



Determining the Mechanics of Living Cells Using AFM

SURI Summer Program 2003

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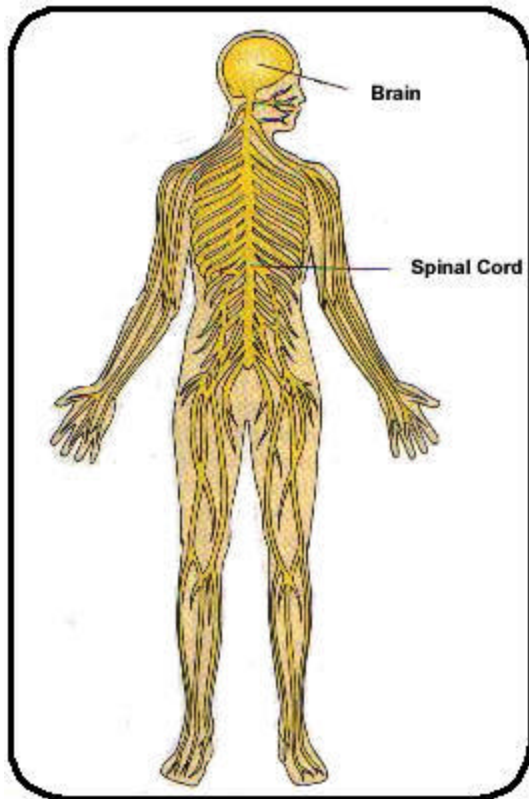
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Outline

- Purpose/Applications
- AFM Basics
- Introduction to Asylum AFM
- Elasticity of PDMS
- Nanoindentation of Yeast Cells
- *Aplysia californica* nerve cell

● ● ● | Purpose/Applications

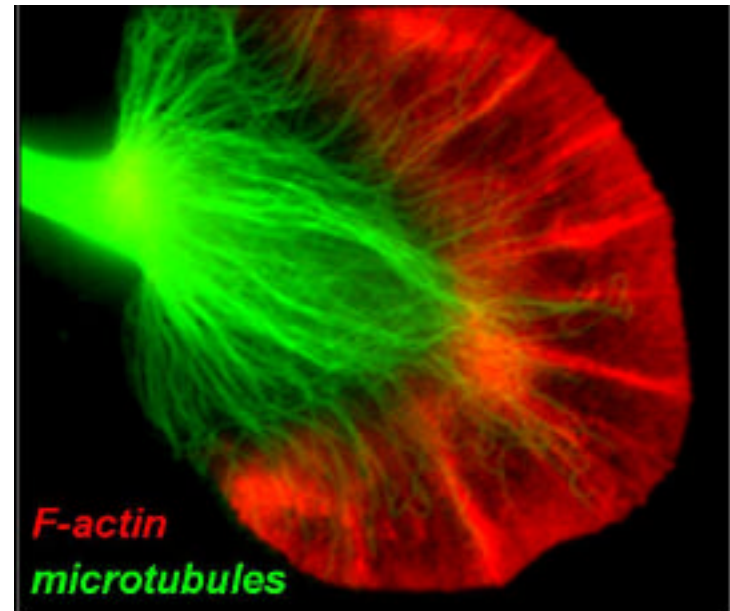


- Understand mechanics of nerve cells
- Develop method of applying forces to cells



