Micro-machinery for Mechano-diagnostics

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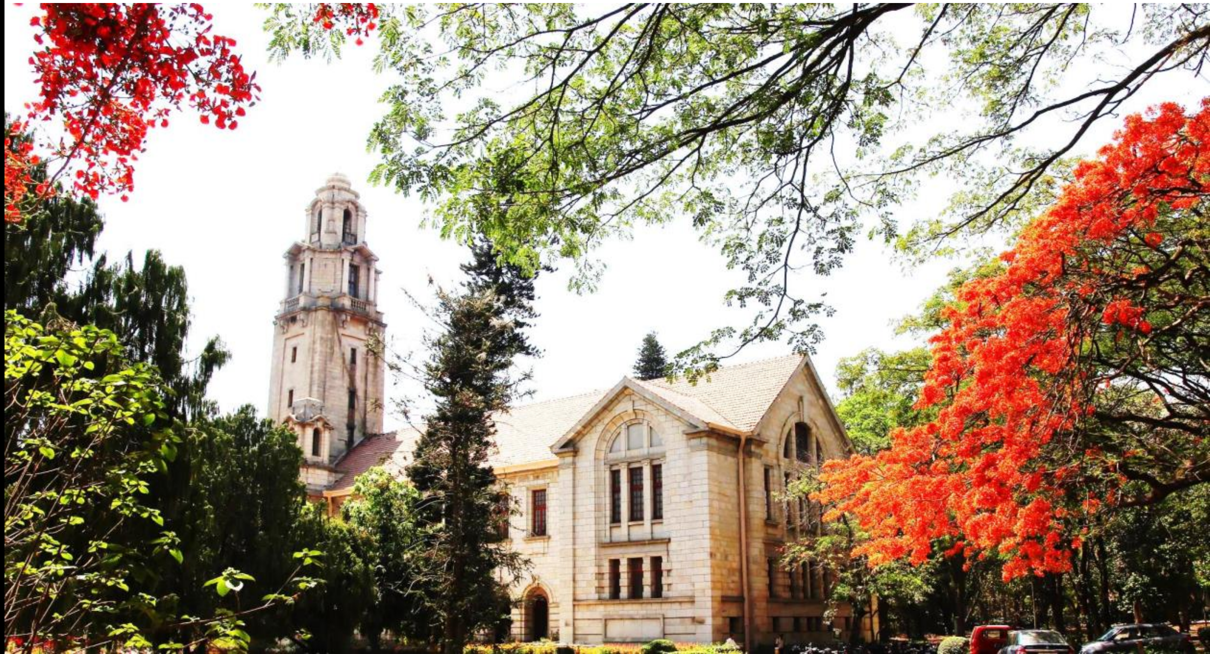
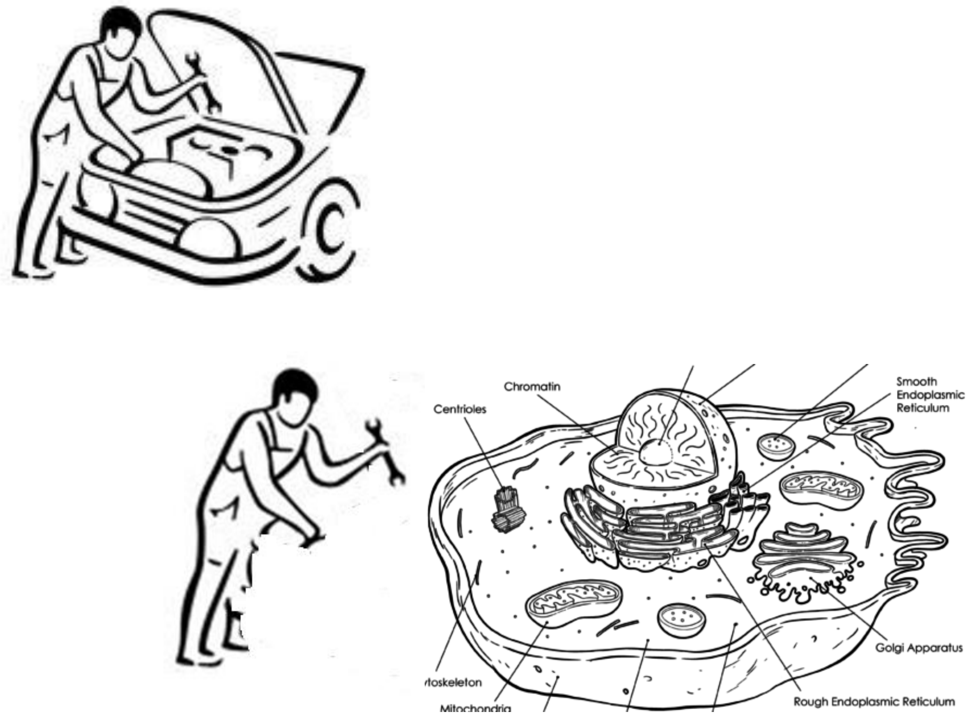


Grasping cells

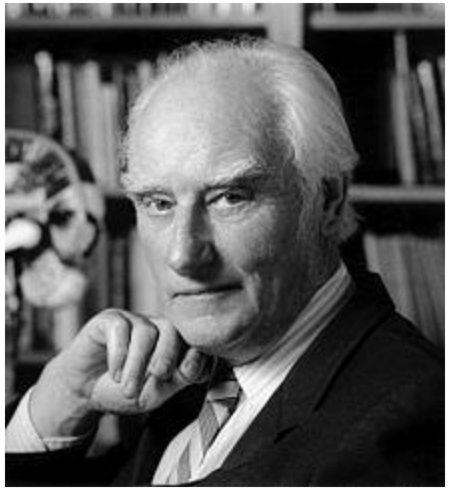
What can we learn if we can literally grasp a cell?

Mechanodiagnostics



A biological cell is a tiny water balloon that is filled with granular material. [an engineer’s view]

“If we were compelled to suggest a model for a cell … we would propose **Mother’s Work Basket** – a jumble of beads and buttons of all shapes and sizes, with pins and threads for good measure, all jostling about and held together by colloidal forces.” – **Francis Crick**



(a) twisting, (b) dragging, and (c) prodding. We shall amplify these in turn. Our work on the two last has been preliminary only.

(A) TWISTING

We have done this in two ways; firstly, by applying a large field parallel to the length of the particle, and then turning this field through a small angle; secondly, by making the particles within the cells into little permanent magnets (by magnetising them in an initial very large field, applied momentarily) and then observing their motion when a relatively small field is applied, usually perpendicular to their length. This latter method has proved to be the more useful, and has been adopted for the greater part of our work.

(B) DRAGGING

A uniform magnetic field produces a pure twist on a magnet. In order to drag it from one place to another a non-uniform field must be used, and to produce appreciable movement, a very high field-gradient is necessary. This implies that the poles of the external magnet must be as near the magnetic particle as possible. To do this so that we can observe the particles during the movement, Dr. H. B. Fell was able to produce tissue cultures on cover-slips no more than 4 mm. in diameter. The polepieces can then be brought up to one side of this culture, and the cells can then be observed with a $\times 20$ objective.

(C) PRODDING

This is really a special case of the first form of twisting. If a cell contains numerous magnetic particles, a strong magnetic field will loosely unite the particles into a rod. This rod, which may be longer than the width of the cell, can be rotated by the field to bear upon the structures of the cell. We

THE PHYSICAL PROPERTIES OF CYTOPLASM

A STUDY BY MEANS OF THE MAGNETIC PARTICLE METHOD

Part I. Experimental

F. H. C. CRICK and A. F. W. HUGHES

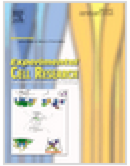
Strangeways Research Laboratory, Cambridge, England

Received October 5, 1949

ELSEVIER

Experimental Cell Research

Volume 1, Issue 1, 1950, Pages 37–80



The physical properties of cytoplasm: A study by means of the magnetic particle method Part I. Experimental

F.H.C. Crick, A.F.W. Hughes

THE PHYSICAL PROPERTIES OF ERYTHROCYTES

WILLIAM SEIFRIZ[1]

Received for publication, August 2, 1926

(With Plates VI and VII)

THE EFFECTS OF SALTS ON THE EXTENSIBILITY OF PROTO-PLASM

WILLIAM SEIFRIZ AND JANET PLOWE

THE TENSION AT THE SURFACE, AND OTHER PROPERTIES OF THE NUCLEATED ERYTHROCYTE

1939

CHARLES HAMILTON NORRIS

Physiological Laboratory, Princeton University, and the Marine Biological Laboratory, Woods Hole, Massachusetts

Biomechanical assays

Biomechanical assays offer a good alternative to biochemical assays in diagnosing disease states and in assessing the efficacy of drugs.

- Less expensive
  - No chemicals
- Small sample size
- Quick
- Portable

Cell biology

Mechanics of solids

Robert Hooke (1635-1703)

The cork cells were observed with this.

"…ceiiinossssttuv…"

"…ut tensio sic vis…"

The force varies as the stretch.

Hooke's law of linear elasticity.

$$\sigma = E\varepsilon$$

CHEM
MECH

Indirect measurements in cell biology, as in engineering,…

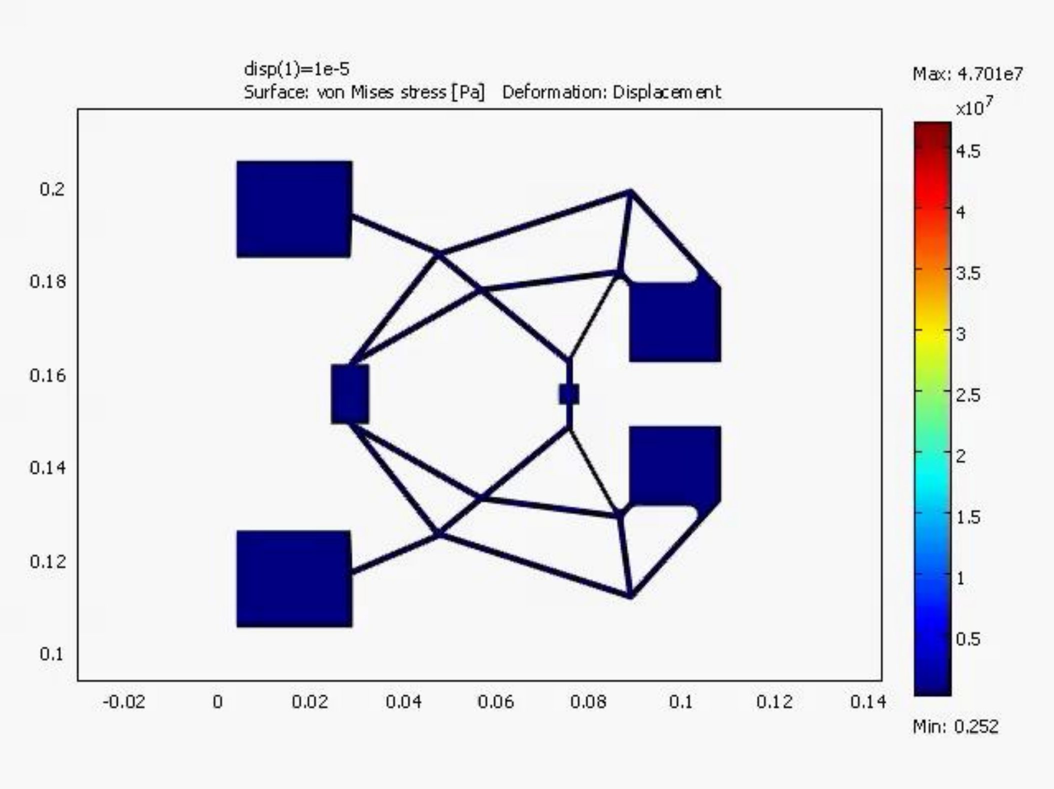
could lead to mechano-diagnostics…

eventually.

A few questions and problems

- How do *we* measure mechanical response of cells?
  - Engineering tools
- What do we make out of mechanical response?
  - Computational techniques
- How do we figure out biological implications, if any?
  - Biological assays


Compliant Mechanisms
macro . meso . micro . nano
DaCM


disp(1)=1e-5
Surface: von Mises stress [Pa]   Deformation: Displacement
Max: 4.701e7
x10^7
4.5
4
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2
1.5
1
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Min: 0.252
0.2
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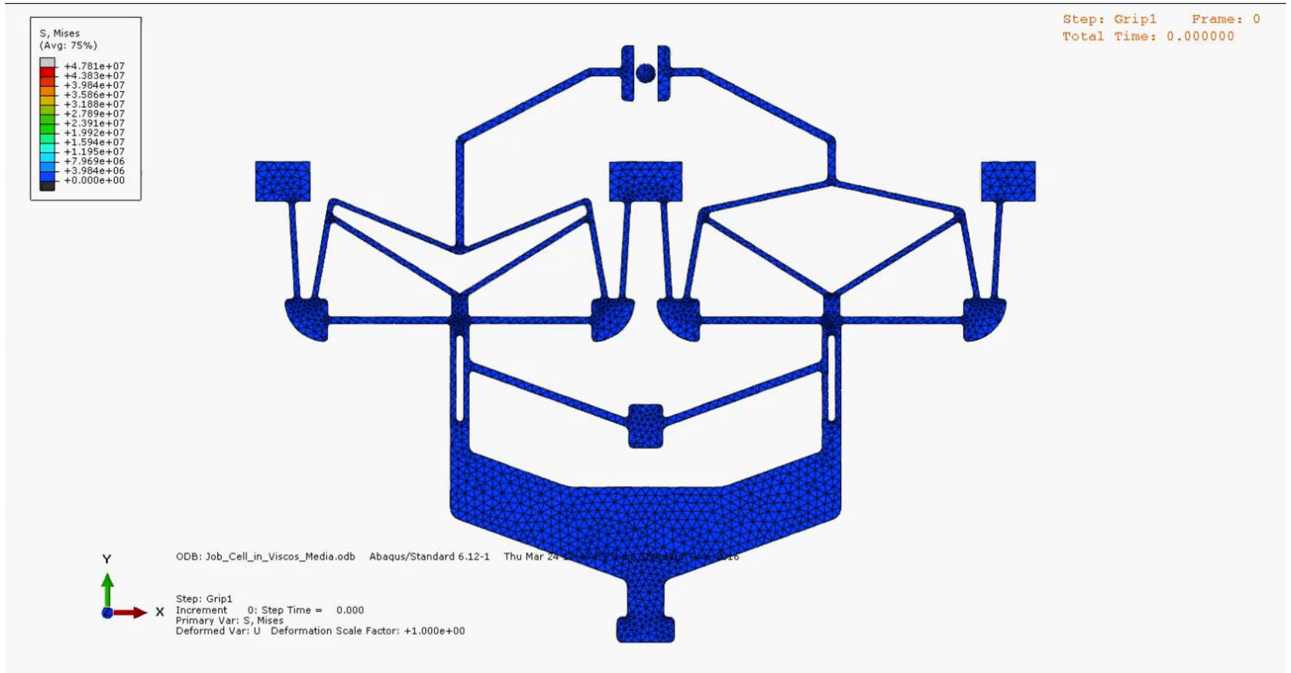
0.1
0.12
0.14


