Recent advances in atomic force microscopy for nanobiological and nanocomposite applications


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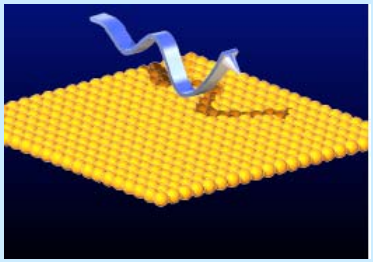
Purdue President France A. Córdova and Prof. Ei-ichi Negishi, Nobel Laureate Chemistry (2010)

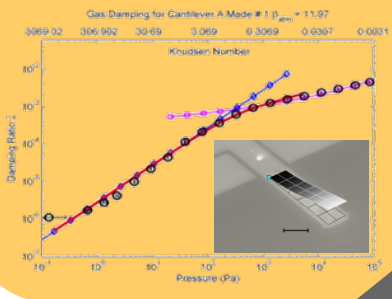
PURDUE UNIVERSITY

Current Projects

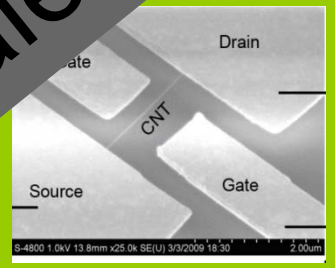
Fundamentals of Atomic Force Microscopy (NSF, NCN, Dow)



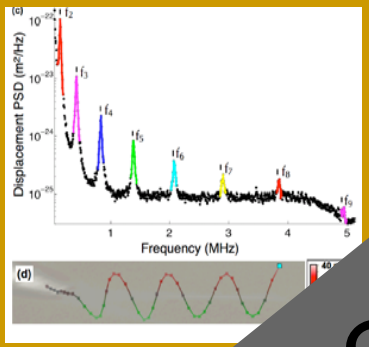
MEMS (NNSA, Sandia)



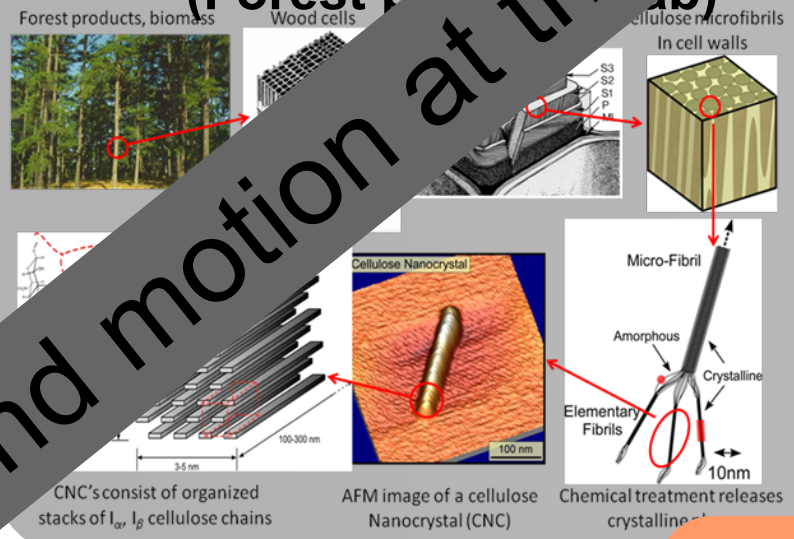
NEMS (NSF)



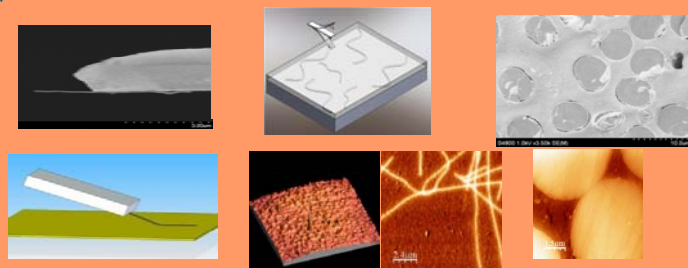
Nanowire resonators for biology (NIH)



Cellulose nan… (Forest p… ap)



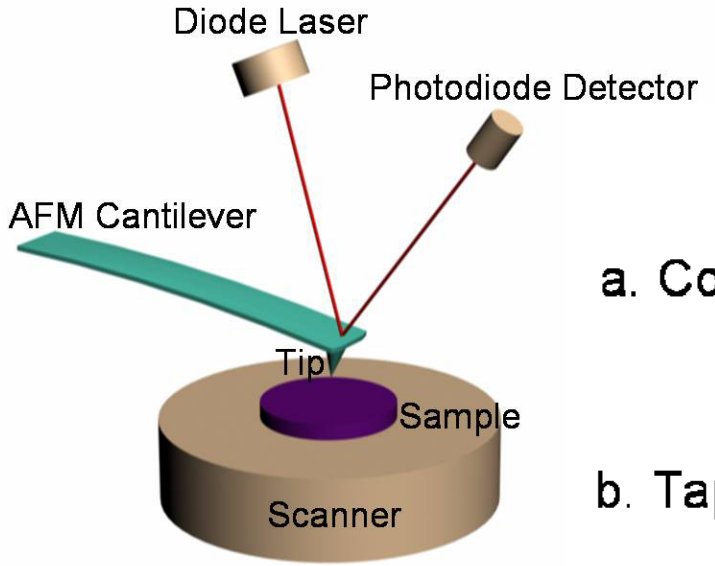
Nanotube/graphene nano-composite studies with AFM (Boeing-NSF-NIRT)

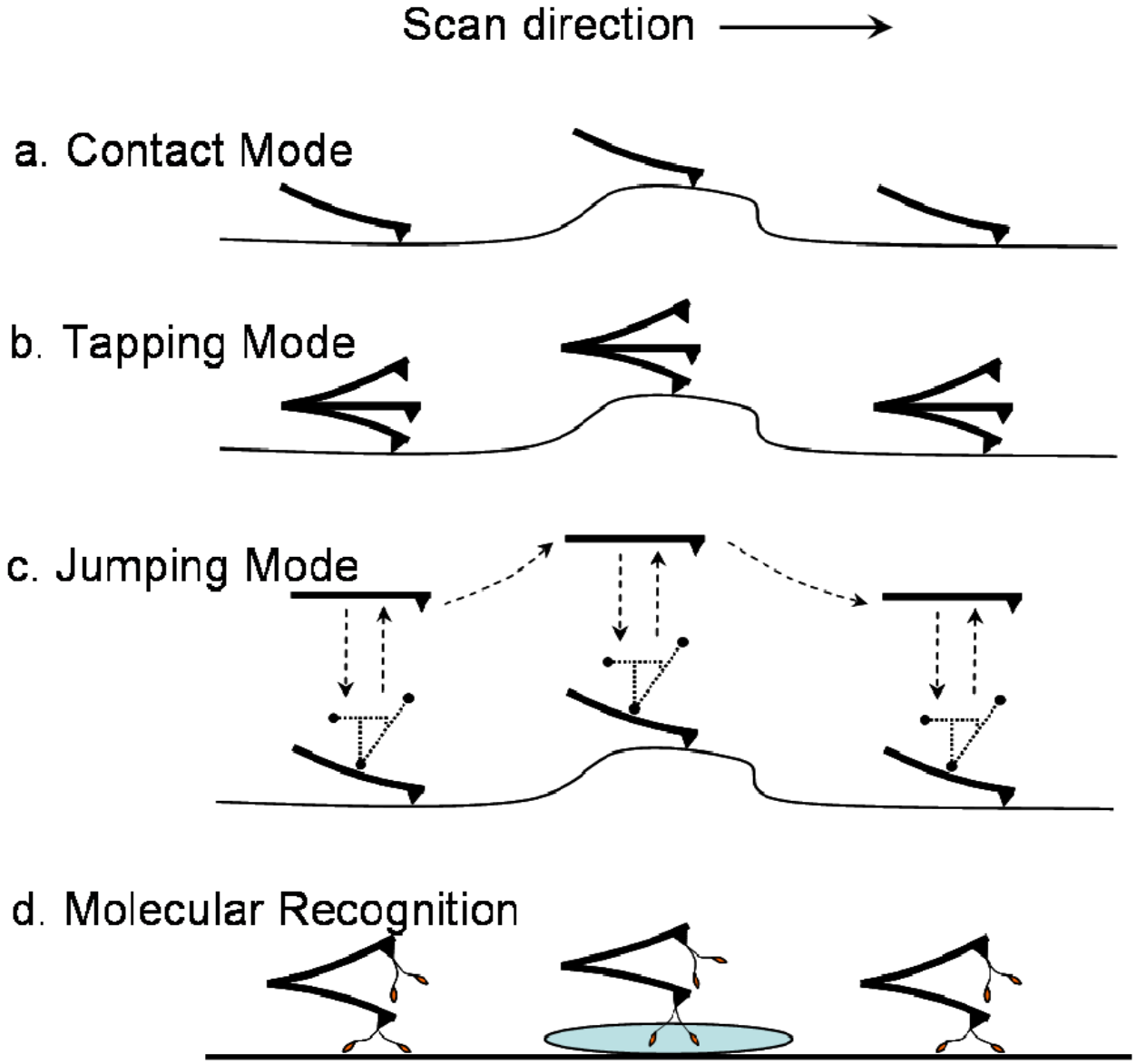


Force and motion at the nanoscale!

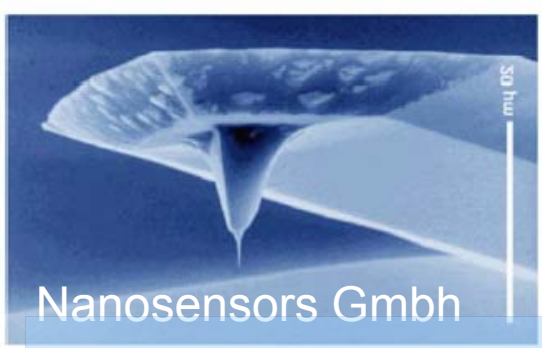
- State-of-the art of dynamic Atomic Force Microscopy
- Nanomechanical property mapping of biological samples
- nanoHUB resources for AFM scientists

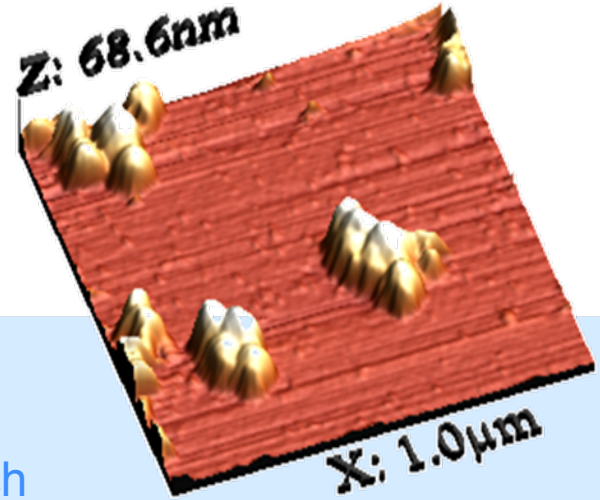
Atomic Force Microscope



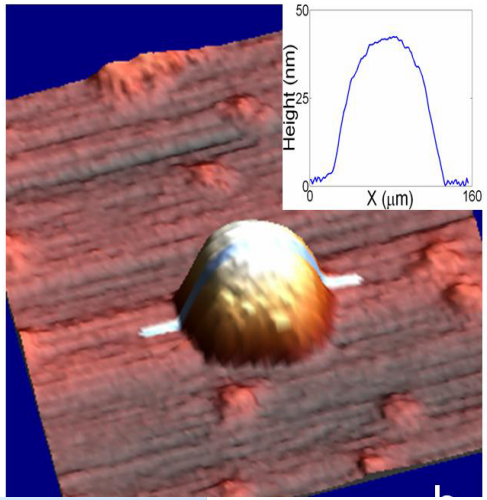


Dynamic Atomic Force Microscopy

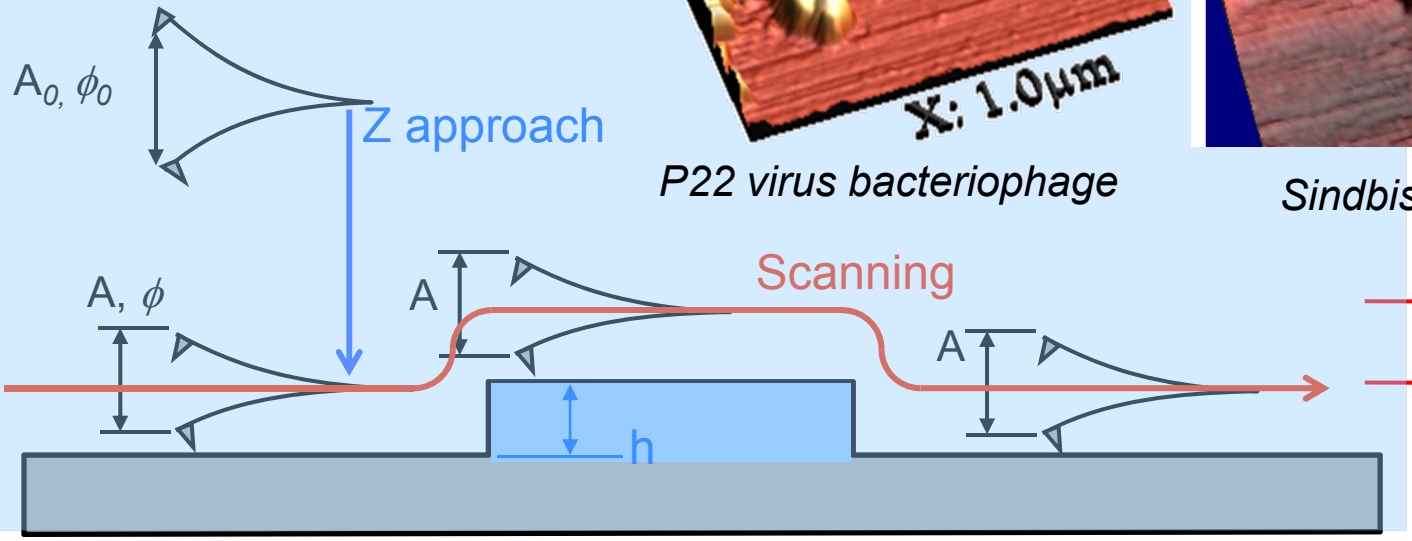




P22 virus bacteriophage



Sindbis virus core



- **Dynamic AFM uses cantilever driven at resonance**
  - Tapping mode regulates the amplitude-Amplitude modulation AM-AFM
  - Frequency modulation (FM) regulates phase, freq shift, and amplitude
  - Has shown tremendous gains in speed and resolution in liquid environments for biology

The driving forces

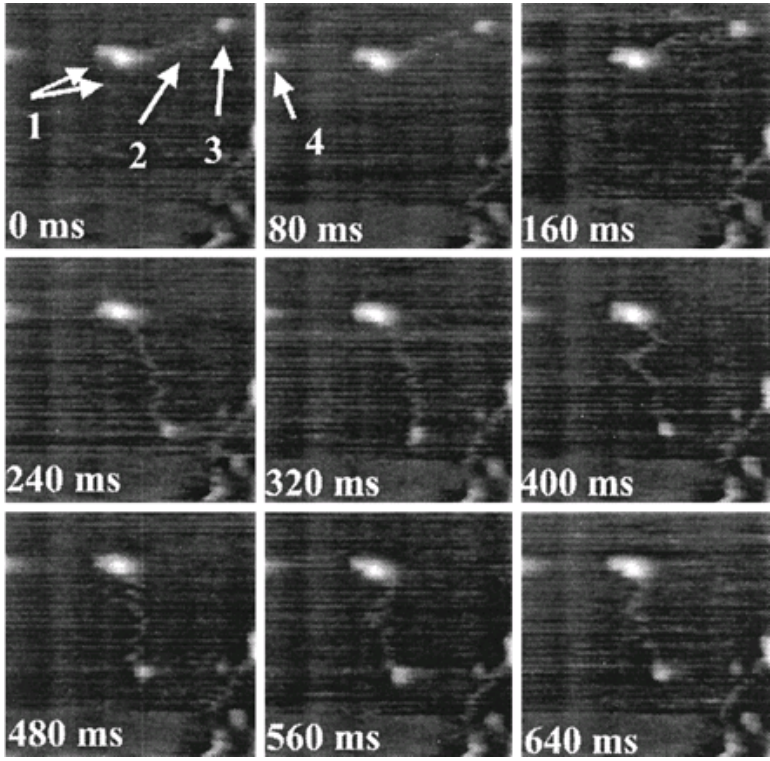
- Primary needs
  - High speed
  - High resolution
  - Material contrast – mechanical, electrical, chemical, thermal to distinguish a variety of samples
  - Quantitative metrology

- Newer needs
  - Subsurface information
  - Live cell imaging
  - Combined instruments (Raman, IR/thermal etc.)

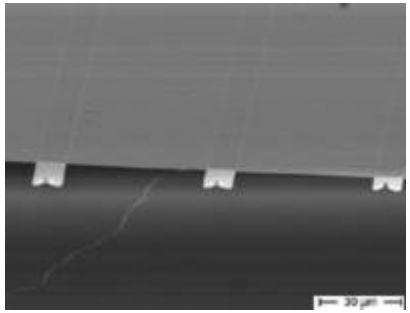
High speed

High speed AM-AFM in liquids

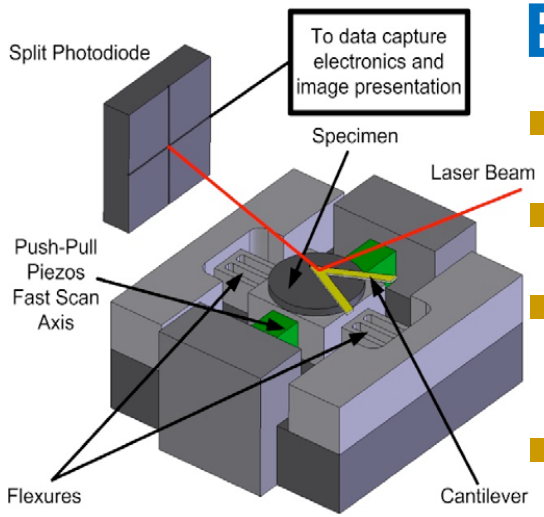
T. Ando et al, 2001, PNAS, Successive images of myosin V on mica in buffer solution. 240 × 240 nm² was imaged 50 times with 100 × 100 pixels. The tip speed is 0.6 mm/s (scan rate, 1.25 kHz), and the frame rate is 12.5/s. The tapping frequency is 620 kHz, A_0=12 nm, A=11.5 nm.