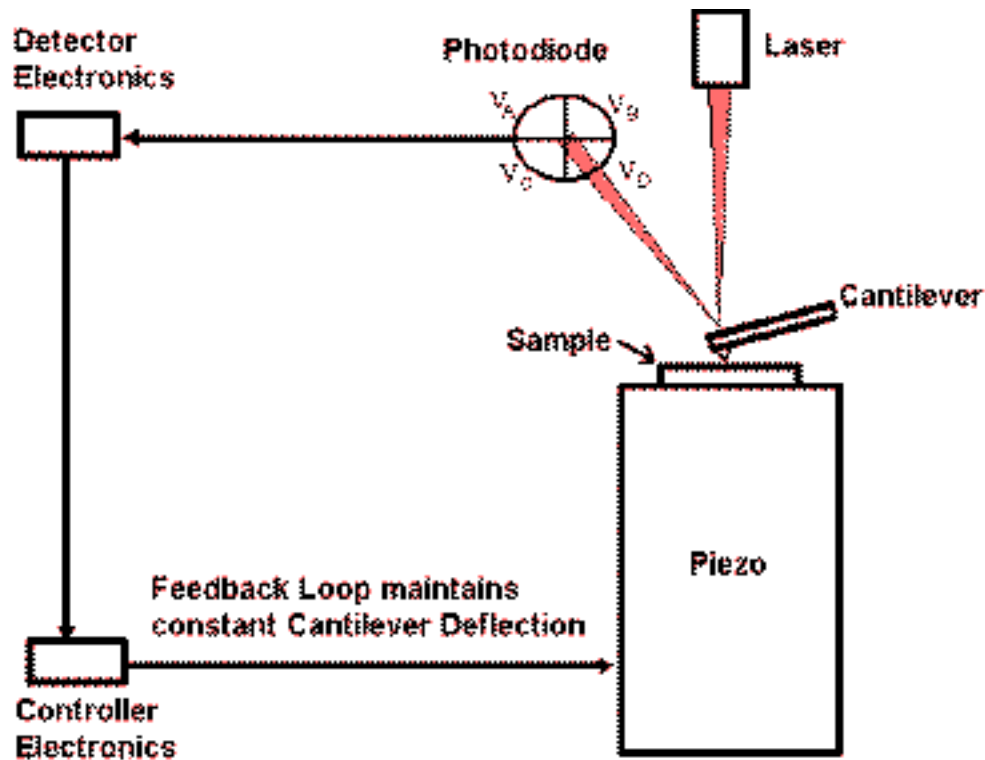
Cell Mechanics of Growth Cone of a Nerve Cell

- Cytoskeleton provides support structure for eukaryotic cells
- Little known of mechanics of the cell.



Prof. Daniel Suter. Purdue University

Atomic Force Microscopy



Courtesy of Jin-Won Park

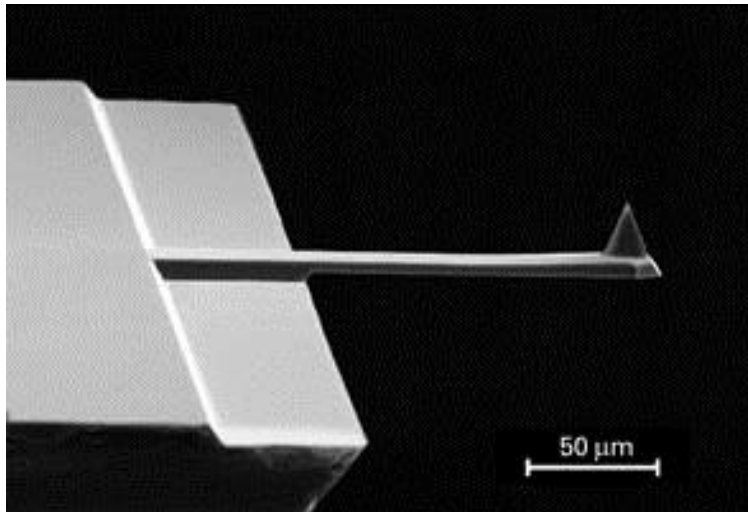
- Sharp tip on cantilever scans surface
- Deflection measured by laser and photodetector
- Uses:
 - Imaging
 - Surface Force Analysis

● ● ● | Asylum AFM



- Shown with Inverted Optical microscope
- x-y piezoelectric scanner in base
- Nanopositioning system corrects for piezo hysteresis