S, Mises
(Avg: 75%)
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+2.789e+07
+2.391e+07
+1.992e+07
+1.594e+07
+1.195e+07
+7.969e+06
+3.984e+06
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Step: Grip1   Frame: 0
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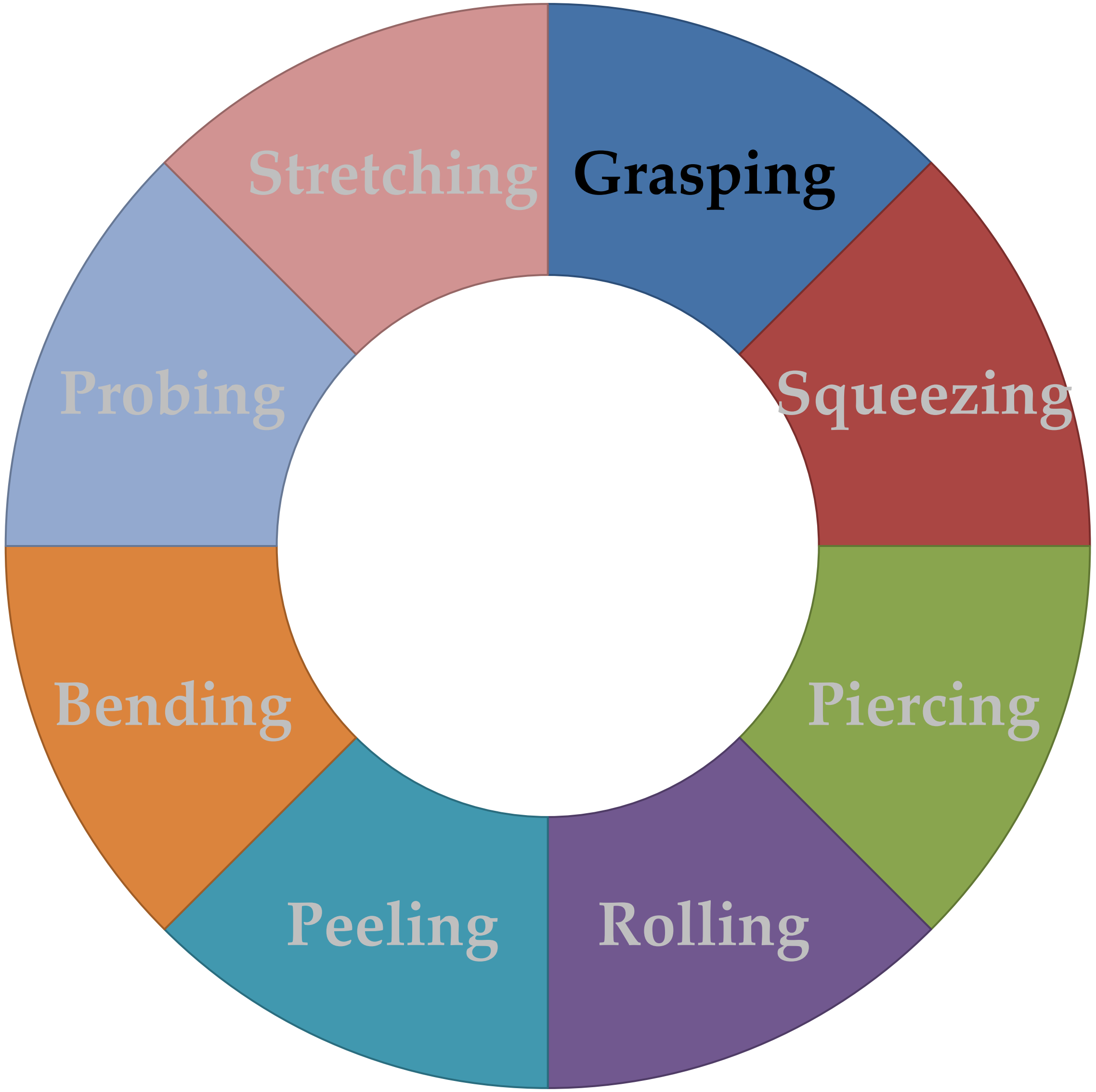
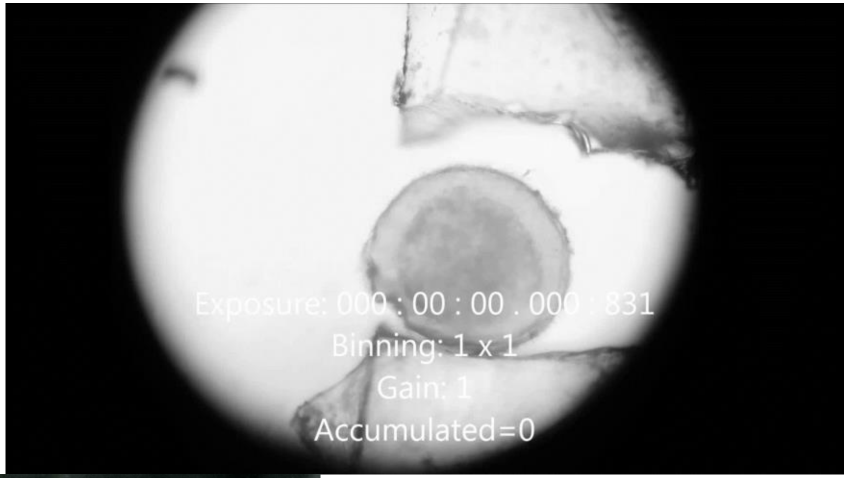
Increment   0: Step Time =   0.000
Primary Var: S, Mises
Deformed Var: U   Deformation Scale Factor: +1.000e+00


Stretching
Grasping
Probing
Squeezing
Bending
Piercing
Peeling
Rolling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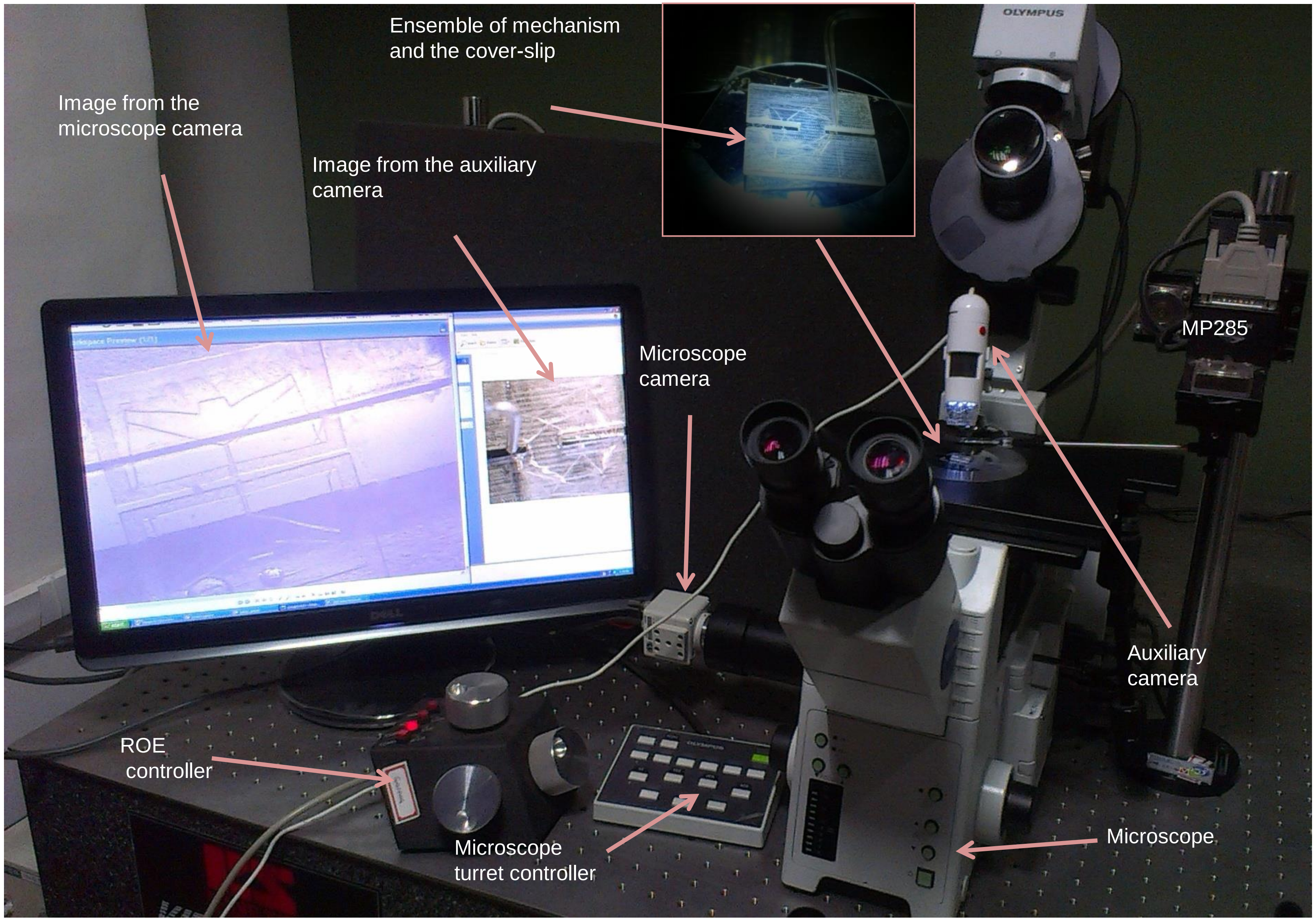
Accumulated=0

Bio-micromanipulation setup



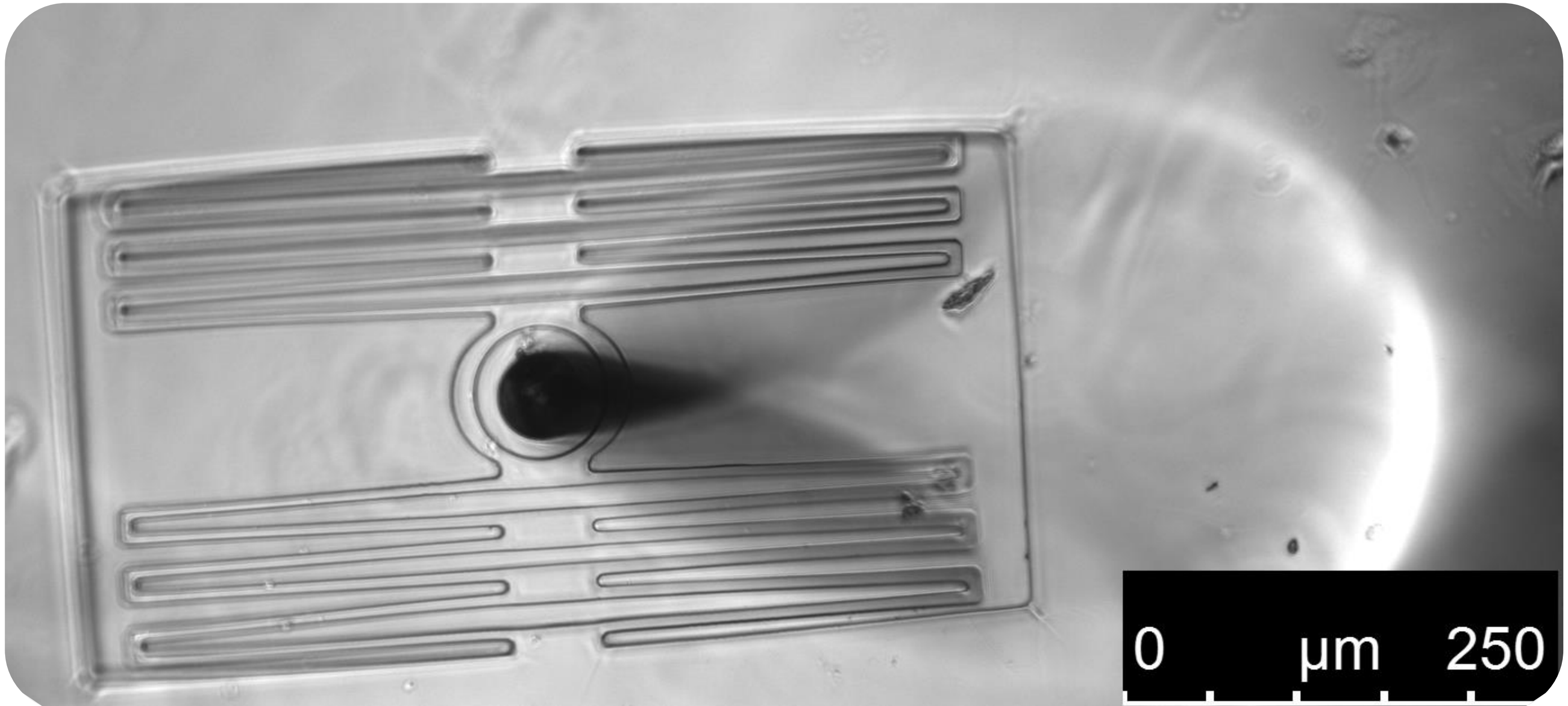
IEEE Transactions on Robotics, Vol. 26, No. 5, 2010; Reddy et al.