- M. Miles et al.
- T. Fukuma et al
- G. Schitter et al
- G. Fantner et al.



www.sclsensortech.com



Enabling technologies

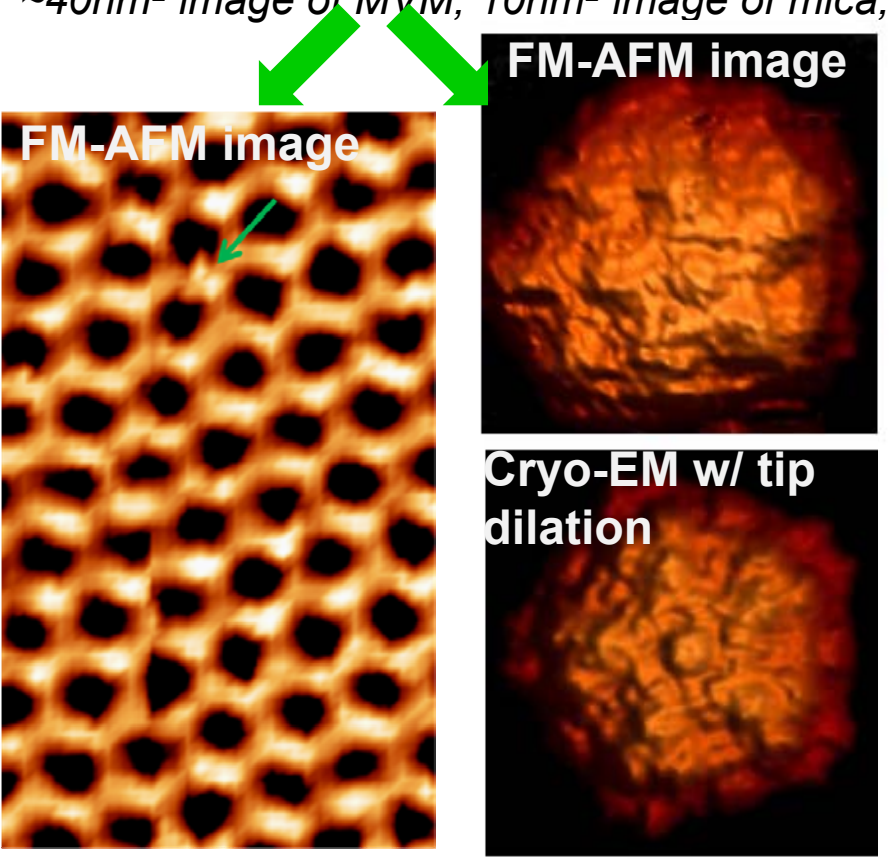
- High bandwidth Scanner, Z piezo,
- Fast phase detection
- Small cantilevers (< 20 microns)
  (also L. Pietrasanta, H. Hansma et al)
- Excitation mechanisms (photothermal, high bandwidth magnetic)

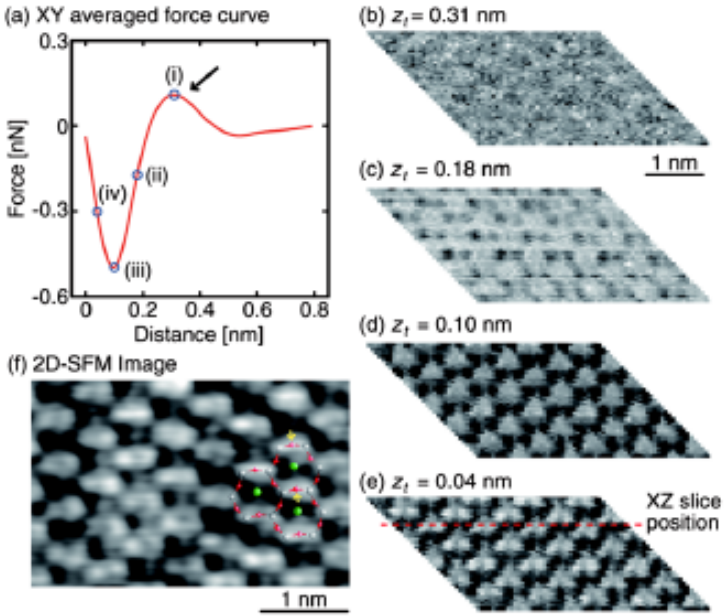
High resolution

- **High resolution FM-AFM in liquids (Fukuma et al., PRL 2010)**

- **High resolution FM-AFM in liquids**

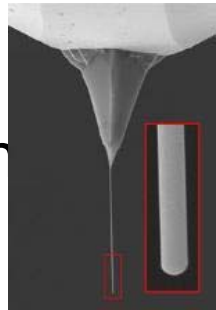
J. Gomez-Herrero, Raman et al, UAM, Madrid (submitted)
~40nm^2 image of MVM, 10nm^2 image of mica, both in PBS





Enabling technologies

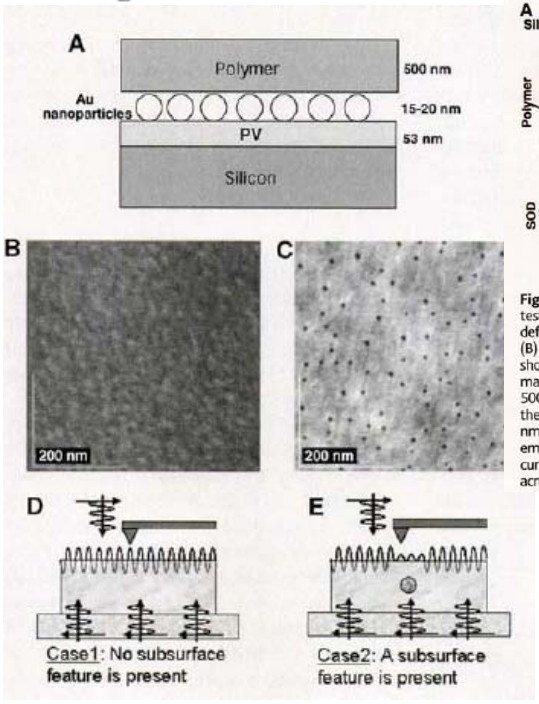
- Laser (transition to thermal limited from shot noise limited)
- Artifact free excitation for FM in liquids (magnetic/photothermal)
- Sharp metallic tips for high contrast EFM



www.nauganeeldes.com

Subsurface imaging using ultrasound

Nanoscale Imaging of Buried Structures via Scanning Near-Field Ultrasound
Gajendra S Shekhawat; Vinayak P Dravid
Science; Oct 7, 2005; 310, 5745; Research Library Core
pg. 89



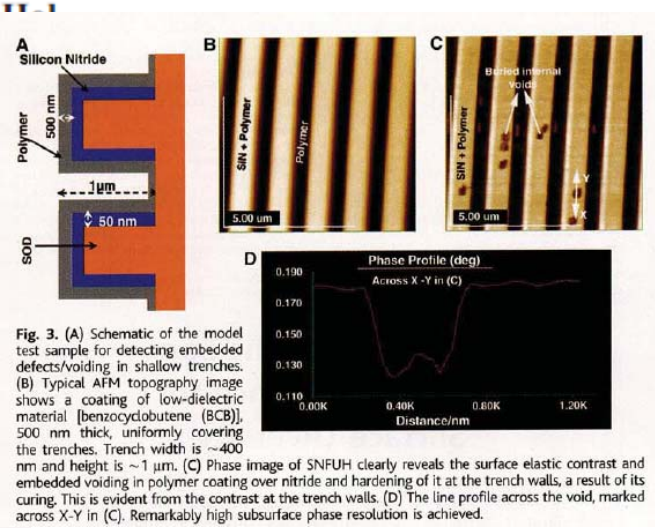


Fig. 3. (A) Schematic of the model test sample for detecting embedded defects/voiding in shallow trenches. (B) Typical AFM topography image shows a coating of low-dielectric material [benzocyclobutene (BCB)], 500 nm thick, uniformly covering the trenches. Trench width is ~400 nm and height is ~1 μm. (C) Phase image of SNFUH clearly reveals the surface elastic contrast and embedded voiding in polymer coating over nitride and hardening of it at the trench walls, a result of its curing. This is evident from the contrast at the trench walls. (D) The line profile across the void, marked across X-Y in (C). Remarkably high subsurface phase resolution is achieved.

LETTERS

Imaging nanoparticles in cells by nanomechanical holography

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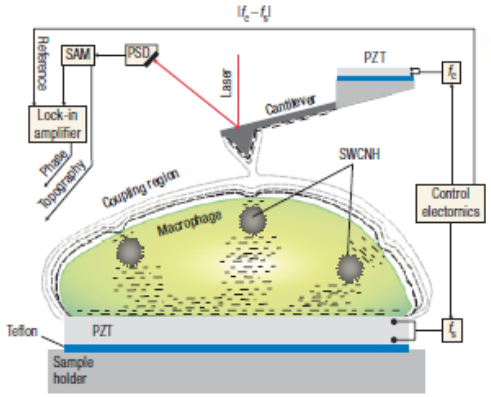
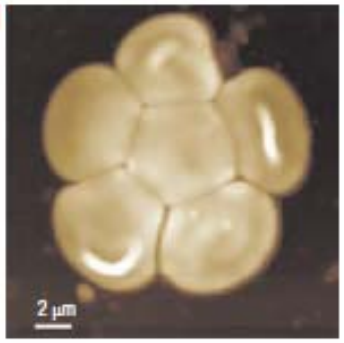
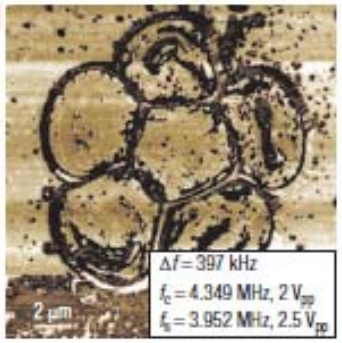




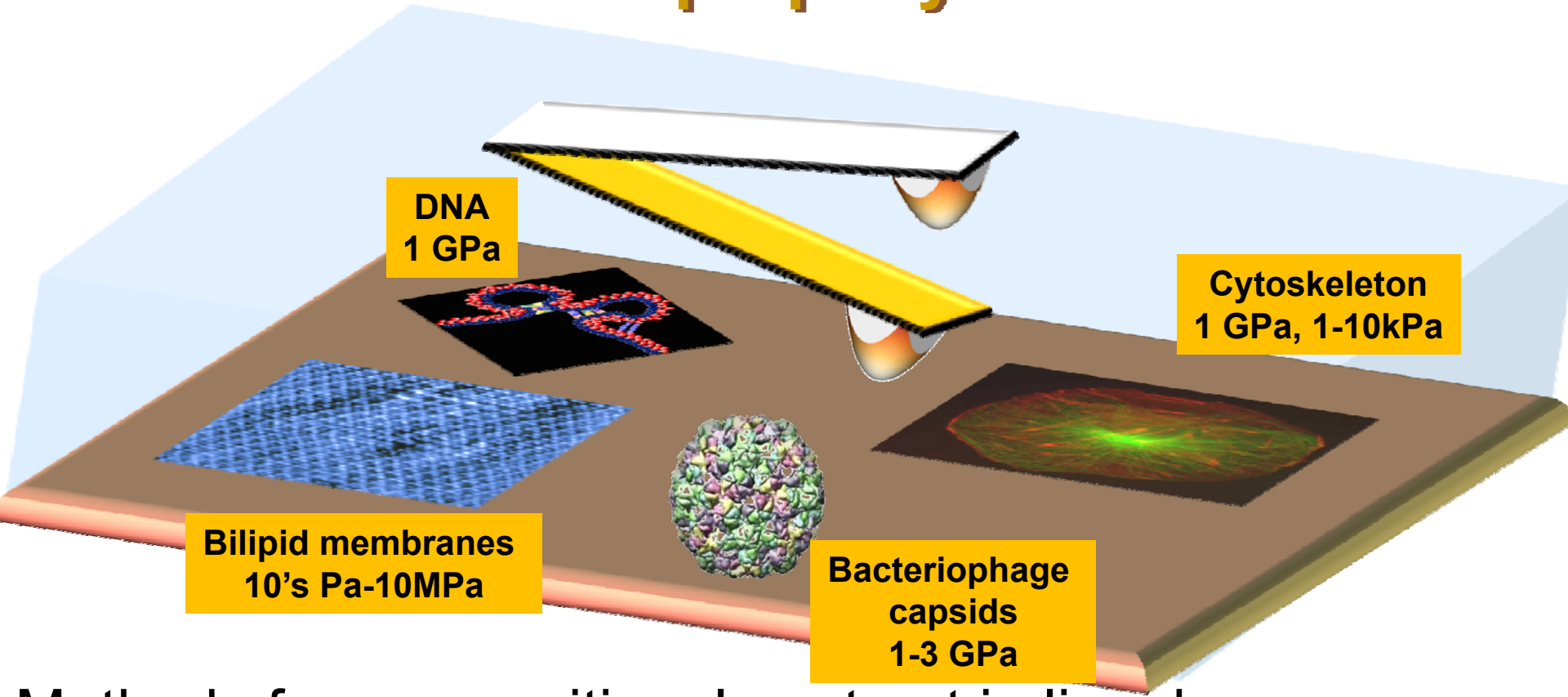


Challenges

- Not clear how mm wave length achieves high resolution
- Not depth sensitive
- Image formation not understood

- State-of-the art of dynamic Atomic Force Microscopy
- Nanomechanical property mapping of biological samples
- nanoHUB resources for AFM scientists

Local mechanical property contrast in dAFM

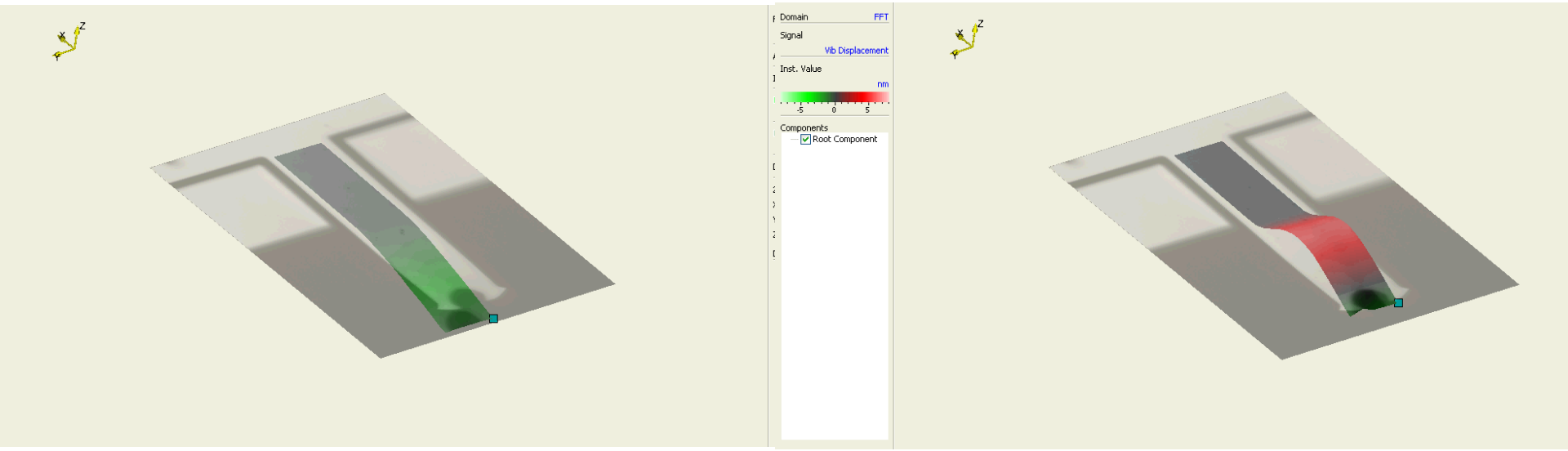


Methods for compositional contrast in liquids

- Second harmonic imaging, Van Noort et al, (Langmuir, 1999), Preiner, Hinterdorfer et al (PRL, 2007, Ultramicroscopy 2009, N.H. Thompson, APL, 2009)
- Momentary excitation (Xu, Melcher, Raman, Reifenberger, PRL, 2009)
- Phase contrast interpreted in liquids (Melcher, Raman, et al., PNAS 2009)
- Bimodal AFM (Martinez, Lozano, Herruzo, Garcia, (Nanotechnology, 2008))

Terminology: eigenmode vs. harmonics

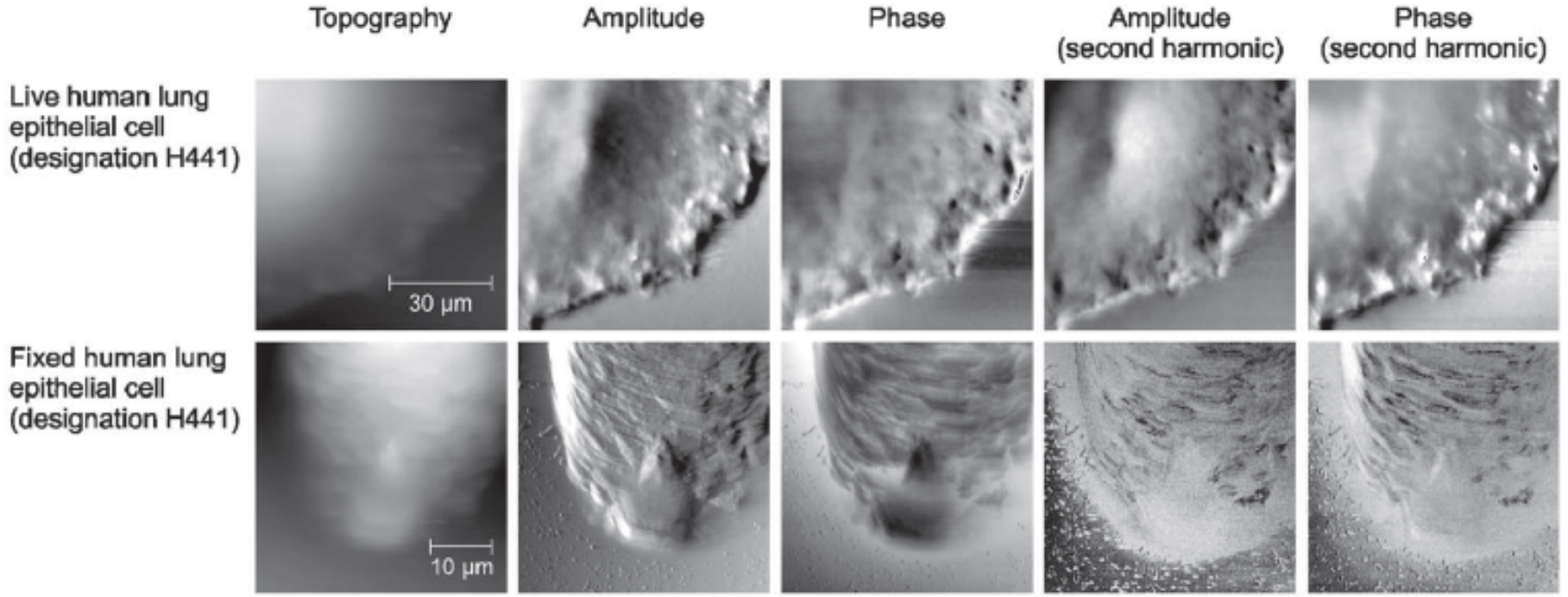
1st eigenmode (f_1 kHz) **2nd eigenmode (f_2~6.5 *f_1 kHz)**