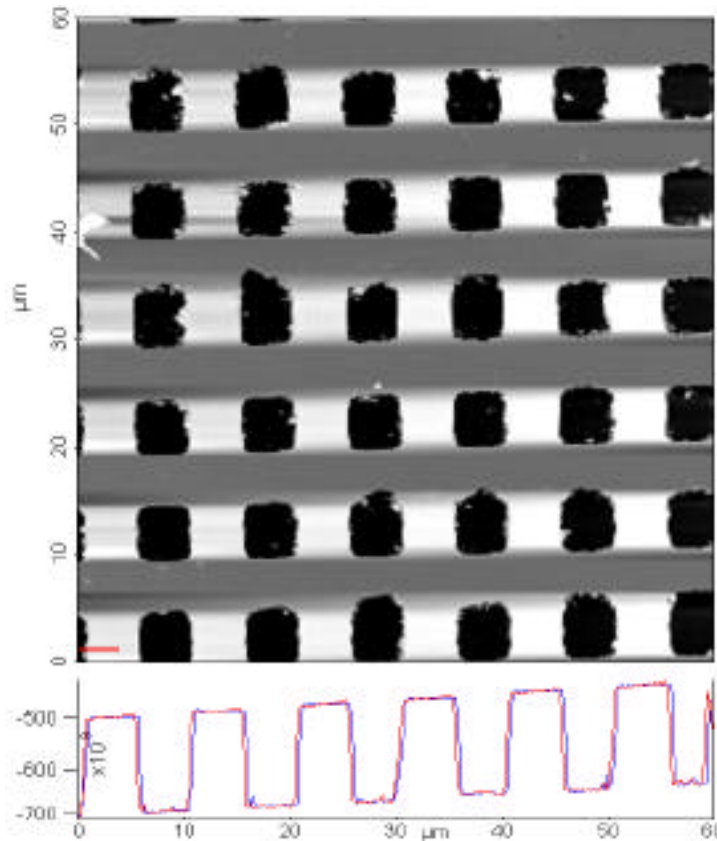
● ● ● | Cantilevers



- Made of Si or SiN
- Tip radius 15 nm
- Calibrate
 - spring constant
 - optical lever sensitivity
 - radius of curvature



