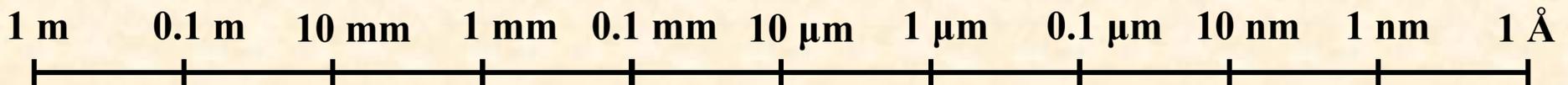
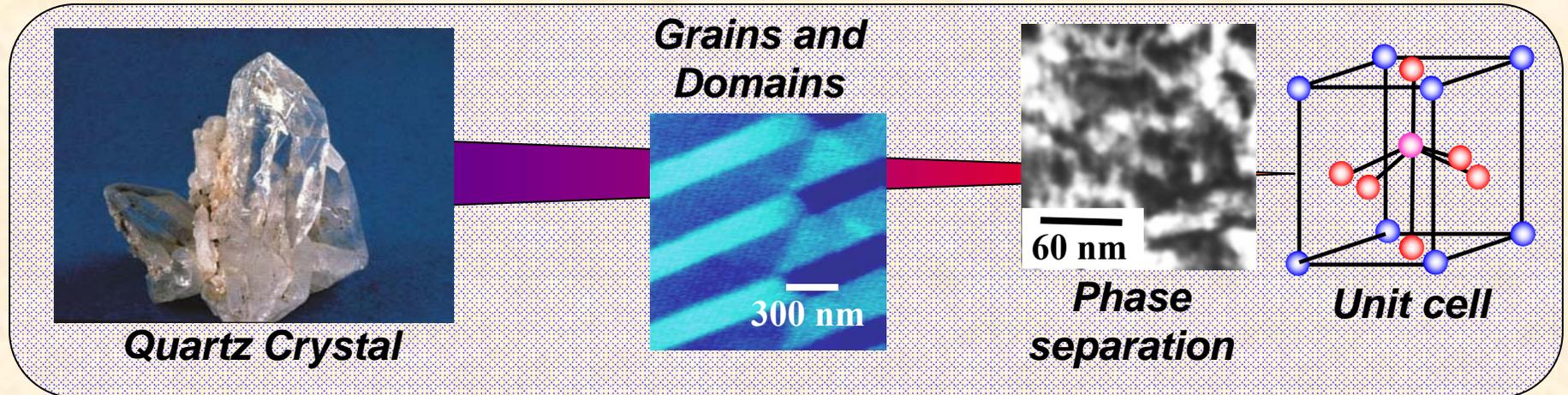