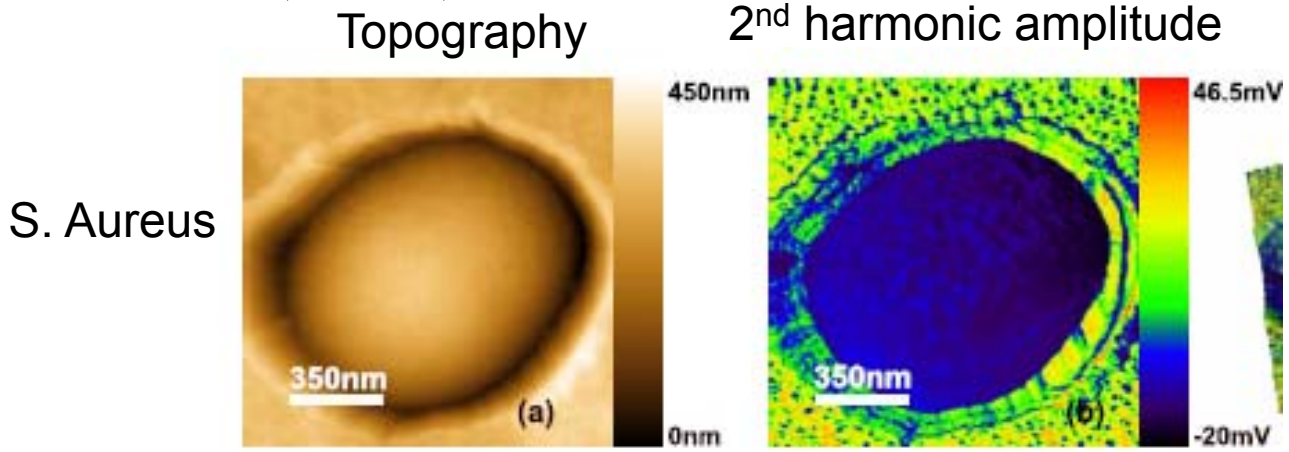
- Each eigenmode is a separate oscillator with its own k_{eff},Q_{eff}, and lever sensitivity
- When driven with a sine wave at a specific frequency f_{dr}, the cantilever vibrates at f_{dr}, however when interacting with samples, its vibrations consist of 1*f_{dr}, 2*f_{dr}, 3*f_{dr}…harmonics

2nd harmonic imaging

- Hinterdorfer et al, Ultramicroscopy 2009



- Thomson et al, APL, 2009



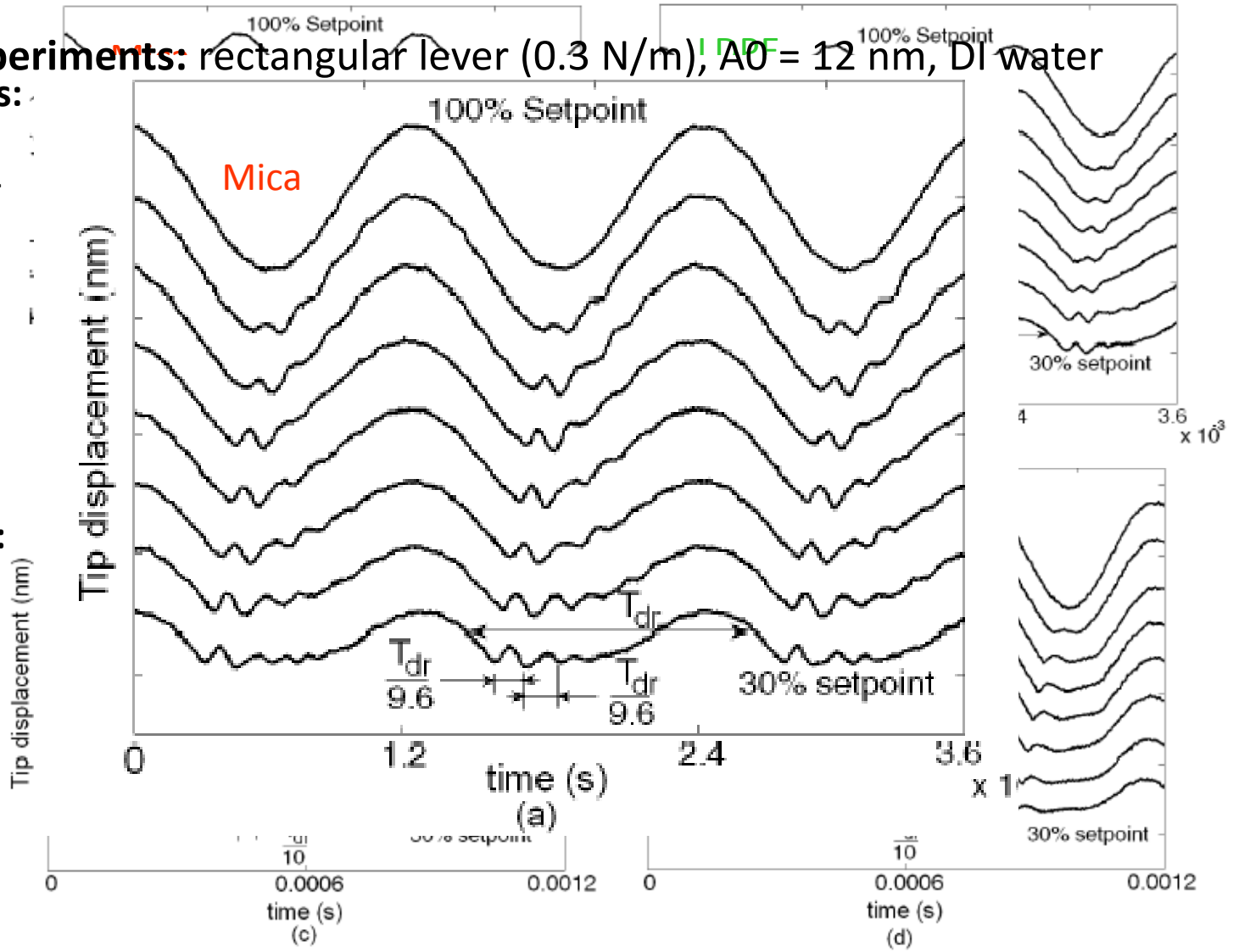
Momentary excitation - experiments

(Basak and Raman, App. Phys. Lett., 2007)

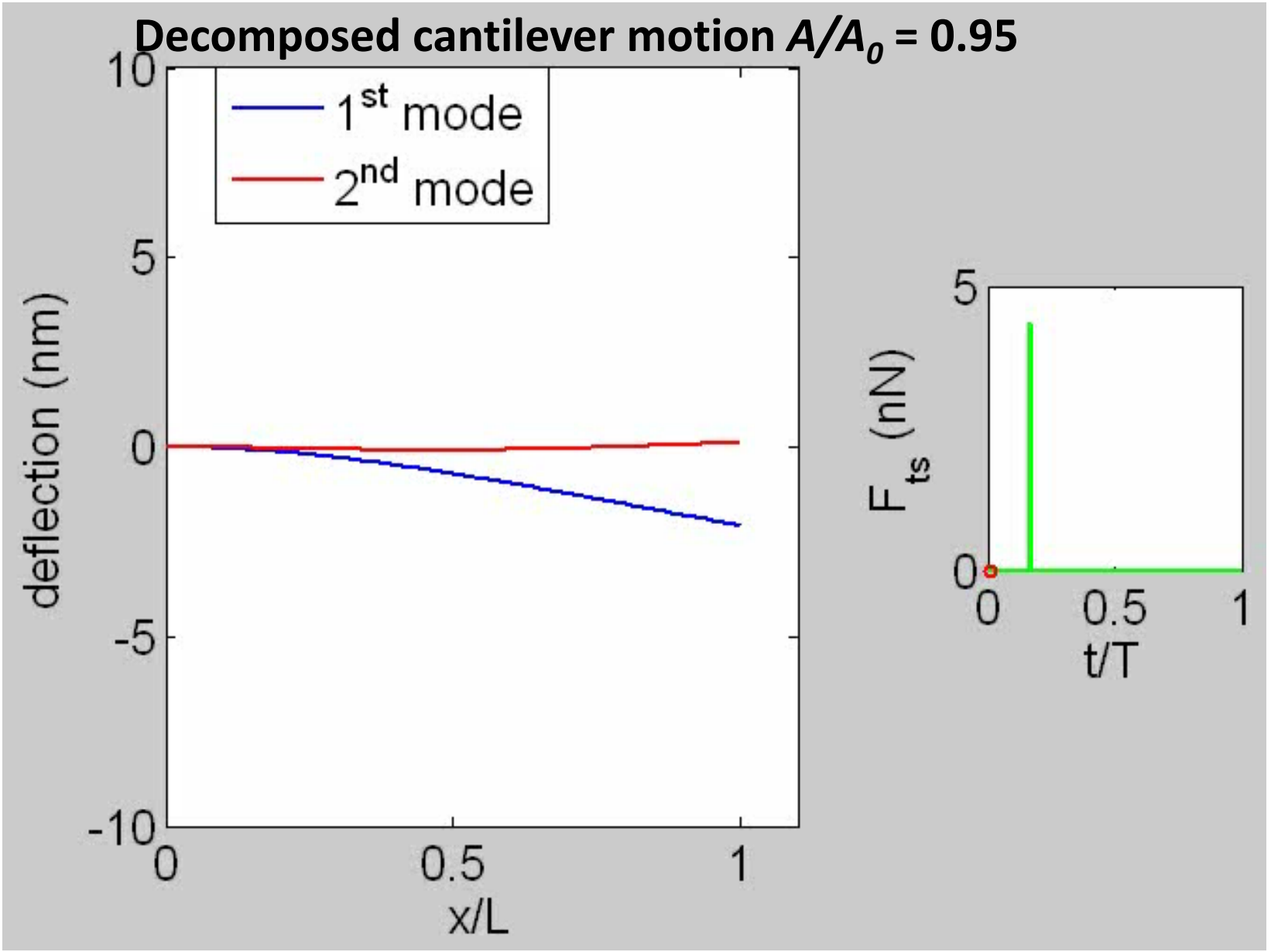
Experiments: rectangular lever (0.3 N/m), A0 = 12 nm, DI water

Experiments:
0.3 N/m
rectangular
lever

Experiments:
0.1 N/m
triangular
lever



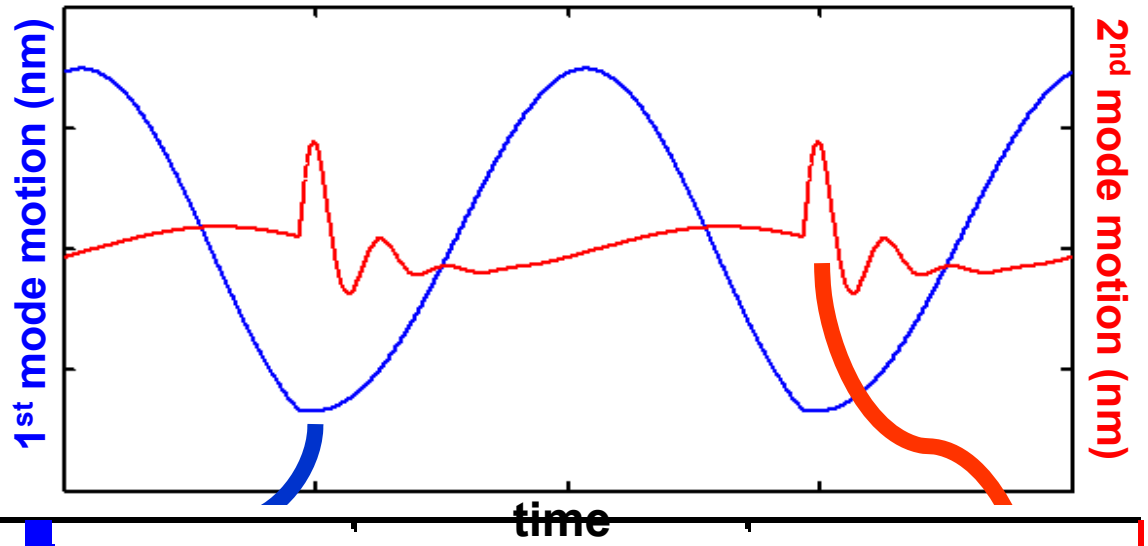
Momentary excitation-theory

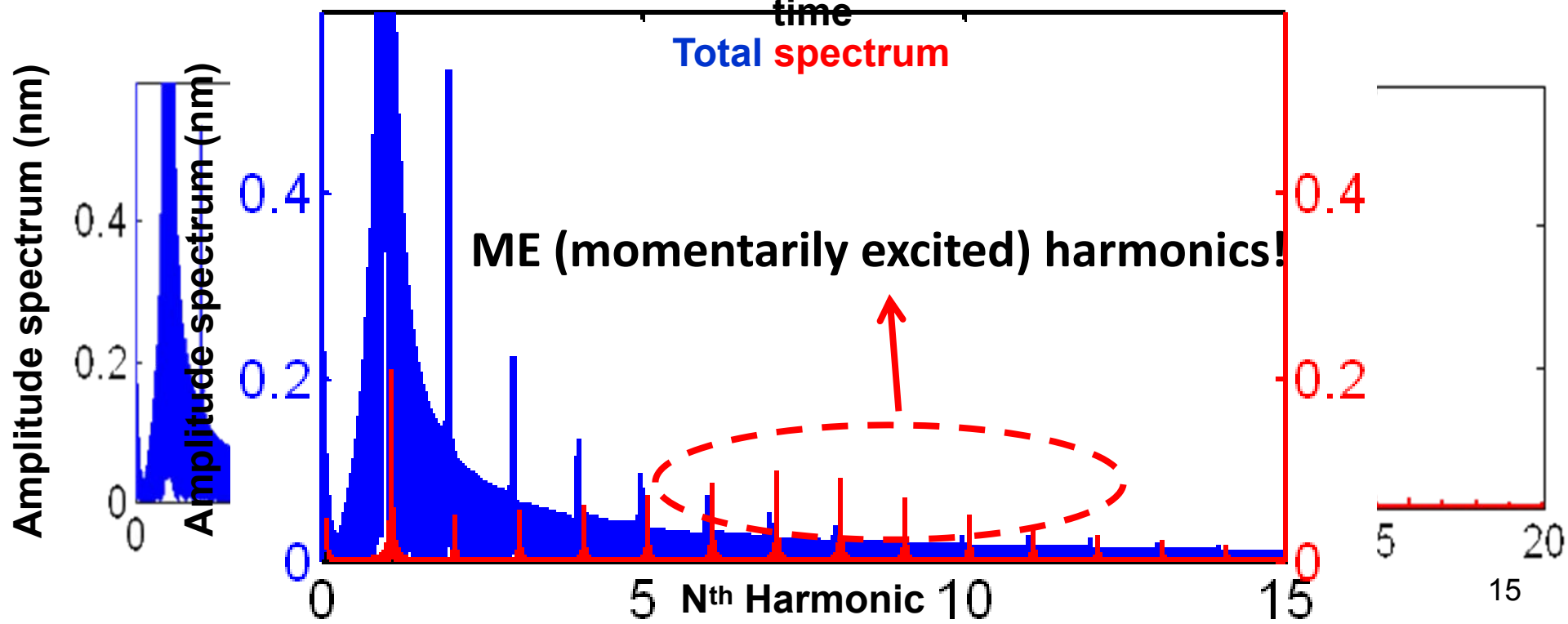


Momentary excitation greater on stiffer samples

Momentarily Excited (ME) Harmonics

Simulations:
MAClever,
A_0=15nm,
A/A_0=0.92



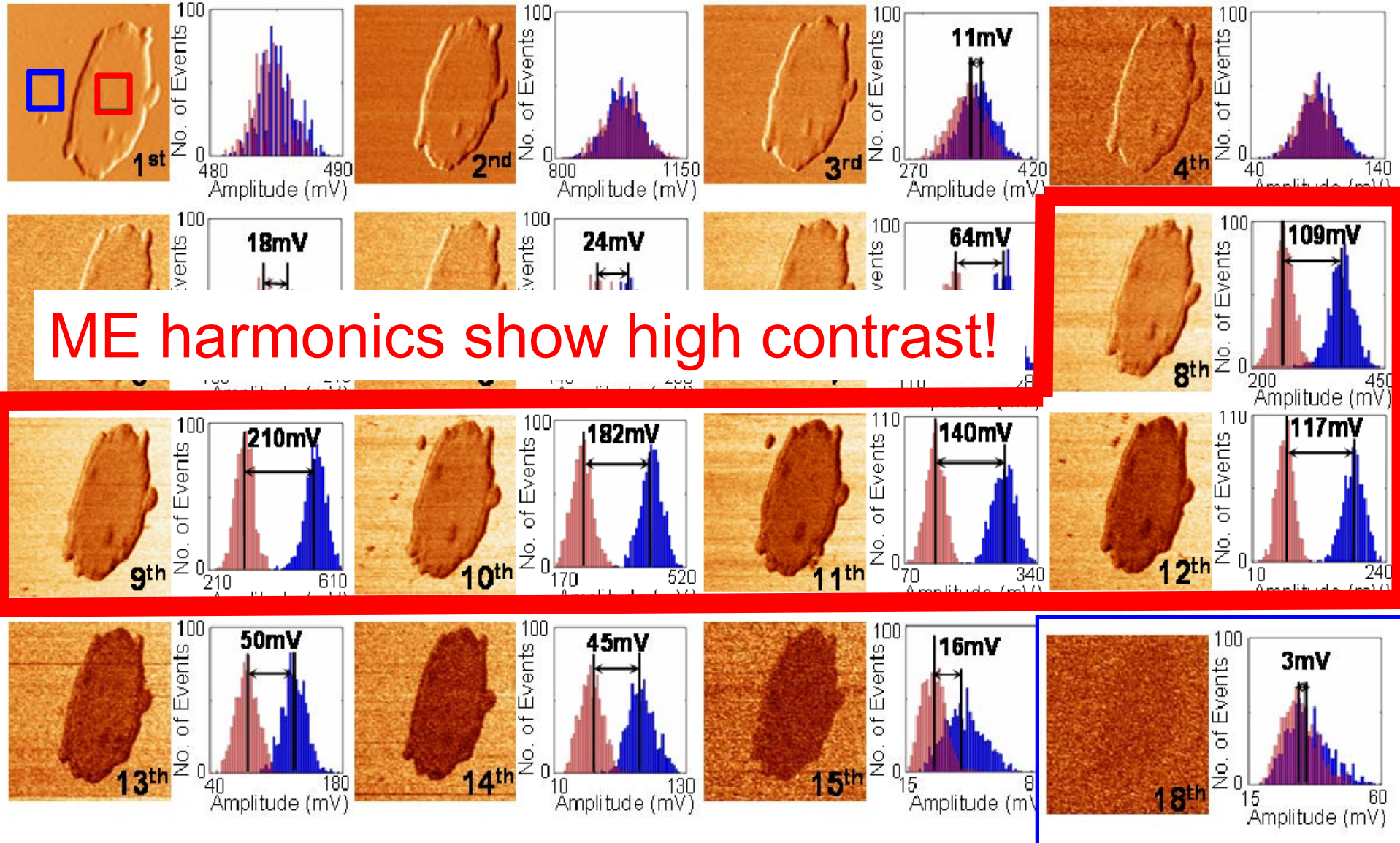


Xu, Melcher, Basak, Reifenberger Raman, **Phys. Rev. Lett.** 2009

Higher Harmonic Imaging

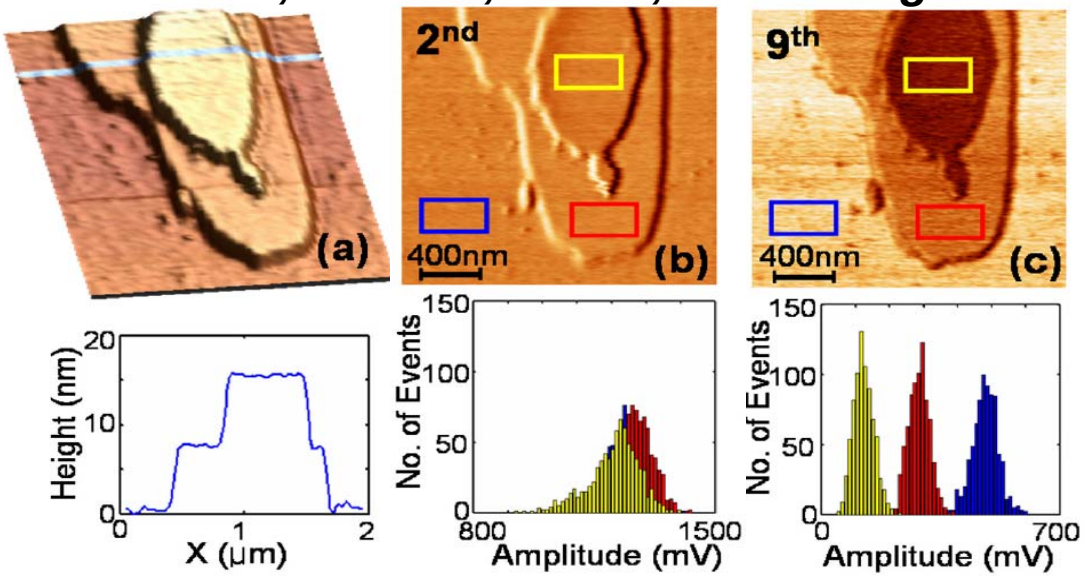
Xu, Melcher, Basak, Reifenberger Raman, **Phys. Rev. Lett.** 2009

