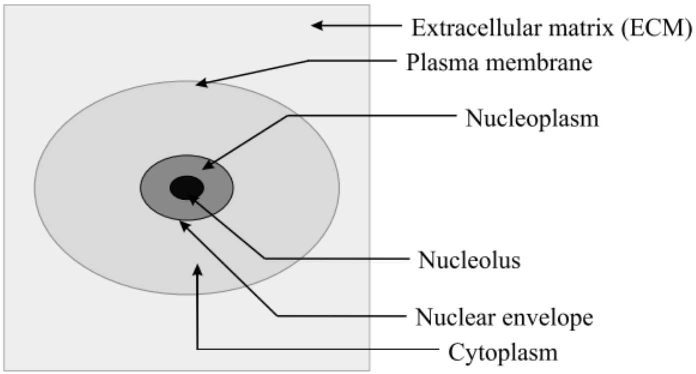
(a-d) Healthy lymphocyte cells
(d-f) Acute Lymphoblastic Leukemia (ALL) cells

Labati, R. D., Piuri, V. & Scotti, F., *IEEE ICIP* (2011) 2045–2048.

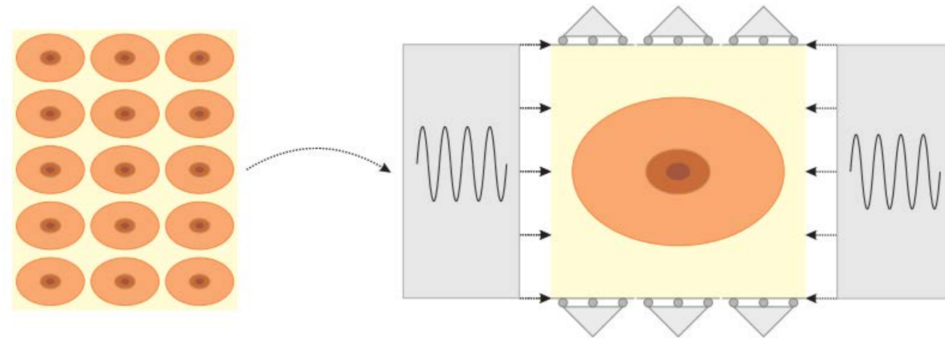
Oncotripsy: The key observation

- Numerous 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 (can reach a diameter of over 50 microns)
- *Mechanical stiffness* of various cell components are found to vary significantly in healthy and diseased tissues [Berman, 2011]
- *Question*: Can cancer cells be *selectively targeted* by harmonic excitation at their resonance frequency? (*oncotripsy*) What are the *therapeutic ranges* of frequency, intensity?

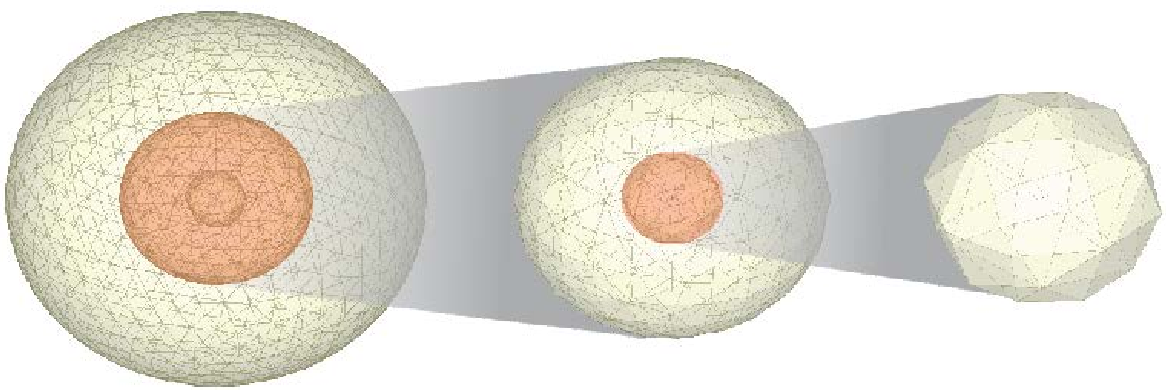
Oncotripsy: The spectral gap



Cell in extracellular matrix



Periodic (Bloch) model of tissue



Finite-element model of
cytoplasm, nucleoplasm and nucleolus

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)

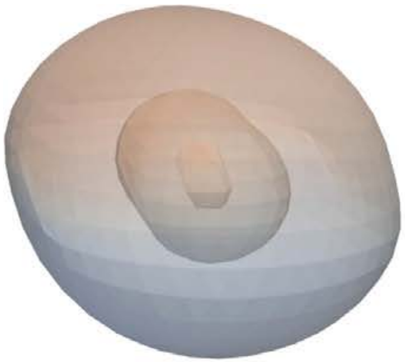
Malignant	κ [kPa]	μ_1 [kPa]	μ_2 [kPa]
Plasma membrane	39.7333	0.41	0.422
Cytoplasm	39.7333	0.41	0.422
Nuclear envelope	239.989	2.41	2.422
Nucleoplasm	239.989	2.41	2.422
Nucleolus	719.967	7.23	7.266
ECM	248.333	5.0	5.0

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

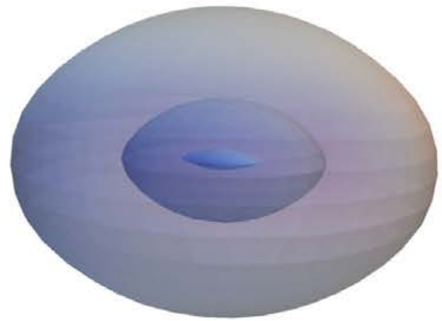
Heyden, S. and Ortiz, M., *JMPS*, **92**:164-175, 2016.
Heyden, S. and Ortiz, M., *CMAME*, **314**, 09 2016.

Oncotripsy: The spectral gap

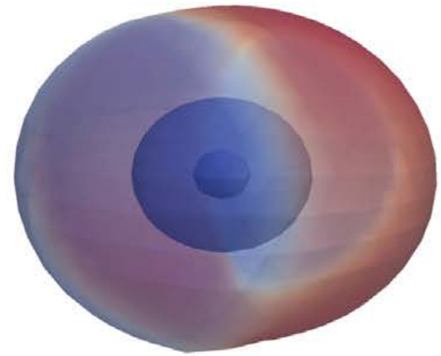
Hepatocellular Carcinoma (HCC)



$\omega = 558031\, rad/s$

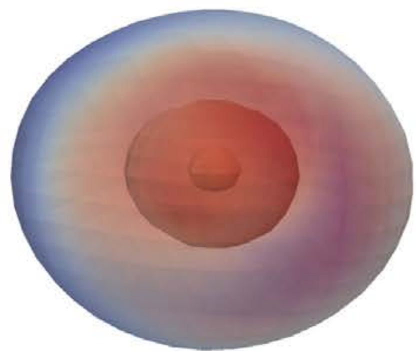


$\omega = 576073\, rad/s$

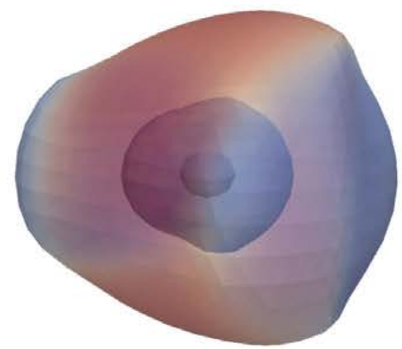


$\omega = 816846\, rad/s$

$\omega = 849764\, rad/s$



$\omega = 979926\, rad/s$

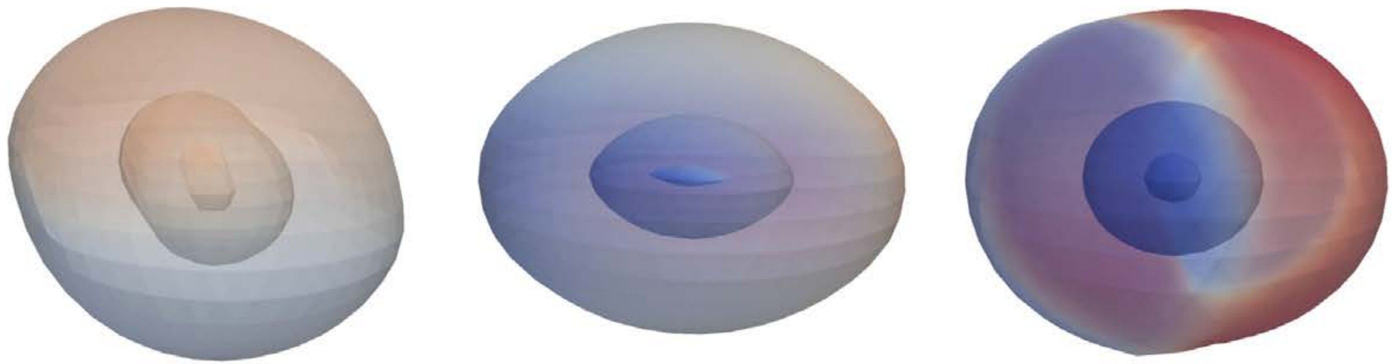


$\omega = 991430\, rad/s$

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)