In situ stretching of adherent cells

A micro-mechanical compliant device for individual cell-stretching, compression, and in-situ force measurement

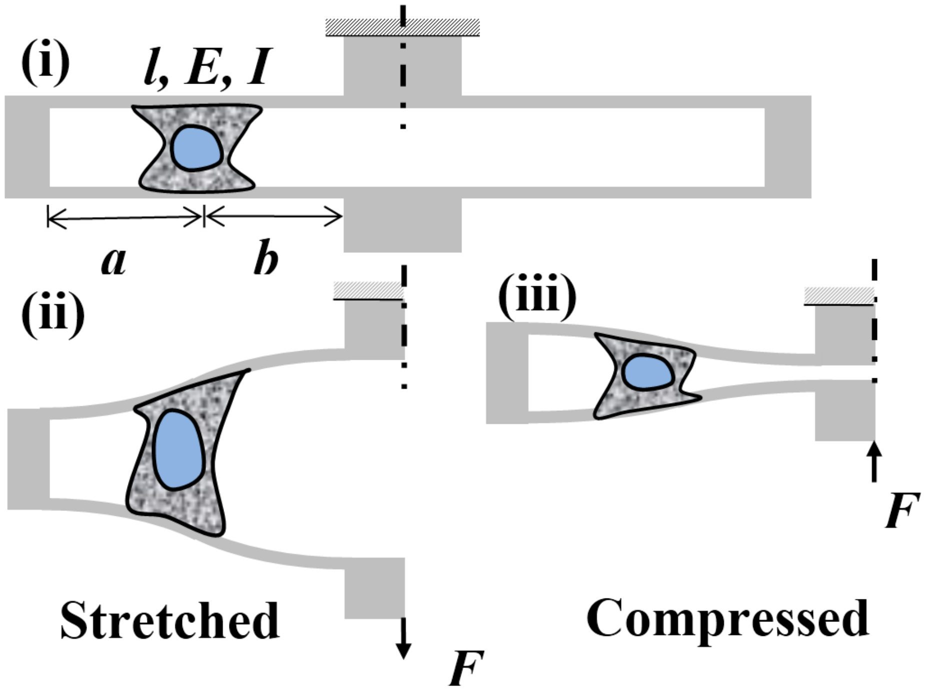
Kollimada et al.

Journal of Micro-Bio Robotics, Vol. 13, 2018, pp. 27-37.

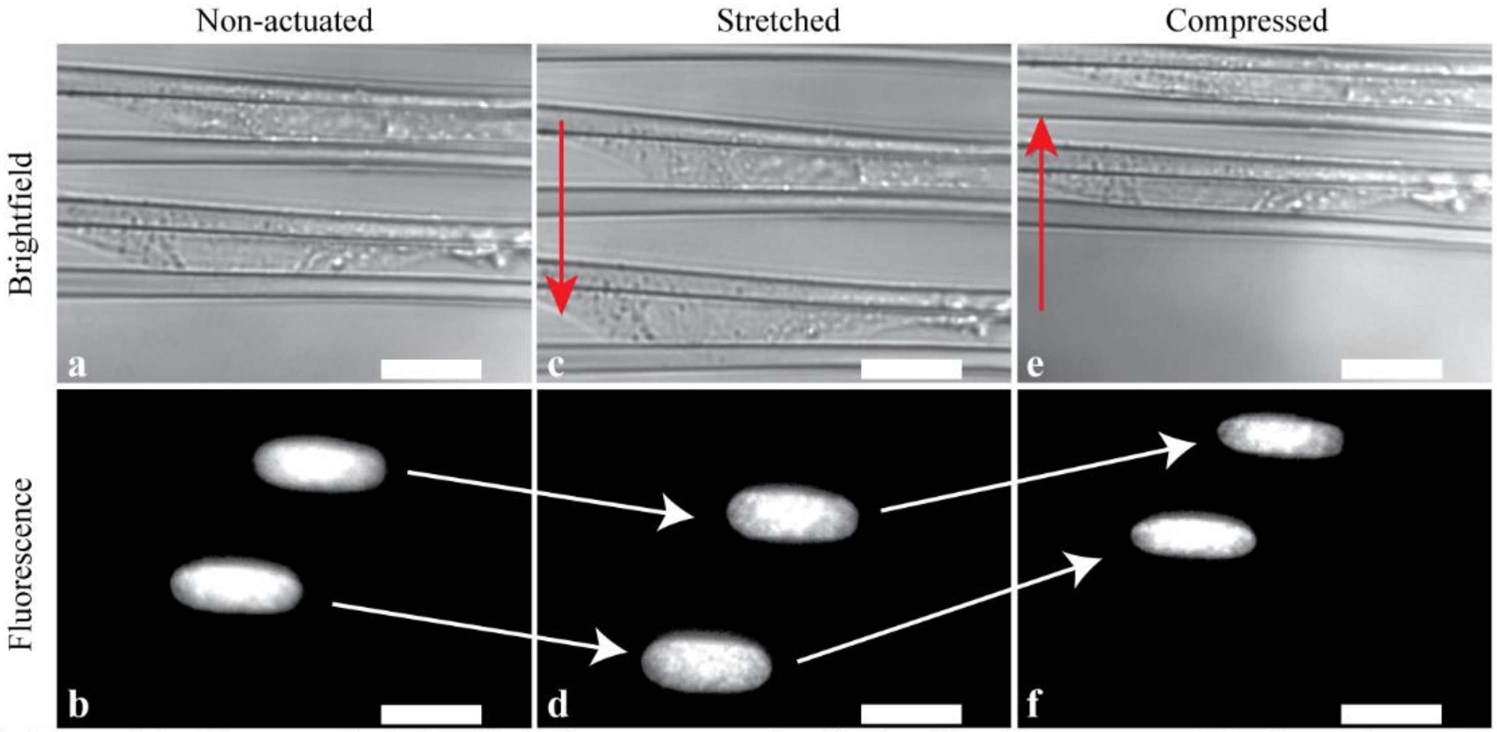


Cell-stretcher can also measure forces.

- Ability to stretch and compress individual cells.
- Scalability- Multiple devices on a 22 mm × 22 mm region.
- Ability to measure forces exerted by cells.
- Transparent material to allow easy imaging of cells.
- Provision for high magnification (20X-60X) imaging.

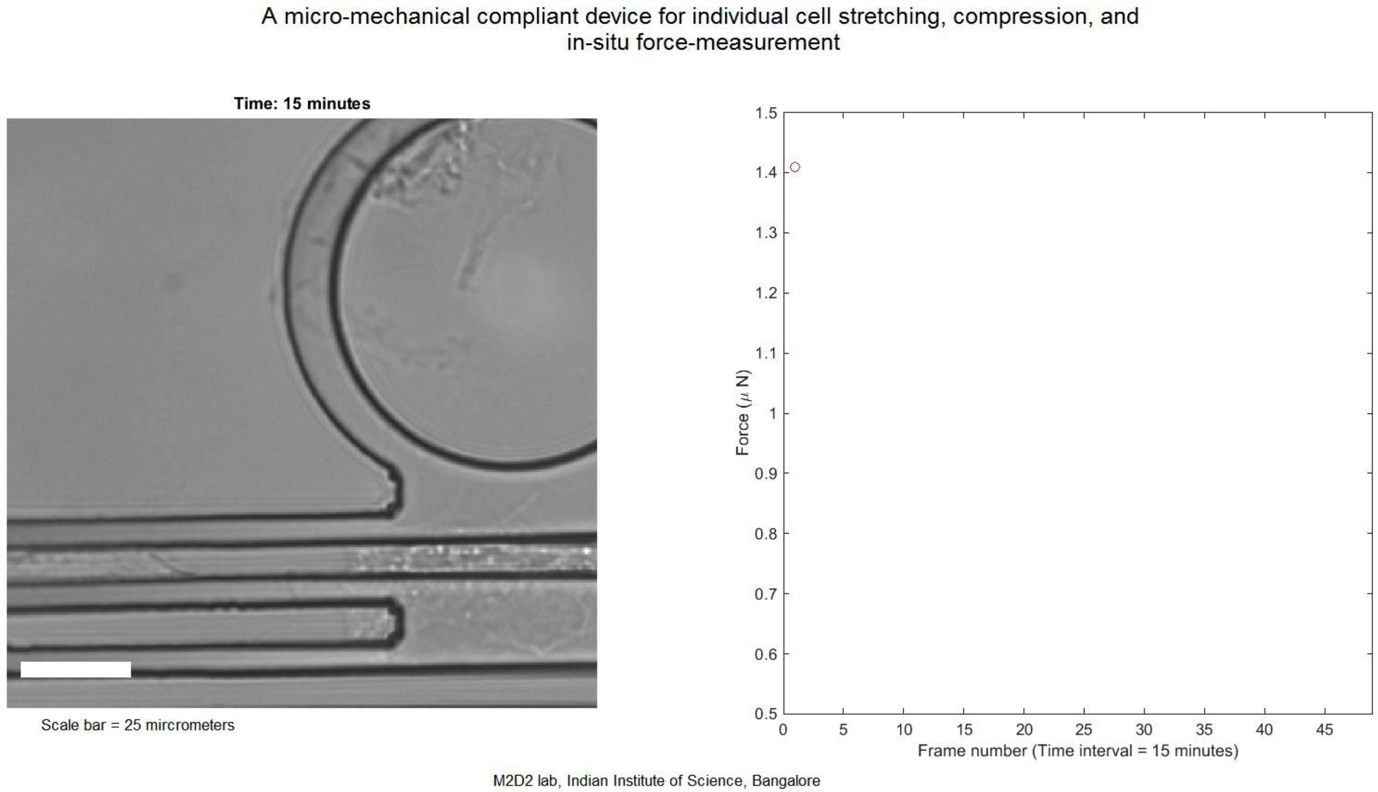


Cells in stretching and compression

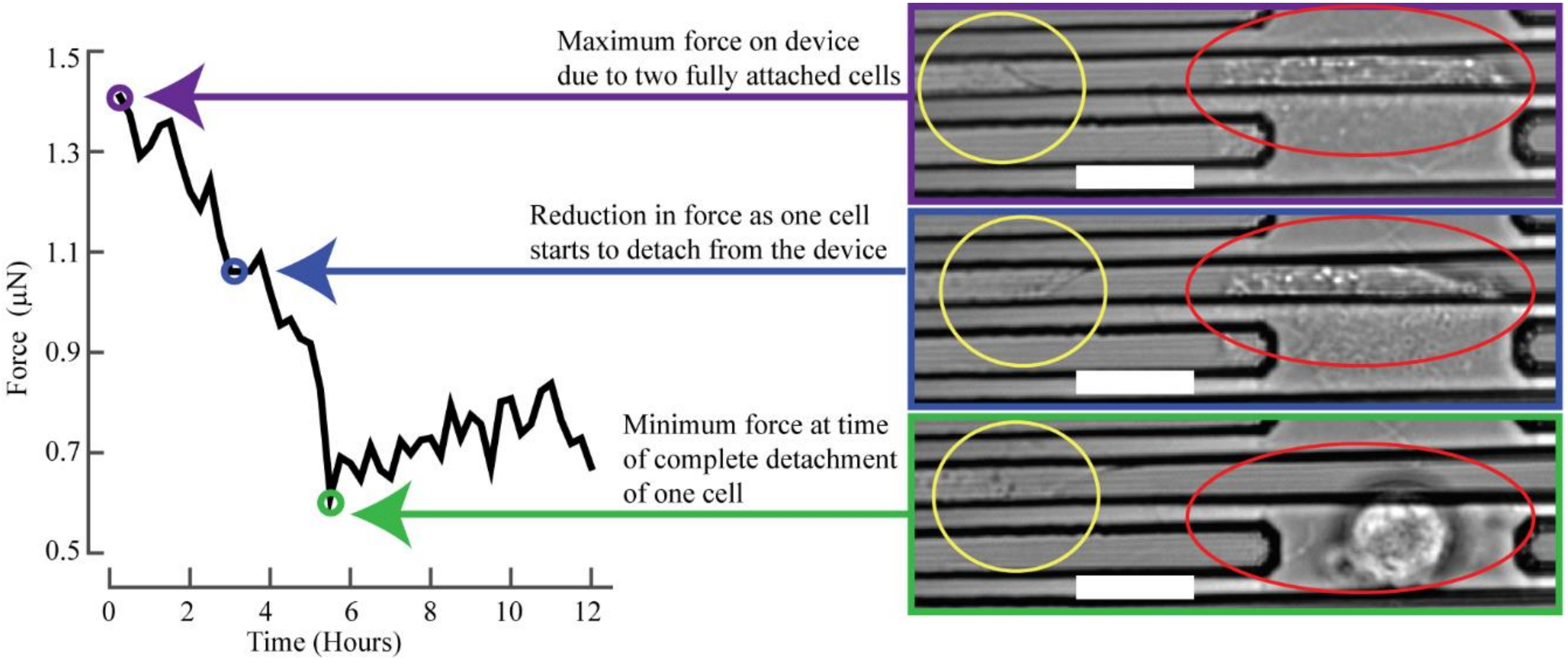


Non-deformed (a, b), stretched (c, d) and compressed cells (e, f) on an actuated device. The red arrows in images (c) and (e) show the direction of actuation in the case of stretching and compression of cells. Brightfield images (a, c, e) show two cells between the folded beams and the fluorescent images (b, d, f) show the cell nuclei. The white arrows show the corresponding nucleus in each image. Scale bar: 25 µm

Real-time force-measurement



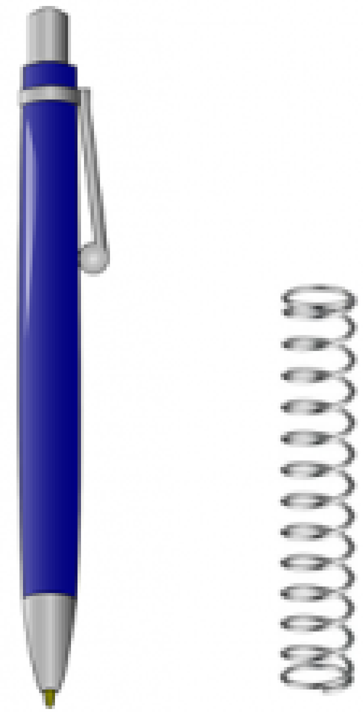
Force plot



Plot of force exerted by two cells (cells shown in yellow and red circles) attached between a single folded beam on a non-actuated device over 12 hours. Maximum force exerted at start of experiment. Steady decrease in force seen as one of the cells (red circle) detaches from the beams during cell division. Images of the cells at time T = 15, 165 and 330 minutes after start of experiment and corresponding force at those time points shown on plot. Scale bar: 25 µm

How flexible is a biological cell?

How flexible is a spring in a ballpoint pen?

