Mechanical Analysis and Characterization of Extracellular Bone Matrix (ECM)

Using Atomic Force Microscopy (AFM) and AFM Based Force-Spectroscopy

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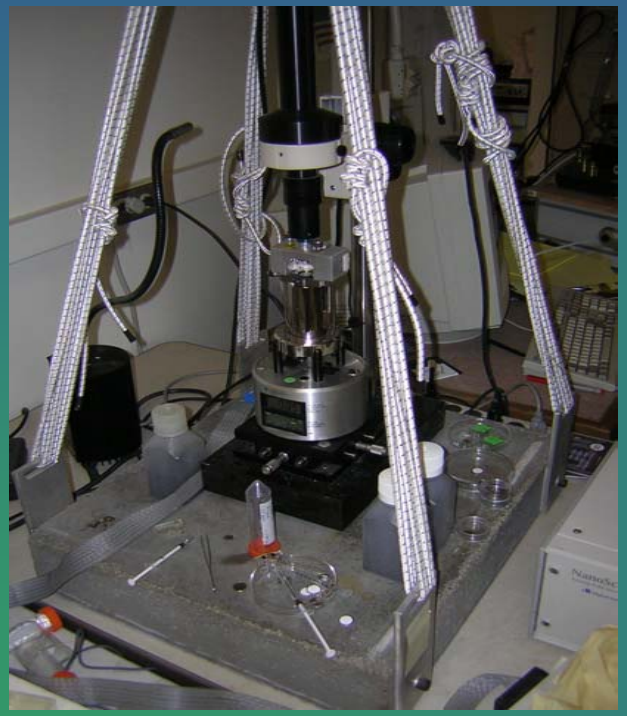
What's The "Big Picture"

- Create an *in vitro* model of Extracellular bone Matrix and test its physico-mechanical properties, using indentation-type AFM. Then compare to *in Vivo* model

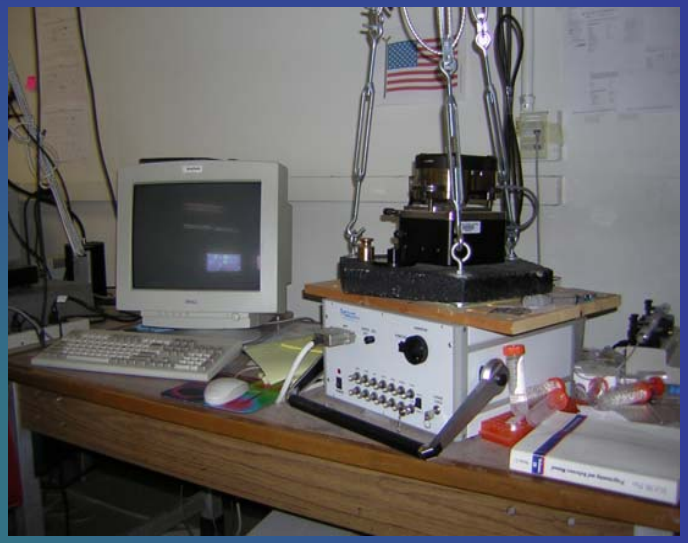
- Understanding mechanical properties of bone will likely lead to advancements in the medical community (ex. Osteoporosis)

