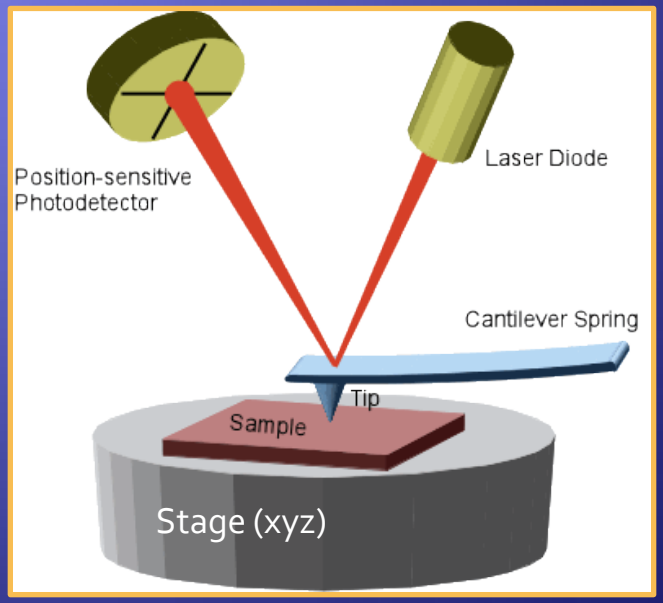
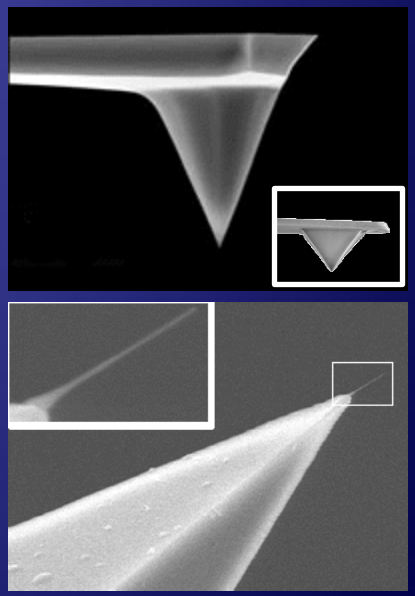
Session 8

ATOMIC FORCE MICROSCOPY

The Approach

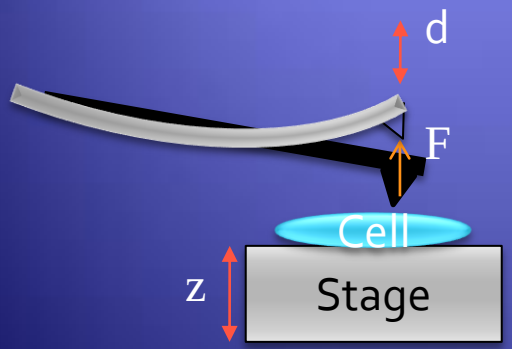
- G. Binning (Stanford) in 1986
- Deflection of a cantilever spring
- Main parts
  - Cantilever tip
  - Laser Diode
  - Photodiode
  - Stage





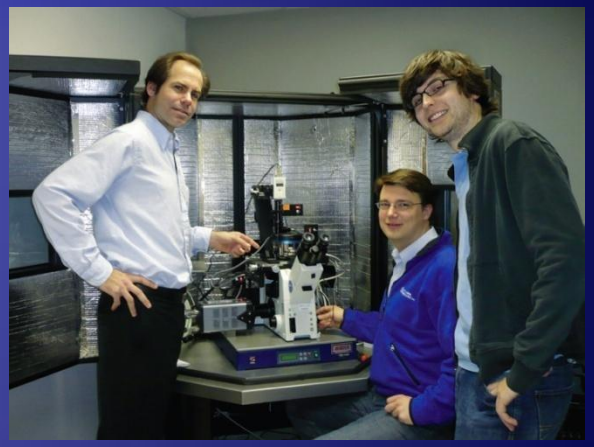
The System

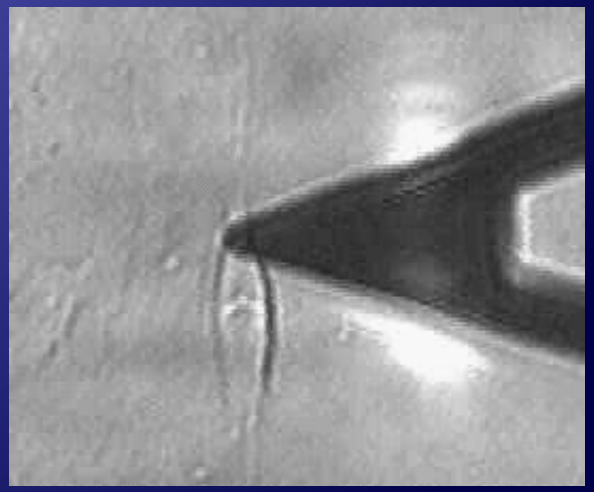
- Configured with optical microscopes
- Cells need softest cantilevers
- Range: 100 μm scan, 10 μm tall



$$F = K * d = \left(\frac{4Ewt^3}{L^3} \right) d$$

F	Loading Force
K	Bending Stiffness (35 nN/μm)
d	Displacement
E	Modulus of Elasticity (100 GPa)
w	Cantilever width (30 μm)
t	Cantilever thickness (1 μm)
L	Cantilever length (150 μm)

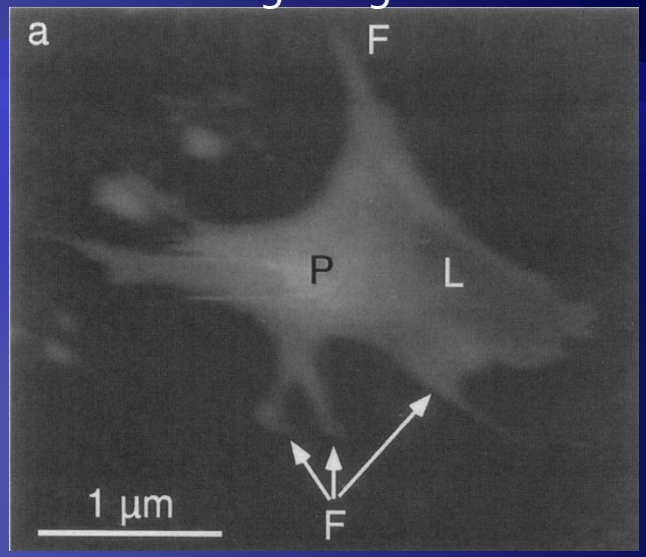




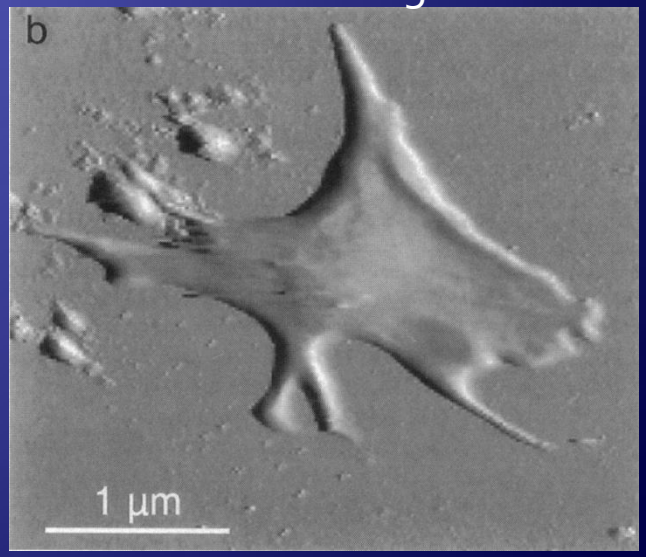
Imaging Mode

- Constant deflection mode (constant force)
- Distance Feedback
  - Height signal : output of the feedback
    - Specimen height (3-4 μm)
  - Deflection signal : residual, uncompensated deflections
    - Provides fine details (100 nm)
  - True Height: A + B
- DC vs. AC mode