Experiment: purple membrane on mica, k_1=0.11 N/m, A_0=15nm, $A_{setpoint}$=97



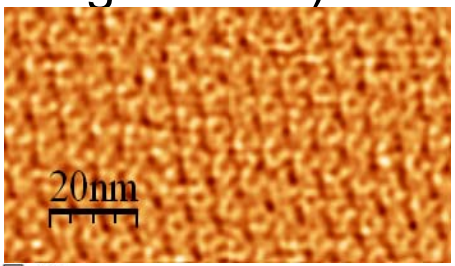
Elasticity contrast for soft samples

Xu, Melcher, Basak, Reifenberger Raman, Phys. Rev. Lett. 2009

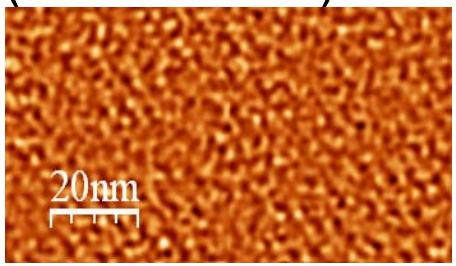


k_1=0.11 N/m, A_0=12.5nm, $A_{setpoint}$=92 %,
buffer: 300 mM KCl, 20 mM Tris-HCl, pH 7.8

Topography (2nd eigenmode)

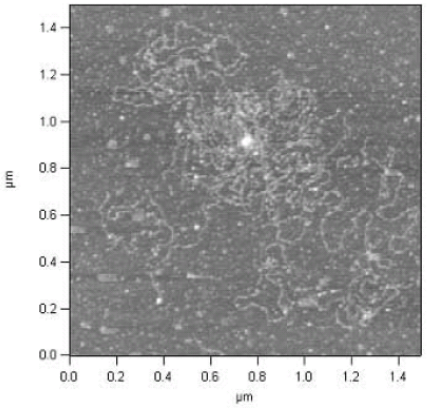


Material property (3rd harmonic)

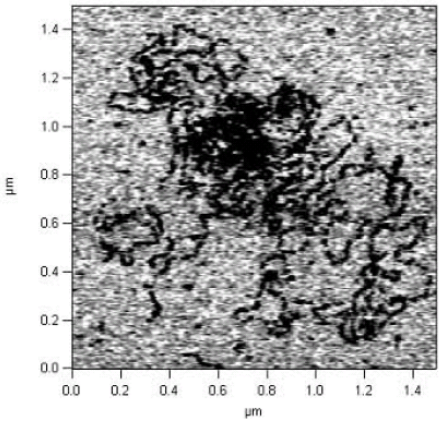


BstE II digested bacteria phage Lambda DNA in buffer (triangular TR400 levers)

Topography



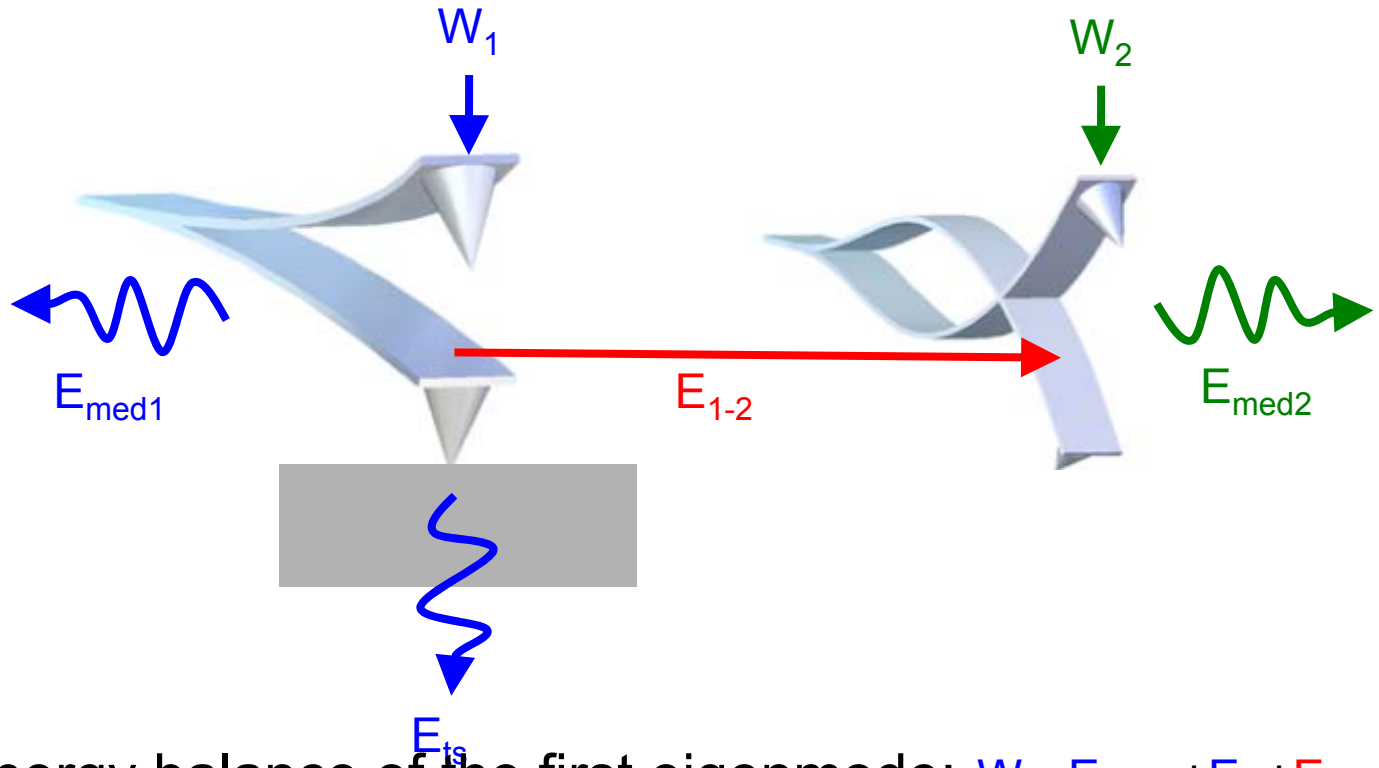
4th Harmonic



k_1= 0.088N/m, f_1=4.4 kHz,
Q_1=1.03; f_2=32.3 kHz, Q_2=3.75;
f_3=96.92 kHz, Q_3=4.73
f_{dr}=31kHz; A_0 ~ 0.3nm; $A_{setpoint}$= 92%

Origins of phase contrast in liquids

Melcher , Carrasco, Xu, de Pablo, Gomez-Herrero, Raman PNAS, 2009



Recall the energy balance of the first eigenmode: $W_1 = E_{med1} + E_{ts} + E_{1-2}$

Approximation:

$$q_1(t) \approx A\cos(\omega_1 t - \phi_1)$$

$$W_1 = \int_0^T F_1 \cos(\omega_1 t)\dot{q}_1 dt$$

$$= A_1 \pi F_1 \sin\phi_1$$

$$E_{med1} = \frac{k_1}{\omega_1 Q_1}\int_0^T \dot{q}_1^{\,2} dt$$

$$= \frac{k_1}{\omega_1 Q_1} A_1^2$$

$$\sin\phi = \frac{A}{A_0}\left[1 + \frac{E_{ts} + E_{1-2}}{E_{med1}}\right]$$

Set-point=constant

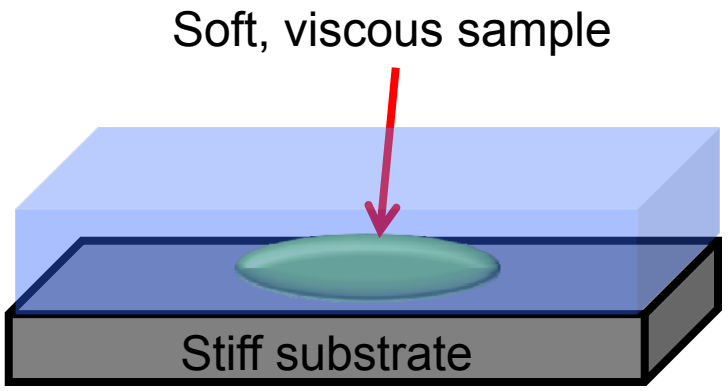
E_{med1} = constant

E_{1-2} is much larger compared to E_{ts}

Experimental considerations

What is the mechanism of phase contrast?

- In high concentration buffer:
  - Electrostatic DLVO forces are screened
  - Conservative forces: elasticity
  - Nonconservative forces: viscoelasticity, solvation shells
- Recall phase lag is proportional to the **total** energy loss: E_{ts} + $E_{1\text{-}2}$
- What is the source of phase contrast?
  - (a) Tip-sample dissipation → biological sample will be bright in phase lag image
  - (b) Energy propagation → substrate will appear bright in phase lag image



Experimental data

Melcher , Raman, et al. (PNAS 2009)

Purple Membrane on **mica substrate**

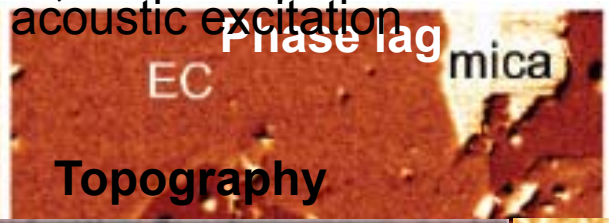
ϕ29 virus capsid on a **glass substrate**

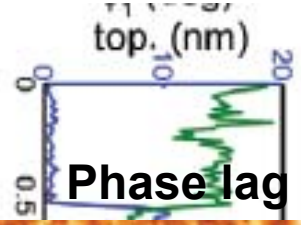
buffer solution: 300 mM KCl, 20 mM Tris-HCl, pH 7.8

buffer: TMS, pH 7.8

Levers: k1 = 0.58, 0.09 N/m, acoustic excitation

Biolever: k1 = 0.03, acoustic excitation

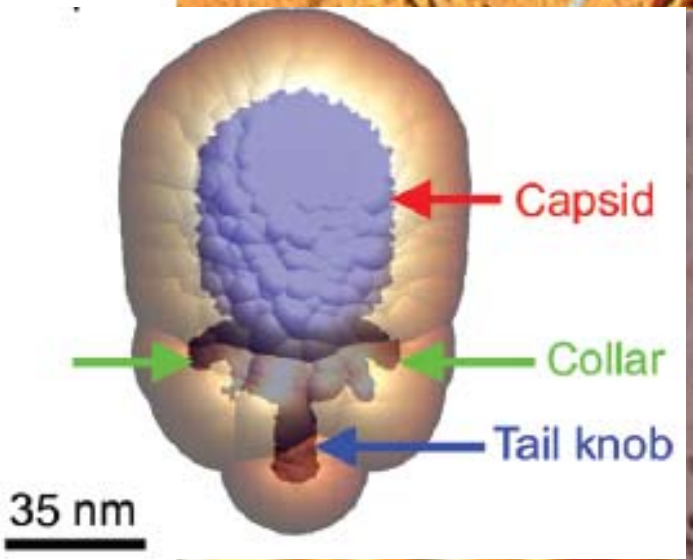


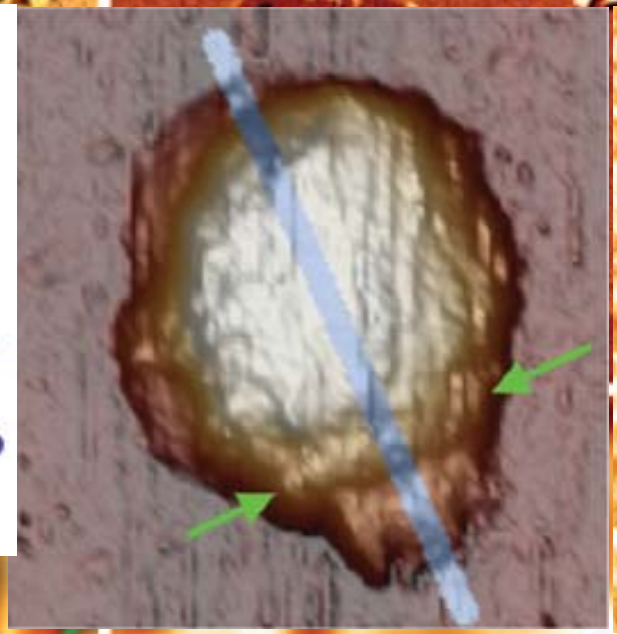


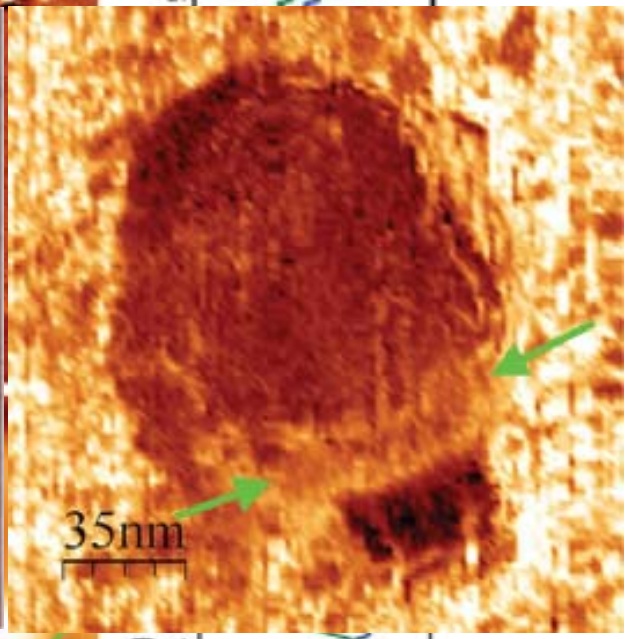
Cryo-EM reconstruction

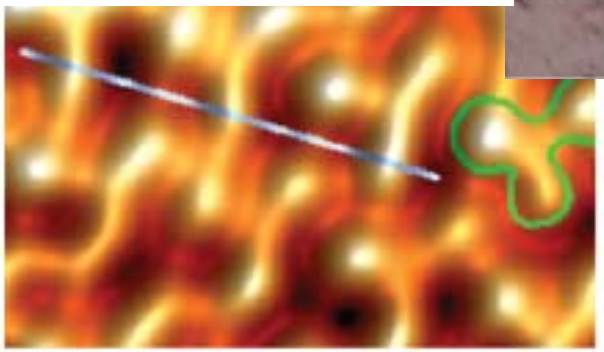
Topography

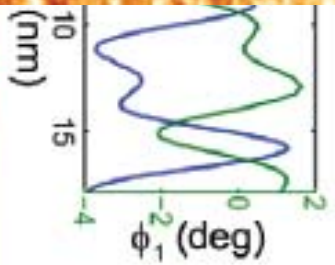
Phase lag



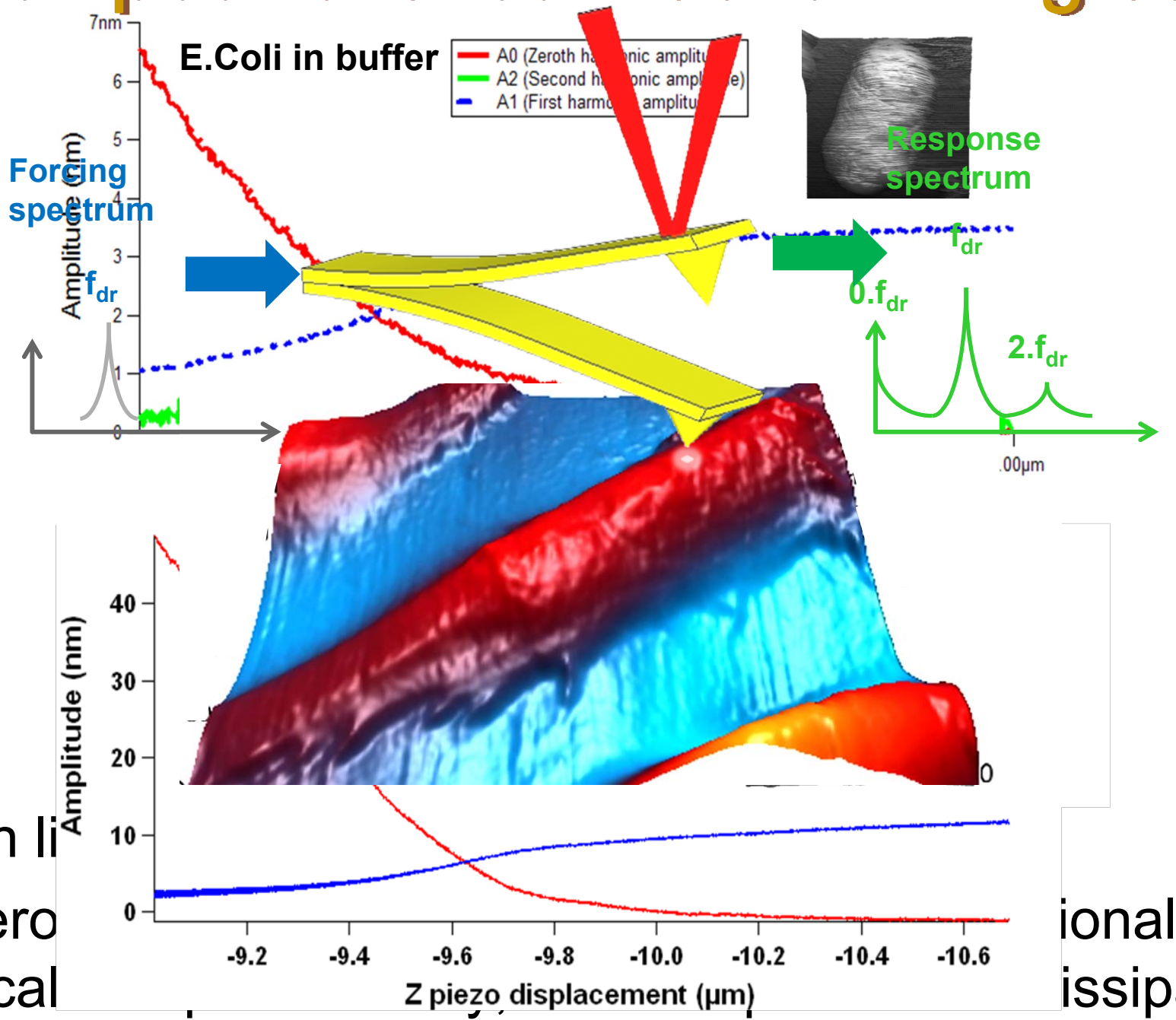






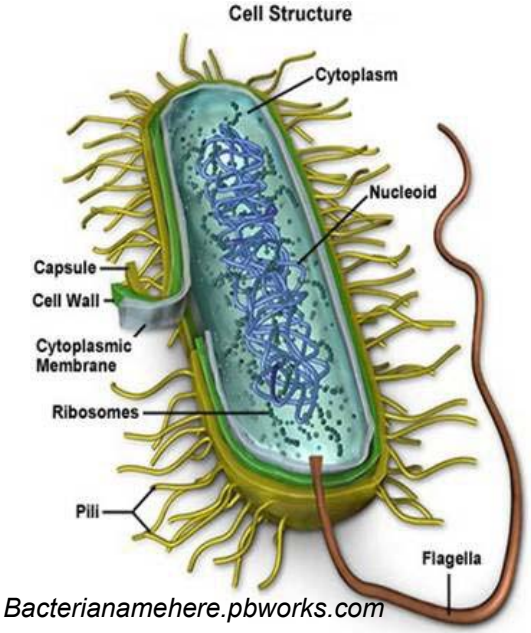


Compositional contrast on living cells



- On li
- Zero ional to local issipation

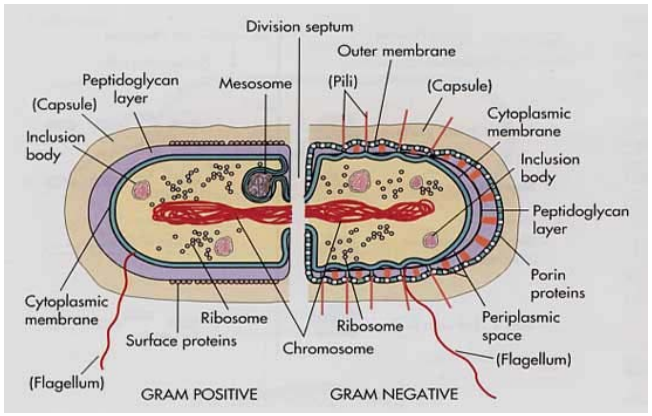
What causes elasticity of cells?