Our Research Objectives

- Investigate mechanical properties of bone ECM, using force-spectroscopy



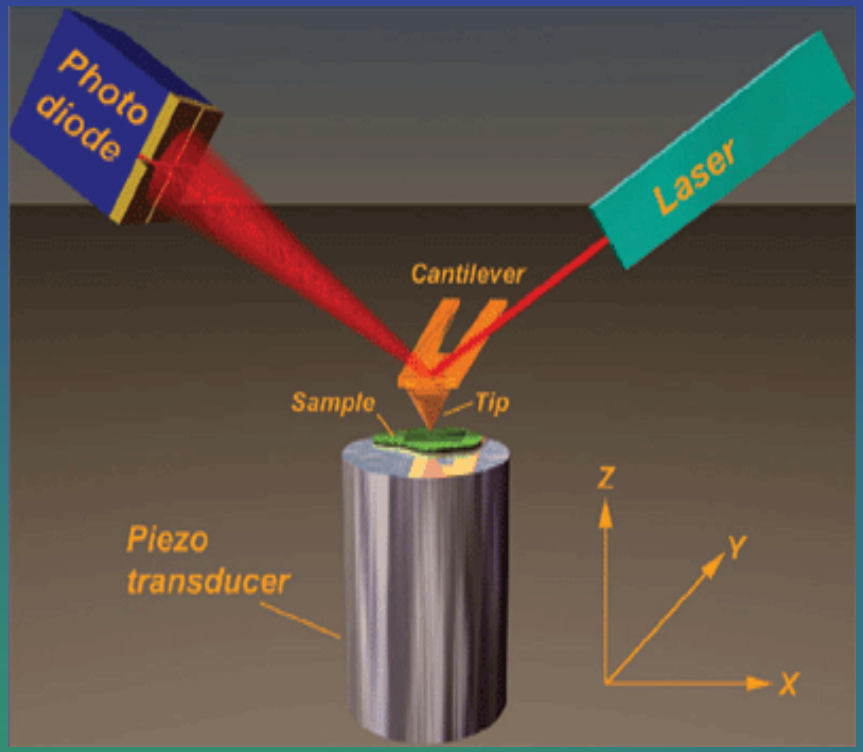
AFM (multimode)