Height signal

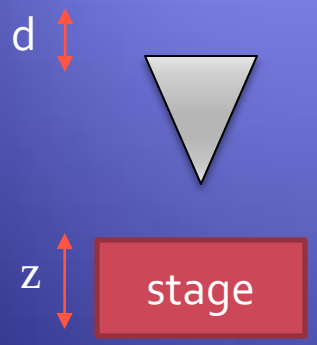


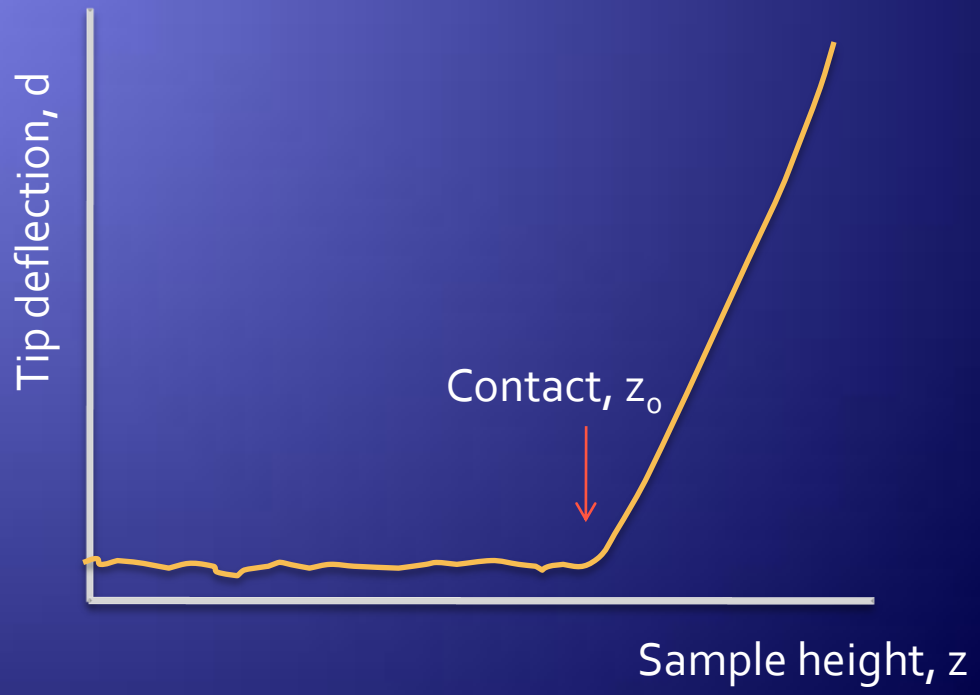
Deflection signal



Force Measurement

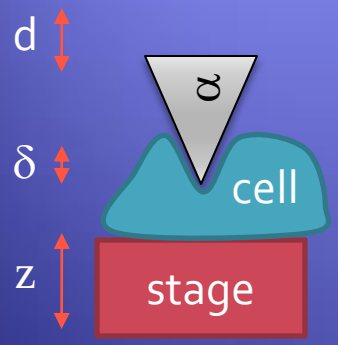
- Force Curve
  - Out of contact, no deflection
  - In contact, deflection proportion to sample height



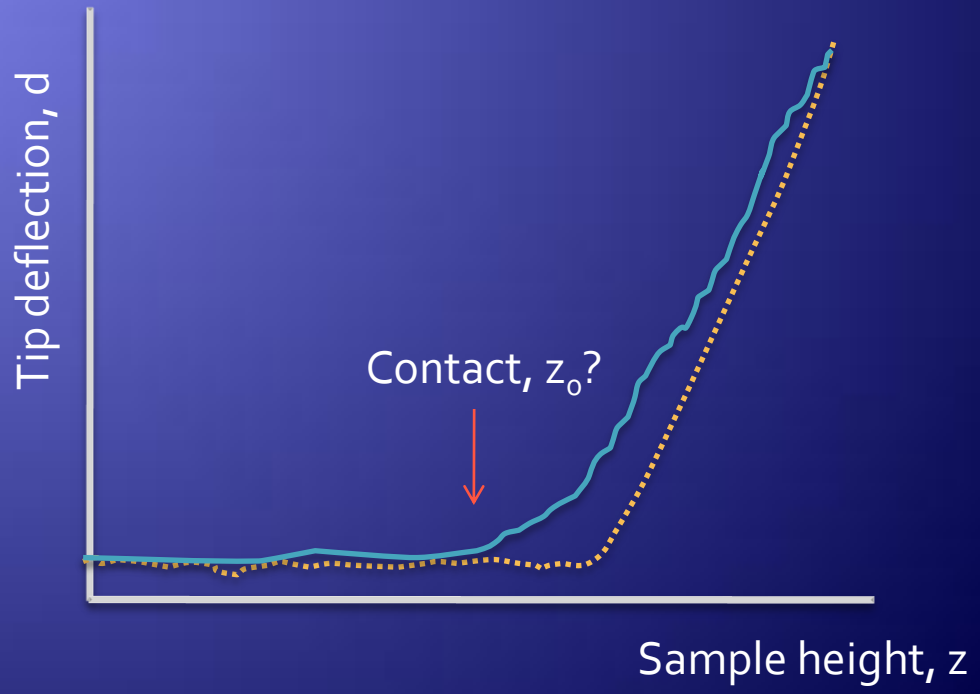


Force Measurement

- Indentation comes from sample compression
  - $d = (z - z_o) - \delta$
  - Hertzian contact for a "conical punch"