# Recent Advances in PFM: Biological Imaging Polymers, and Imaging in Liquid Environment

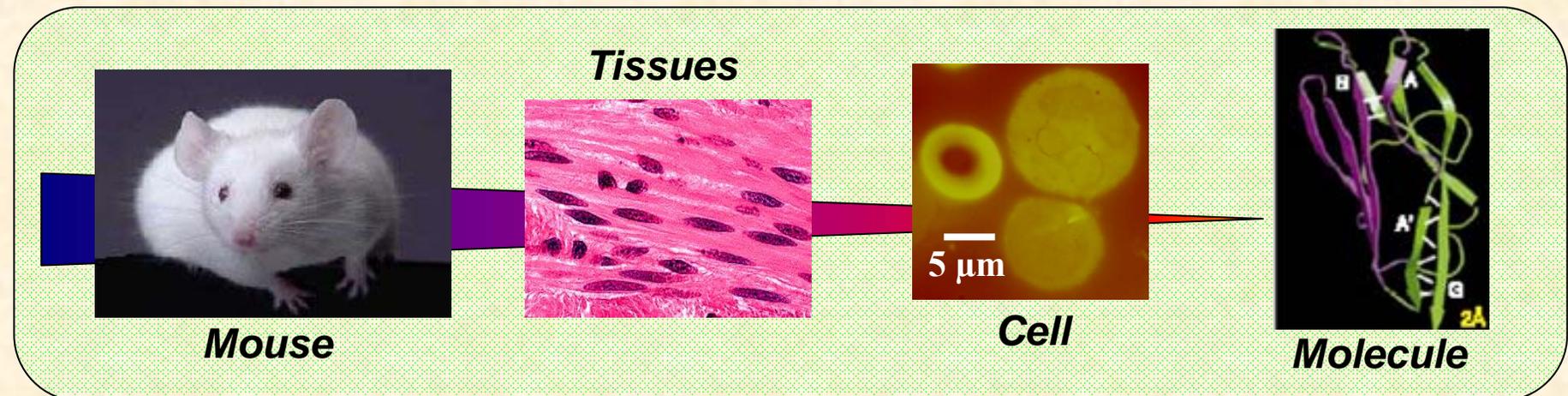
Sergei V. Kalinin

Material Sciences and Technology Division and The Center for Nanophase Materials Sciences,  
Oak Ridge National Laboratory