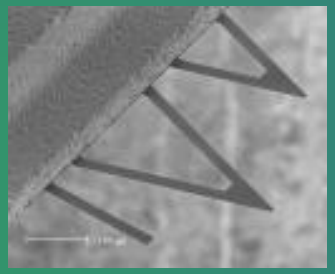
MFP

- Acquire data with MFP and analyze compression graphs

Basic Principles/Theory for Atomic Force Microscope and Molecular Force Puller



www.ifr.ac.uk/.../images/AFM1.gif



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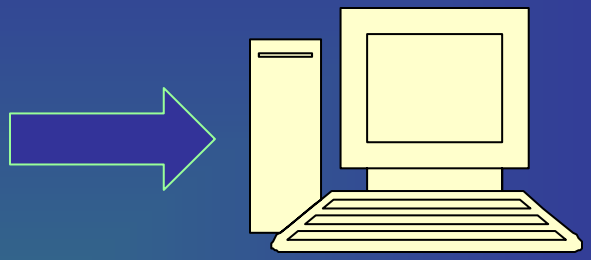
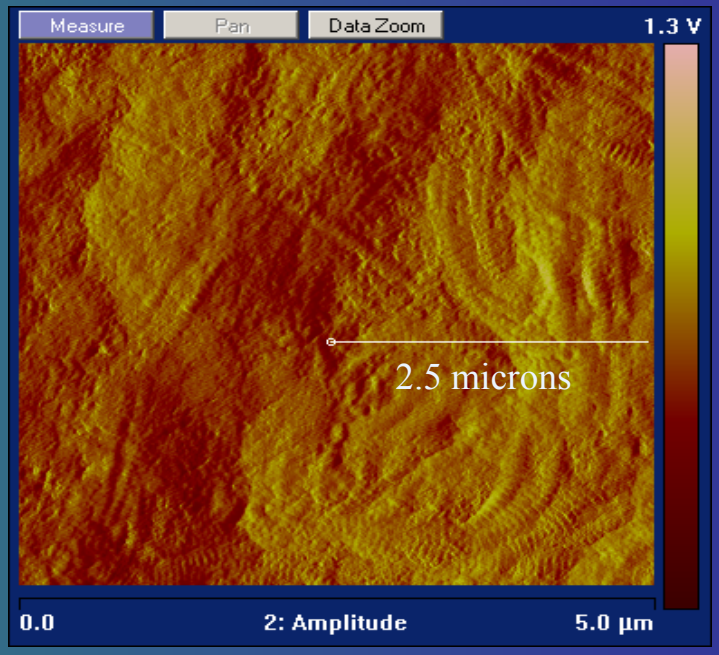
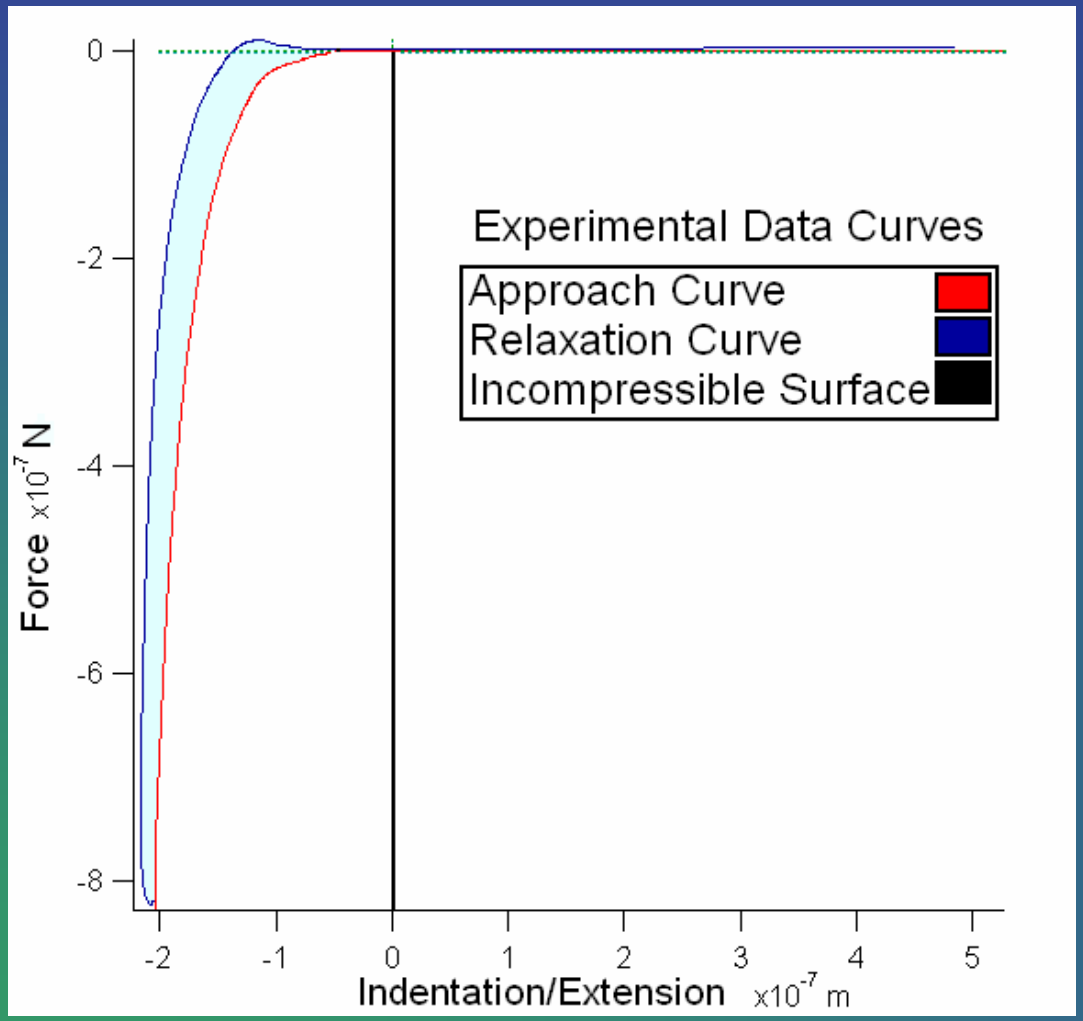