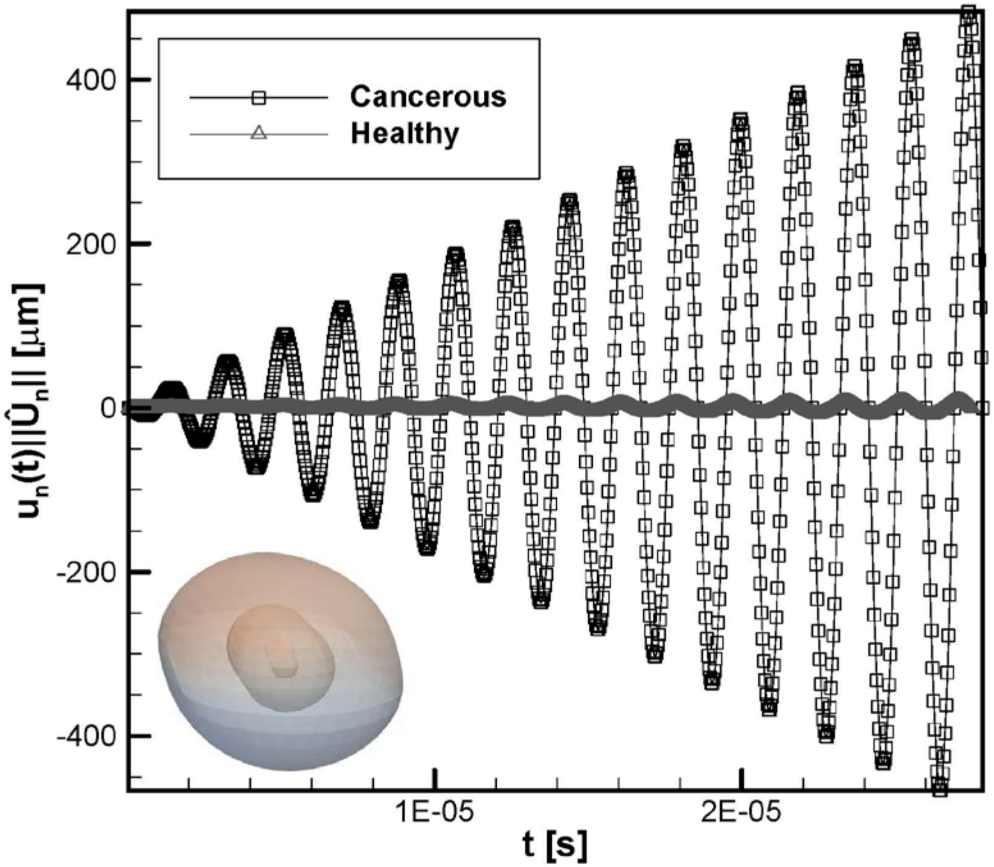
$\omega = 501576\, rad/s$ $\quad \omega = 502250\, rad/s$ $\quad \omega = 508795\, rad/s$

	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

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 showing vastly different growth rates: Malignant cells can be brought to lysis first!

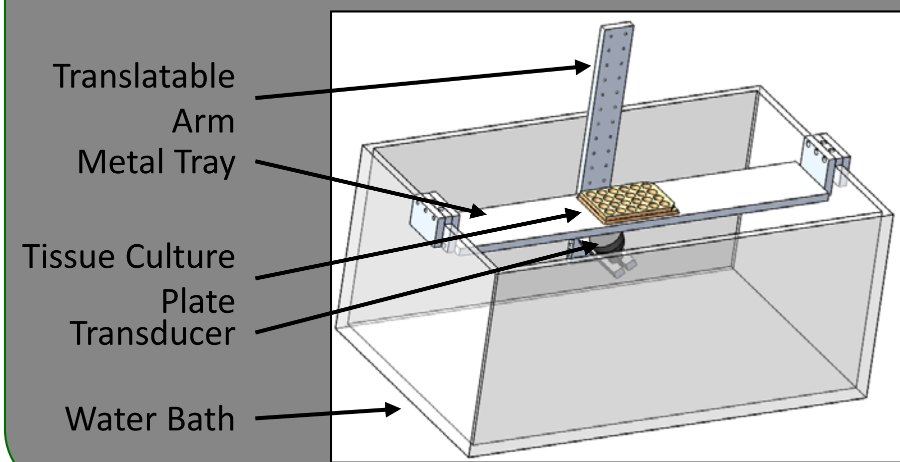
Heyden, S. and Ortiz, M., *JMPS*, **92**:164-175, 2016.
Heyden, S. and Ortiz, M., *CMAME*, **314**, 09 2016.

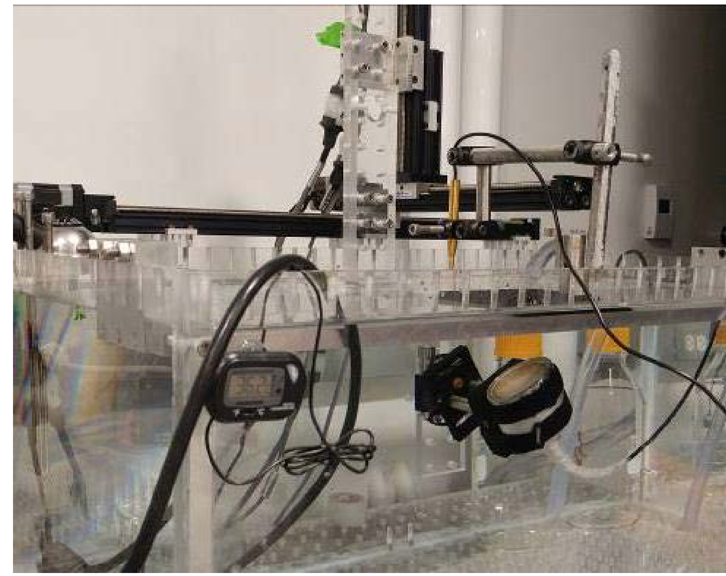
Oncotripsy: The spectral gap

- Computational studies of HCC give natural frequencies of ~80 kHz (malignant) and ~43 kHz (healthy): *Ultrasound range*
- 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...)

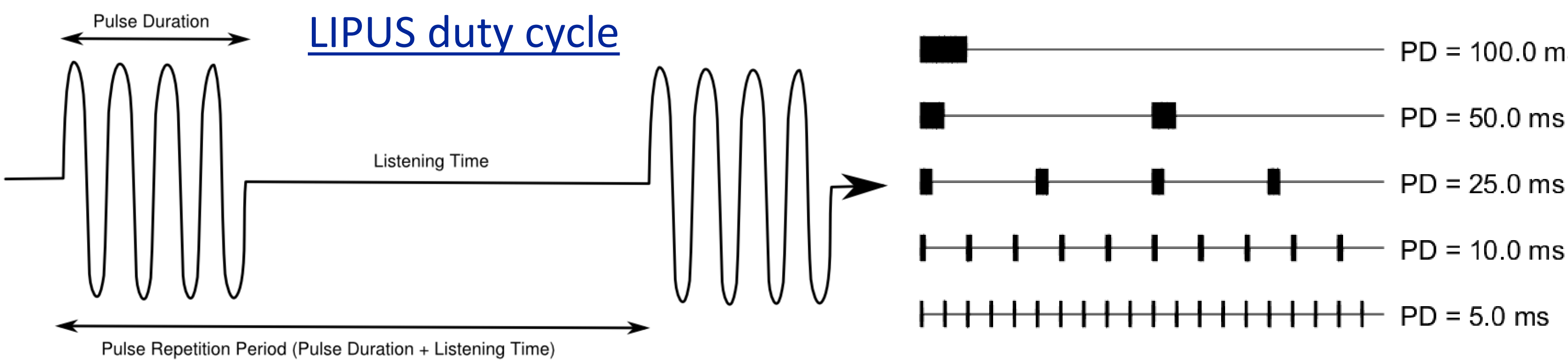
In vitro testing of cells in suspension

In-vitro experimental setup:



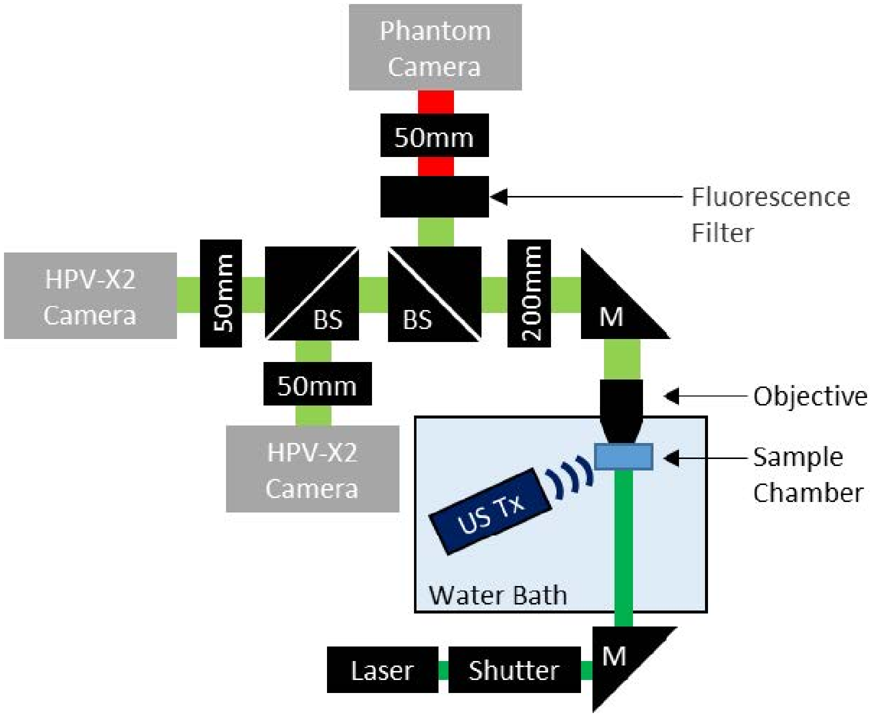


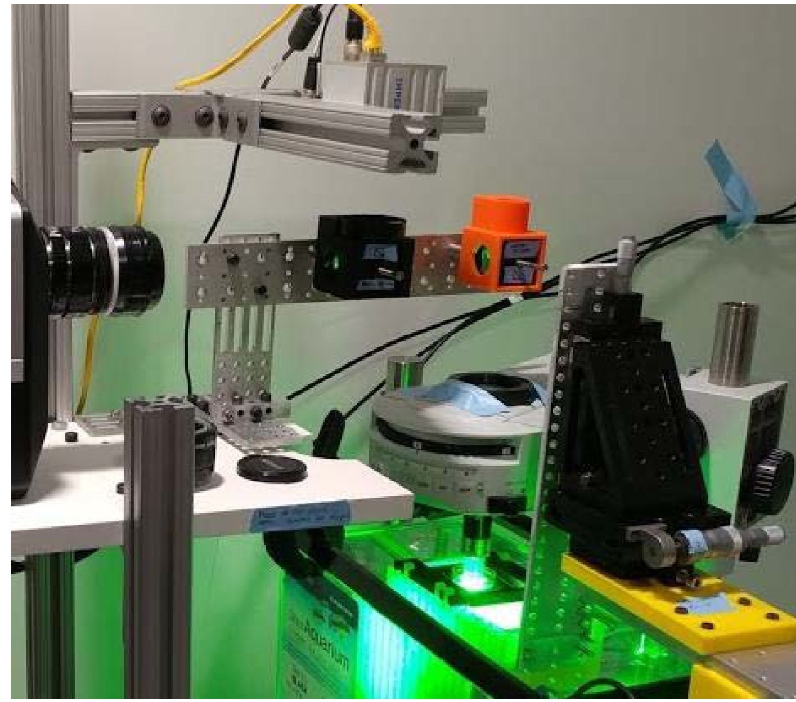
LIPUS duty cycle



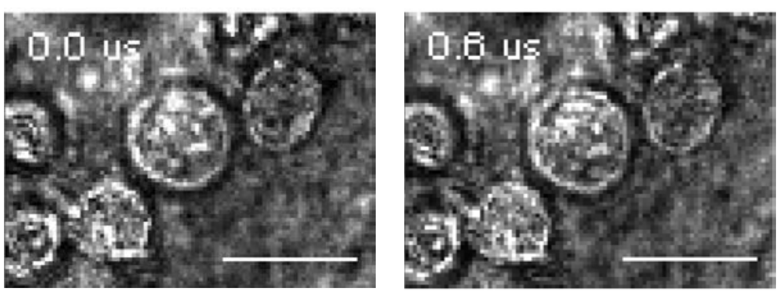
D. Mittelstein *et al.* (2019) (manuscript)