$$F = \frac{2}{\pi}\tan(\alpha)\left(\frac{E}{1-\nu^2}\right)\delta^2$$



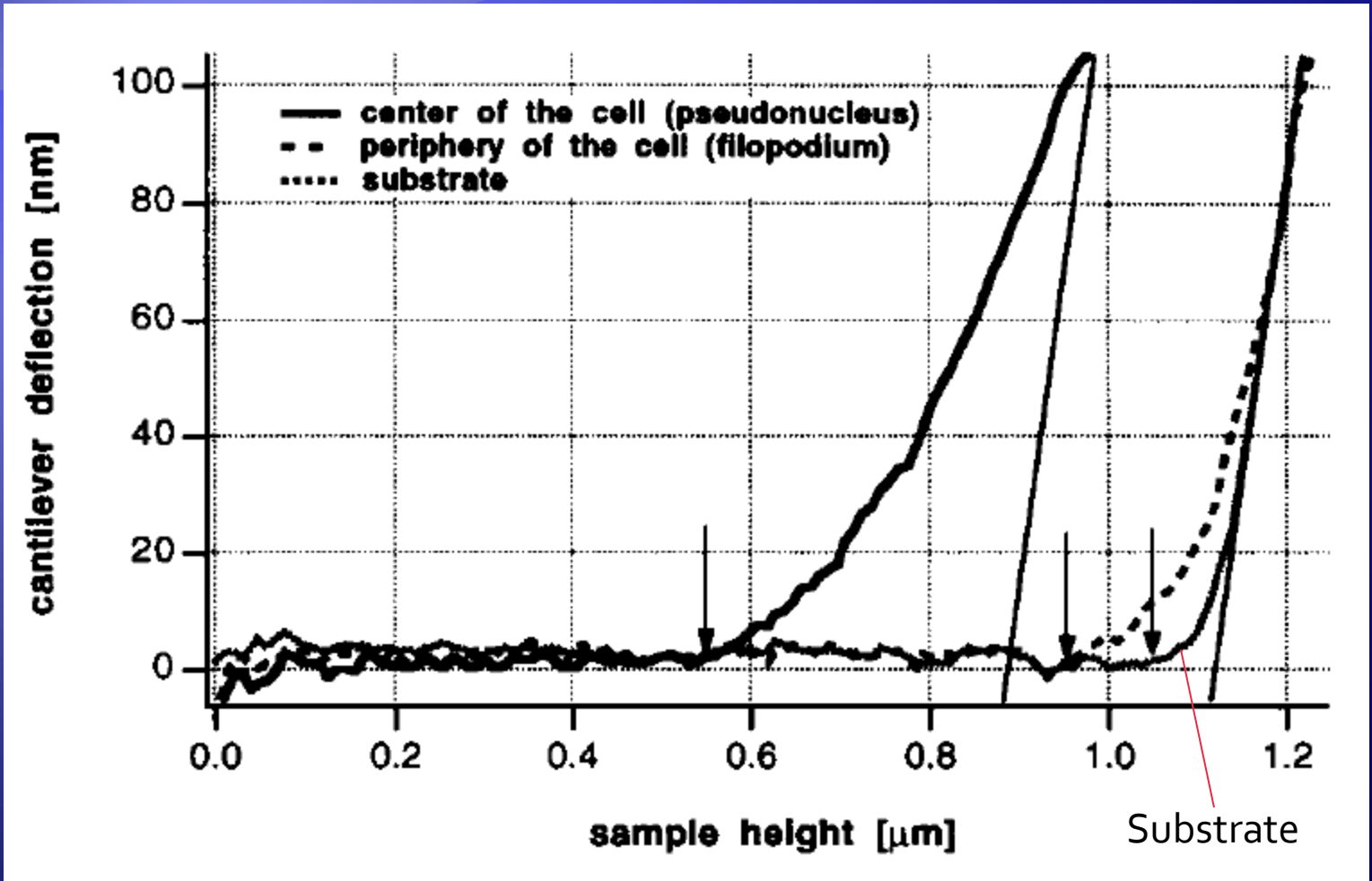
Force Measurement

- Sample Stiffness

$$z\text{-}z_0 = d + \sqrt{\frac{2dK(1-\nu^2)}{\pi \tan(\alpha) E}}$$

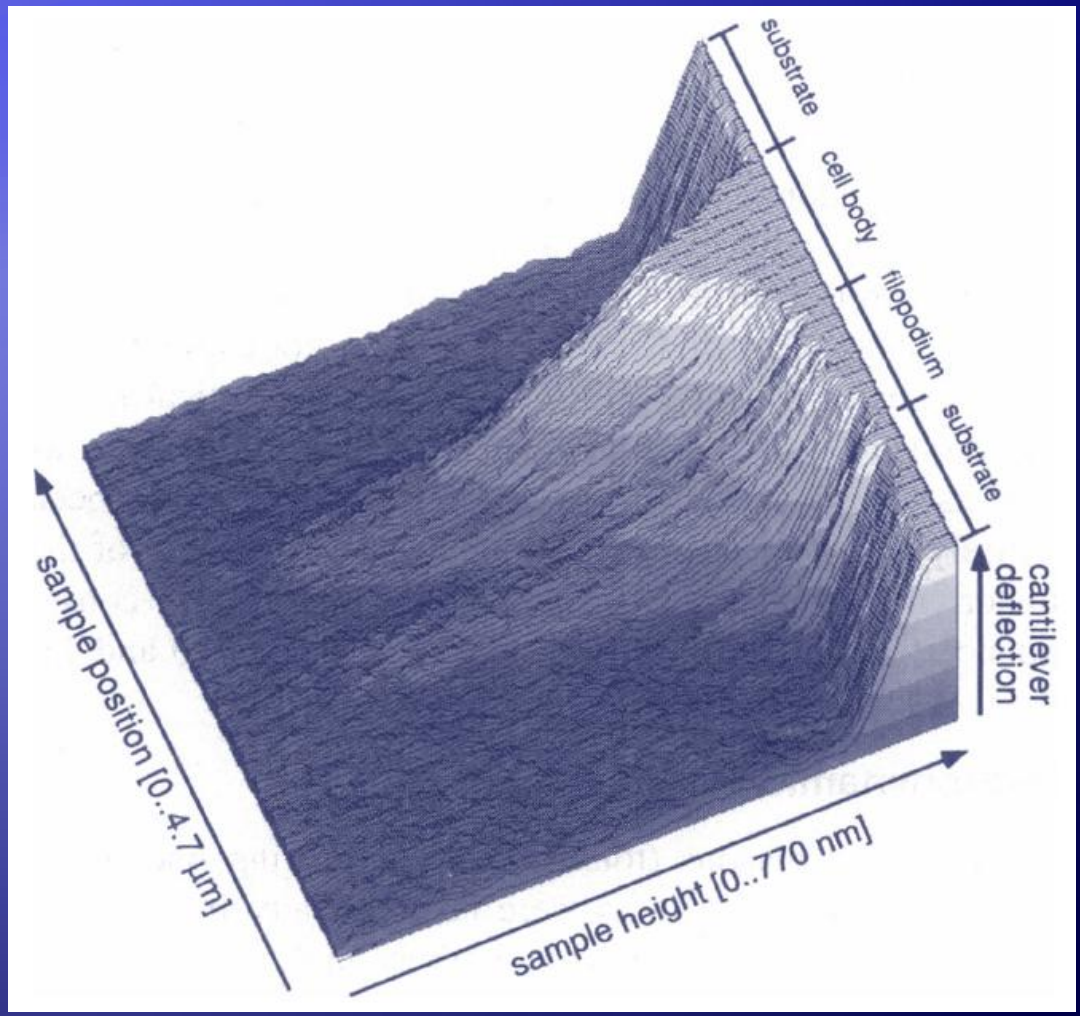
- Measurement for sample height (z_o) not easy
- Two fit-parameters: E and z_o