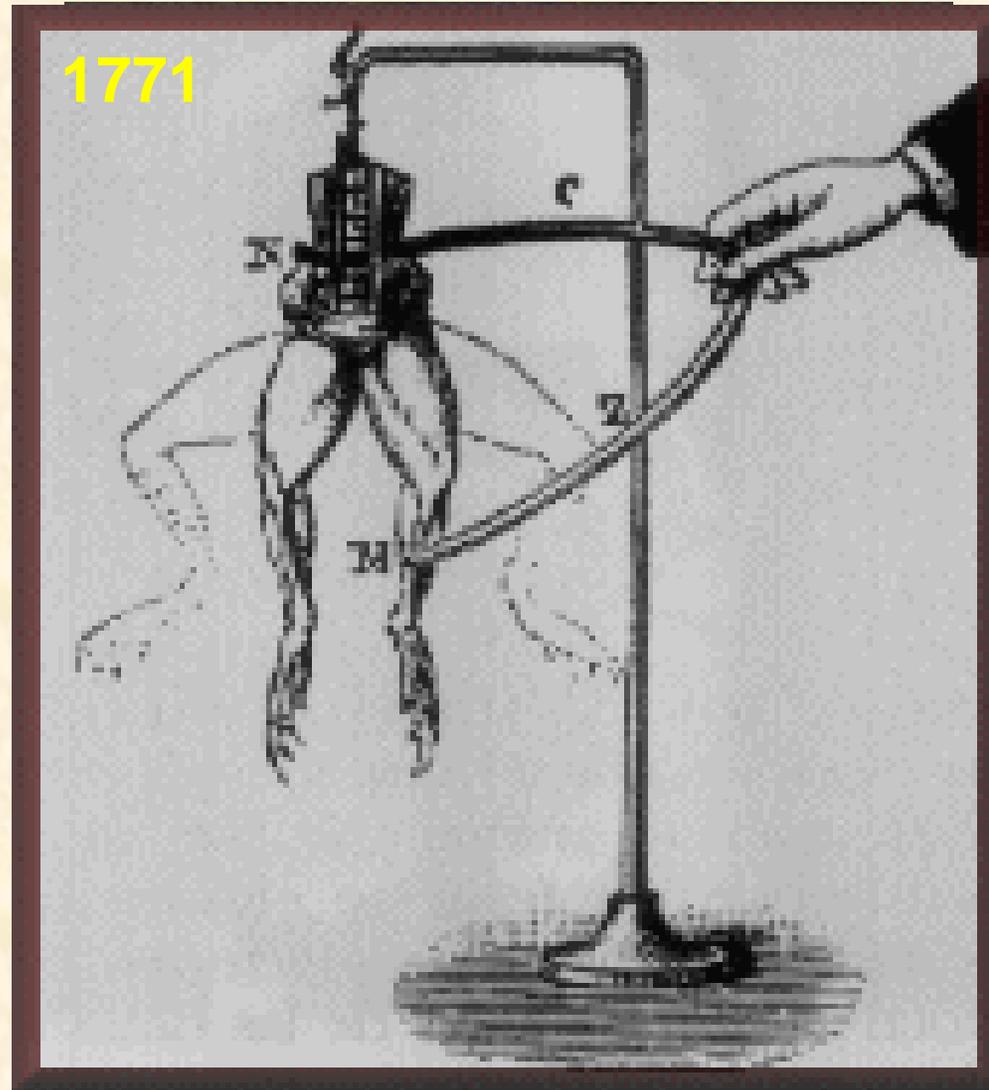
# Electromechanics: The Next Frontier



**We need Scanning Probe Microscopy to image elastic, electromechanical and electrical behavior !**



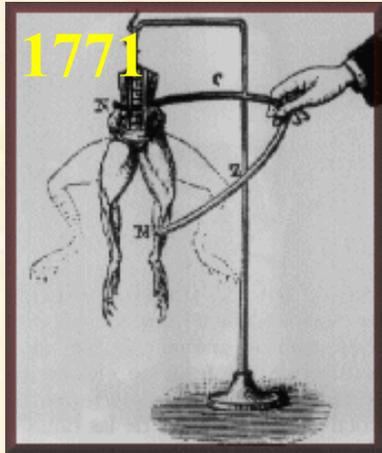
# *Electromechanics and the Origins of Physics*



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# Electromechanical Phenomena in Biosystems



1771: Galvani – discovery of electricity

1942: Wul - Ferroelectricity in  $\text{BaTiO}_3$

1957: Fukada – piezoelectricity in bones

1996: Gruverman - Piezoresponse Force Microscopy

Since 1955, it was shown that many biomaterials, including collagen and cellulose, are piezoelectric. This piezoelectric activity was postulated to be directly related to biochemical functionality.

## *Bones remodel to respond to external stimuli (Wolff law):*

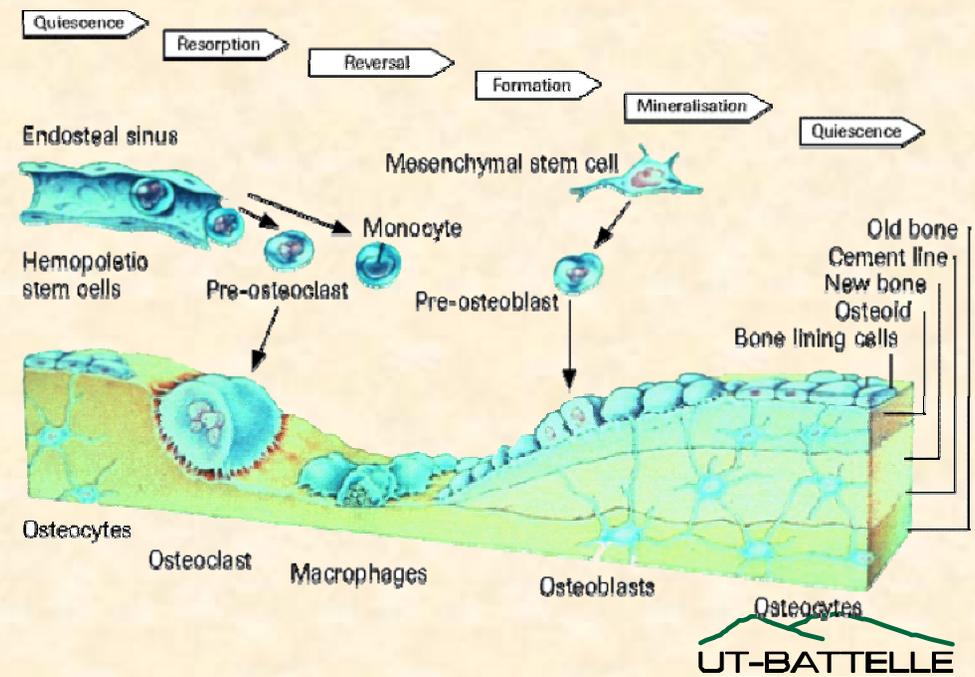
**Bones:**  $d_{24} = 2-10 \text{ pC/N}$  (similar to quartz)

As we know, bones regenerate

**Teeth:**  $d_{24} = 0.2 \text{ pC/N}$

And teeth don't...