In vitro testing of cells in suspension



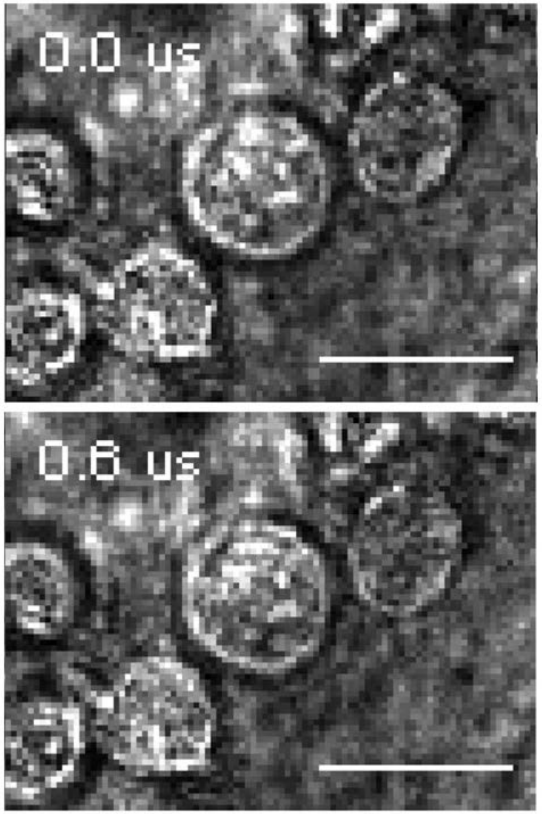


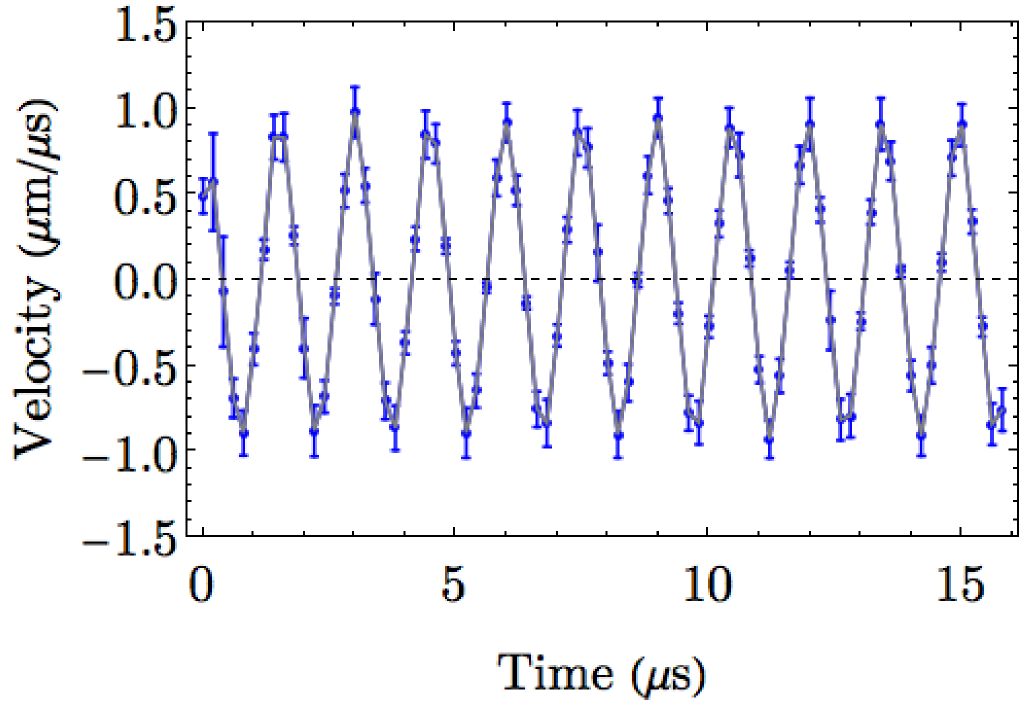
High-speed camera setup



D. Mittelstein *et al.* (2019) (manuscript)

In vitro testing of cells in suspension



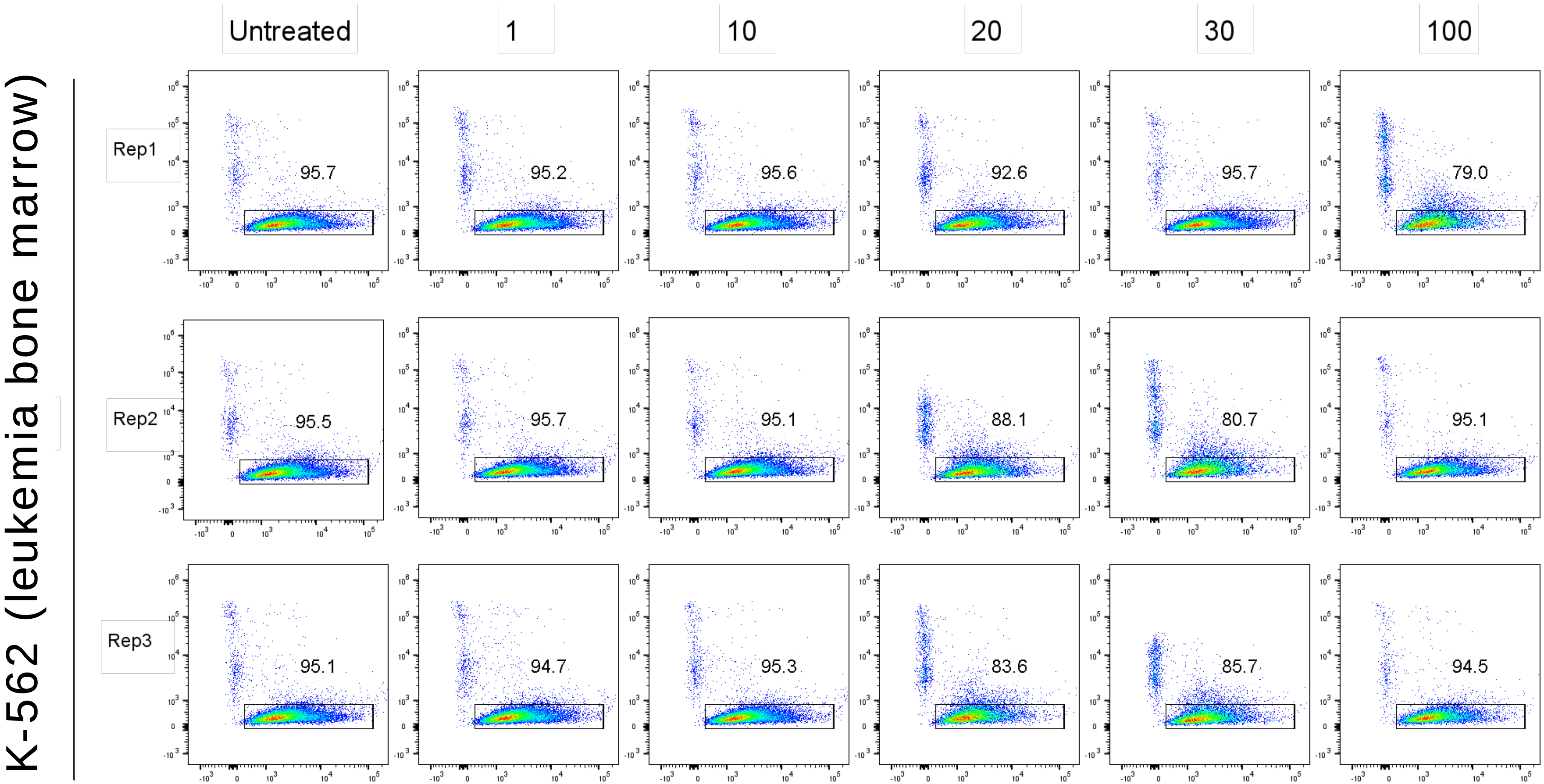


High frame-rate camera recordings showing minimal K-562 cell distortion after 100 ms of 670 kHz ultrasound exposure (scale bar 20 microns)

Data reduction from video showing nearly harmonic motion of the cell

Video courtesy of D. Mittelstein (2019)