Regional difference in cell stiffness

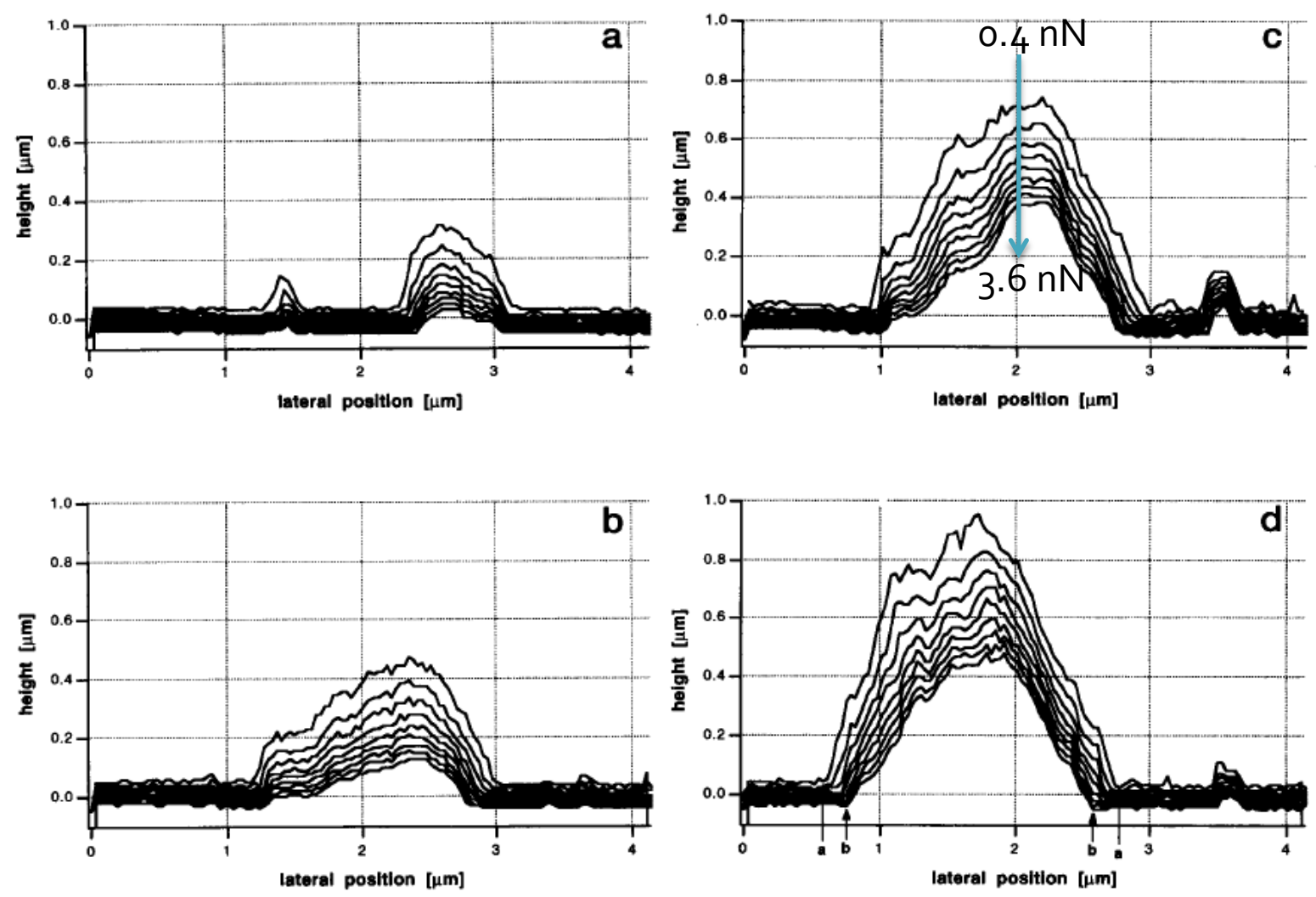


Line Scan

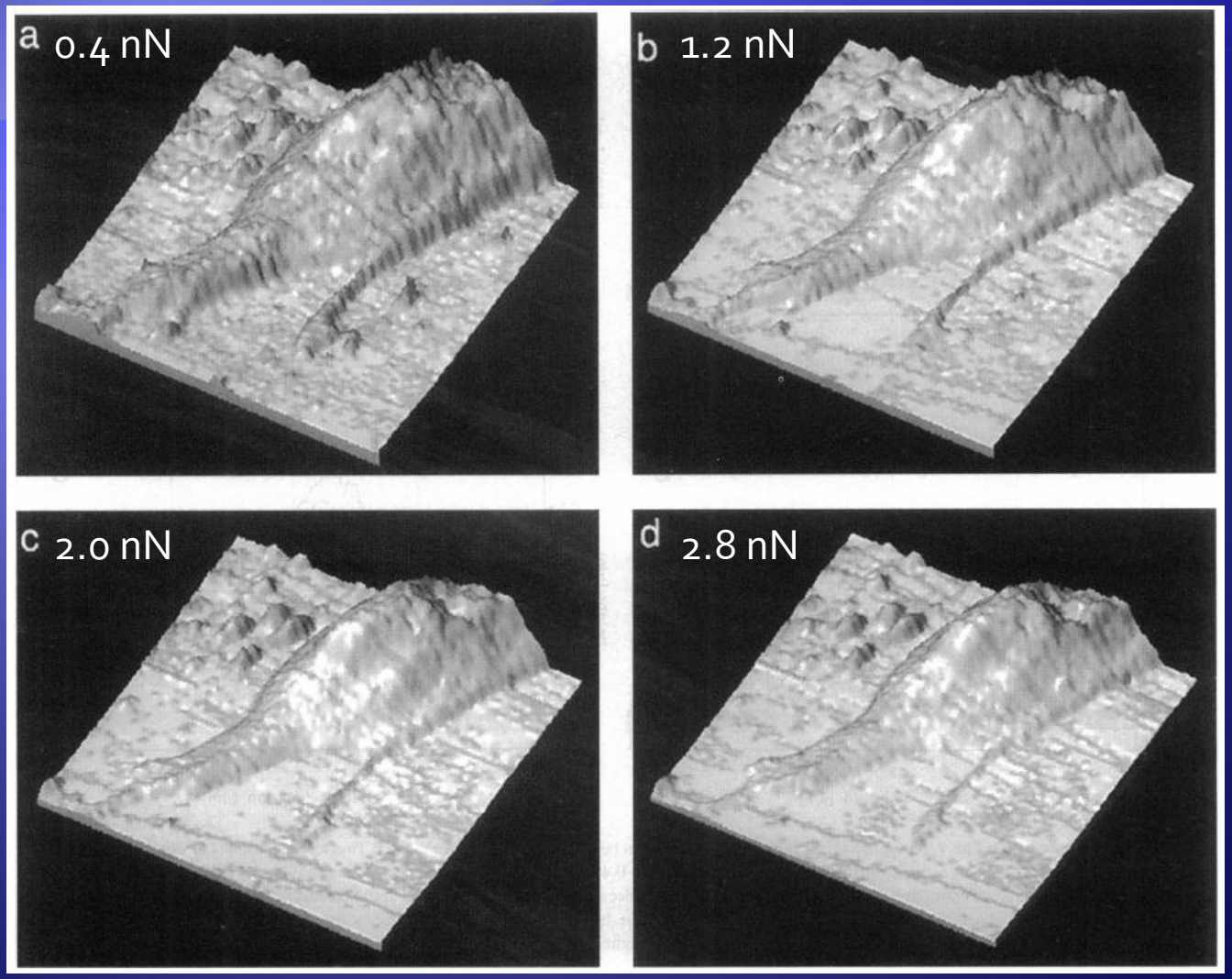
- Stacked force curves
- Shows height of cell along scan
- Cell body: more compressible
- Periphery: less compressible