Software interface



Native ECM 5X5 micron AFM scan

Young's Modulus

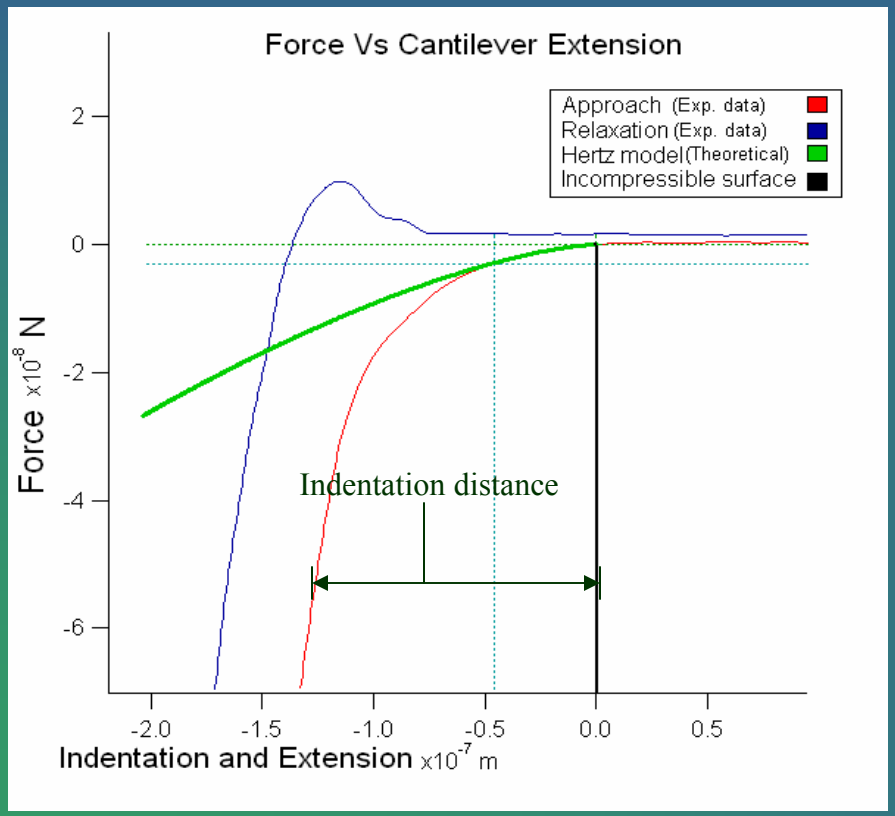


- Young's Modulus quantifies the general term "stiffness"
- Defined as: the rate of change of stress to strain of the approach curve (red)
- In units Pascal (N/m^2)

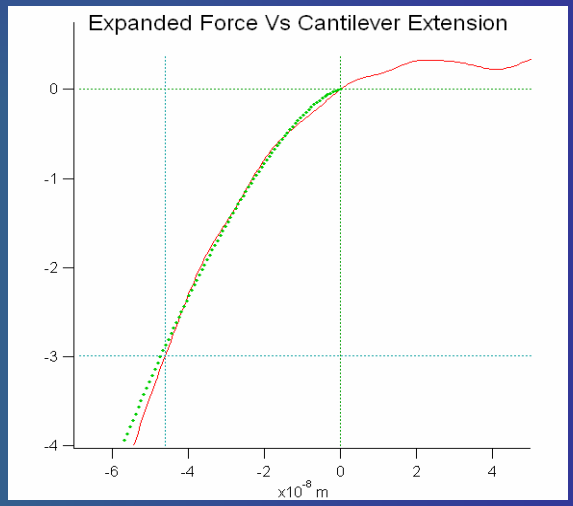
Data Analysis

Calculating Young's Modulus with Curve fitting

- A modified Hertz model used to fit data curve
- Where F is force (Y), E is Young's Modulus, R is probe radius, V is Poisson's ratio, I is indentation