Flow cytometry measurements

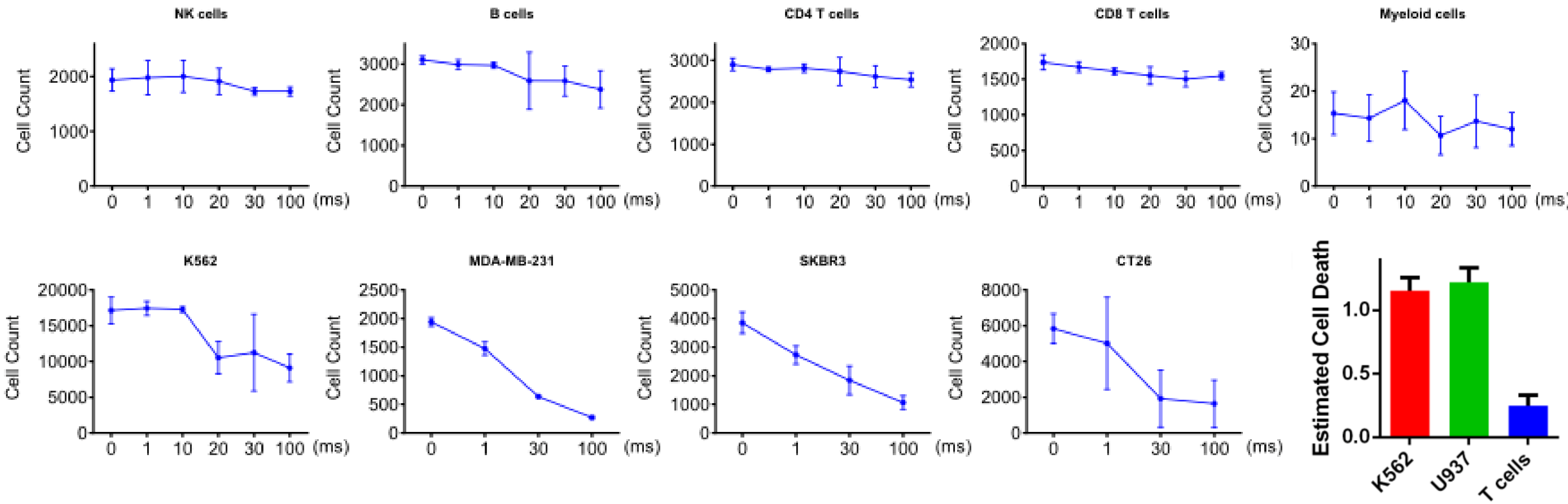


Double fluorescence dot plots from cytometry analysis

Dead-cell fractions as a function of exposure and duty cycle

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

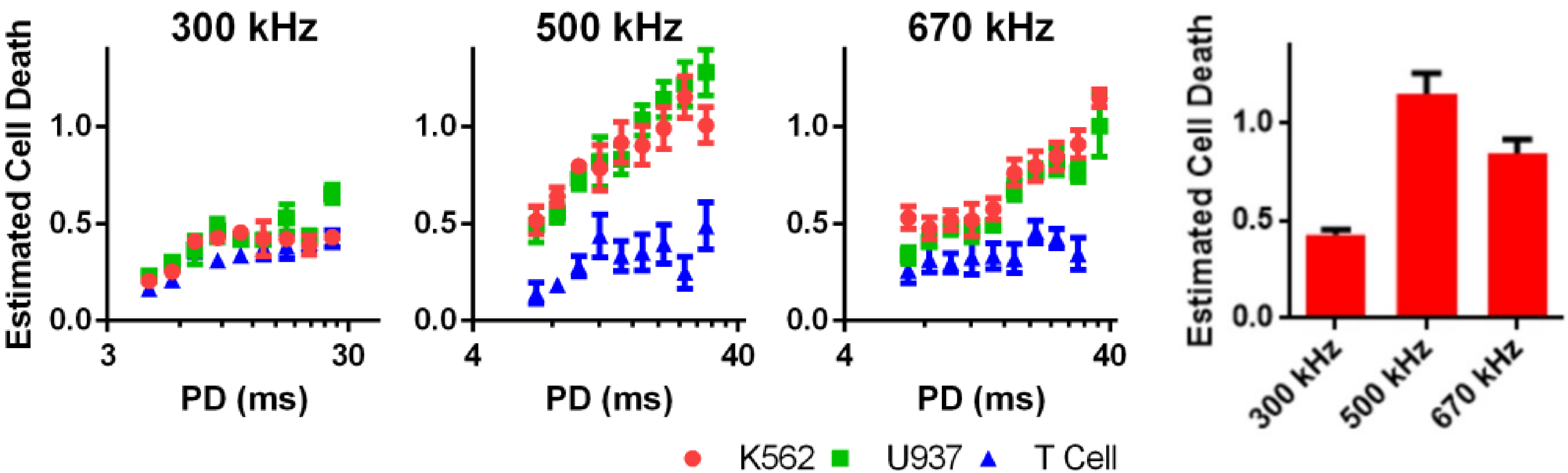
Flow cytometry measurements



Cell counts at 500 kHz, 20 ms PD,
on healthy cell models (row 1) and cancer cell models (row 2),
demonstrate significant therapeutic index

Sources: D. Mittelstein *et al.* (2019) (manuscript),
Lee, P. and Ye, J., *City of Hope*, 2019.

Flow cytometry measurements



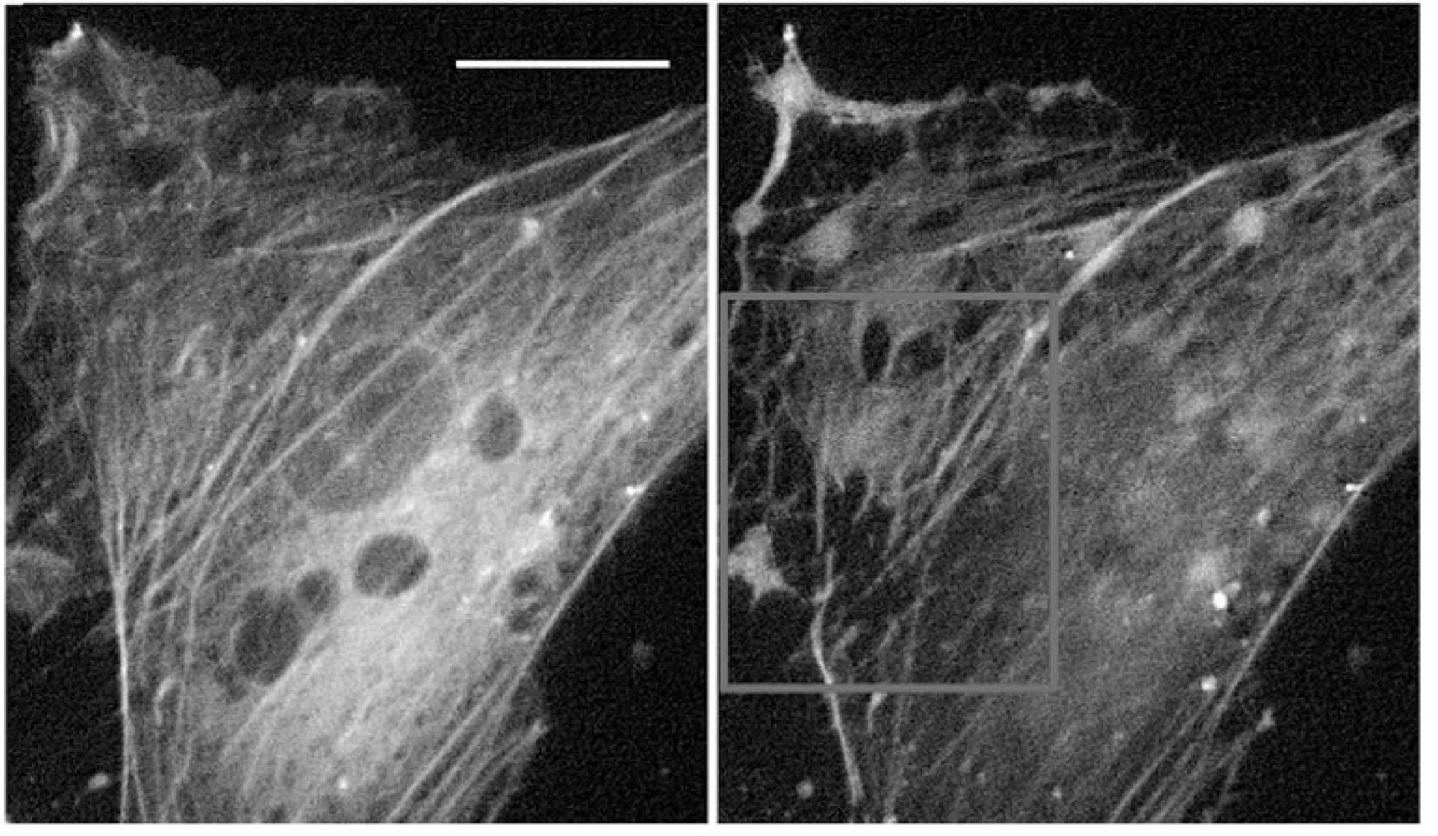
Cell death patterns for different cell types
assessed in high-throughput screen
as a function of insonation frequency and pulse duration

Sources: D. Mittelstein *et al.* (2019) (manuscript),
Lee, P. and Ye, J. *City of Hope*, 2019.

In vitro testing of cells in suspension

- Cell death in response to ultrasound exhibits *frequency-dependence, peak frequency*
- Targeted US induces *highly selective cell death*, demonstrating significant therapeutic index
- These observations bear out *oncotripsy*
- *But*: Cell death dependent on pulse duration, despite constant energy deposited
- Cell death requires a *large number of pulses*
- *Hypothesis*: Cells in aqueous suspension behave as *internal resonators*, die by slow accumulation of *damage to the cytoskeleton* (fatigue)

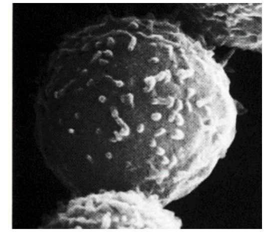
Response of actin network to US

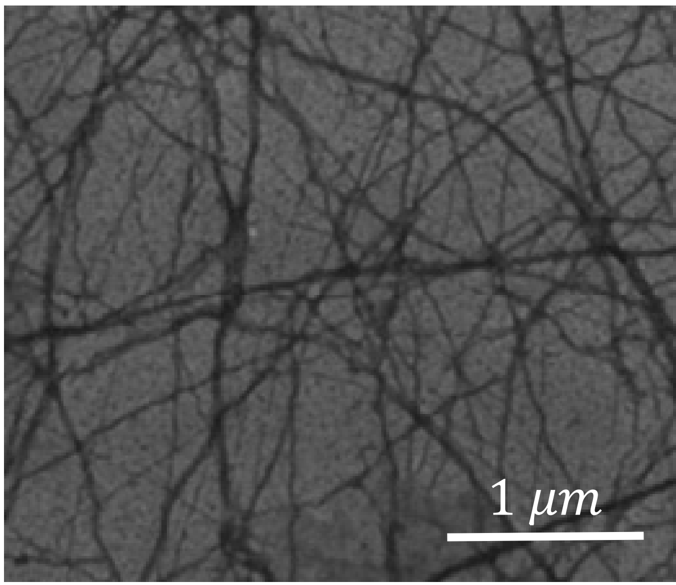


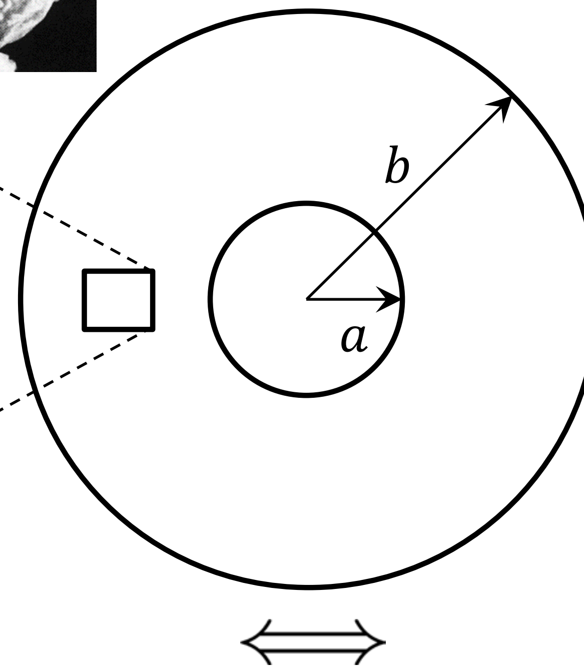
Cell actin network subjected to high-intensity US, progressively disassembles within 3 min exposure (Mizrahi et al., 2012)