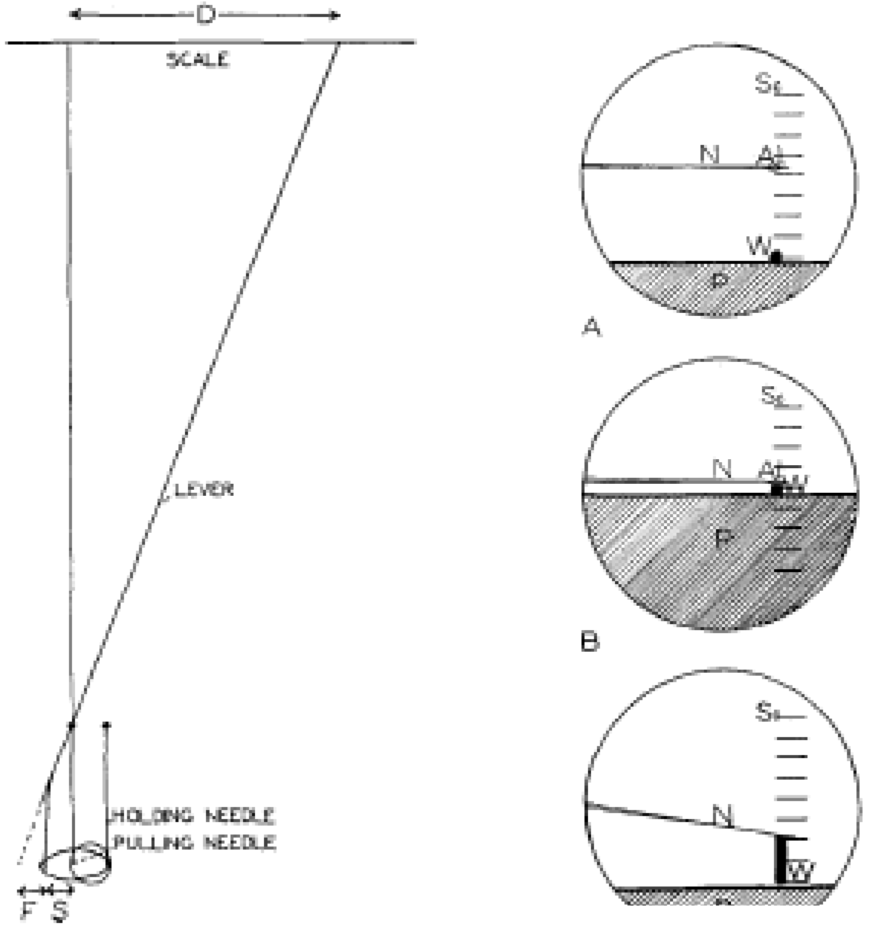


To compress it by 1 cm, how much force do you apply?

1 N/cm = 100 N/m

The spring constant of a cell is around 0.001 N/m.

Measuring forces



Cell-stiffness

1.4 dynes/cm

= 0.0014 N/m

= 1.4 mN/m

= 1.4 μN/mm

= 1.4 pN/nm

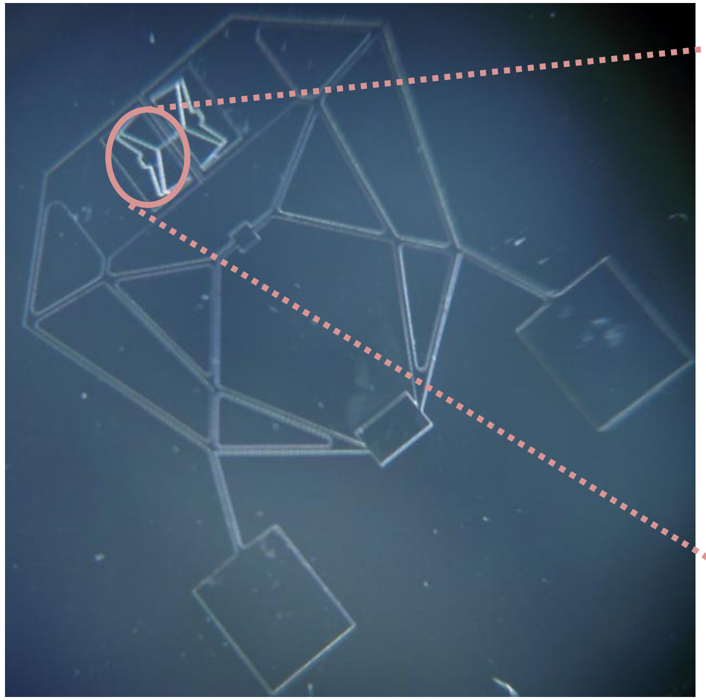
1939

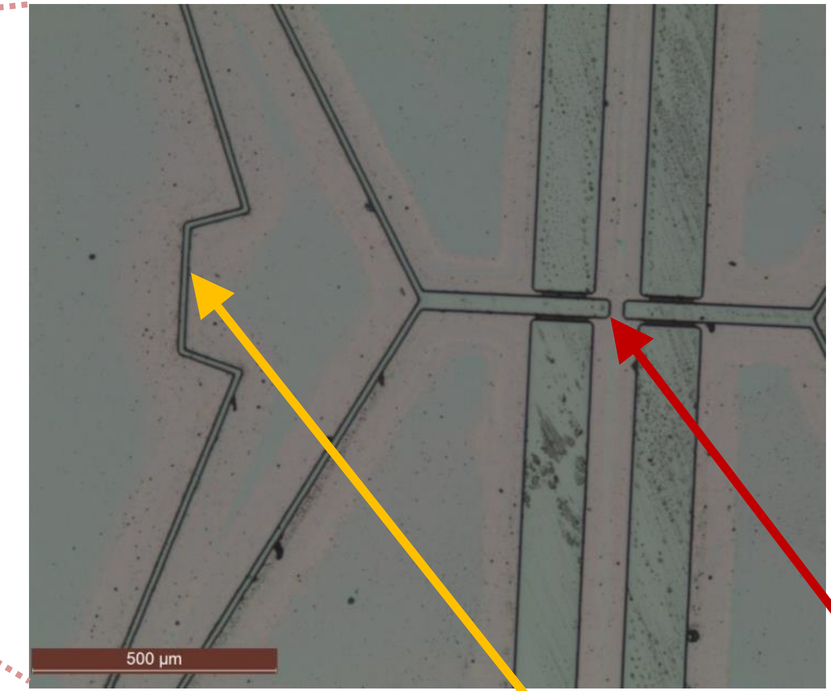
THE TENSION AT THE SURFACE, AND OTHER PHYSICAL PROPERTIES OF THE NUCLEATED ERYTHROCYTE [1]

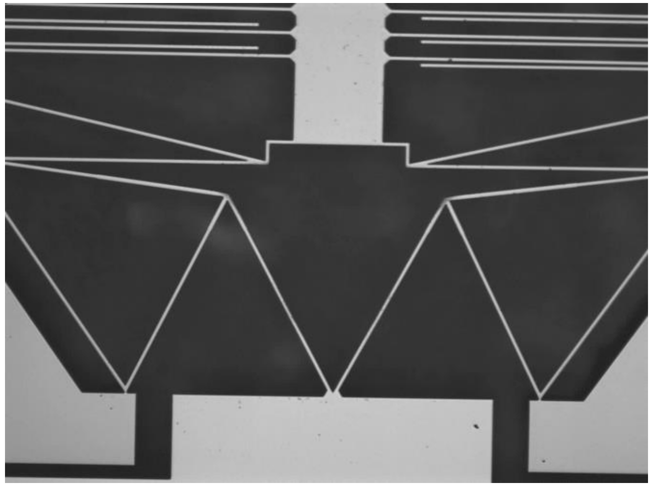
CHARLES HAMILTON NORRIS

Physiological Laboratory, Princeton University, and the Marine Biological Laboratory, Woods Hole, Massachusetts

SU-8 micro-gripper

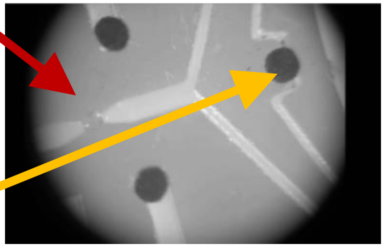






Mechanical amplification

Cell is here.



Measure there.

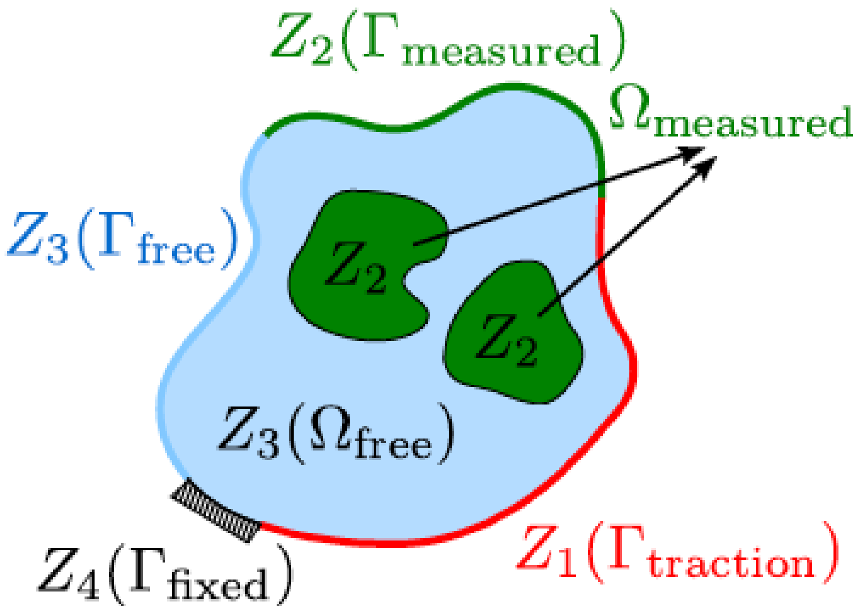
Bhargav, et al., J. ISSS, 3(2), pp. 7-14.

Int. J. Numer. Meth. Engng 2008; **76**:1645–1677; Reddy and Ananthasuresh

1st inverse problem

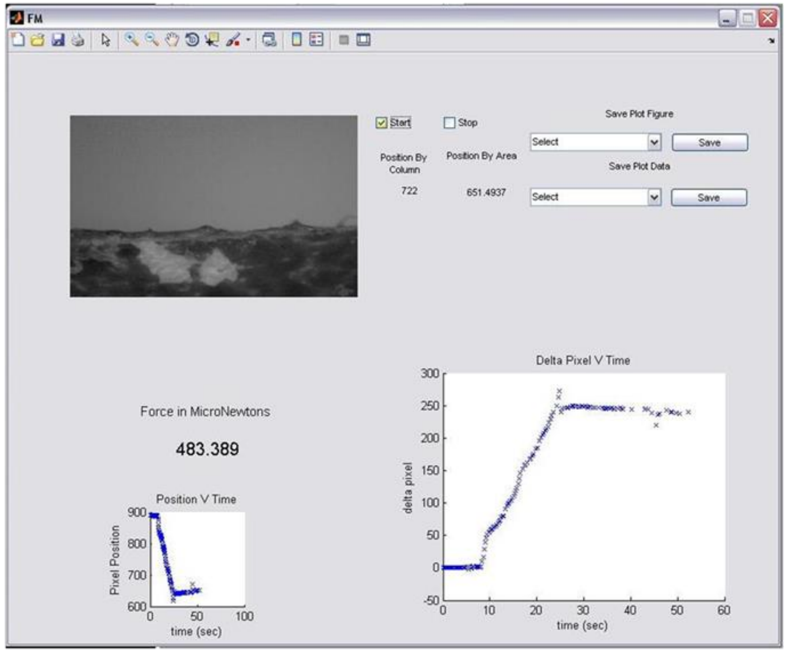
- Governing equation:
  $\nabla.\mathbf{T} + \mathbf{b} = 0$ on Ω
- Cauchy data:
  $\mathbf{u} = \mathbf{u}_m$ and $\bar{\mathbf{t}} = \mathbf{0}$ on Z_2
- Boundary condition:
  $\mathbf{u} = \mathbf{0}$ on Γ_{fixed}
- Constitutive relation:
  $\mathbf{T} = \lambda\, tr(\tilde{\mathbf{E}})\mathbf{I} + 2\mu\tilde{\mathbf{E}}$
- Green's strain tensor:
  $\tilde{\mathbf{E}} = \frac{1}{2}(\nabla\mathbf{u} + \nabla\mathbf{u}^{\mathrm{T}} + \nabla\mathbf{u}^{\mathrm{T}}\nabla\mathbf{u})$

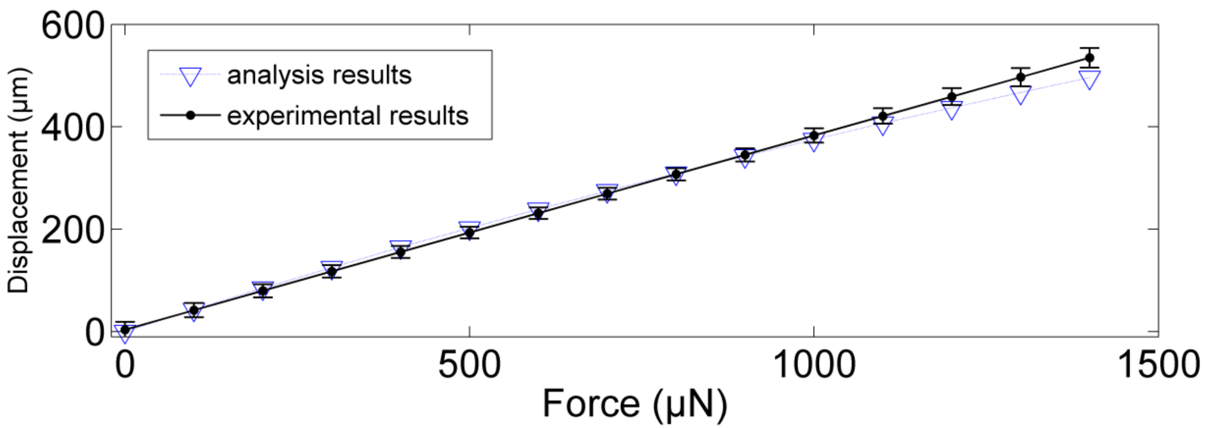
Cauchy's problem in elasticity



Zone	Name	Displacements	Forces
Z_1	Force(traction) zone	Not known	Unknown
Z_2	Measured zone	Known (measured)	Known (Zero)
Z_3	Free zone	Not Known	Known (Zero)
Z_4	Fixed zone	Known (specified)	Unknown