Bacterianamehere.pbworks.com

Gram negative bacteria

- Surface tension (membrane stress) due to osmotic/turgor pressure
- Bending stiffness of sacculus
- AFM will also probe soft bending response of pili and flagella
- Cytoskeleton!

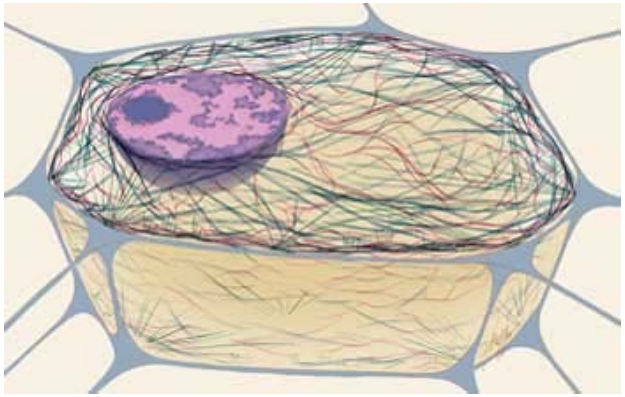


Micro.digitialproteus.com

Gram positive bacteria

- Bending stiffness of cell wall
- Turgor pressure

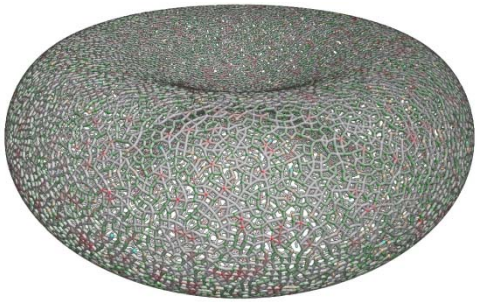
Stiffness maps of cells will reveal completely different patterns depending on
(a) type of cell, its morphogenetic state and
(b) conditions under which it is probed



www.nih.gov: blue- microtubules, red- intermediate Filaments , green- actin filaments

Mammalian eukaryotic cells

- Cytoskeleton
- Osmotic pressure

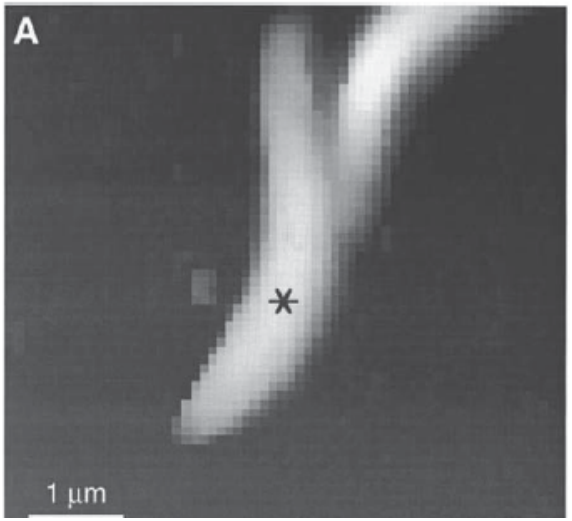


Red blood cells

- Osmotic pressure
- Boundary conditions!
- Spectrin network

AFM Mapping properties of live cells

- M. Radmacher et al. (PRE, 2000)



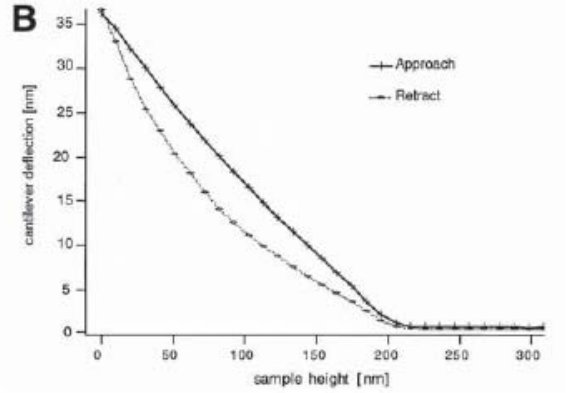


FIG. 7. Force mapping on intact bacteria. (a) Reconstructed height signal of intact bacteria. (b) Force versus distance curve during approach to bacterium at point marked by the asterisk. Note that the linear relationship agrees with the theoretical approach (Sec. II), predicting a linear force-indentation relation for a cylindrical shell, and with the model experiment described in Sec. III.

- McNally et al. (J. Neuroscience methods, 2010)

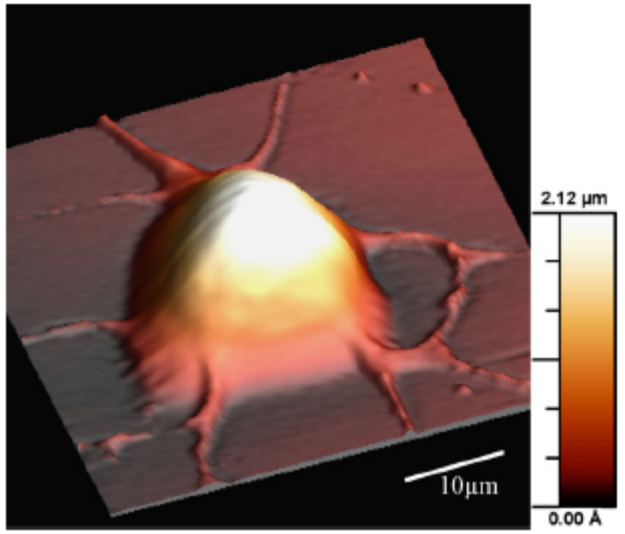
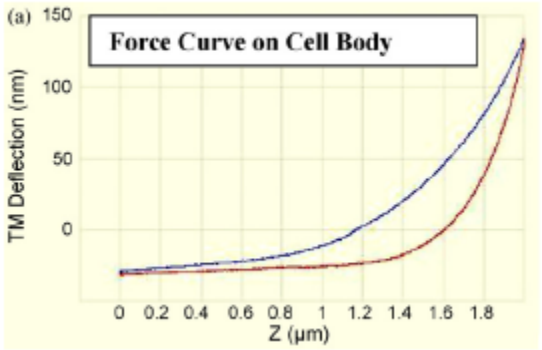
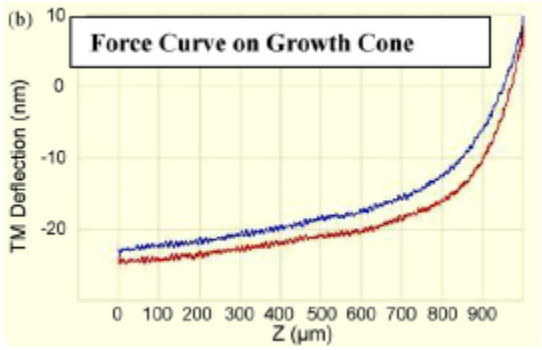


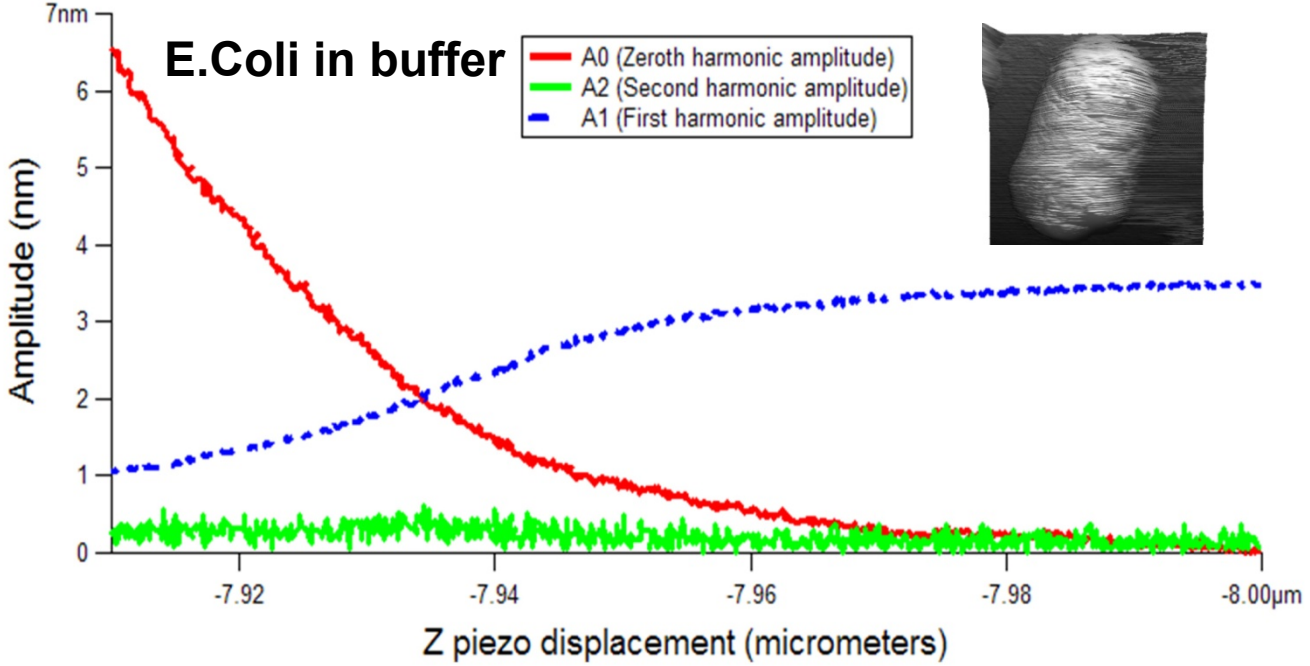
Fig. 3. Sympathetic neural cell body imaged in tapping mode with the Bioscope II.

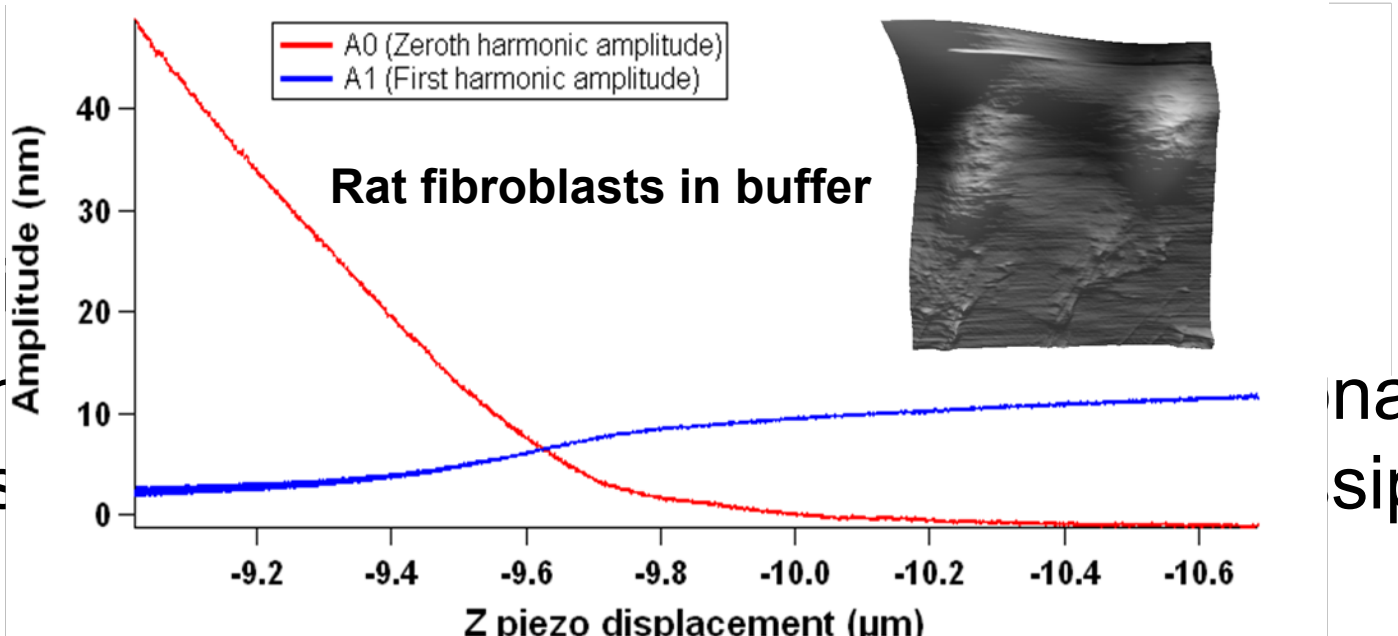




- Existing methods are slow,
- low resolution

Compositional contrast on living cells

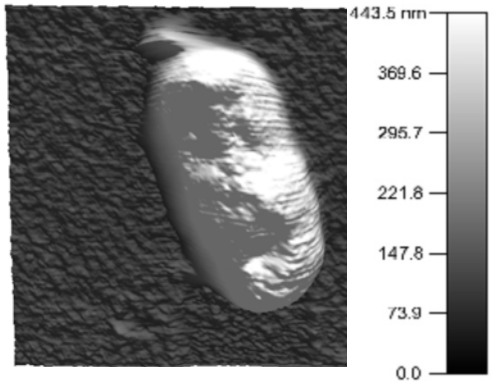




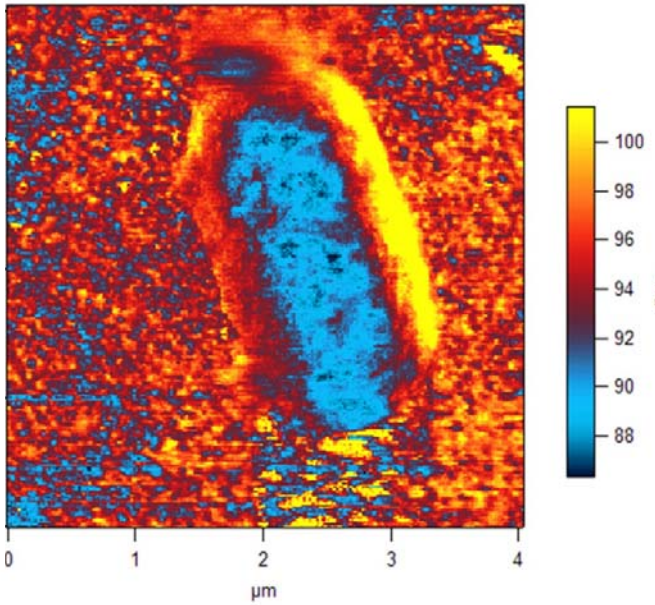
- On liv
- Zeroth nal to local s sipation