**A number of approaches for bone treatments (osteoporosis, fracture) were suggested using periodic fields**



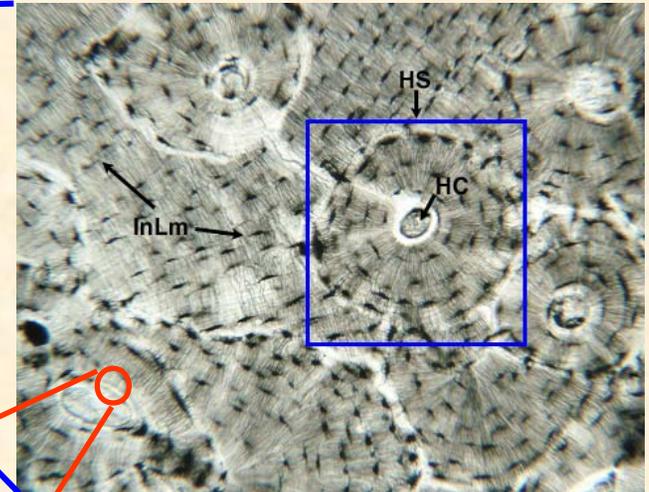
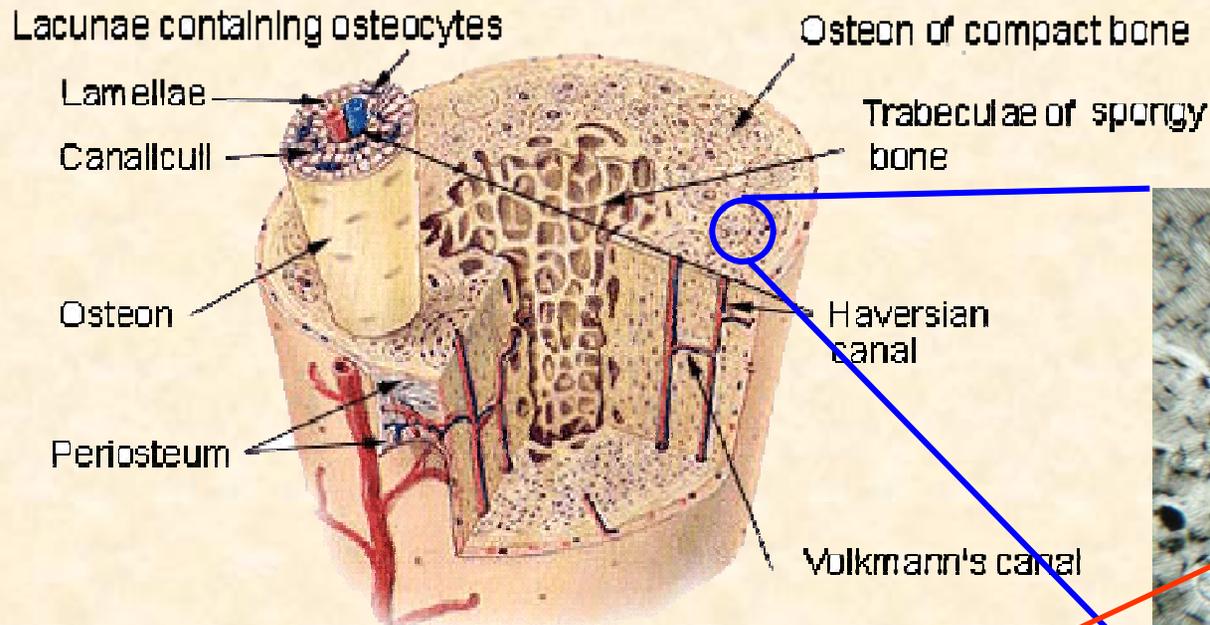
# *Piezoelectricity and Extinction of Dinosaurs*