2D Scan

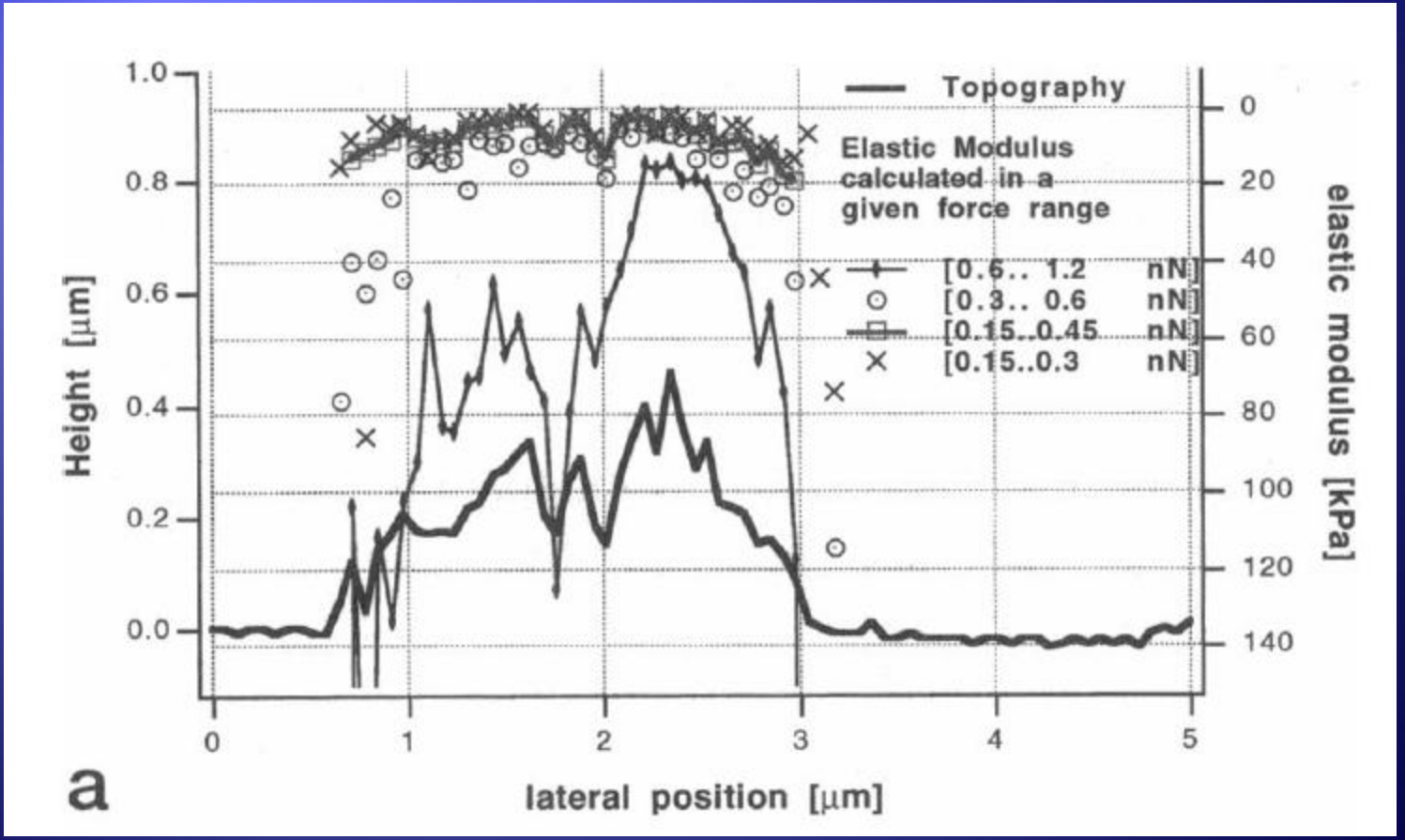

a
b
c
d
0.4 nN
3.6 nN
height [µm]
lateral position [µm]
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
a
b