Elastic stiffness maps of E. Coli

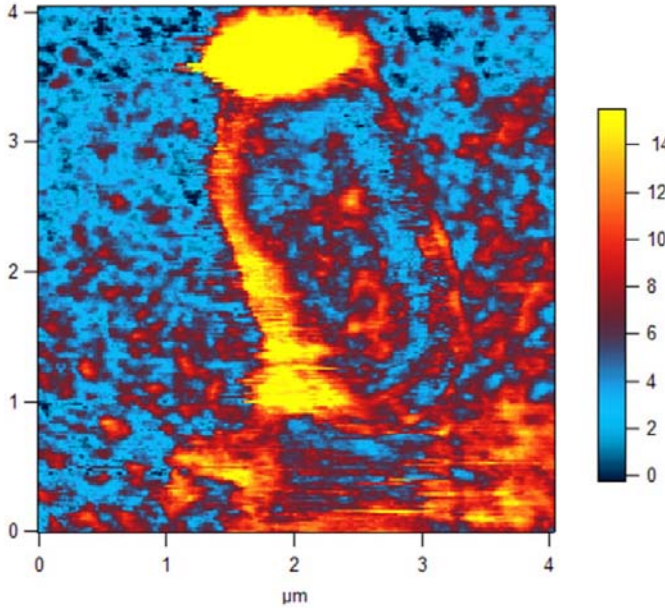
topography



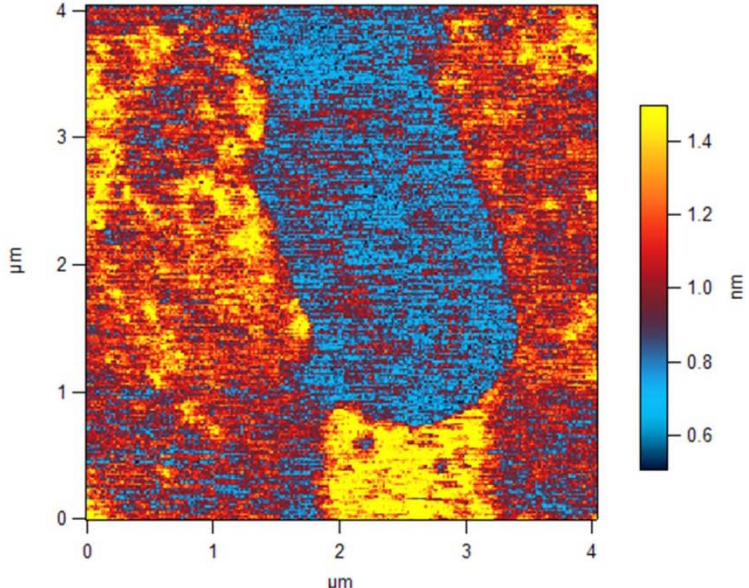
Phase lag



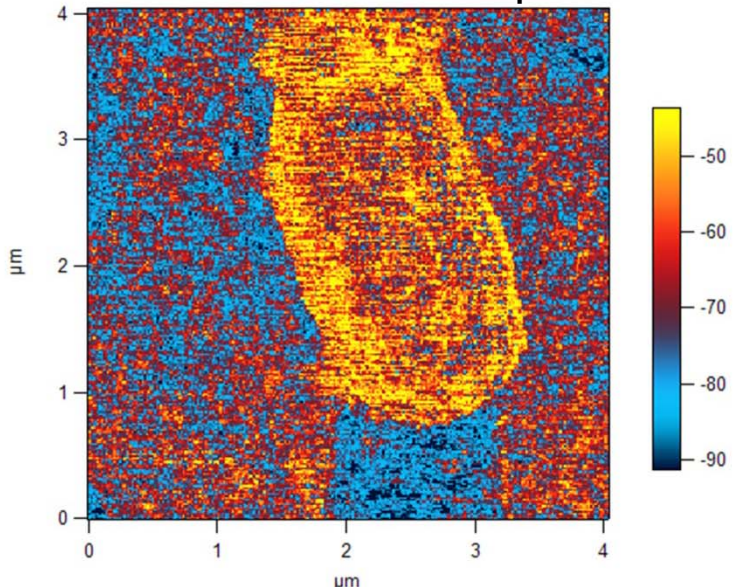
Zeroth harmonic



2nd harmonic amplitude

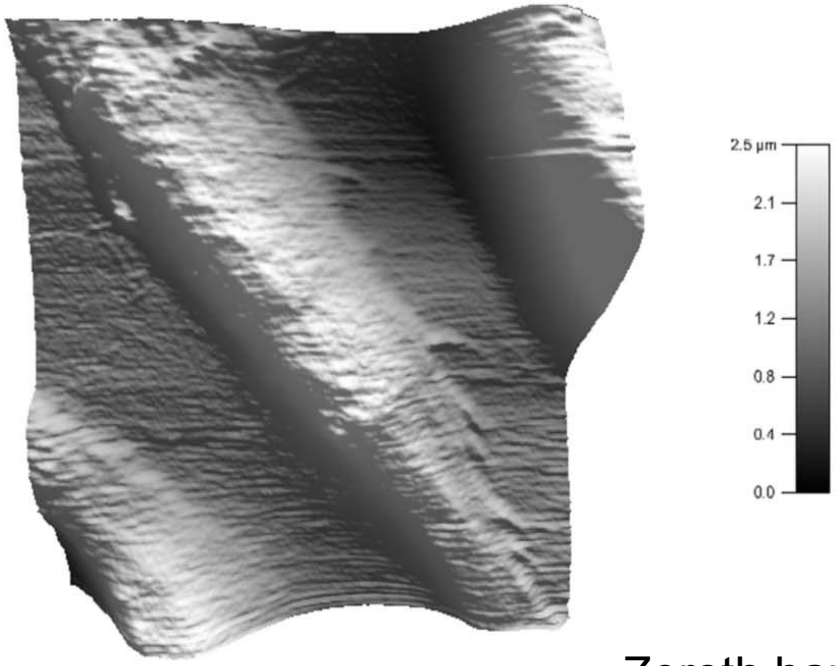


2nd harmonic phase

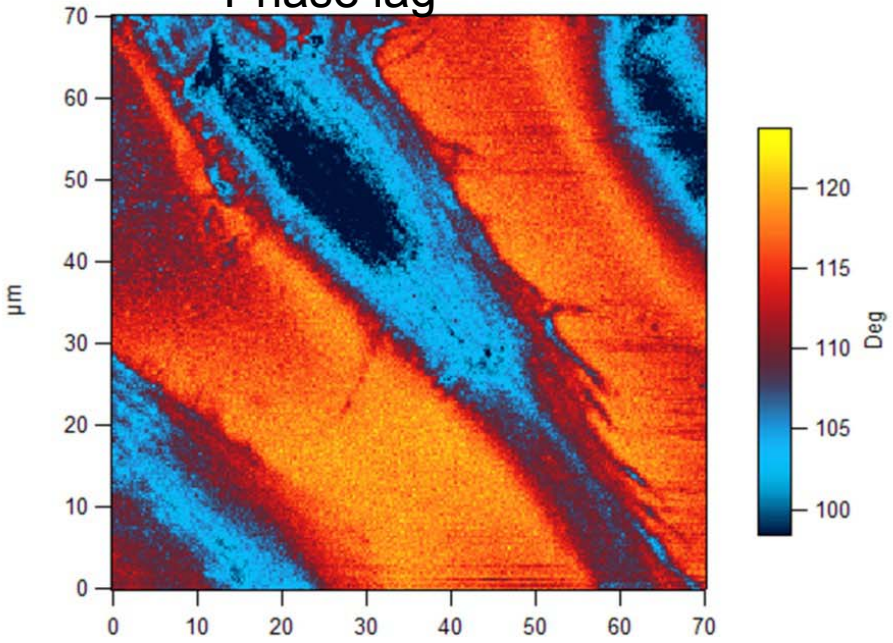


Local elastic modulus variation in fibroblasts

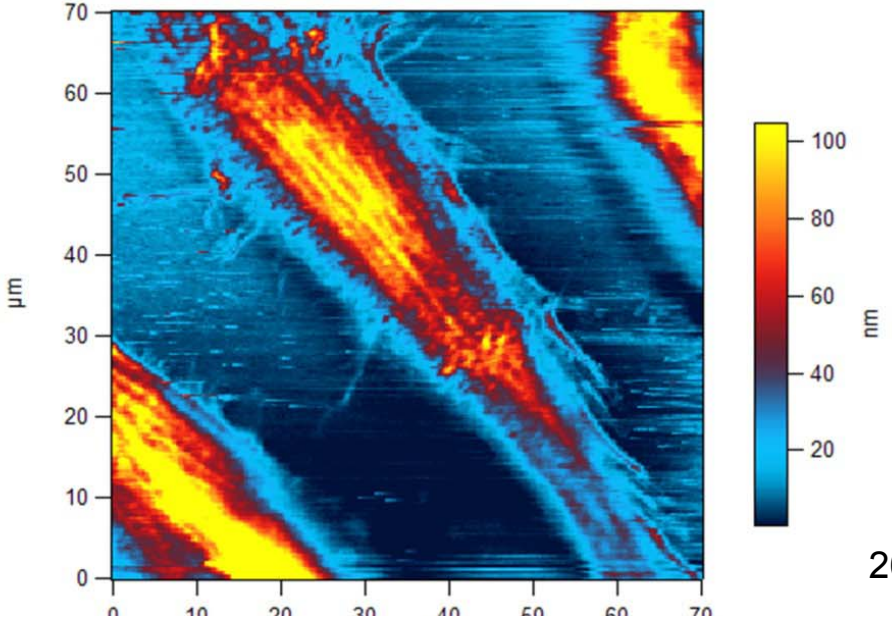
topography



Phase lag

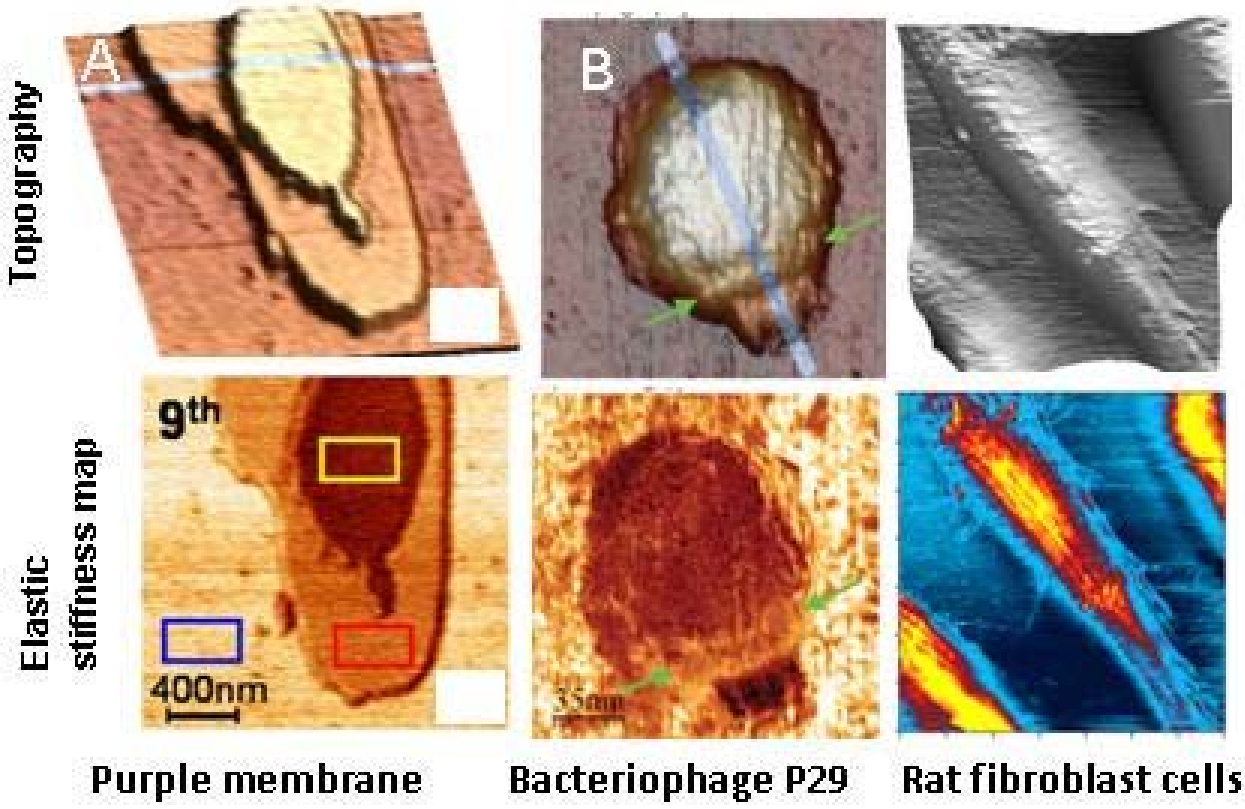


Zeroth harmonic



Conclusions

- Many technological advances for high speed, high resolution, and high material contrast
- Quantitative metrology remains a challenge
- Lots of new modalities emerging – subsurface, combined instruments



- State-of-the art of dynamic Atomic Force Microscopy
- Nanomechanical property mapping of biological samples
- nanoHUB resources for AFM scientists

nanoHUB resources for AFM

Virtual environment for dynamic AFM (VEDA) is a open-source, free cyber-infrastructure enabled online simulator for AFM available on nanohub (www.nanohub.org). VEDA funded by Network for Computational Nanotechnology and Dow AFM division

D. Kiracofe, J. Melcher, S. Hu and A. Raman, *Cover feature , Rev. Sci. Inst*, 2008

- **~1000 users; ~10,000 of simulations run**
- **Used by all major AFM companies**
- **Air/vacuum/water simulations**
- **AM/FM-AFM, bimodal, multi-frequency**

3 AFM Manufacturers:

Veeco

Agilent Technologies

Mechanics

AFM TECHNOLOGY LEADERSHIP
1999-2010

I have been using VEDA …
… found it to be extremely useful. …
… enabled us to make better choices in designing new probes.
… used VEDA as a check on other calculations.

Roger Proksch
Asylum Research

New Partnership

DOW

Virtual AFM

Mechanics

Materials

Virtual Atomistic
Tip and Surface

ME597/PHYS 570 Fundamentals of Atomic Force Microscopy on nanoHUB

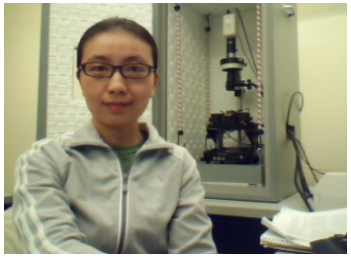
- By Ron Reifenberger[1], Arvind Raman[2]

  1. Department of Physics, Purdue University, West Lafayette, IN; 2. Mechanical Engineering, Purdue University, West Lafayette, IN;

Ron Reifenberger

- A full semester course for students interested in learning the fundamentals underlying Atomic Force Microscopy
- Problem sets using VEDA
- >2000 people follow the lectures through nanoHUB

Acknowledgements



Xin Xu

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Shuiqing Hu (Veeco-Bruker)

Sudipta Basak

Carolina Carrasco Pulido

Pedro Jose de Pablo

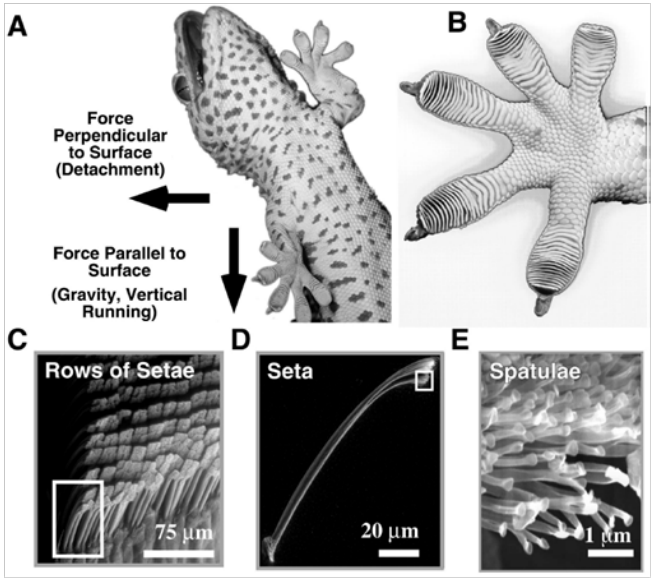
Julio Gomez-Herrero

Extra slides

Nanocomposites.

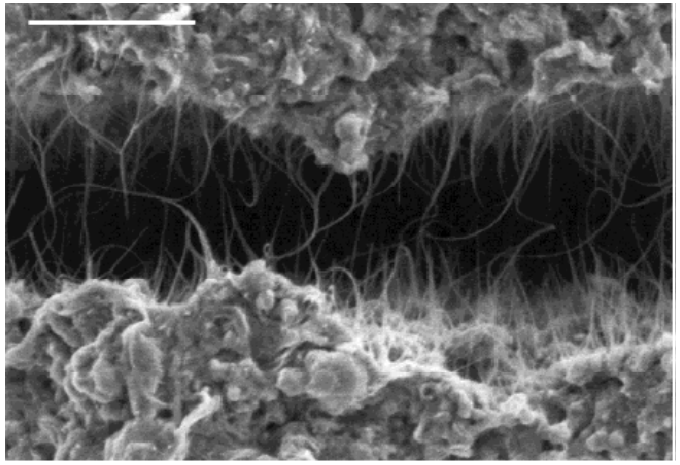
Nanophysics of stiction: why is it important?

Reversible Adhesion



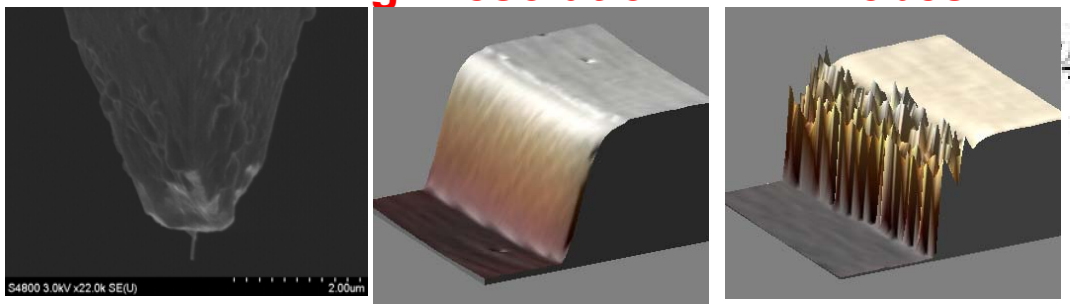
Anatomy of Gecko Foot
Autumn & Peattie Integr. Comp. Biol. (2002)

Reinforced Nanocomposites



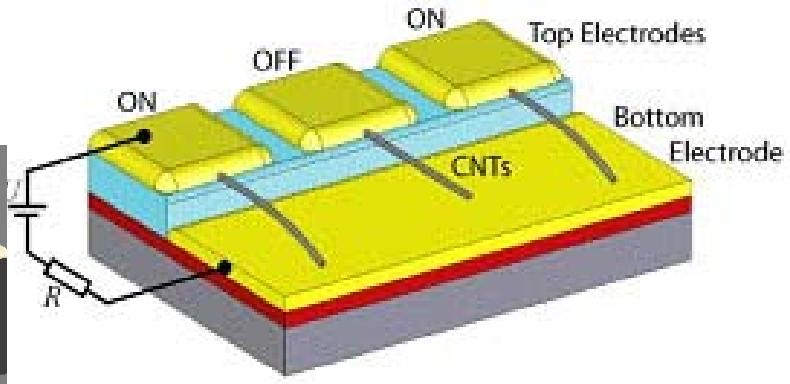
Tensile-Loaded CNT-reinforced epoxy
Ajayan et al, Adv. Mat. (2000)

High-resolution AFM Probes



Strus, Raman, et al , Nanotechnology (2006)

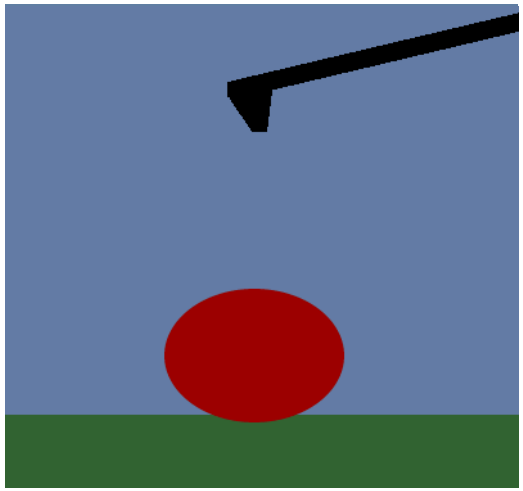
Nanoswitches/Nanotweezers



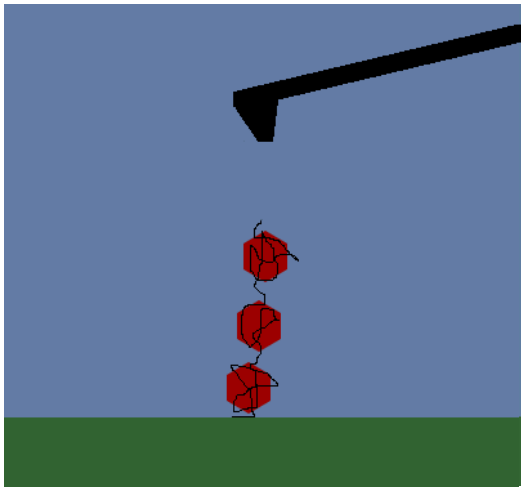
Nano-electro-mechanical CNT Switches
Espinosa et al, Small (2006)