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

High-cycle fatigue model







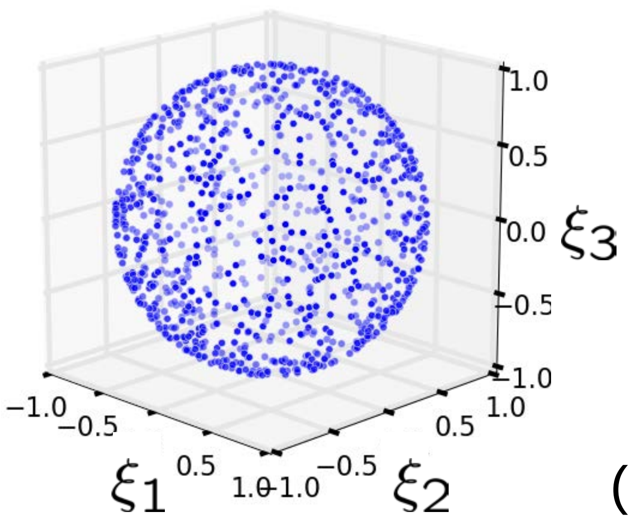
Electron micrograph of F-actin
(Gardel et al., 2008)

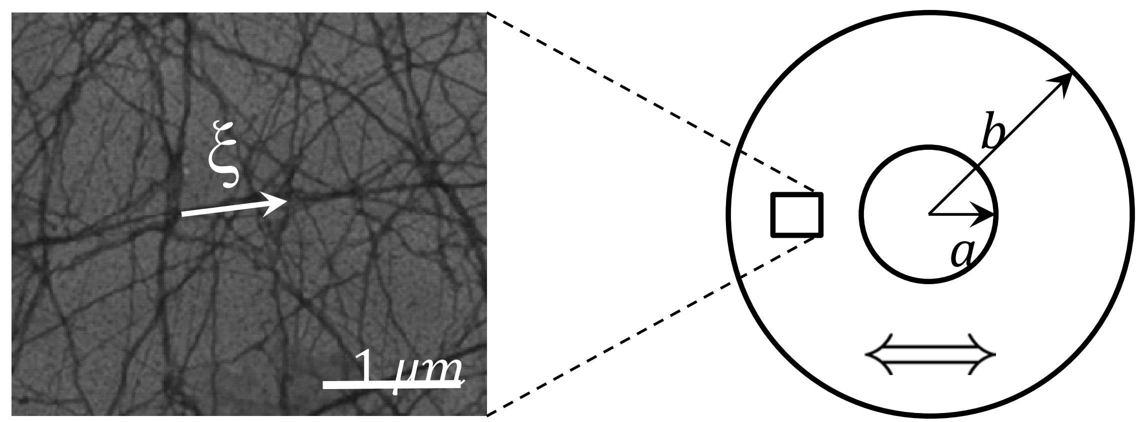
$$v(t) = V \sin \omega t$$

Globular cell moving with fluid particle velocity

M.L. Gardel *et al*. volume 89 of Methods in Cell Biology,
Chapter 19, pages 487-519. Elsevier Inc., 2008

High-cycle fatigue model





(Gardel *et al*., 2008)

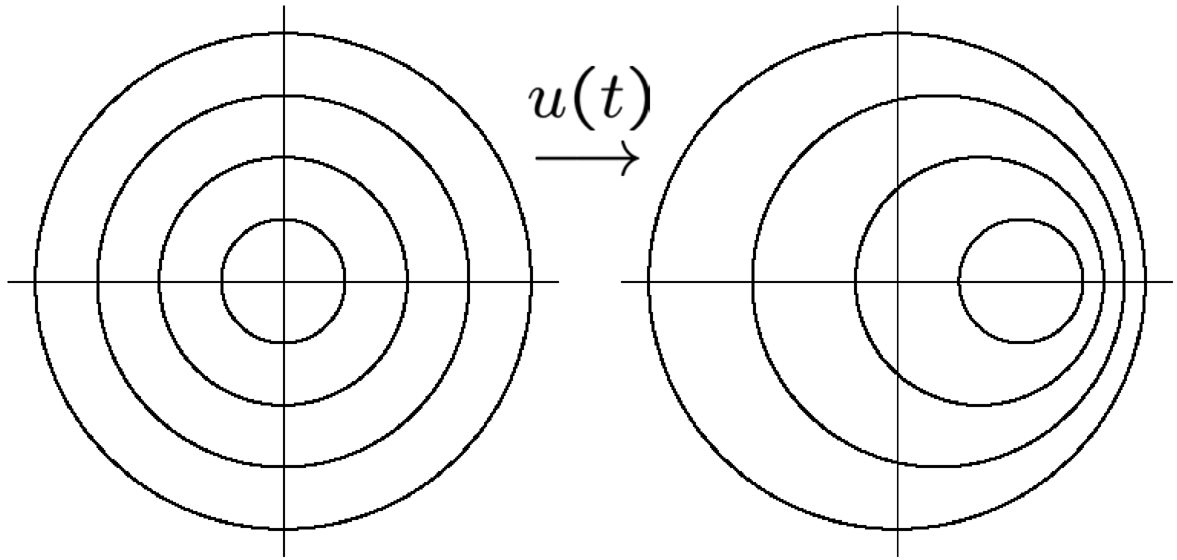
$v(t) = V \sin \omega t$

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

$$\int_{S^2} p(\xi)\Big(\frac{\mu(T)}{2}(1-q(\xi))^2\Big(\lambda^2(\xi)+\lambda^{-2}(\xi)\Big)+\frac{\beta}{2}q^2(\xi)\Big)\,d\Omega$$