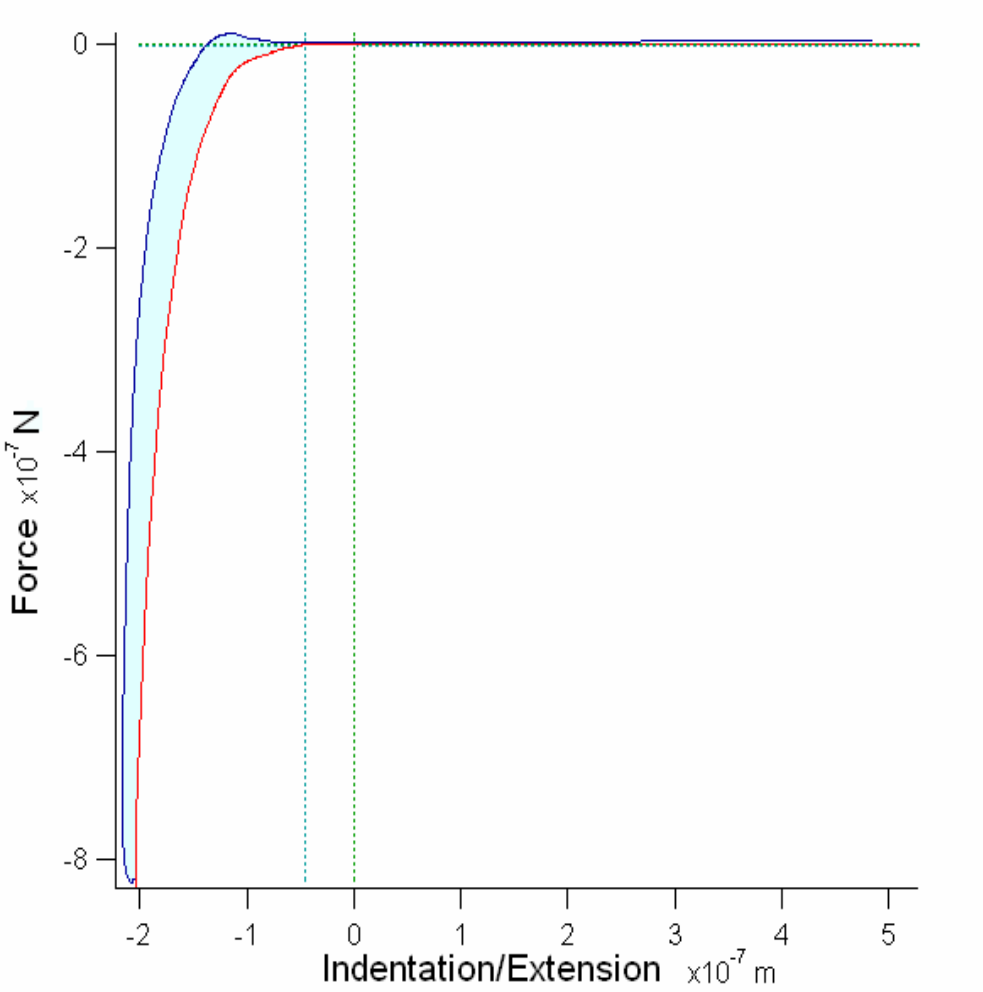
- Young's modulus for expanded region is the derivative of Hertz model



$$F = \frac{4E_y R^{1/2}}{3(1-V^2)} I^{3/2}$$

Data Analysis

Measuring Energy Dissipation of Plastic Region



- The ECM acts as a viscoelastic solid
- Unlike an elastic solid a viscoelastic material, after being compressed, doesn't immediately "spring back" to its original size and/or shape
- The light blue area depicts the plastic region
- Measuring this area and converting from N → J gives a dissipation of energy value