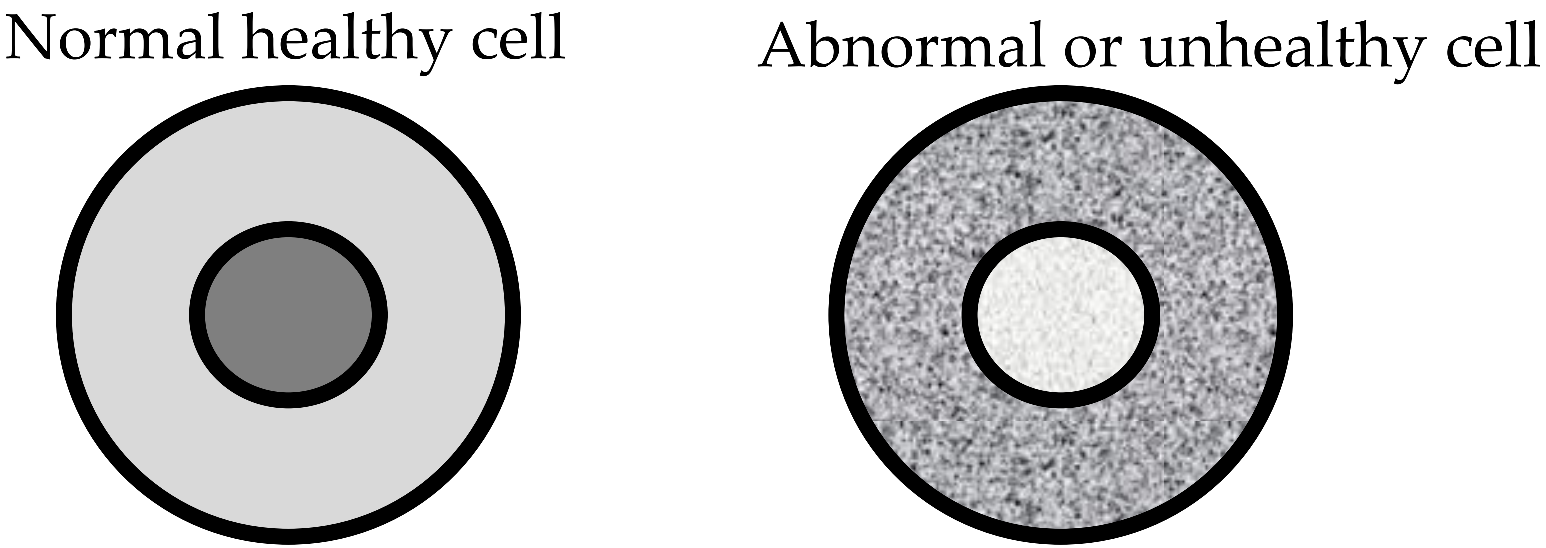
A vision-based force-sensor





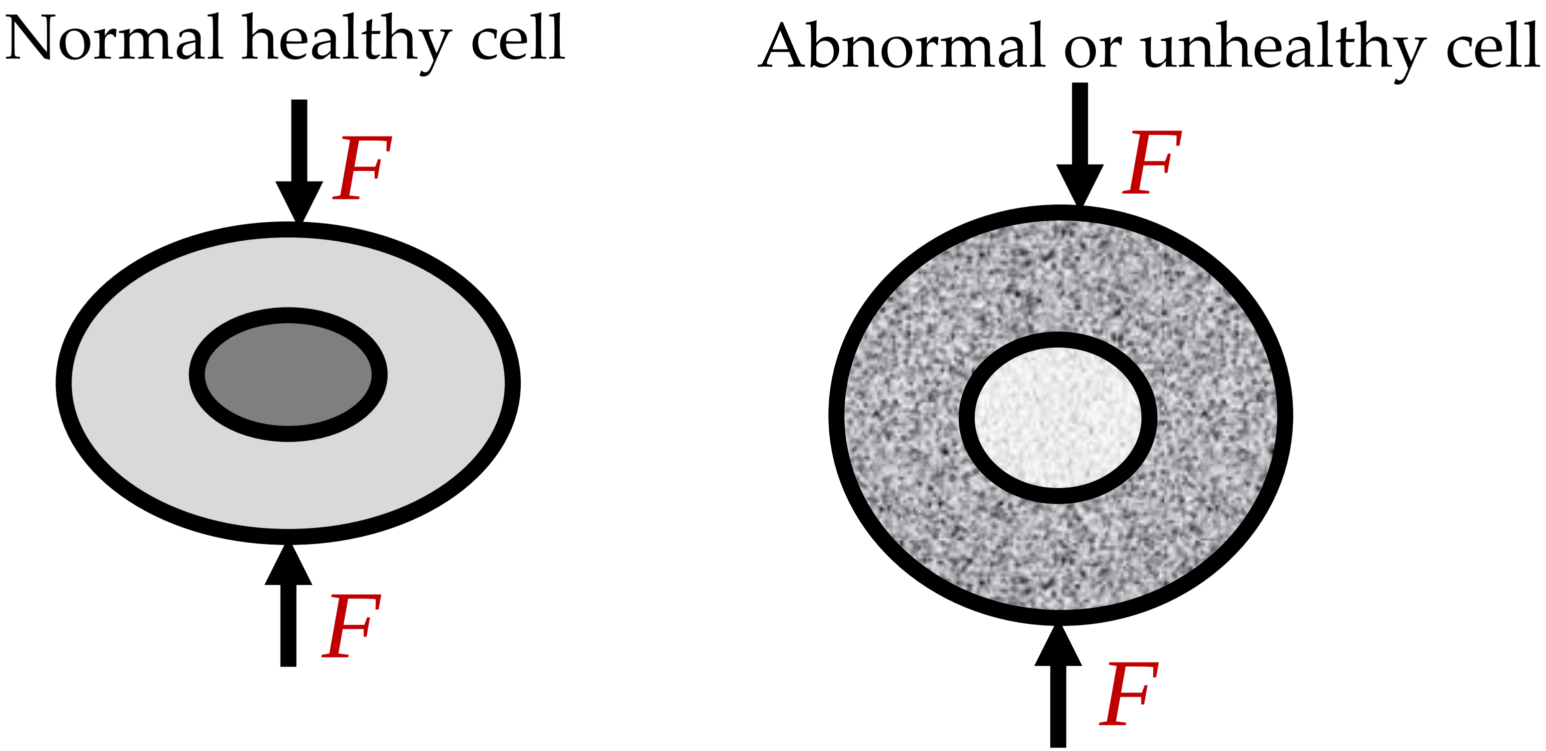
Outline of the talk

- How do *we* measure mechanical response of cells?
  - Engineering tools
- What do we make out of mechanical response?
  - Computational techniques
- How do we figure out biological implications, if any?
  - Biological assays



Mechanical responses of these two cells are likely to be different.

Stiffness, strength, stability, viscoelastic behavior, resonance frequency…



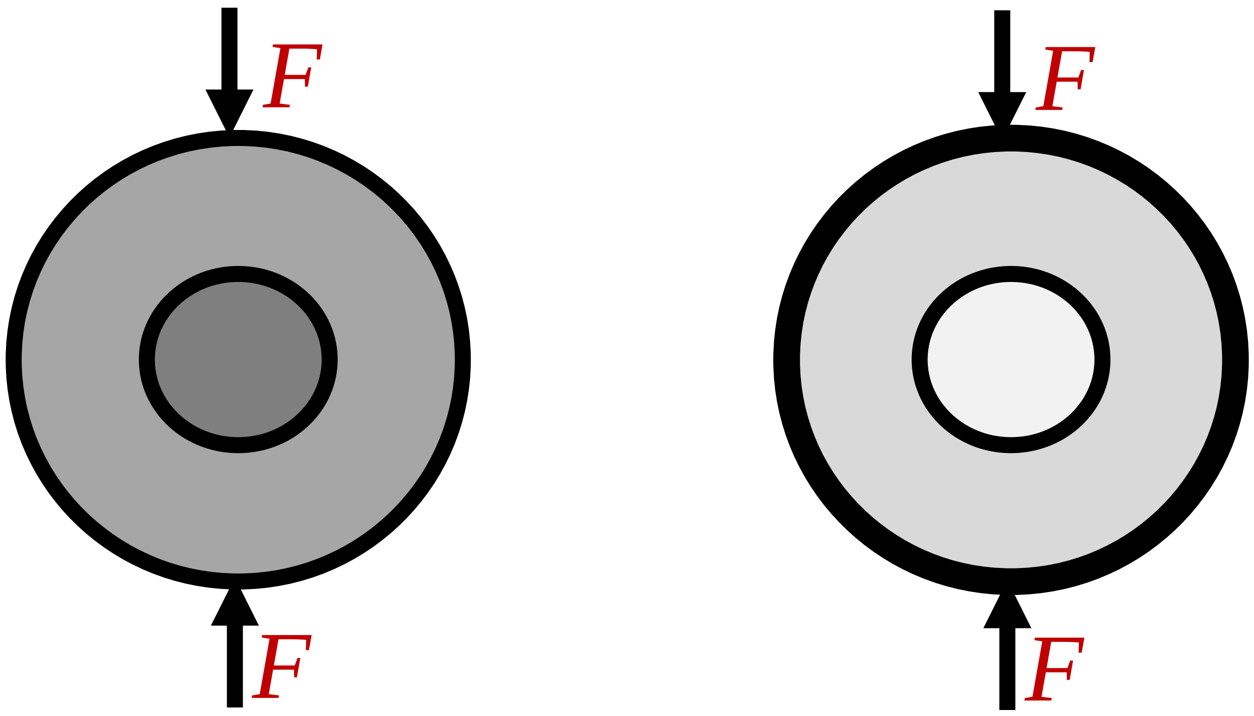
Bulk stiffness measured in N/m.

Unhealthy cell is stiffer than the healthy cell.

Is this enough for diagnostics?

Specificity is important in biology.

- When stiffness is different, how much is it different?
- In what mode is it different?
- What changed the bulk stiffness?
  - Which organelle contributed to the change?



These two may show the same bulk stiffness.
How do we tell them apart?

DtN map: an inverse problem

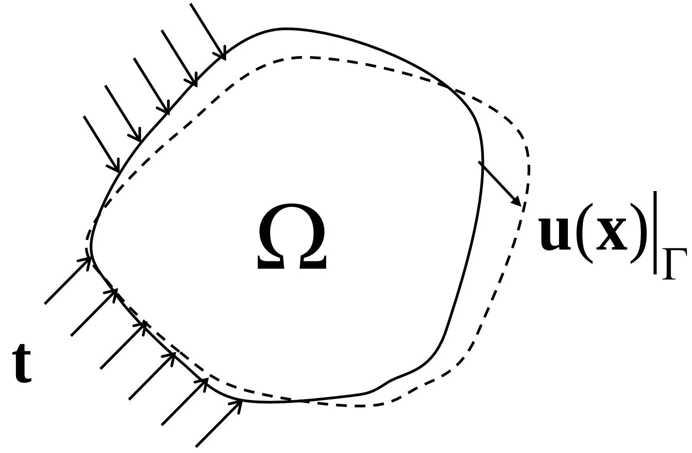
$$\nabla \cdot \boldsymbol{\tau} = 0 \quad \text{in} \quad \Omega$$

$$\boldsymbol{\tau} = \lambda \, \mathrm{tr}(\boldsymbol{\varepsilon})\mathbf{I} + 2\mu\boldsymbol{\varepsilon}$$

$$\boldsymbol{\varepsilon} = \frac{1}{2}\left(\nabla\mathbf{u} + \nabla\mathbf{u}^{\mathrm{T}}\right)$$

$$\mathbf{t} = \mathbf{t}^{*} \quad \text{on} \quad \Gamma$$

$$\mathbf{u} = \mathbf{u}^{*} \quad \text{on} \quad \Gamma$$

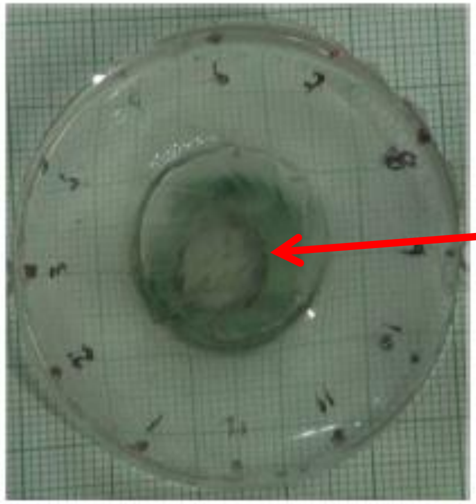


Dirichlet-to-Neumann map:

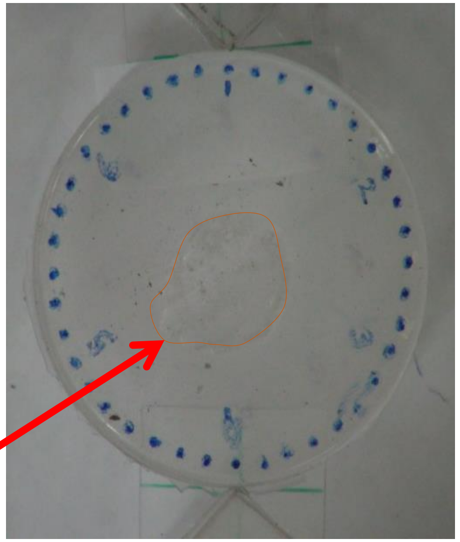
$$M_{\lambda,\mu} : \mathbf{u}|_{\Gamma} \rightarrow \mathbf{t}$$

$$\text{where} \quad t_i = \frac{1}{2}\sum_{j,k,l=1}^{3} n_j C_{ijkl}\left(\frac{\partial u_k}{\partial x_l} + \frac{\partial u_l}{\partial x_k}\right)\Bigg|_{\Gamma}$$

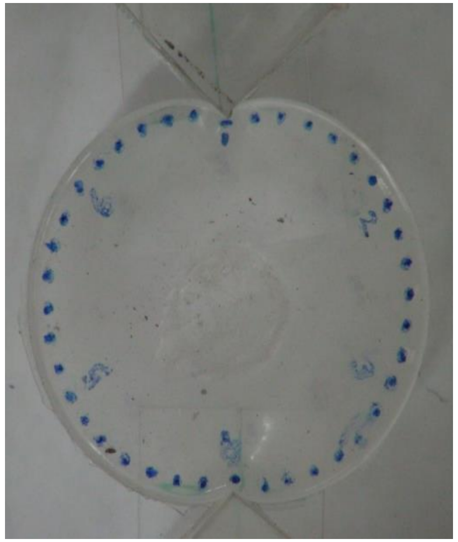
Can we tell which has a stiff inclusion by feeling only the boundary?