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

High-cycle fatigue model

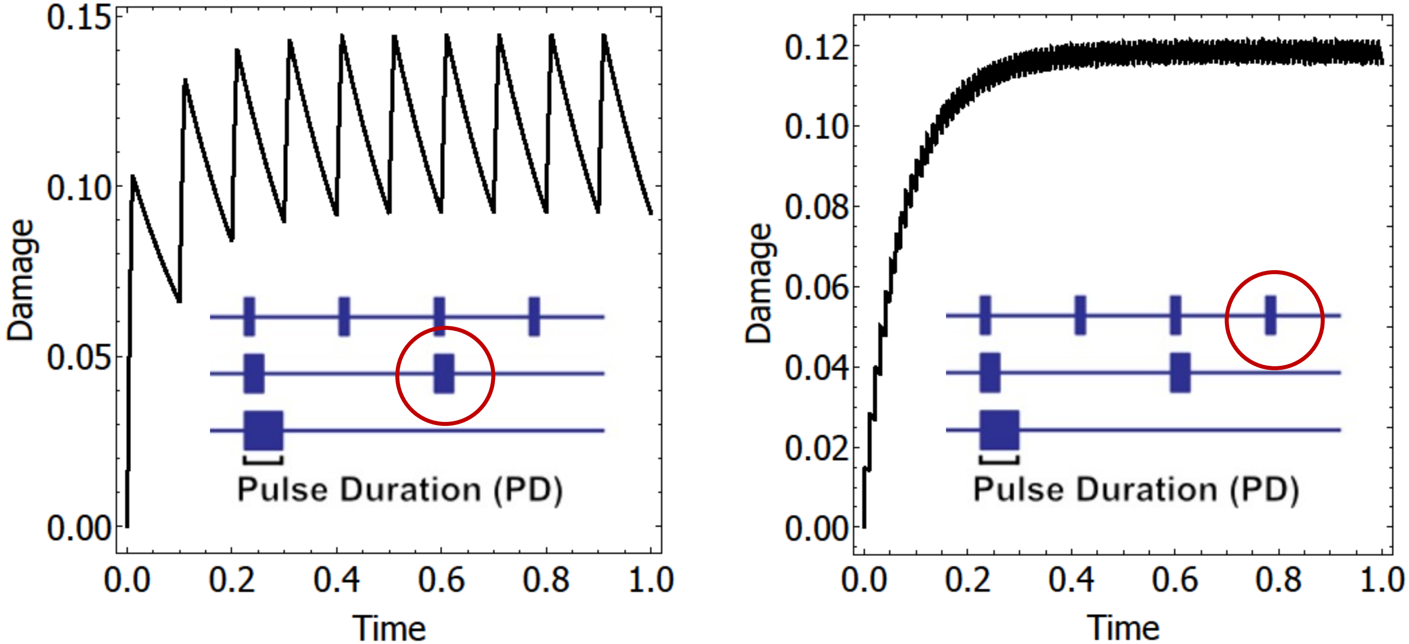


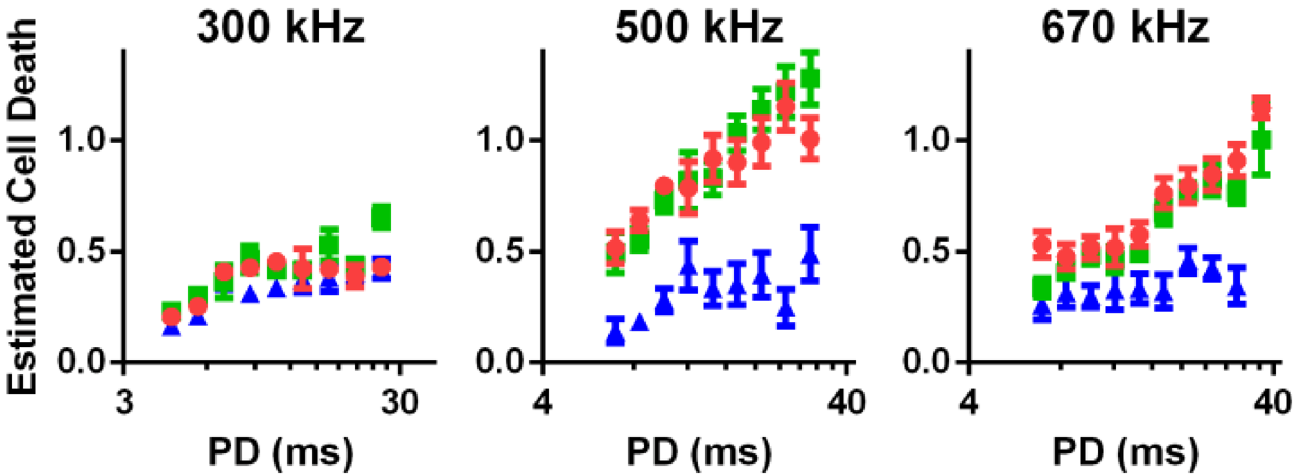
- Reduced (two d.o.f.) dynamical model:

$$\left.\begin{aligned} &m\ddot{u}(t) + c\dot{u}(t) + (1 - q(t))^2 k u(t) = -m\dot{v}(t) \\ &\alpha\dot{q}(t) + \beta q(t) = (1 - q(t))\frac{k}{2}u^2(t) \end{aligned}\right\}$$

Michael Ortiz
SEMTA 2019

High-cycle fatigue model





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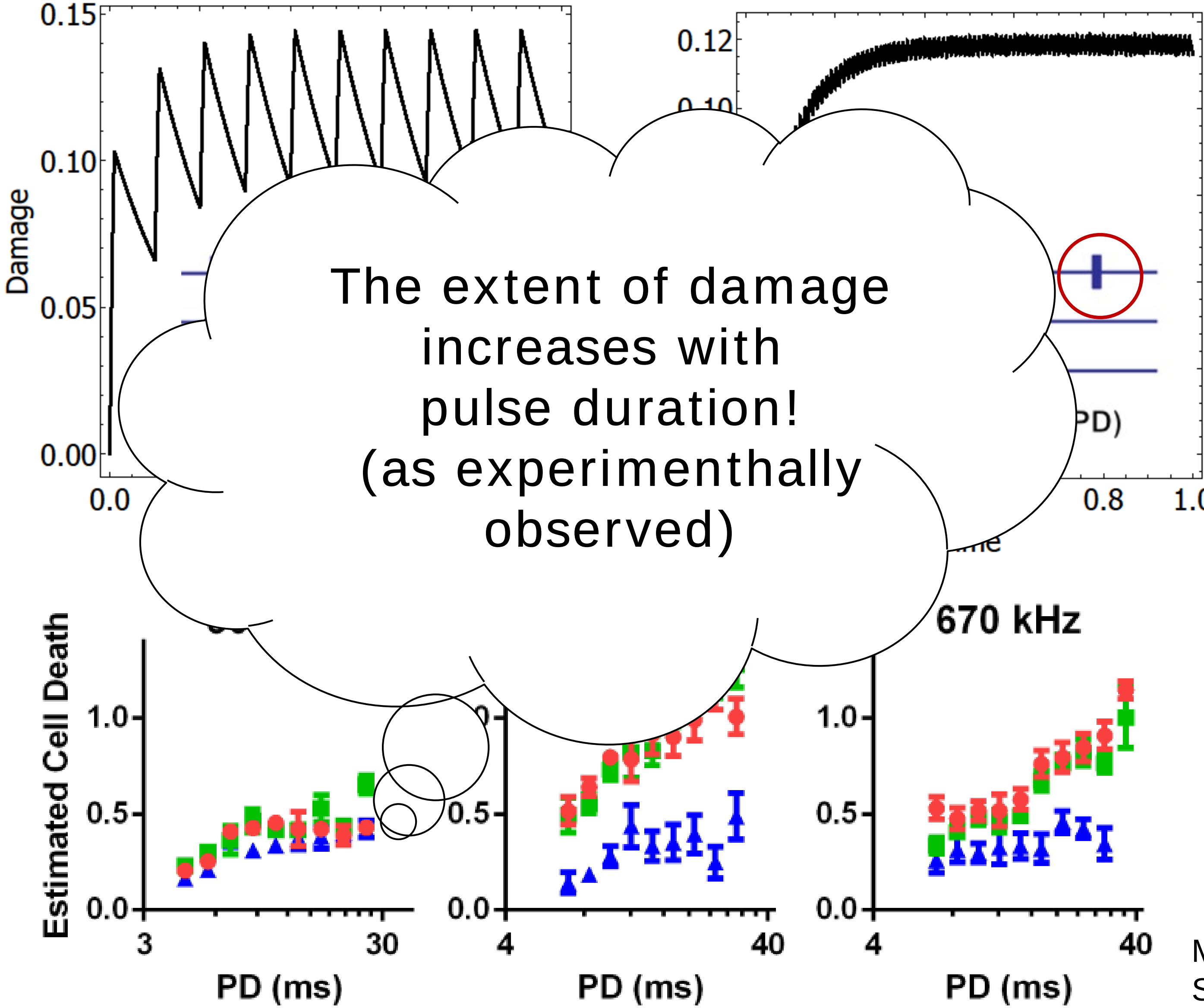
High-cycle fatigue model