Summary

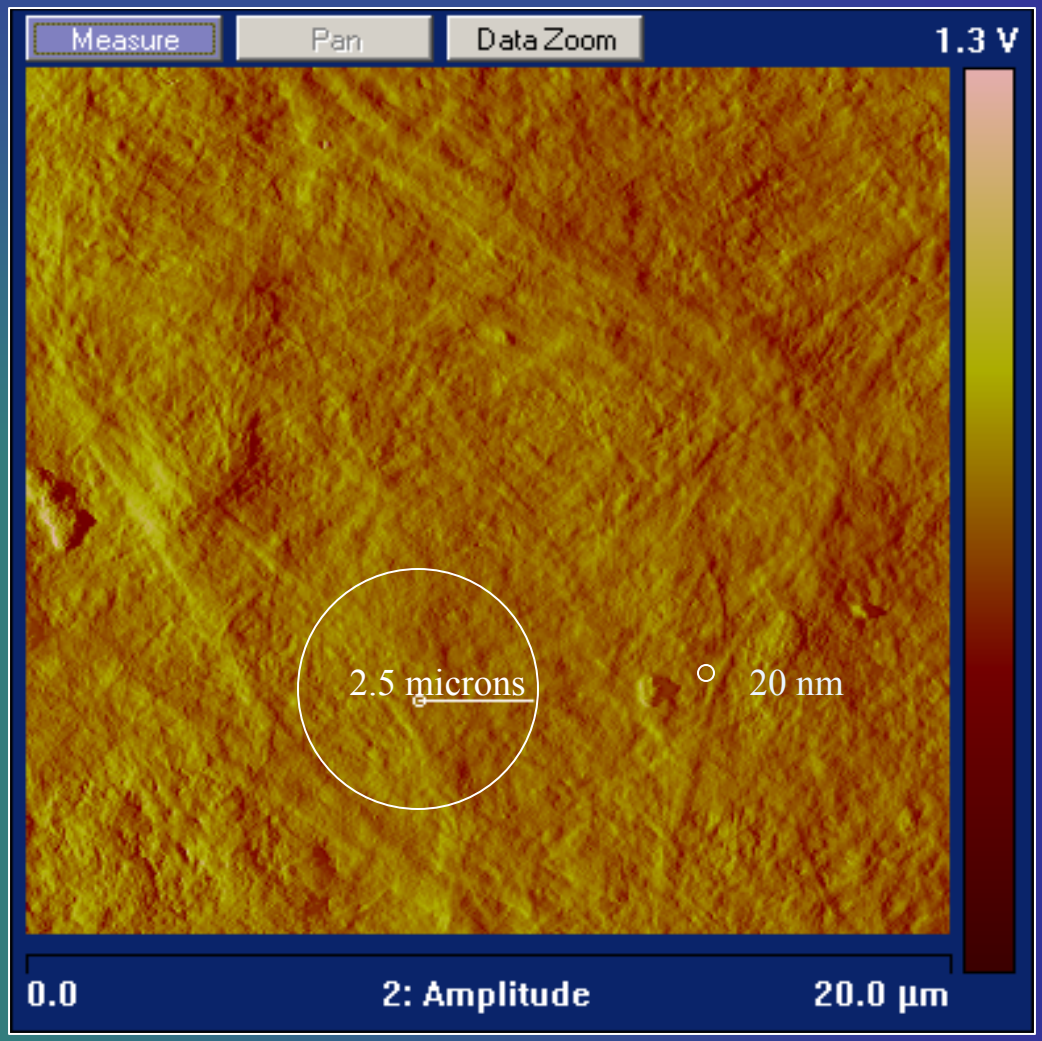
Research Achievements

- Gained basic understanding of bone structure/biology
- Learned how to operate AFM and MFP
- Gained insight as to how ECM reacts mechanically under cyclic loading

- Collected data- 480 force/indentation curves, using MFP (force spectroscopy technique)
- Used data analysis software to normalize data (fix axis scaling)
- Found Young's modulus, and Energy dissipation values for data curves. Analyzed these values by graphing them vs. timescale

Future Directions

- Compare our research (*in vitro*) with *in vivo*
- Compare data of submicron and macroscopic (millimeter scale) testing
- Experiment with indentation using sharp tip cantilever (nominal radius 20 nm) compare results to current experiment (probe radius 2.5 microns)



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INSET program facilitators