Constant Force Topography

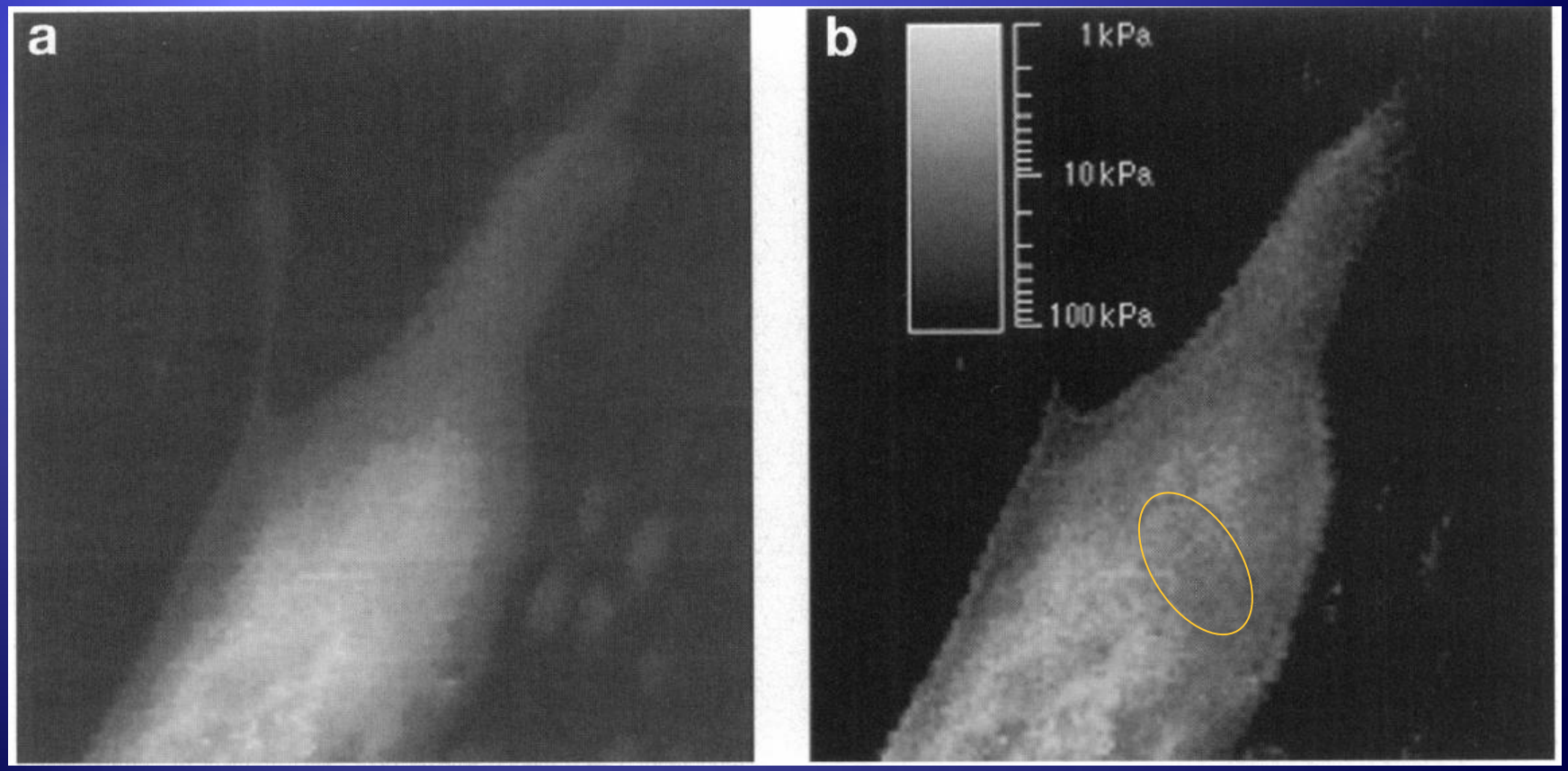


Feel the substrate

- Low loads – surface High loads – CSK

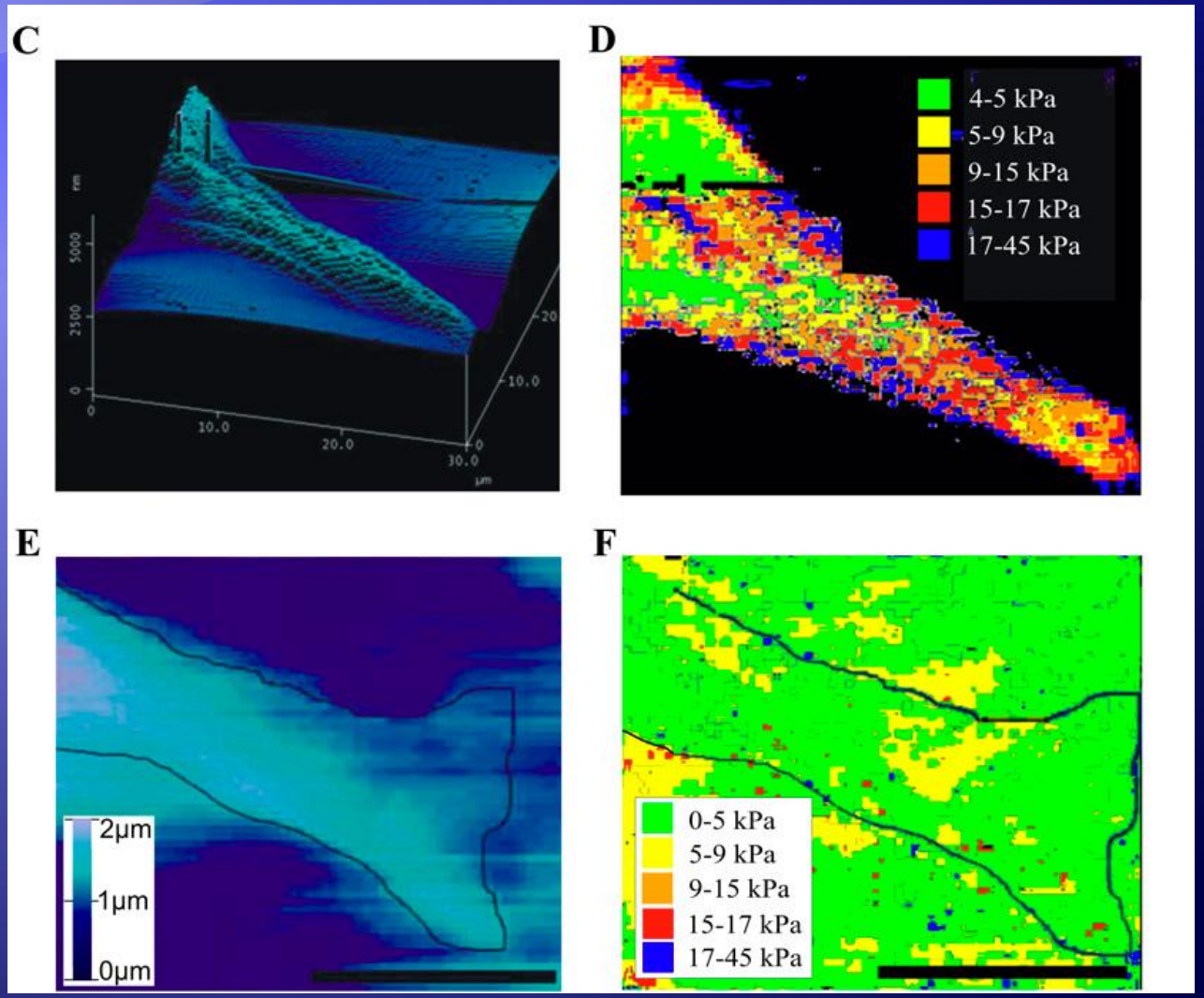


Elastic Tomography



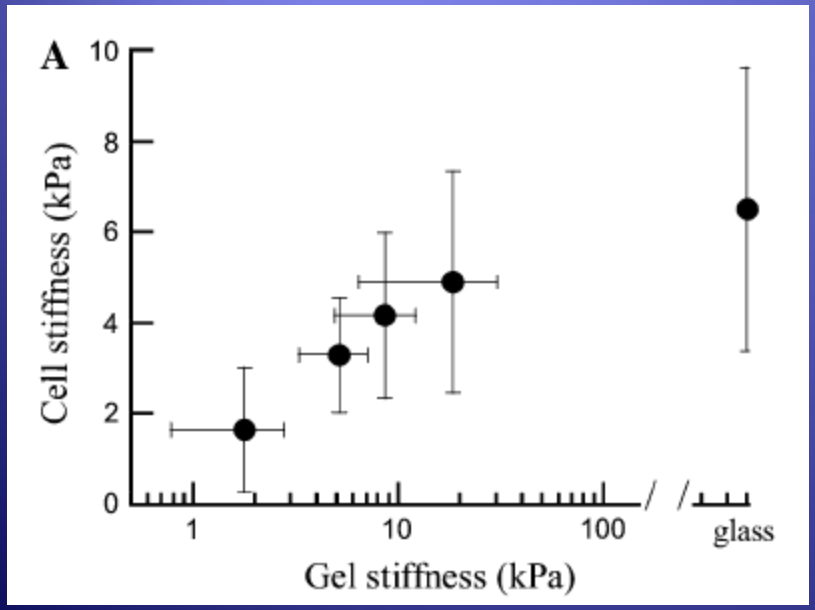
Stiffness Matching

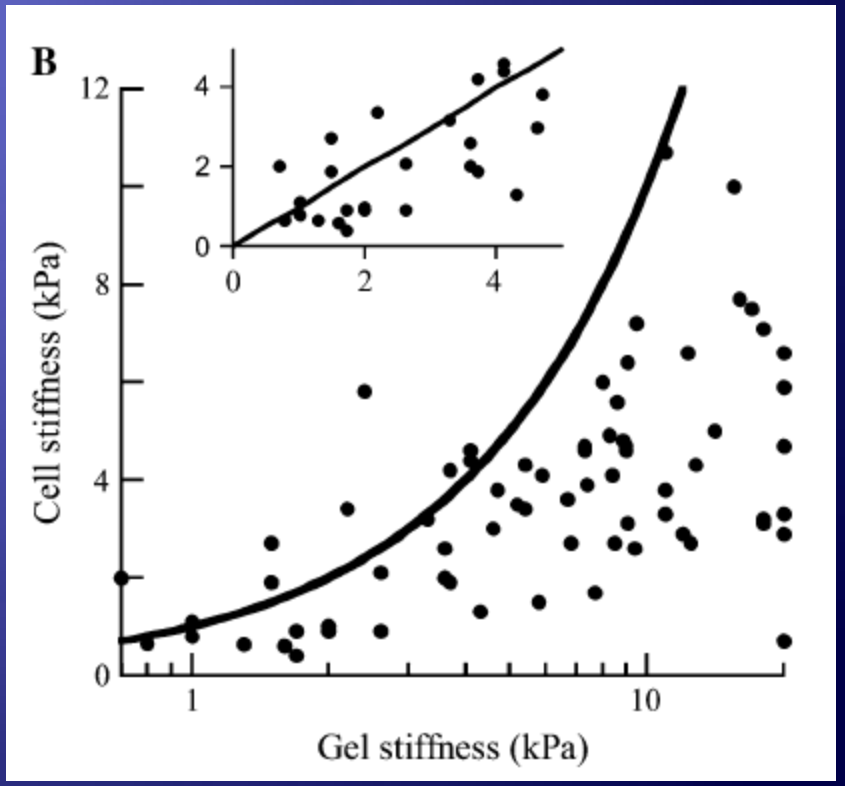
- Glass
  (1 GPa)
- Gel
  (5 kPa)



Stiffness regimes

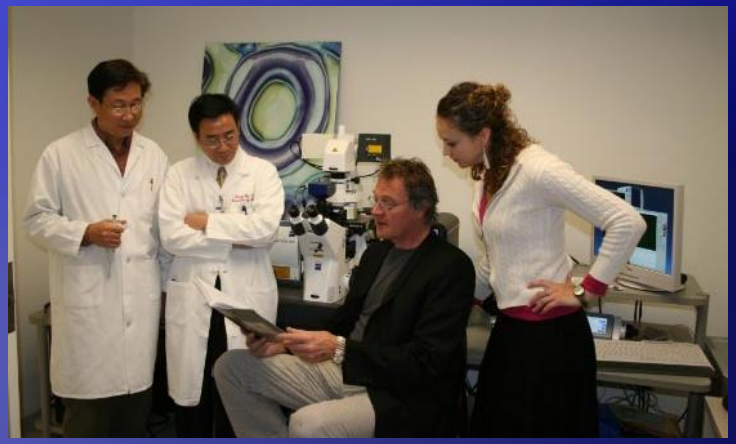
- For 1-5 kPa, cell stiffness matches substrate
- For >5 kPa, cells are softer than substrate