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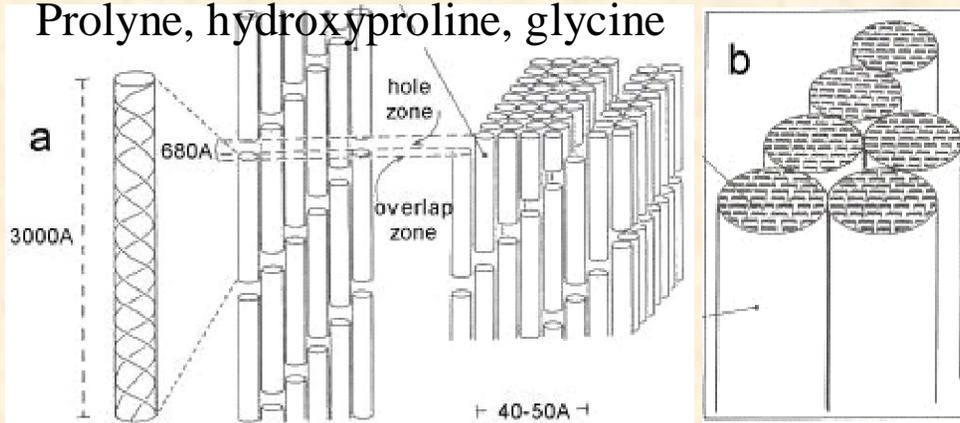
  