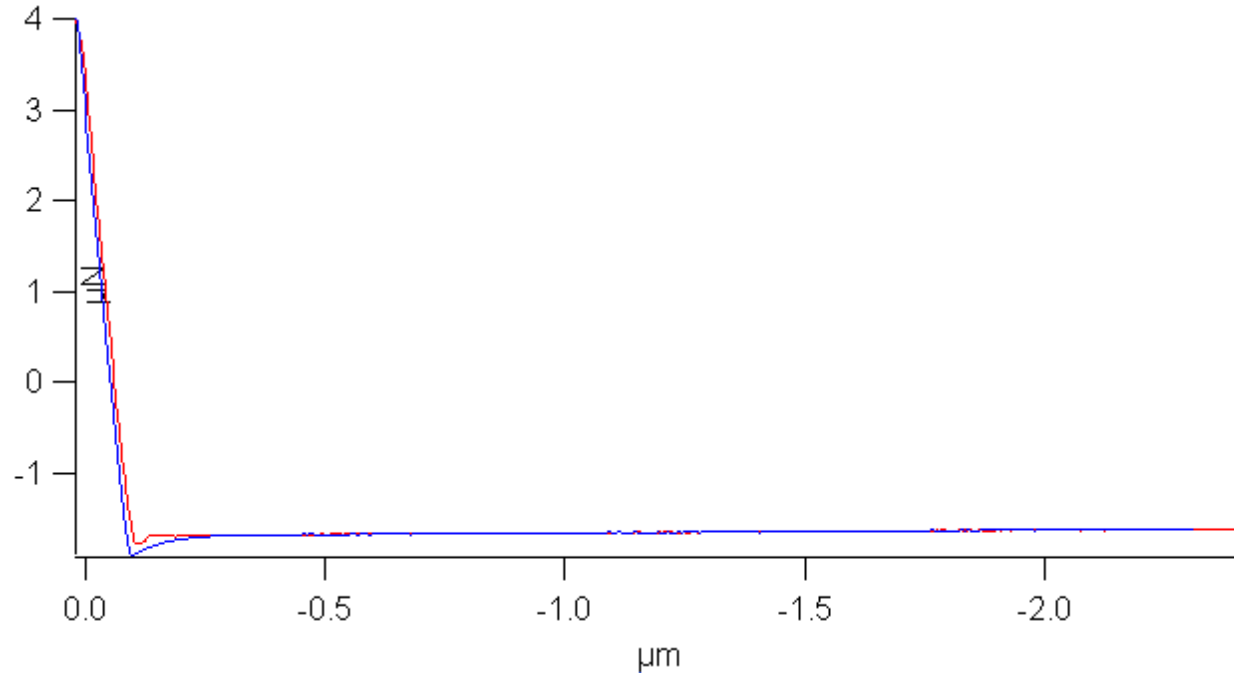
Imaging with AFM



- Calibration grid
- 5_μm wells evenly spaced
- 200 nm deep



Force Curves

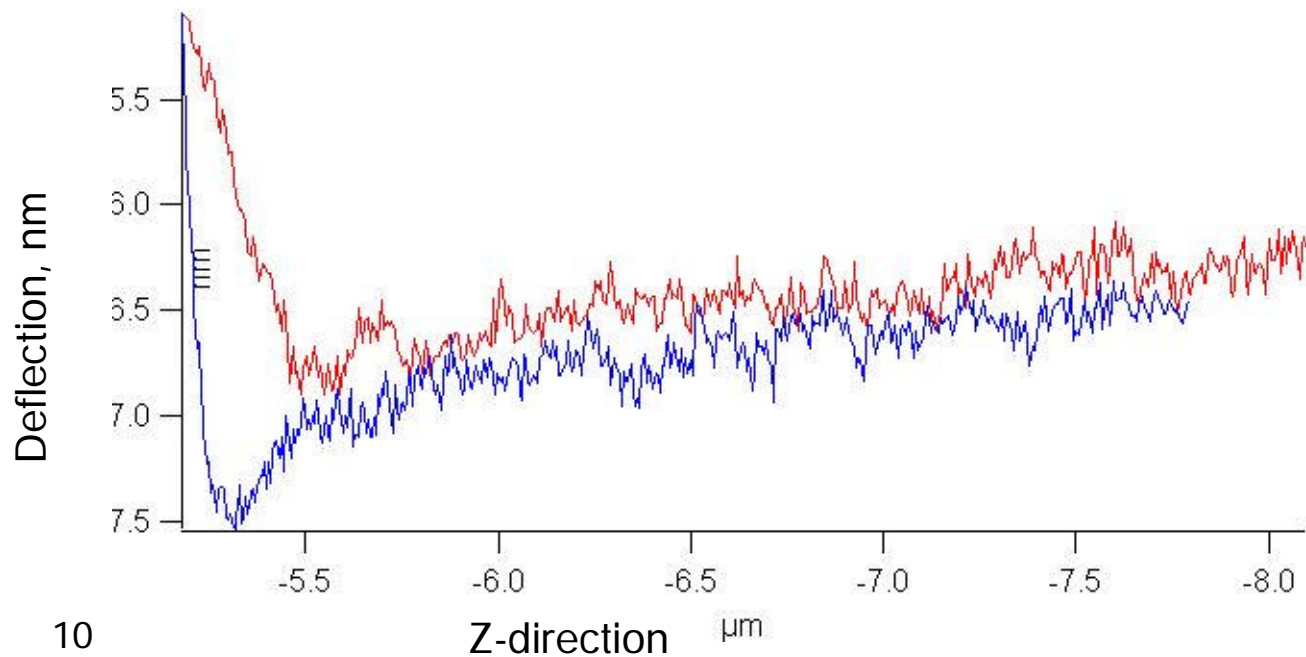


- Deflection vs. LVDT (z-position)
- Typical hard surface force curve
- Used to calculate optical lever sensitivity



Elasticity of PDMS

- Polydimethylsiloxane – thin film on Si wafer
- Calculate Young's modulus by nanoindentation of polymer





Elasticity of PDMS

- Hertzian models

$$F_{cone} = \frac{2}{\pi} (\tan \alpha) E^* \delta^2$$

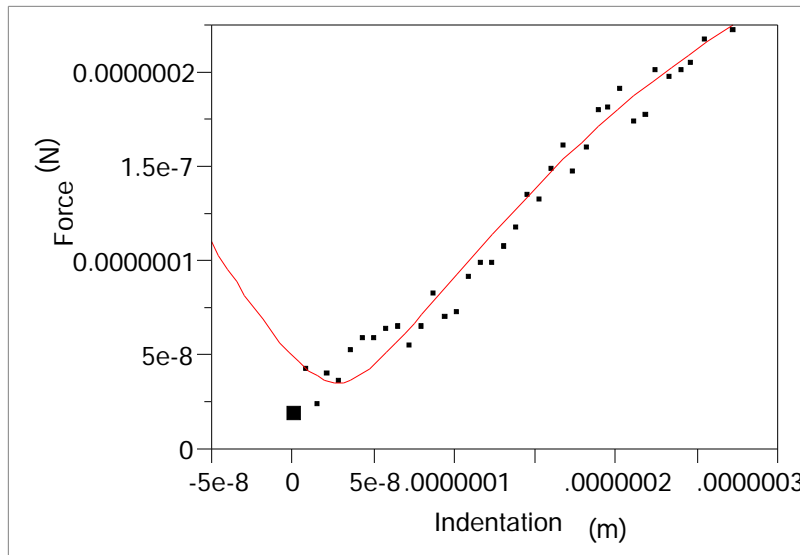
$$E^* = \frac{E}{1-\nu^2}$$

$$F_{paraboloid} = \frac{4}{3} E^* R^{1/2} \delta^{3/2}$$

Where; α = half-opening angle, E^* = surface elastic constant
 R = radius of curvature of tip, δ = indentation depth, ν =
Poisson ratio, E = Young's modulus