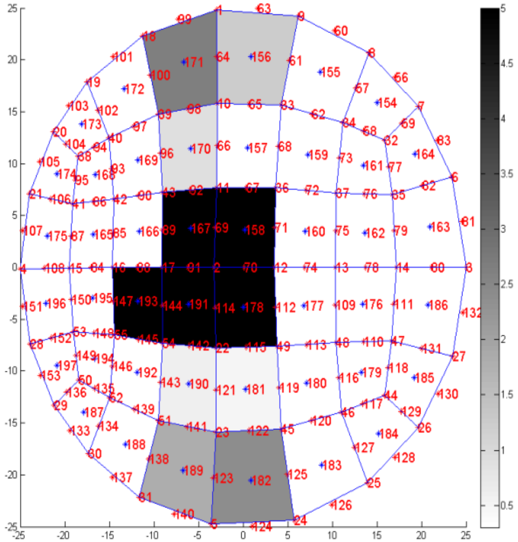
Stiffer inclusion



Undeformed configuration



Deformed configuration



Estimated elastic map

Uniqueness is guaranteed if…

- Multiple datasets of force and displacements are considered on the boundary.
- How many datasets?

Inverse Problems in Science and Engineering

A numerical approach to determine the sufficiency of given boundary data sets for uniquely estimating interior elastic properties

A. Narayana Reddy [a] & G.K. Ananthasuresh [a]

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- How do *we* measure mechanical response of cells?
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- What do we make out of mechanical response?
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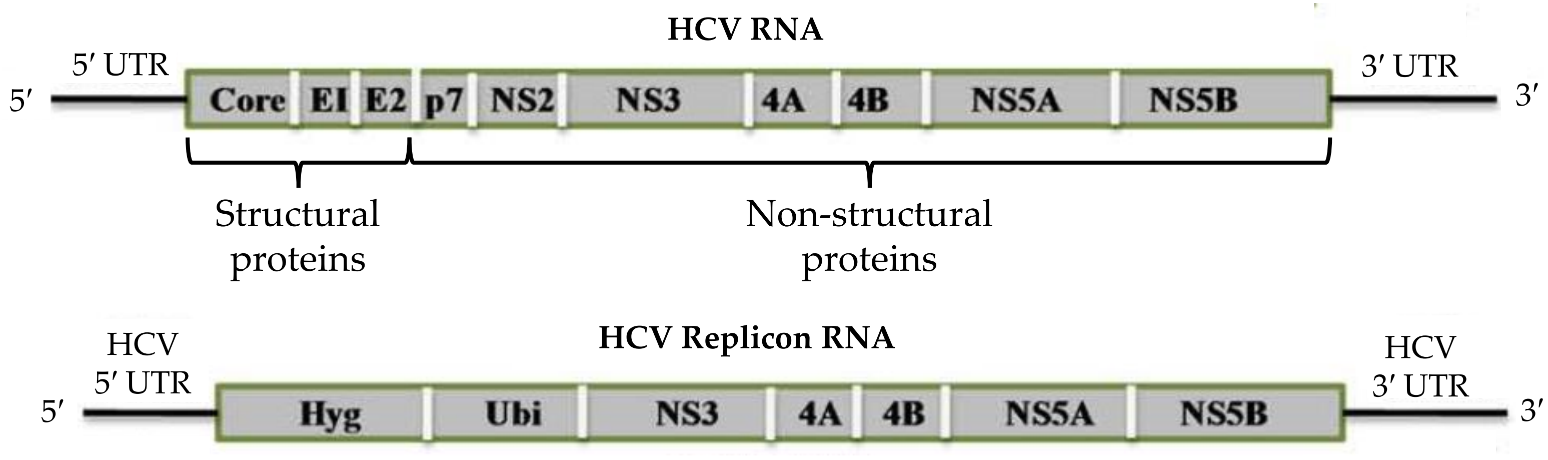
Investigating the changes in biomechanics of liver cells upon Hepatitis C Virus infection

Sreenath Balakrishnan

G.K. Ananthasuresh

Saumitra Das

Model system: Huh7 harboring HCV replicon



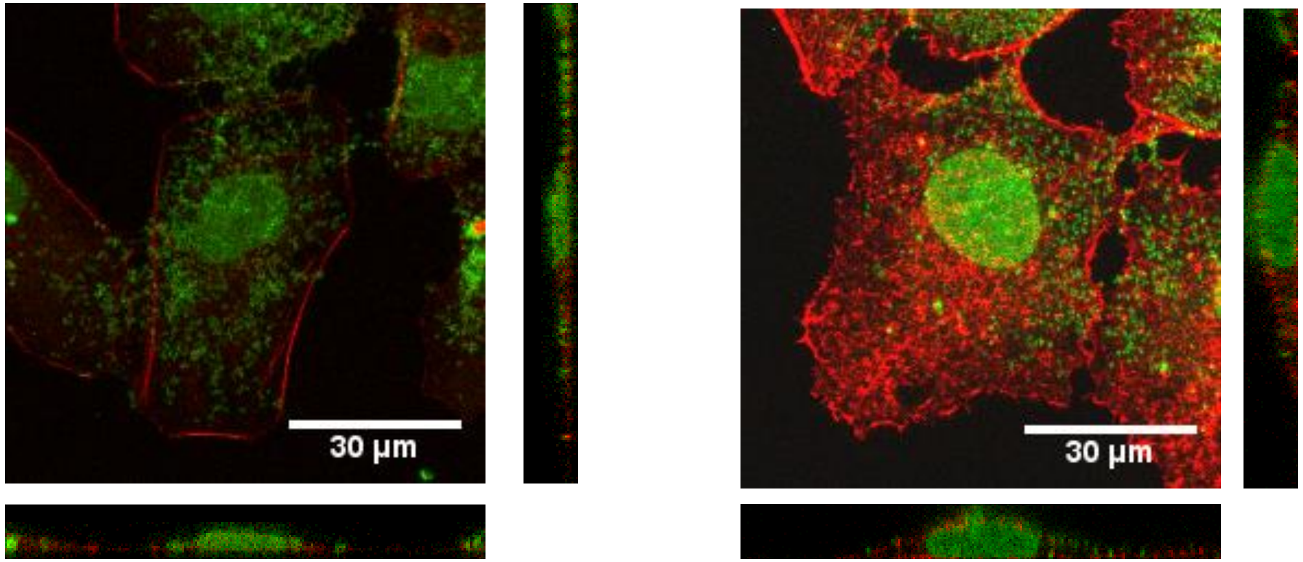
Adapted from Shivaprasad et al. *Journal of virology* (2015)

Huh7

Huh7 harbouring HCV Replicon

Nuclear morphology

Huh7 HCV replicon

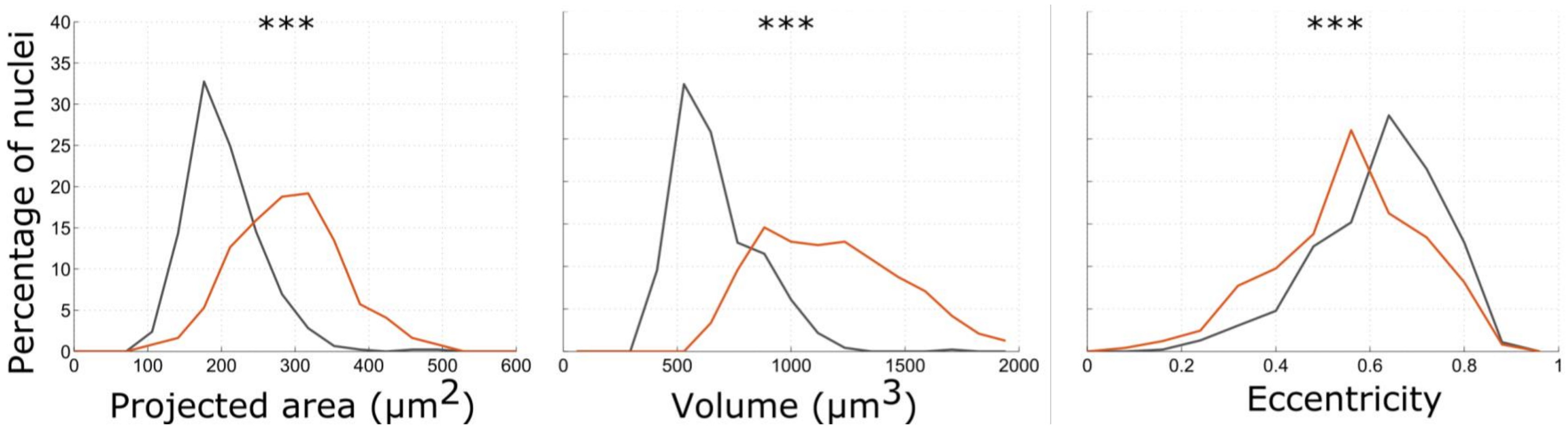


Nucleus

Actin

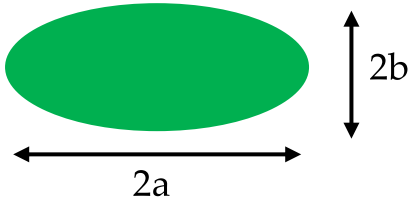
The nuclei of HCV replicon cells appear to have larger area and height.

Morphological changes



Huh7 (N = 461)

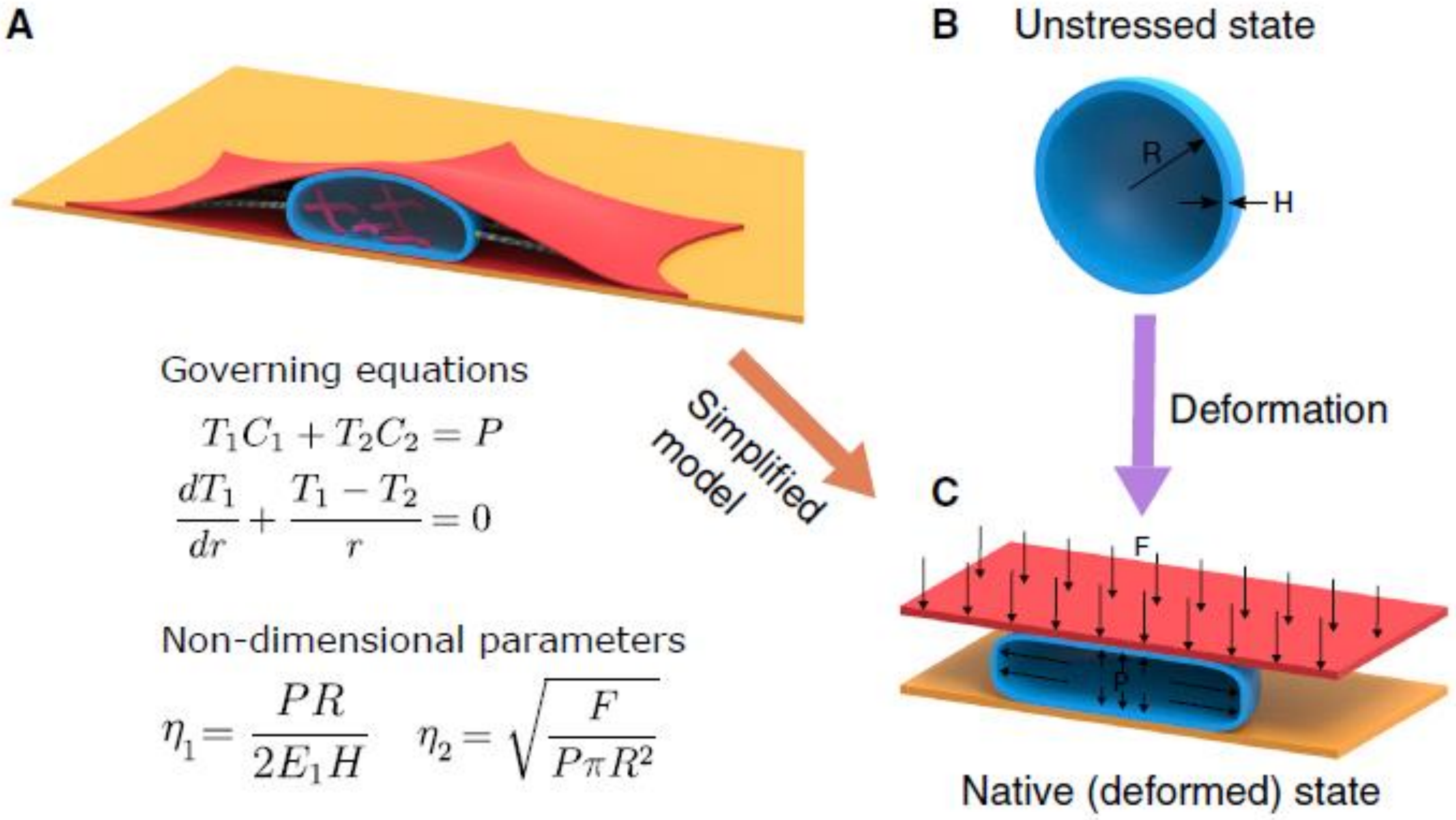
HCV replicon (N = 327)



Eccentricity = $\sqrt{1 - b^2/a^2}$

HCV proteins increase area and volume, and decrease the eccentricity of the nucleus.