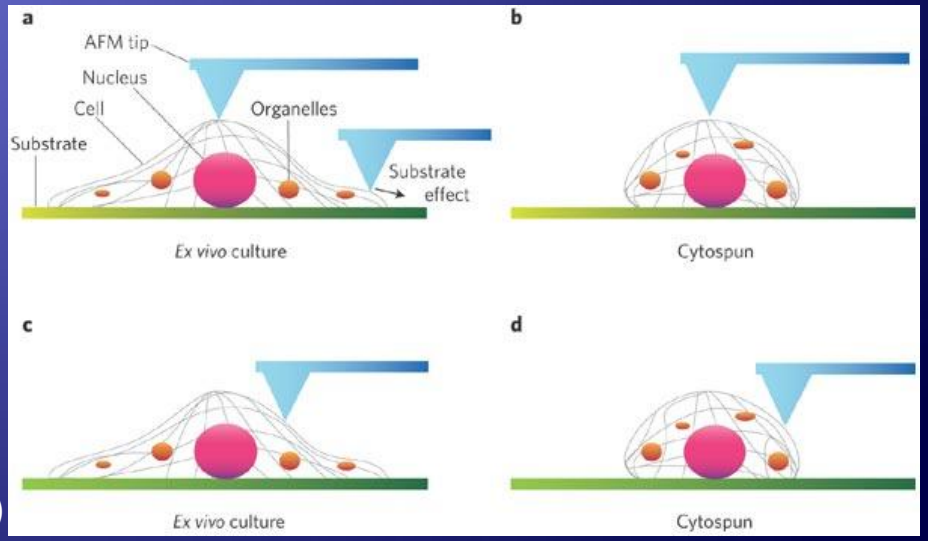


The softness of cancer

- Method to distinguish between metastatic cancer and normal cells
- Cancer needs to be flexible to migrate through tissue to get into blood stream



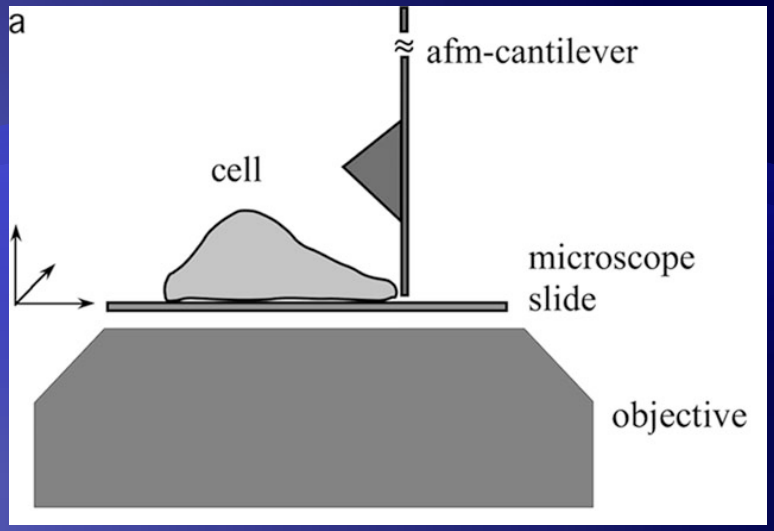
Jian Yu Rao, Yu-Sheng Jin, and James Gimzewski , and Sarah Cross (UCLA)

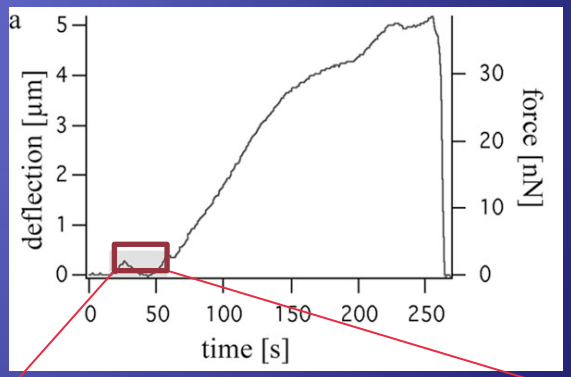


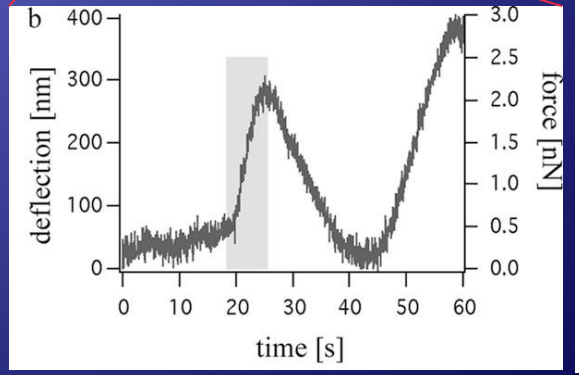
Nature Nanotech. **2,** 780–783 (2007)

Force of protrusion

- Sideways mounted AFM
- Stall force detected
  - F = 1.18 ± 0.35 nN
- Elastic Brownian Ratchet
  - 3 µm x 0.2 µm AFM contact area
  - 4 pN force per filament
  - 100 actin filaments pushing
  - 2 kPa lamellipodia pressure







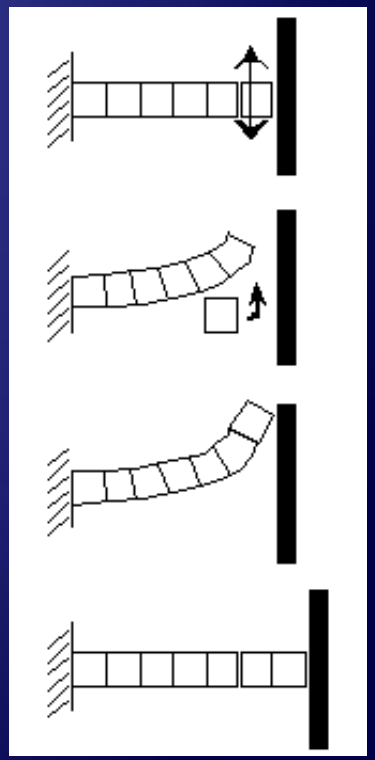
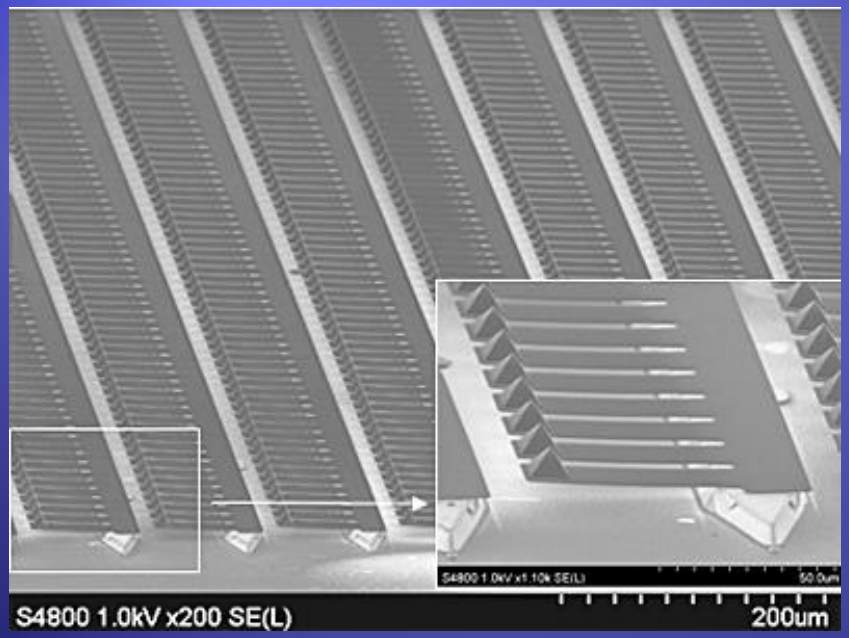
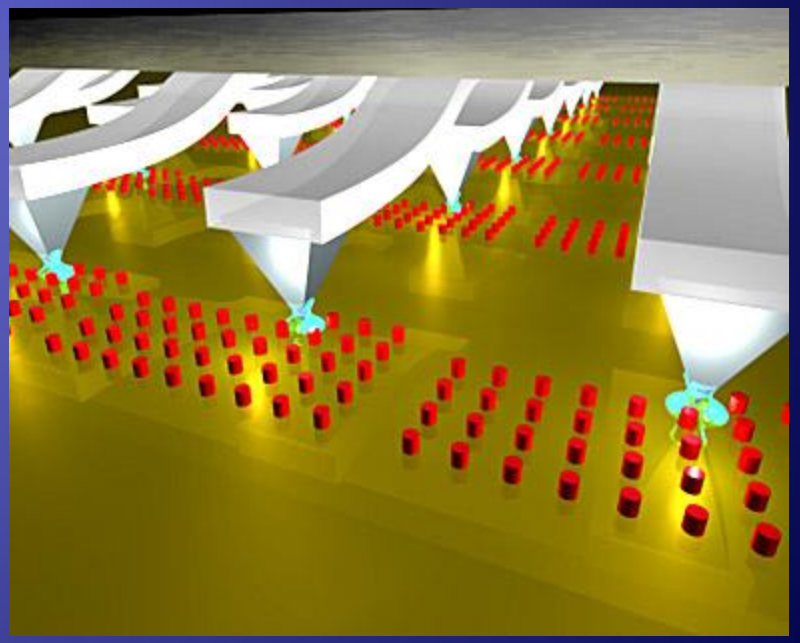