Elasticity of PDMS



- Paraboloid fit
(F vs. $\delta^{3/2}$)

$$F_{paraboloid} = \frac{4}{3} E^* R^{1/2} \delta^{3/2}$$

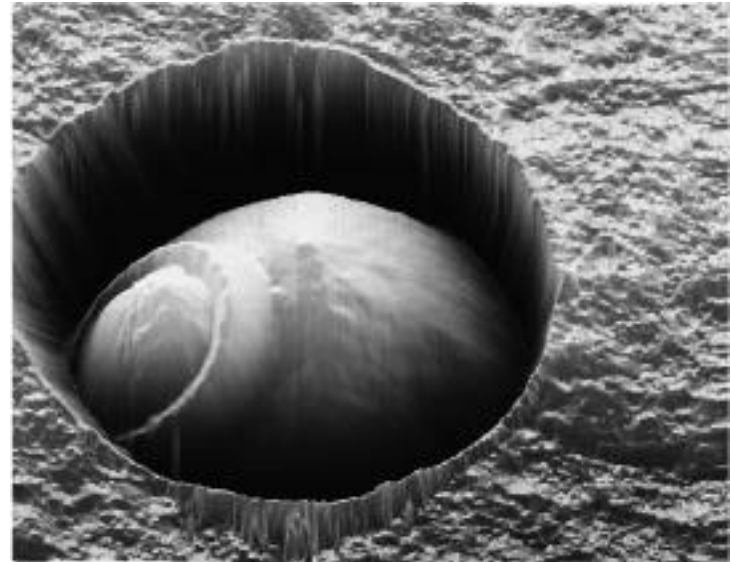
- $F = 2963039.8 \delta^{3/2}$
- $R = 20 \text{ nm}; \nu = 0.5$

- $E = 6.85 \text{ MPa}$



Nanoindentation of Yeast

- *Saccharomyces cerevisiae* – Brewer's yeast
- Cell wall = stronger surface than animal cell membranes
- Trap yeast in nanoporous membrane

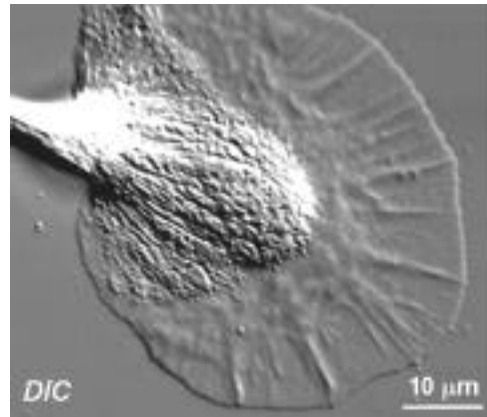


Touhami, et al. Langmuir 2003, 19:4539.

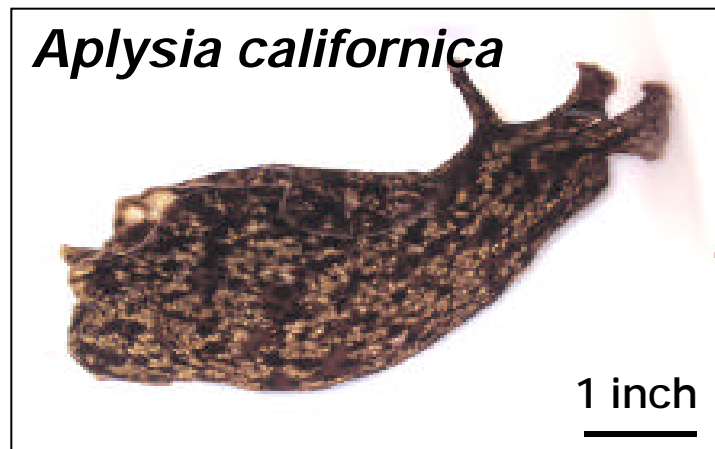


Aplysia californica

Growth cone
of neuron



Sea slug



- Cells easily maintained at room temp
- Actin filaments in growth cone
- Land tip on growth cone and examine forces exerted by cell.



Acknowledgements

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- Asylum Research