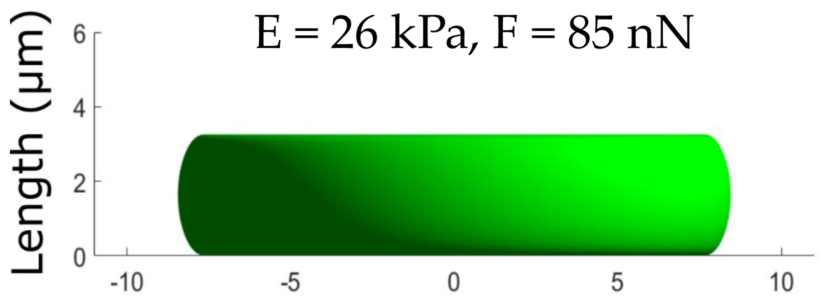
Mechanical modeling

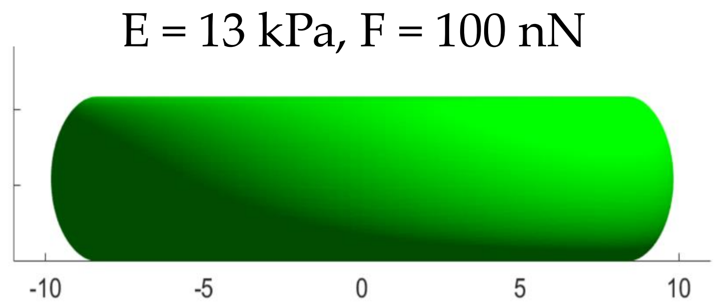


Balakrishnan et al., Biophysical Journal, 116(7), April 2019, pp. 1328-1339

Estimating nuclear modulus

P = 450 kPa, R = 5.7 μm, t = 120 nm



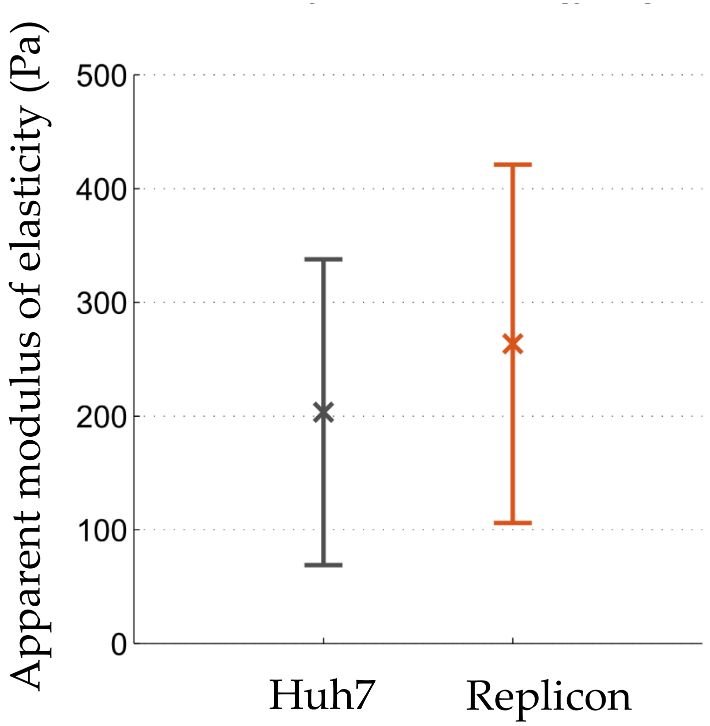


	Huh7	
	Experimental (mean)	Simulation
Projected area (μm^2)	220	224
Volume (μm^3)	667	701

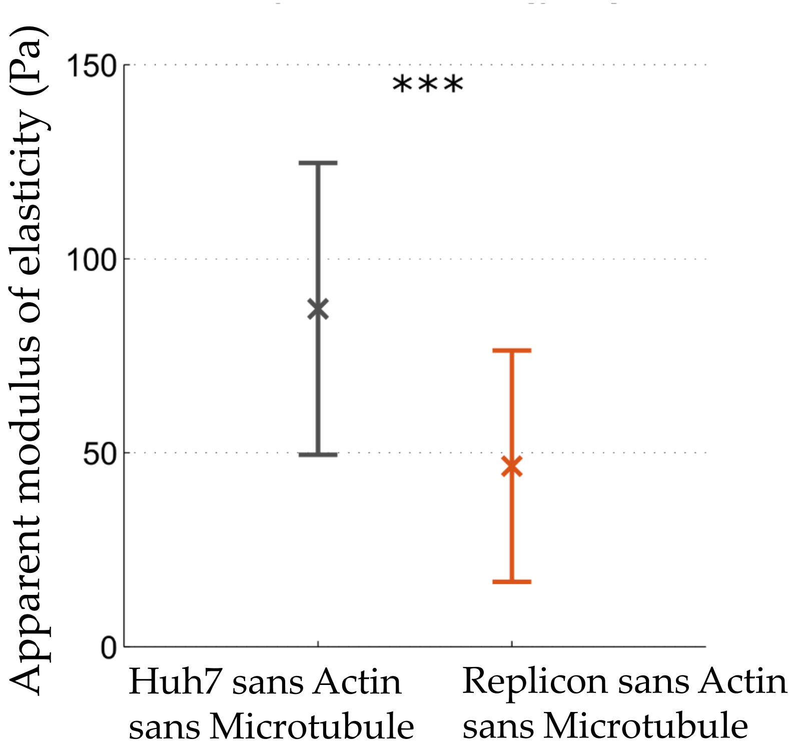
HCV replicon		
Experimental (mean)	Simulation (E = 13 kPa, F=85 nN)	Simulation (E = 13 kPa, F=100 nN)
312	299	303
1218	1573	1234

The increase in area and volume of the nucleus can be due to reduction in modulus of the nucleus and an increase in pretension of cortical actin.

Change in nuclear stiffness using AFM



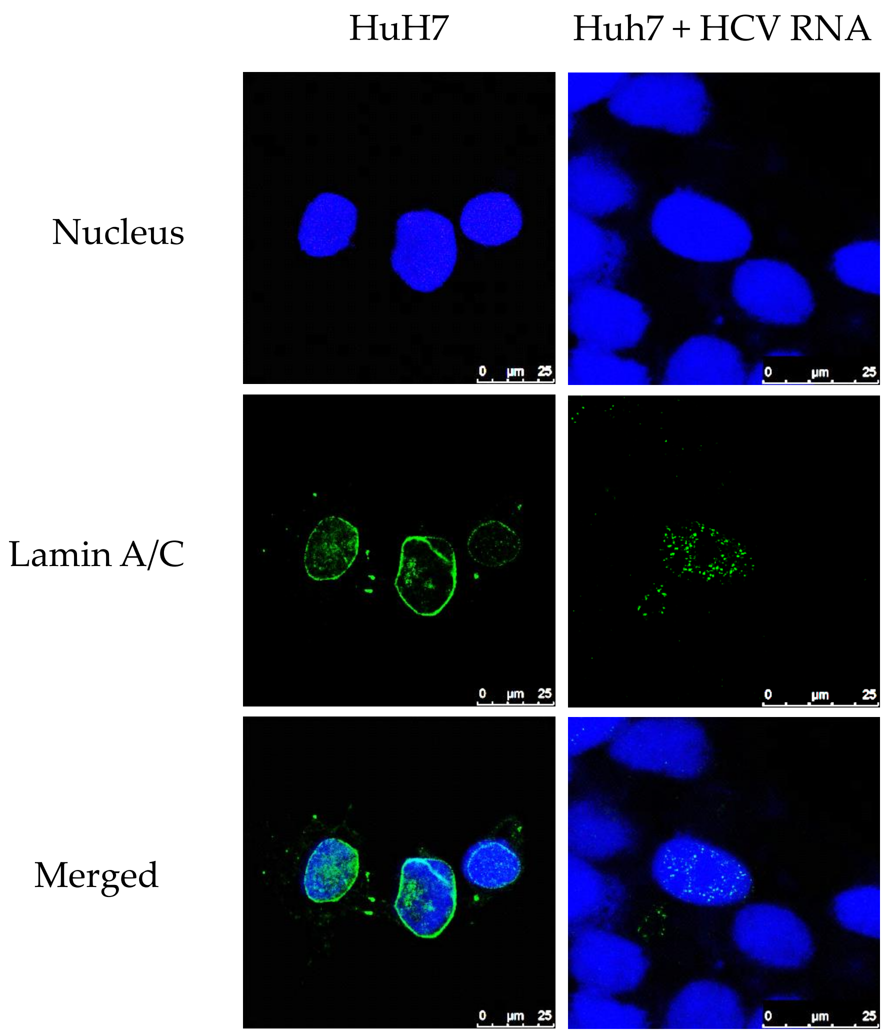
N = 21 N = 23



N = 34 N = 20

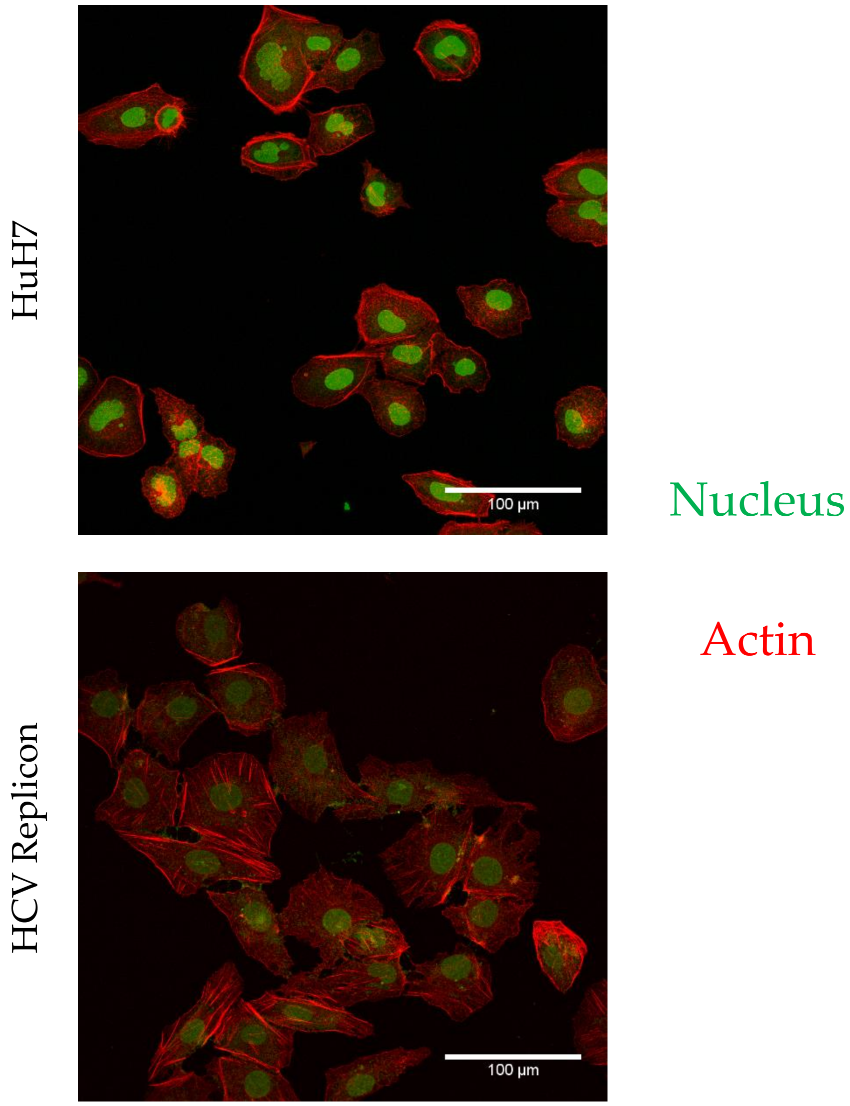
HCV proteins decrease the stiffness of the nucleus and marginally increase the stiffness of the cell.

Lamin A/C and β-actin expression levels in HCV replicon cell line



Lamin A.C is down-regulated; Beta-actin is up-regulated.

Inferred mechanically first.

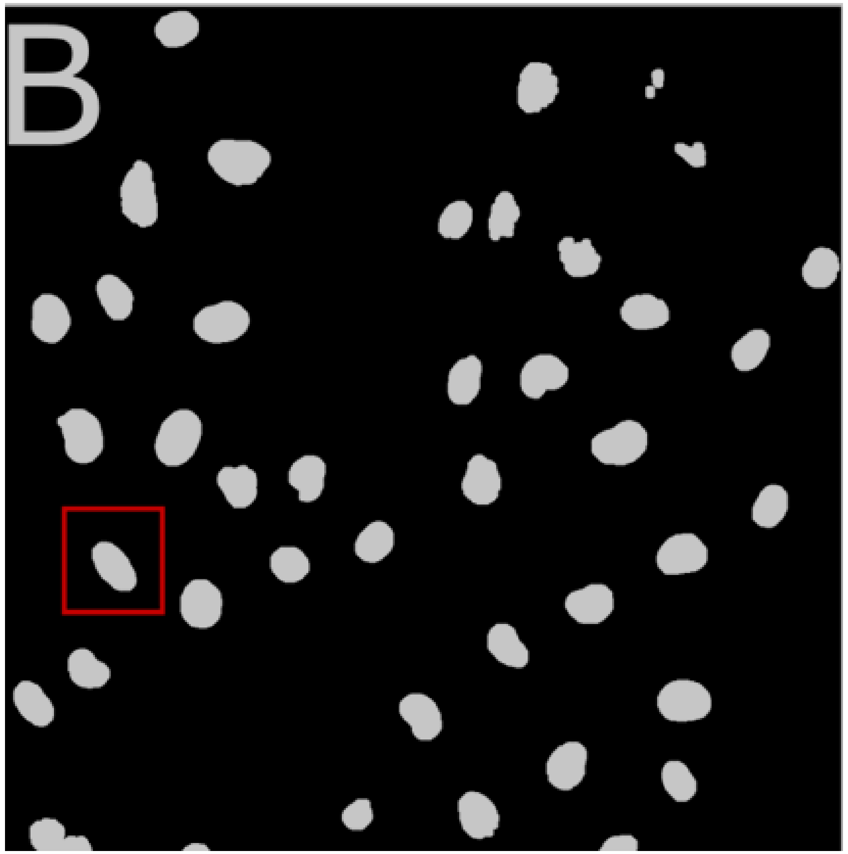


Cell heterogeneity and mechanics

Cell heterogeneity might have underlying mechanics reasoning….