Nanomechanical peeling of 1D nanostructures using AFM

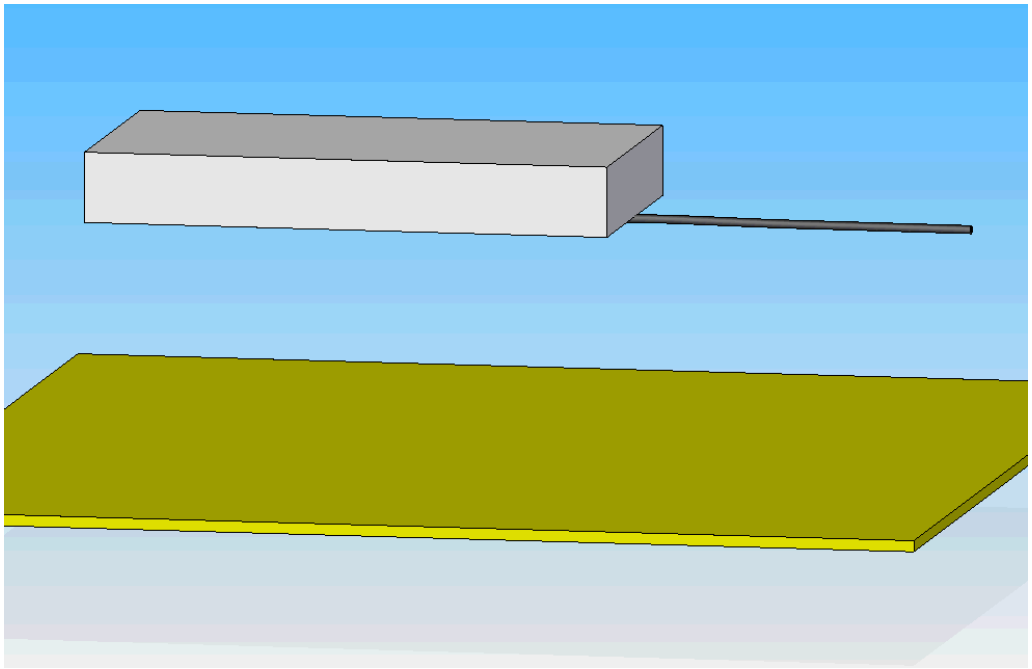
AFM Pushing



AFM Pulling



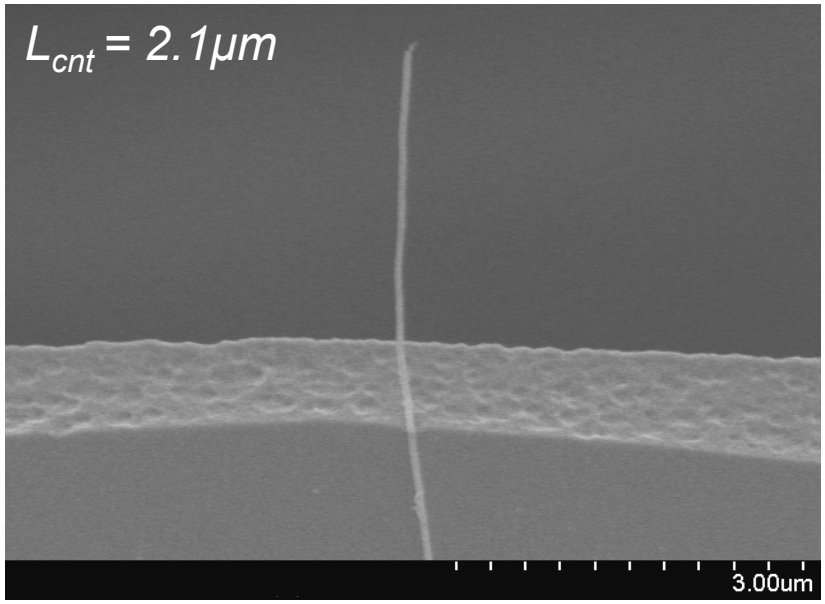
AFM Peeling



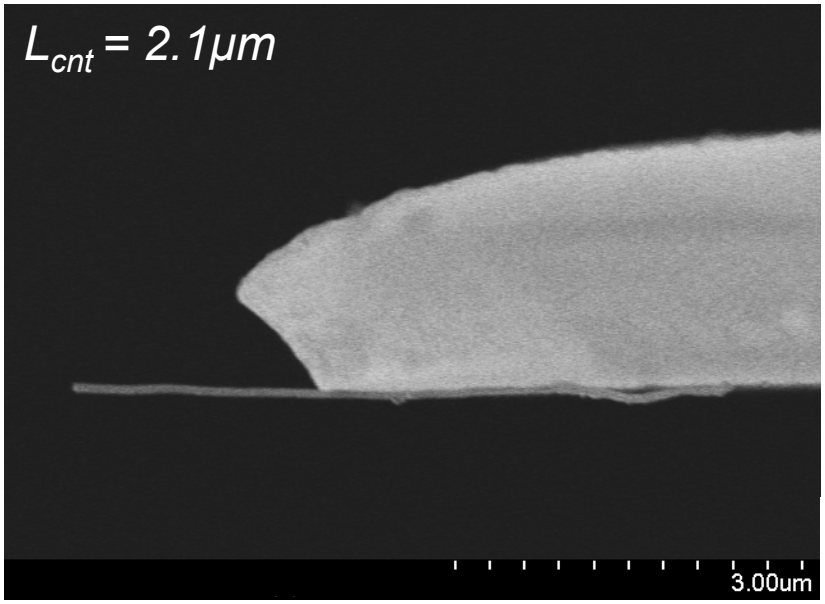
Strus, Raman, Pipes et al, Nano Letters (2008)
Strus, Raman, Pipes et al, Comp. Sci. Tech (2009)

Experiments: Probe Preparation

Bottom View

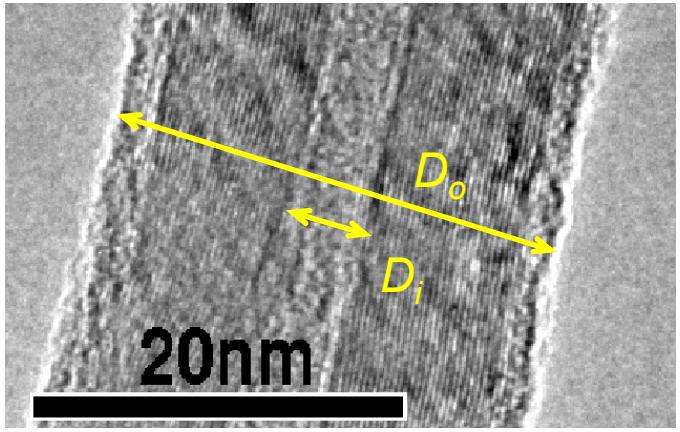


Side View



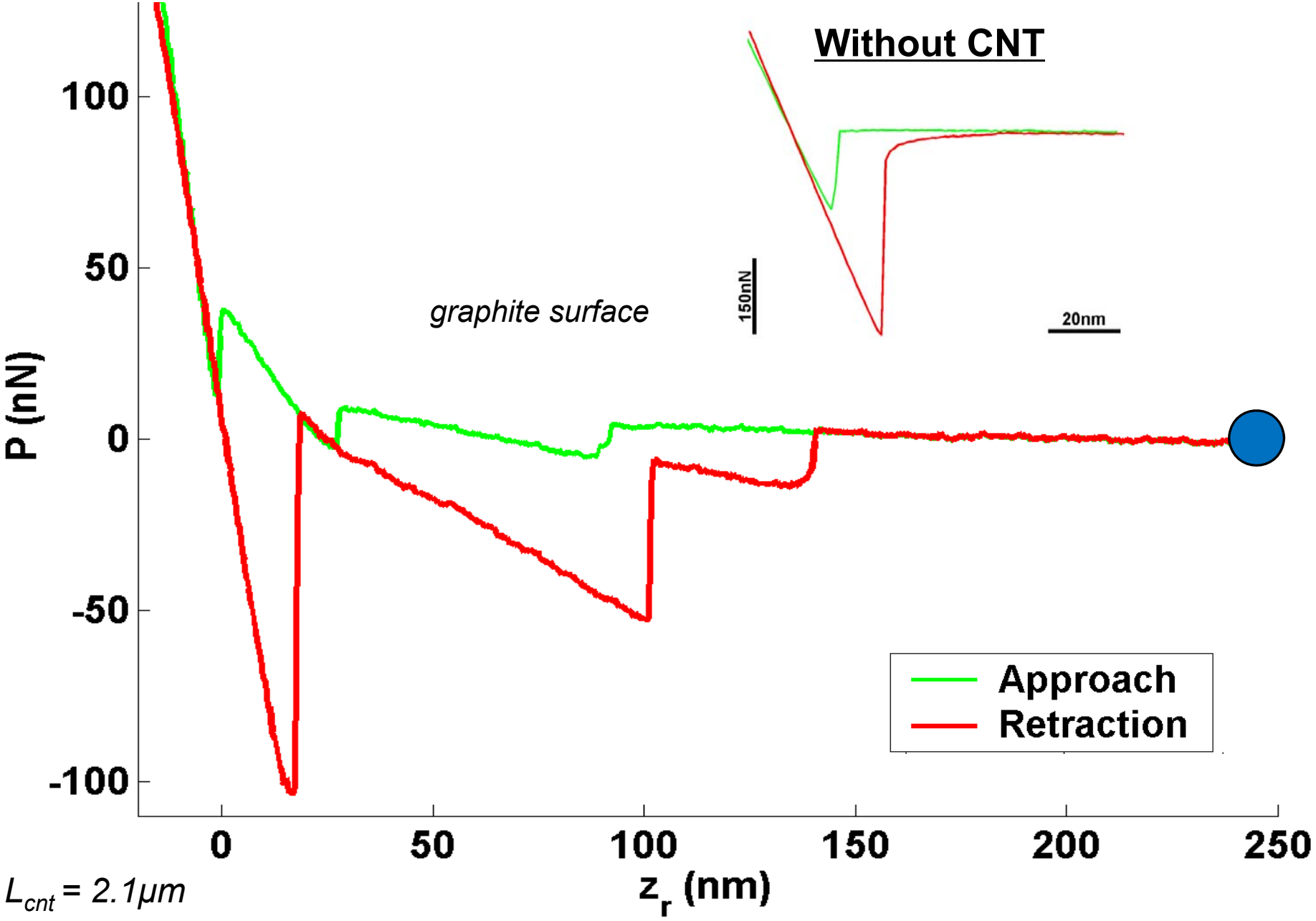
- Coat cantilever with nickel (≈ 10-15nm)
- Typical cantilever stiffnesses: *5 – 20 N/m*
- Grow CNTs on wire via CVD process
- Attach MWCNTs in a dark-field optical microscope by applying ≈10mA current to weld CNT in place
- Typical MWCNT lengths: *0.5-8μm*
- CNTs with small deviations from straightness

Typical TEM



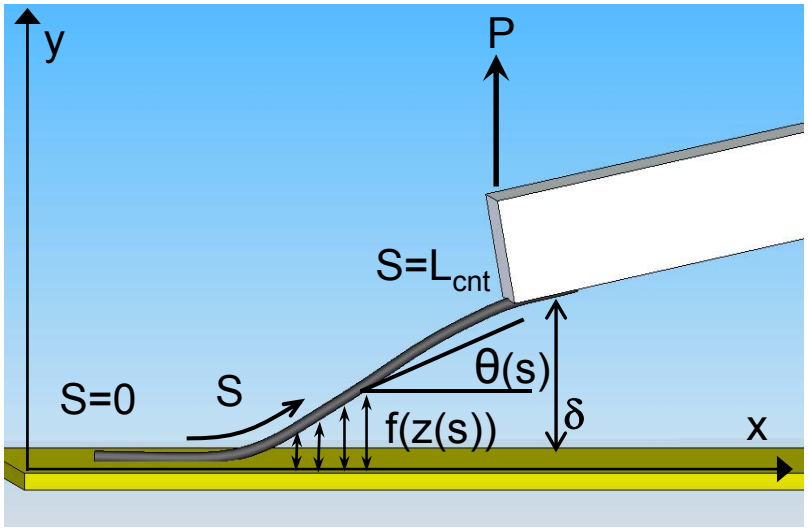
D_i = 10nm +/- 2nm
D_o = 37nm +/- 5nm

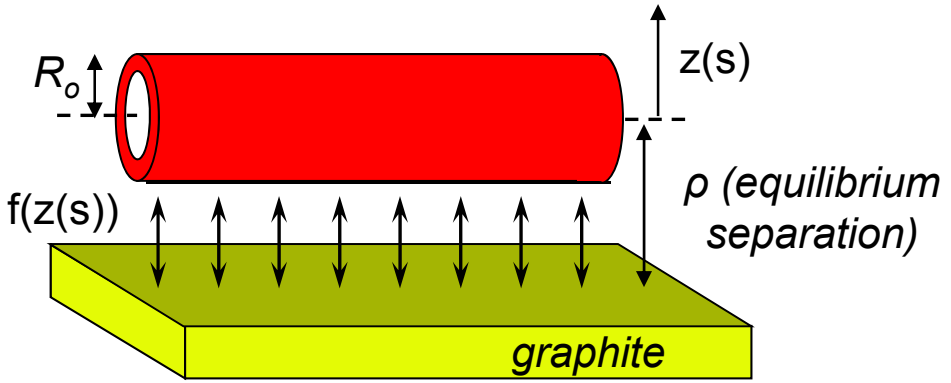
Experimental Peeling of Straight CNTs



L_{cnt} = 2.1μm

Adhesive Nanomechanics of the Elastica





$$\bar{s} = \frac{s}{L_{cnt}} \qquad \bar{z} = \frac{z}{L_{cnt}} \qquad \bar{\gamma}(s) = \frac{L_{cnt}^{\,2}}{EI}\gamma(\bar{s})$$

- Following the procedure of *Oyharcabal and Frisch Phys. Rev. E (2005)*

3 ODEs

$$\frac{d^2\theta}{d\bar{s}^2}(\bar{s}) = -\bar{\gamma}(\bar{s})\cos\theta(\bar{s}) \qquad \frac{d\bar{\gamma}}{d\bar{s}}(\bar{s}) = \bar{f}(\bar{s}) \qquad \frac{dz}{d\bar{s}}(\bar{s}) = \sin\theta(\bar{s})$$

moment balance — *shear balance* — *inextensibility*

Girafalco et al Phys. Rev. B (2000)

$$\bar{f}(\bar{s}) = \frac{dE_I}{d\bar{z}}(\bar{s}) = 6.119\alpha\left(\frac{L_{cnt}}{R_o-\rho}\right)\left(\left(\frac{1}{1+0.9179\frac{L_{cnt}}{R_o-\rho}\bar{z}(\bar{s})}\right)^5 + \left(\frac{1}{1+0.9179\frac{L_{cnt}}{R_o-\rho}\bar{z}(\bar{s})}\right)^{11}\right)$$

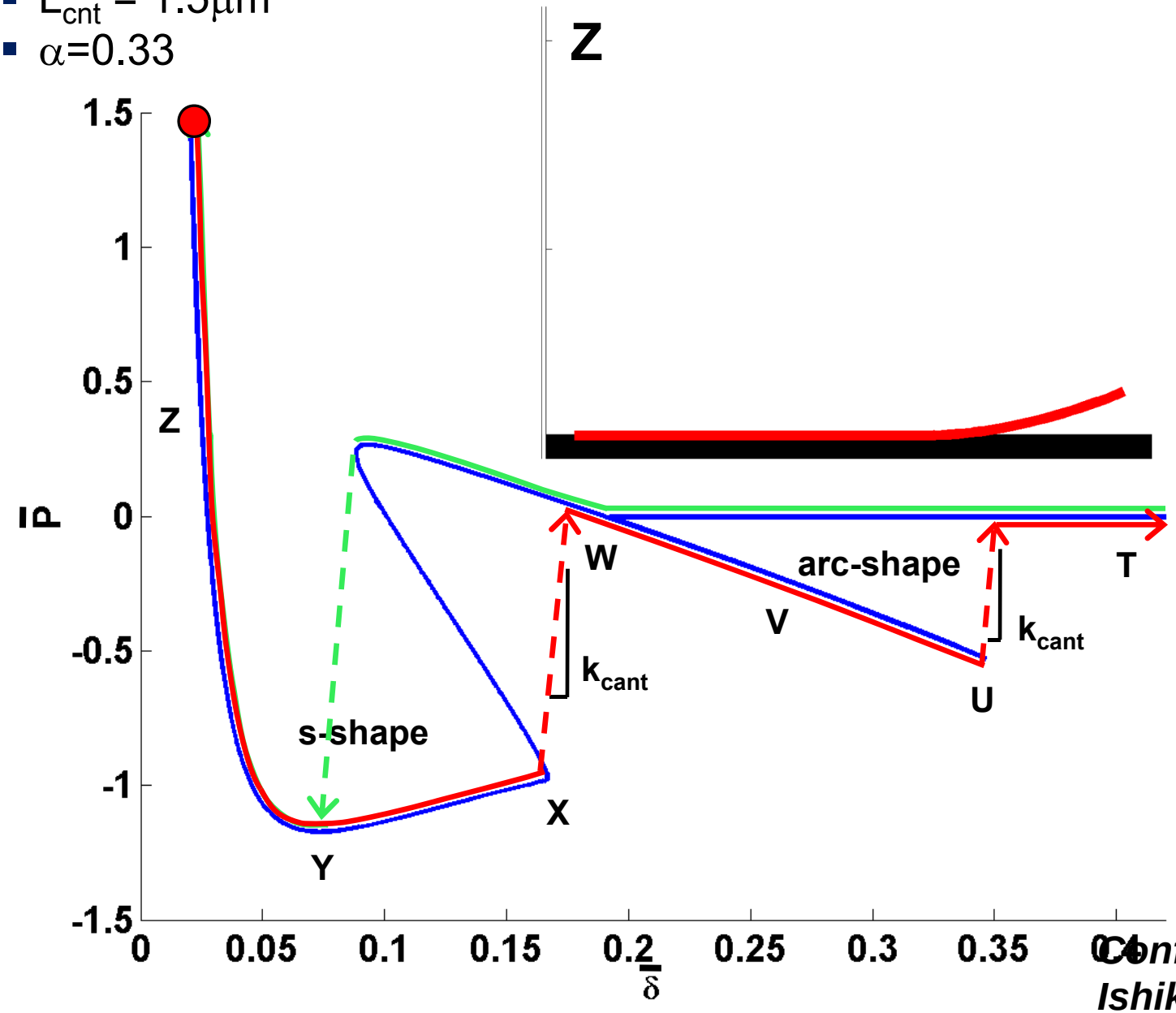
$$\alpha = \frac{\Phi_o L_{cnt}^{\,2}}{EI}$$

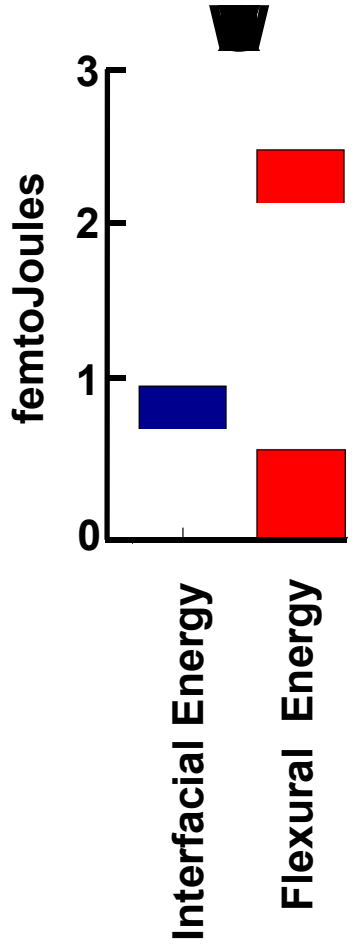
- Ratio between CNT-surface adhesion and bending stiffness
- Long SWCNTs have large α while short MWCNTs have small α