Figure 1. A 2D nano Print Array

The Future?

- Nanoink's 2D nano PrintArray for nanoscale patterns

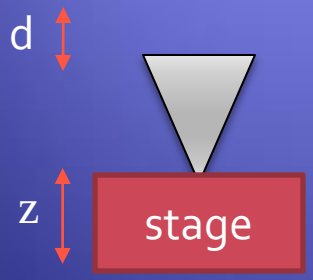


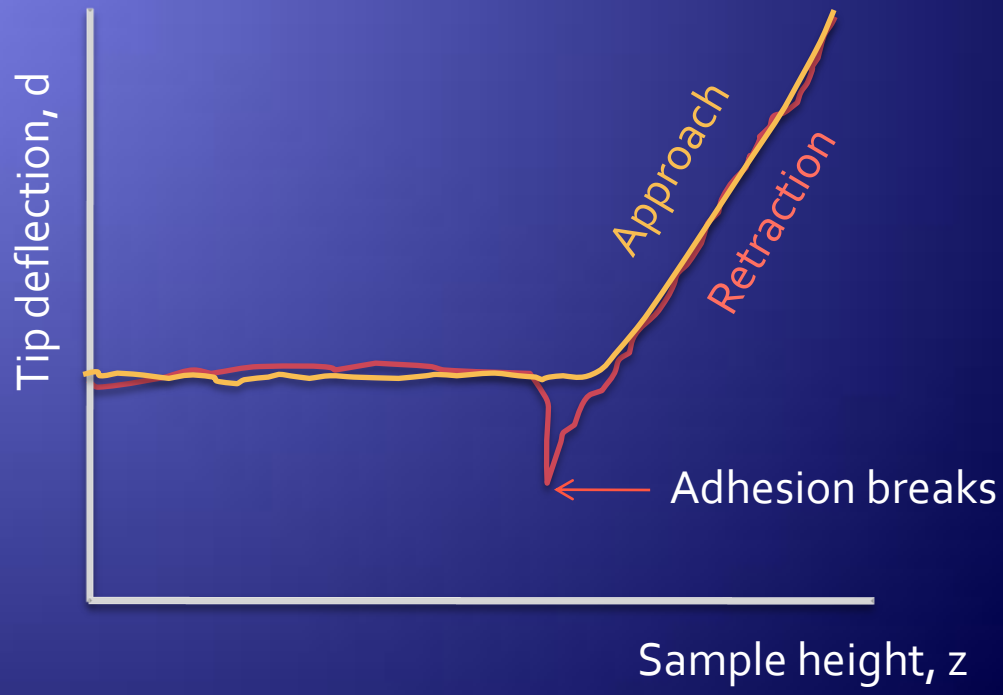


- High throughput cell measurements?

Adhesion Force Measurement

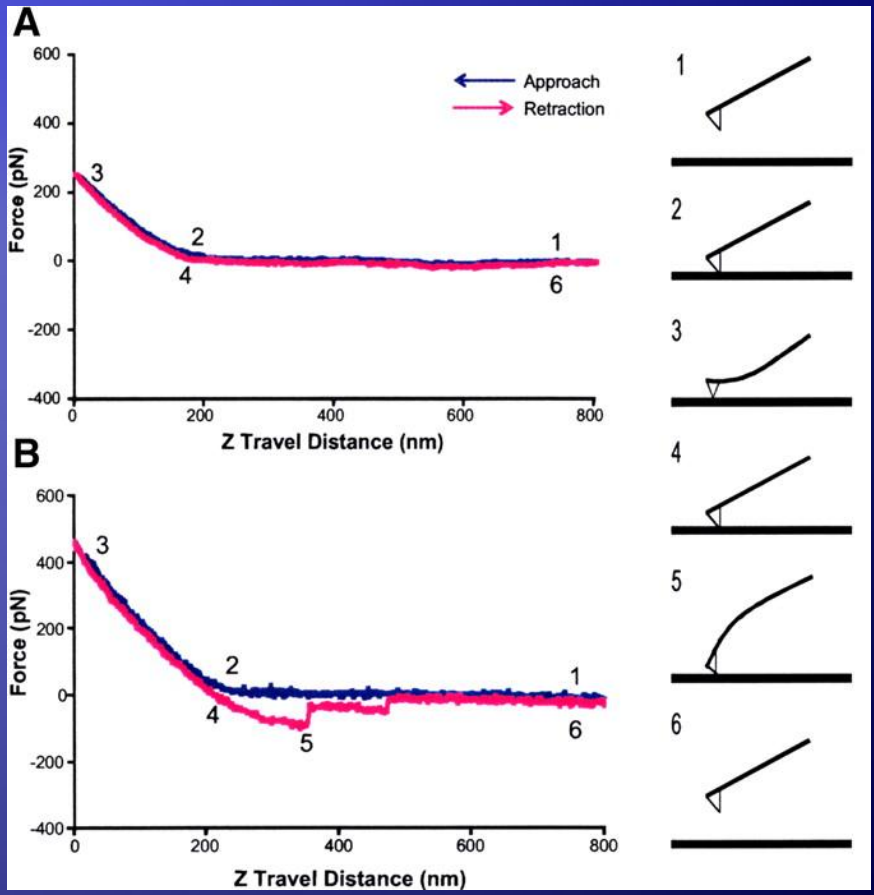
- If adhesive, tip sticks during retraction
- Van der waals forces hold onto the tip





Integrin Adhesion Forces

- Fibronectin and $\alpha_5\beta_1$-integrin on vascular smooth muscle cells
- Individual bond force is 39 ± 8 pN
- Cells have multiple bonds at each focal adhesion due to integrin clustering



Am J Physiol Heart Circ Physiol 289: H2526-H2535, 2005.