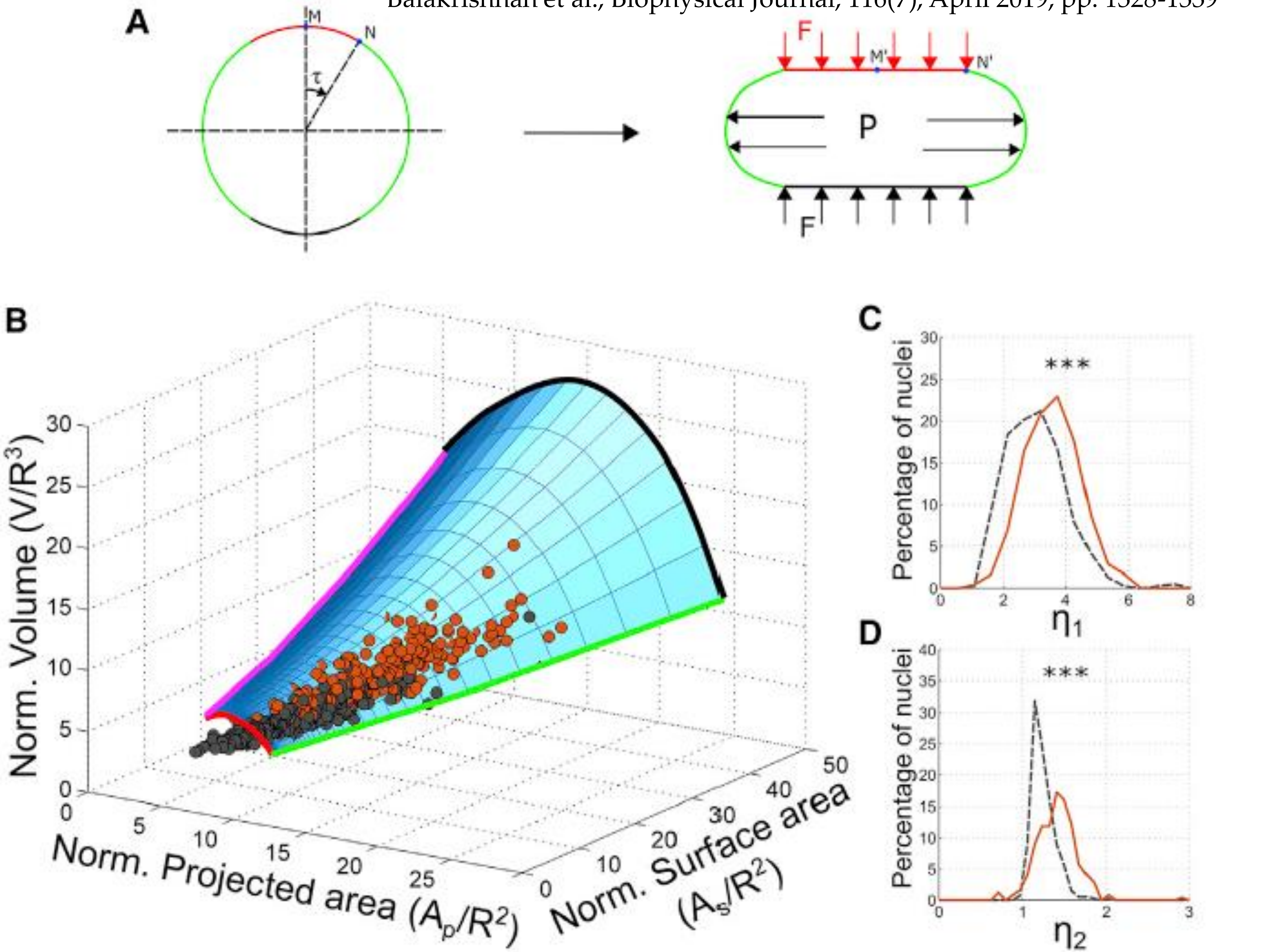
because biological cells cannot escape physics!

Nuclei of HuH7 cells



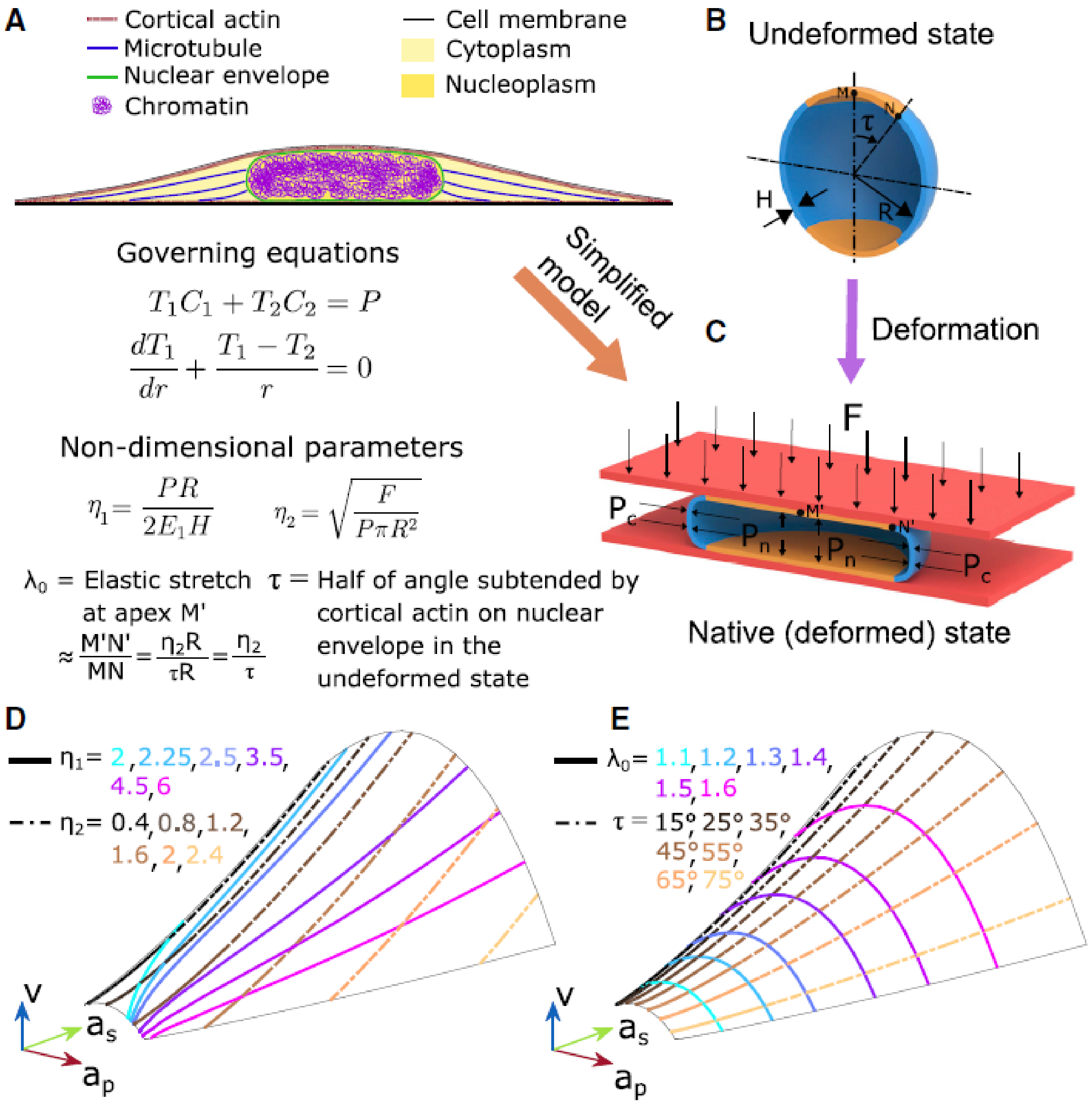
Heterogeneity!

Balakrishnan et al., 116(7), April 2019, pp. 1328-1339

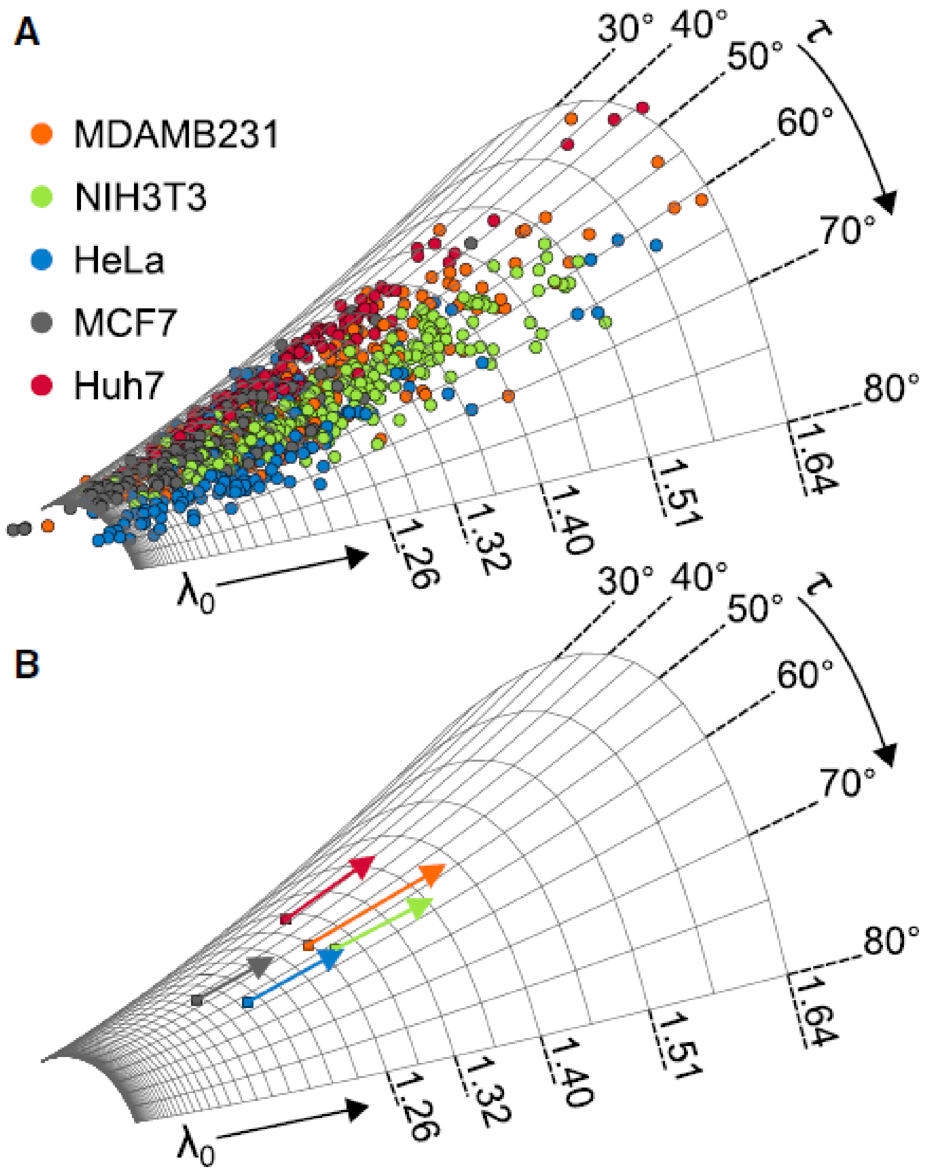

A
M
N
τ
F
M'
N'
P
F
B
Norm. Volume (V/R³)
Norm. Projected area (A_p/R²)
Norm. Surface area (A_s/R²)
C
Percentage of nuclei
***
η1
D
Percentage of nuclei
***
η2

Telltale signs from cell nuclei

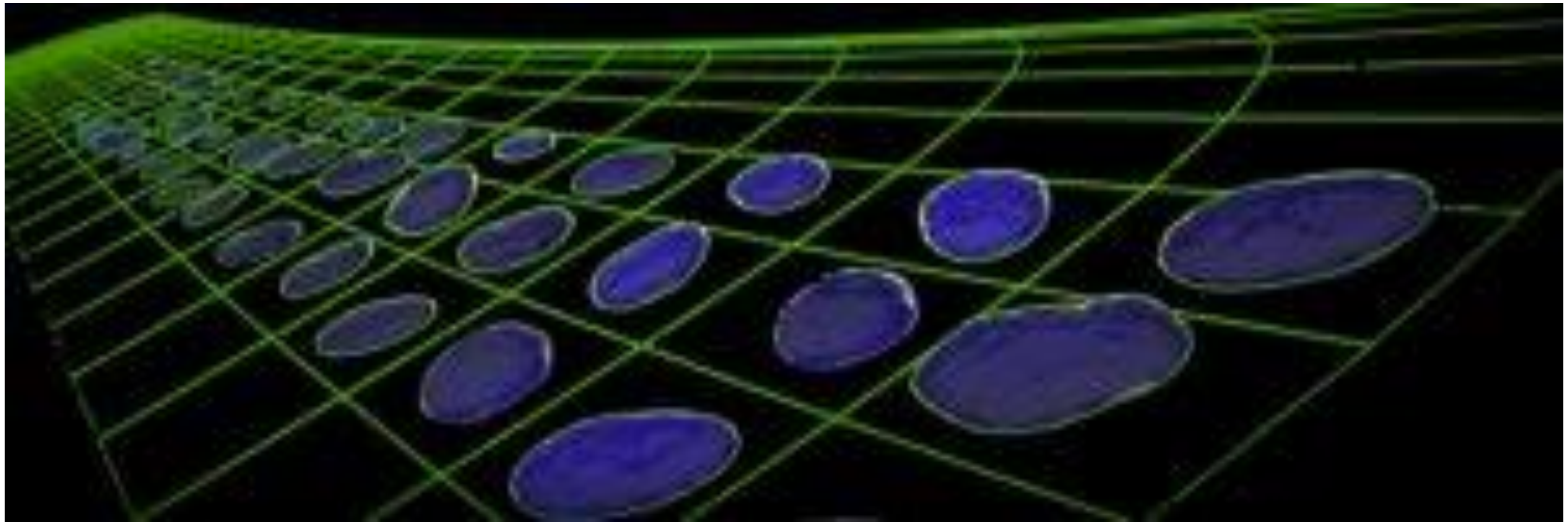
- Flatness index, τ
- Size factor, λ



Biophysical Journal, Vol. 120, 4698–4709, November 2, 2021, 2021


A
MDAMB231
NIH3T3
HeLa
MCF7
Huh7
30°
40°
50°
60°
70°
80°
τ
1.26
1.32
1.40
1.51
1.64
λ0
B
30°
40°
50°
60°
70°
80°
τ
1.26
1.32
1.40
1.51
1.64
λ0

Heterogeneity falls in line!



Balakrishnan et al., Biophysical Journal, 116(7), April 2019, pp. 1328-1339

Main points

- Mechanical response can be measured with miniature tools.
- We aim to establish specificity to develop mechano-diagonostics.
- We can try to understand the underlying biological mechanisms.
- We can mechanically intervene.
- We can discern some biological processes using mechanics-aided insights.

CHEM MECH

Indirect measurements in cell biology, as in engineering,…

could lead to mechano-diagnostics…

eventually.

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