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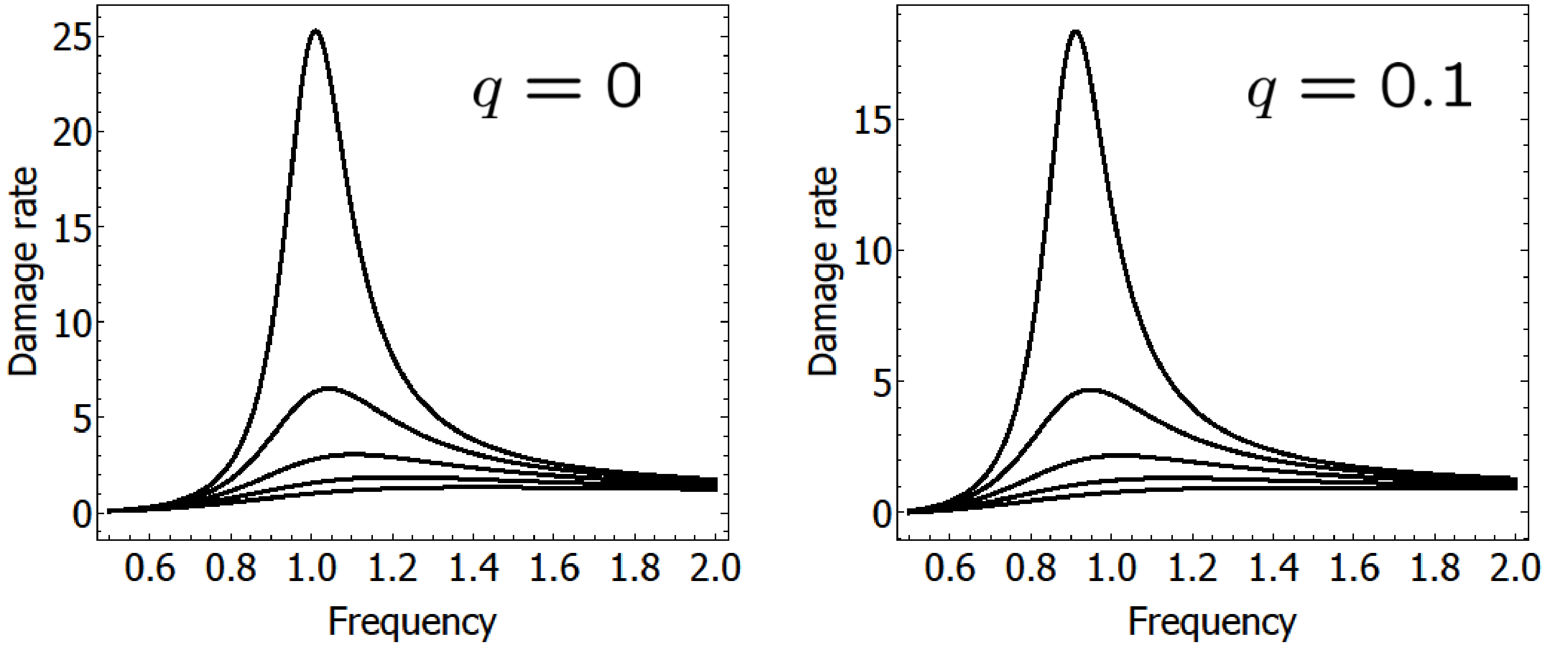
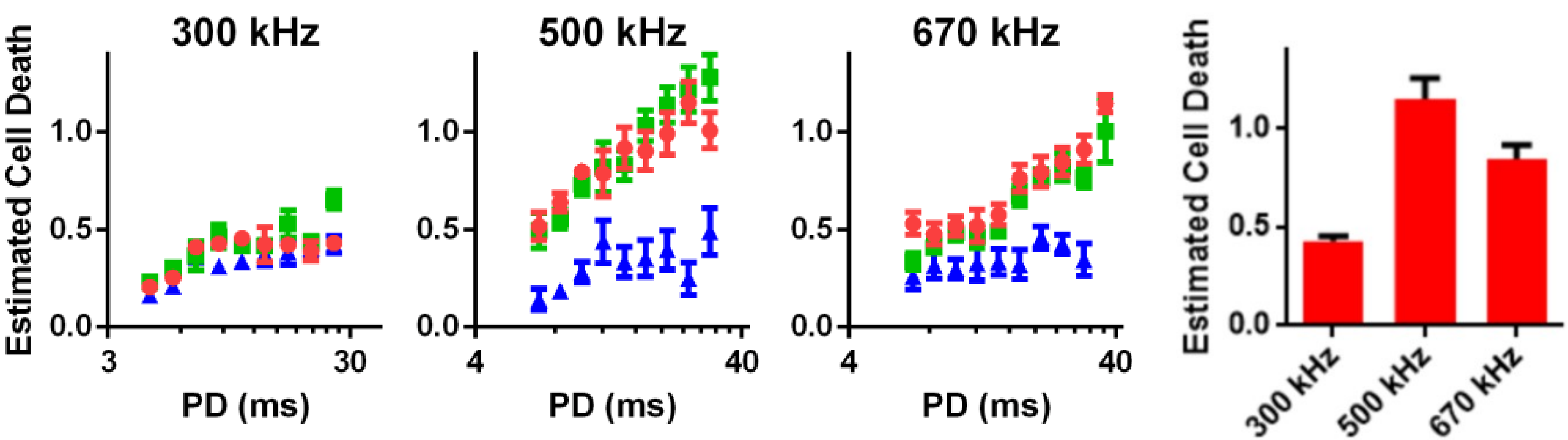
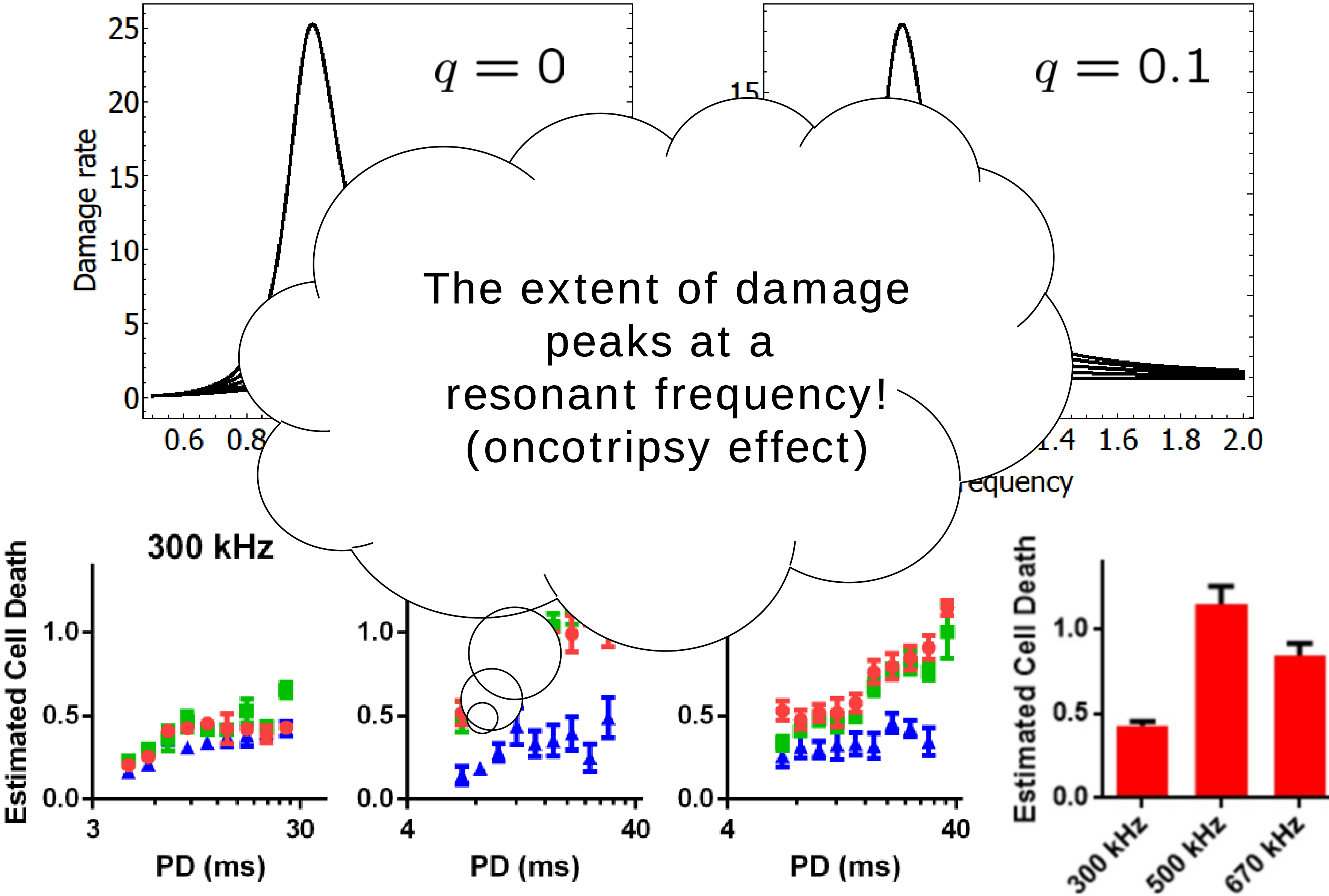
High-cycle fatigue model





High-cycle fatigue model



Concluding remarks

- A simple dynamical model of *oncotripsy* captures and quantifies all the observed experimental trends pertaining to the response of cells in aqueous suspension to low-intensity pulsed ultrasound

Thank you!