- See full derivation in ***Strus et al, Nano Letters (2008)***

Typical Static Peeling Model for MWCNTs

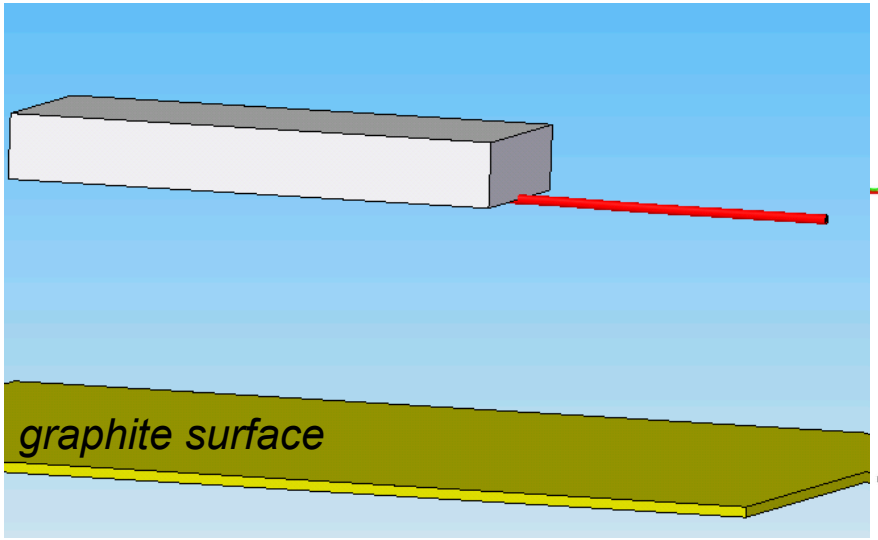
- L_{cnt} = 1.5μm
- α=0.33





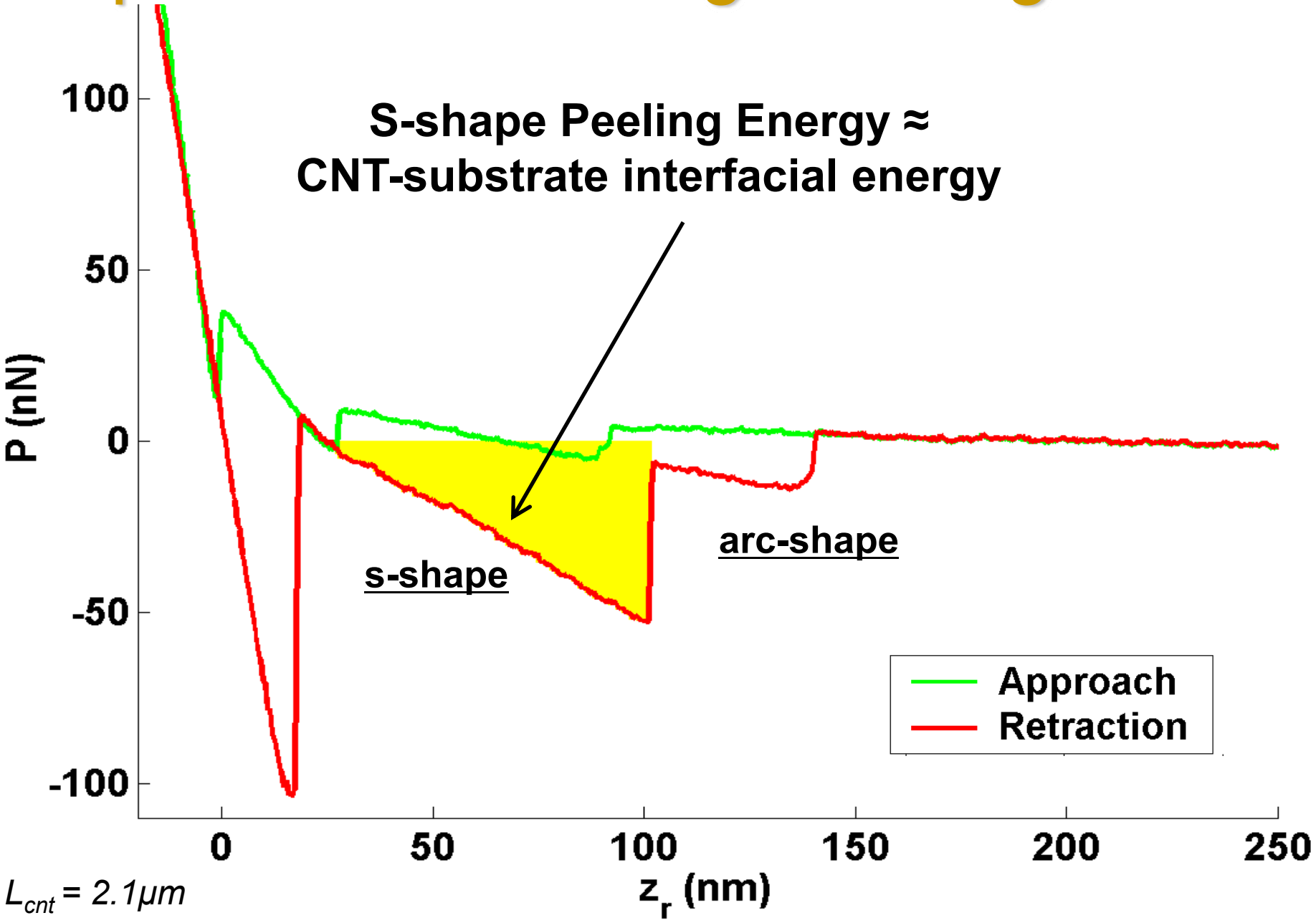
Confirmed by Ishikawa et al, APL 2008

Experimental Peeling of Straight CNTs



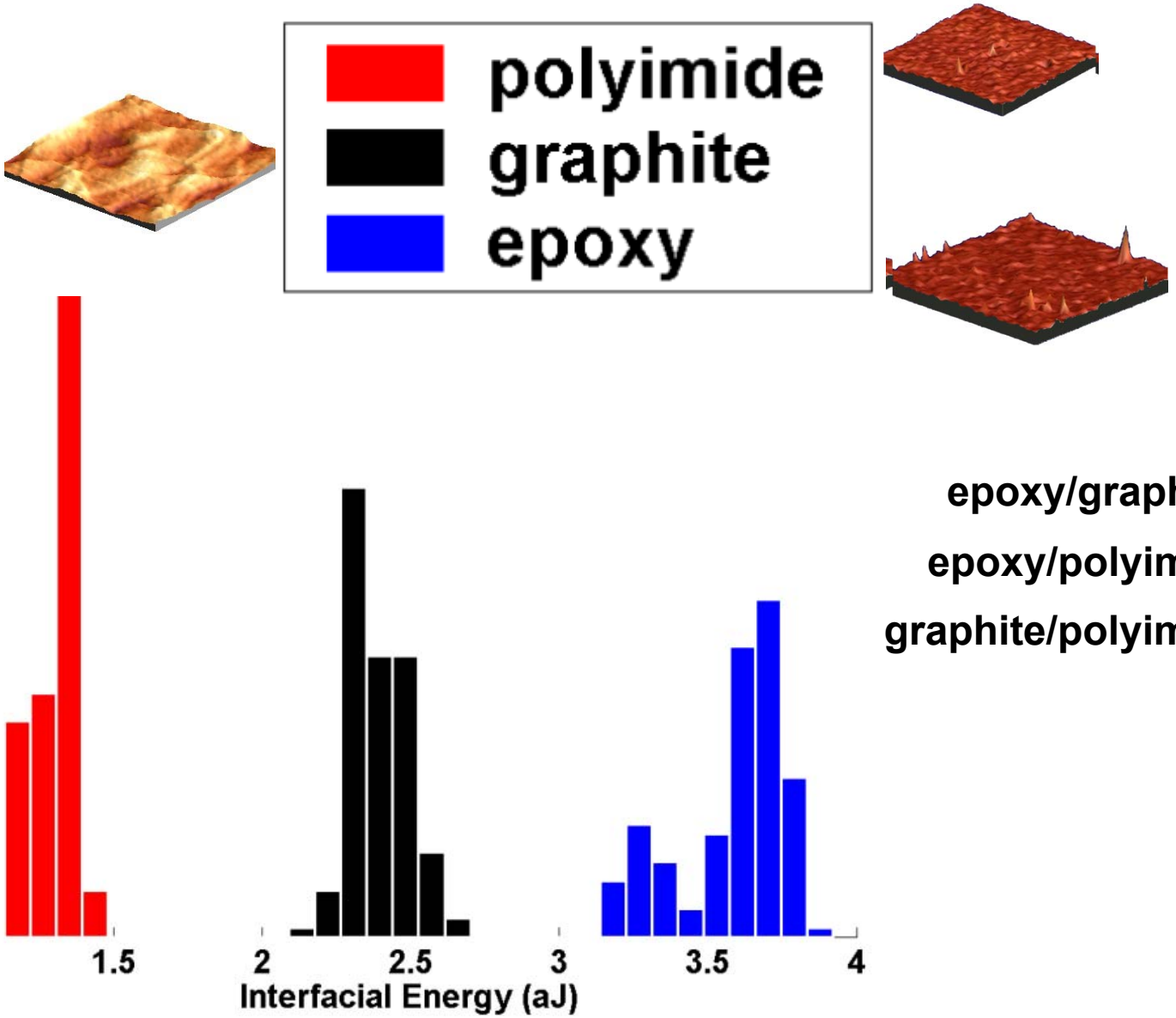
L_{cnt} = 2.1μm

Experimental Peeling of Straight CNTs



L_{cnt} = 2.1μm

CNT-Substrate Interfacial Energy on Different Materials



epoxy/graphite = 1.5

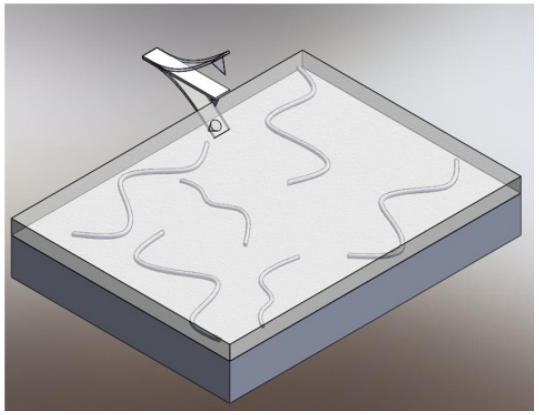
epoxy/polyimide = 2.7

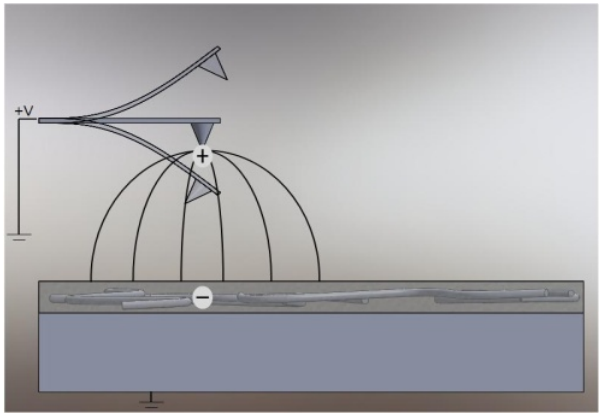
graphite/polyimide = 1.8

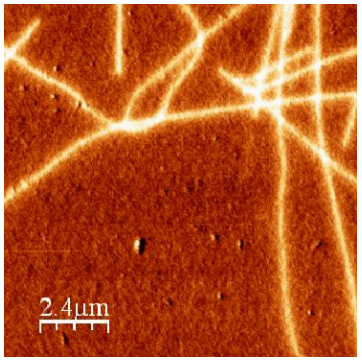
L_{cnt} = *2.1μm*

5 sample locations on each material x 100 test per location

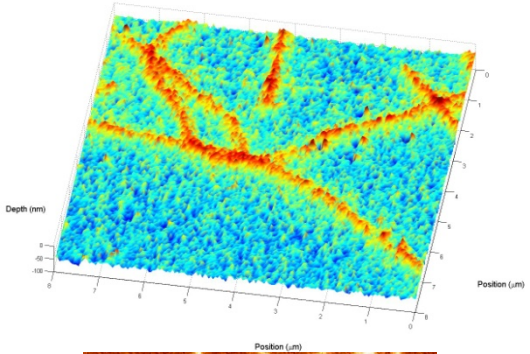
Scanning Polarization Microscopy for subsurface characterisation of CNT nanocomposites

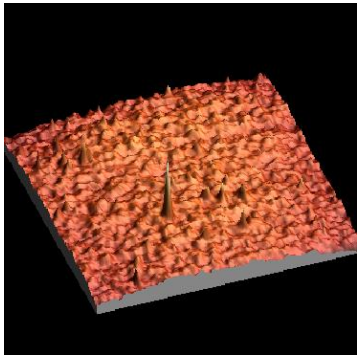


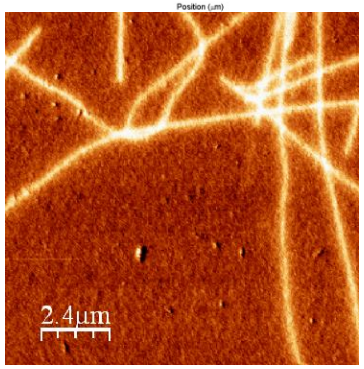




2D
3D



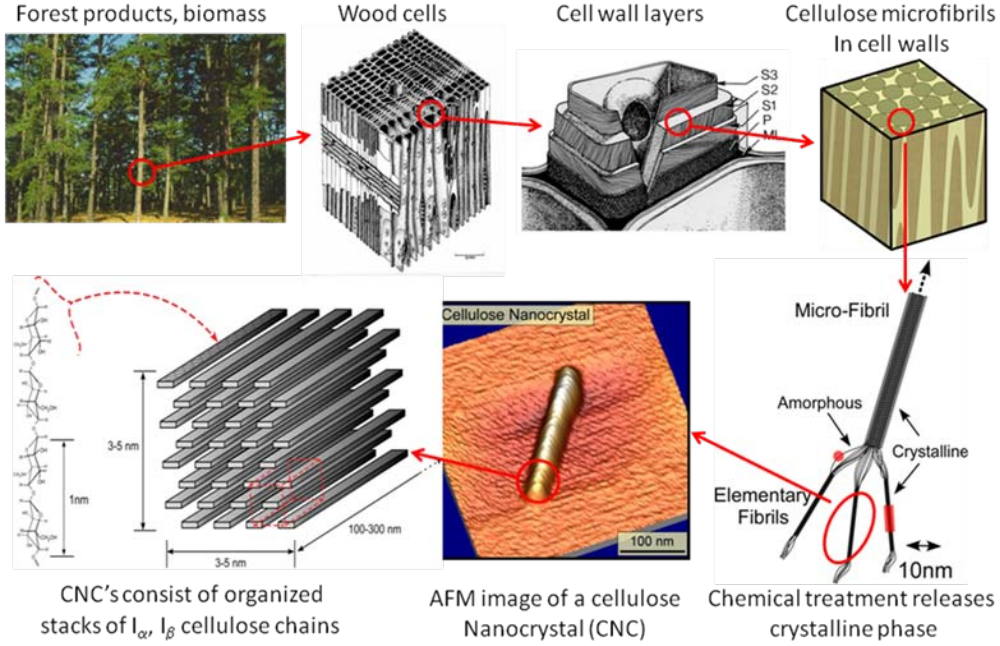




Topography

Phase Lag

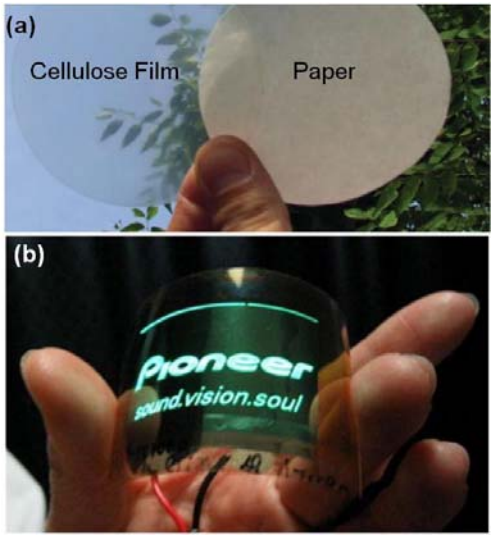
Nanomechanics of cellulose nanocrystals



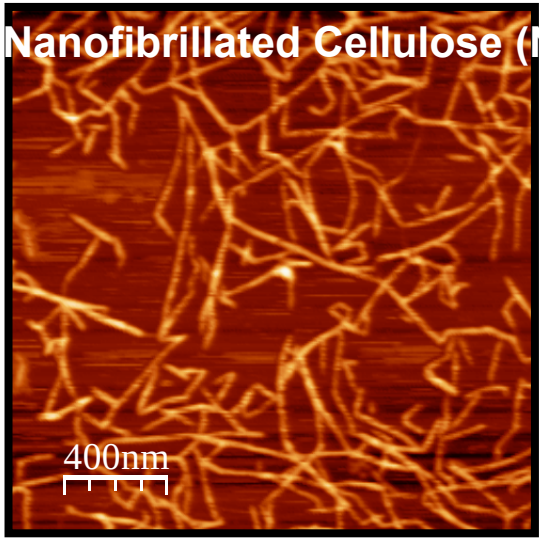
CNC's consist of organized stacks of I_α, I_β cellulose chains

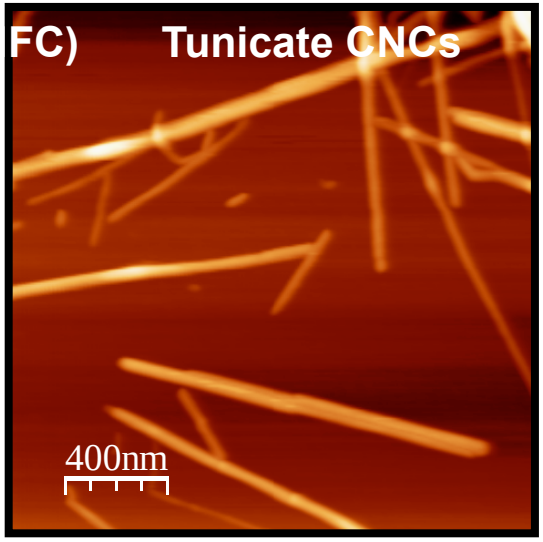
AFM image of a cellulose Nanocrystal (CNC)

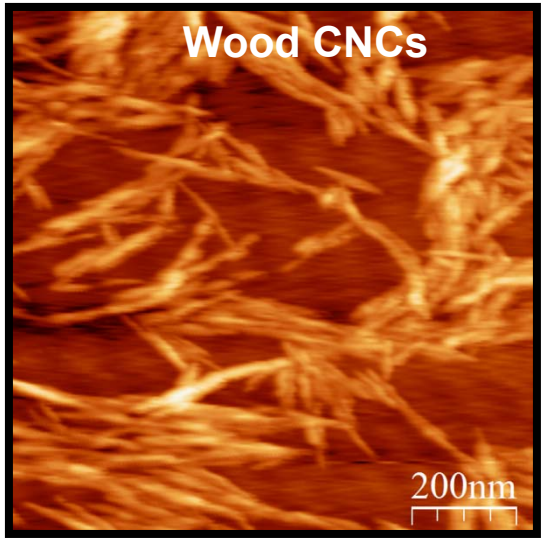
Chemical treatment releases crystalline phase



Applications of cellulose nanoparticles. (a) transparent paper for packaging, , and (b) Luminescence of an organic light-emitting diode deposited onto a flexible, low-CTE and optically transparent wood–cellulose nanocomposite.







Transverse modulus and surface energy of nanoparticle using AFM