UT-BATTELLE

# Piezoelectricity of Bones



**Piezoelectric collagen:** triple helix

Prolyne, hydroxyproline, glycine



**Bones are non-uniform:**

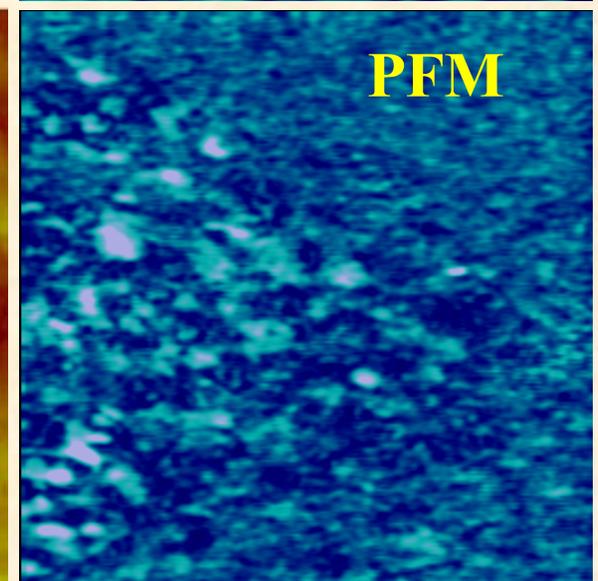
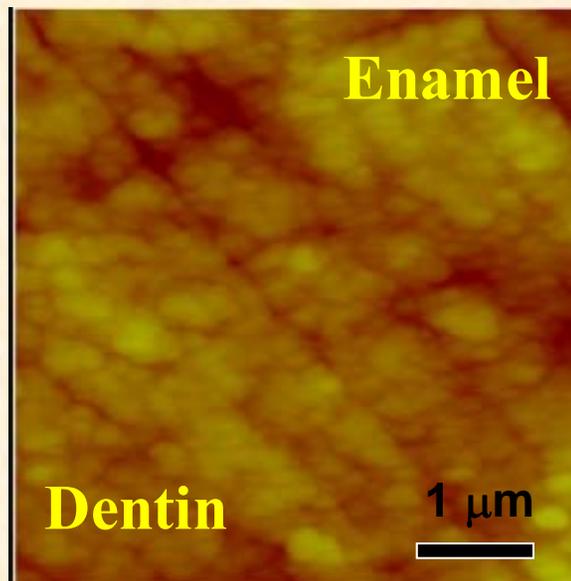
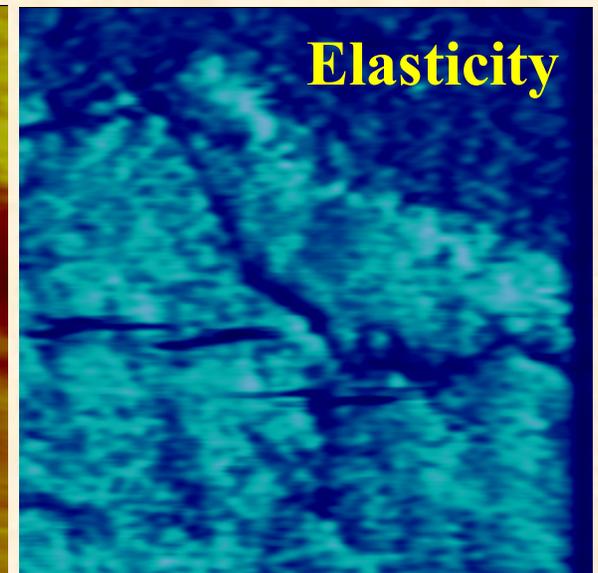
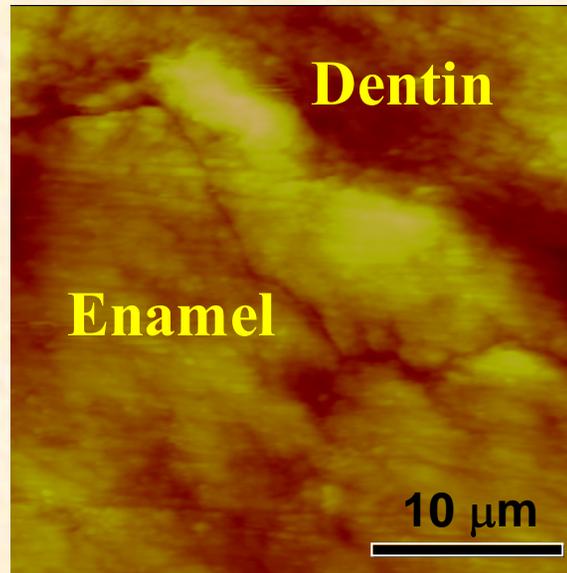
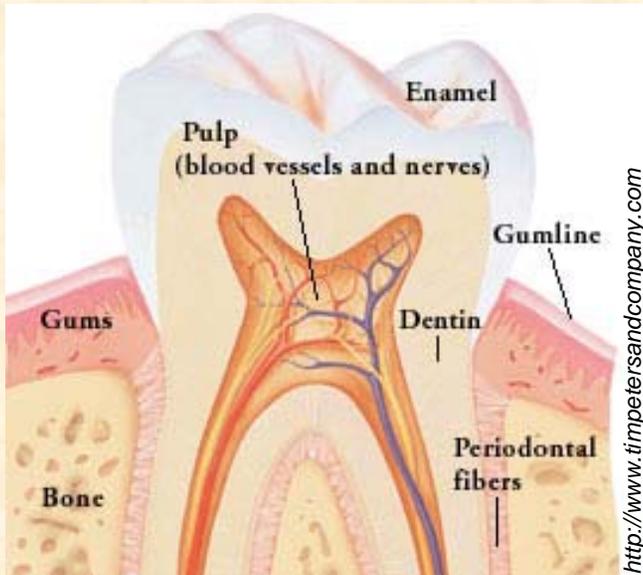
Macroscopic piezoelectric measurements are not reproducible

**On sub-micron level:**

20-30 nanometer apatite lamellae  
~15 nanometer collagen fibrils

**The detailed structure is virtually unknown...**

# Dentin-Enamel-Junction as a Sample Model

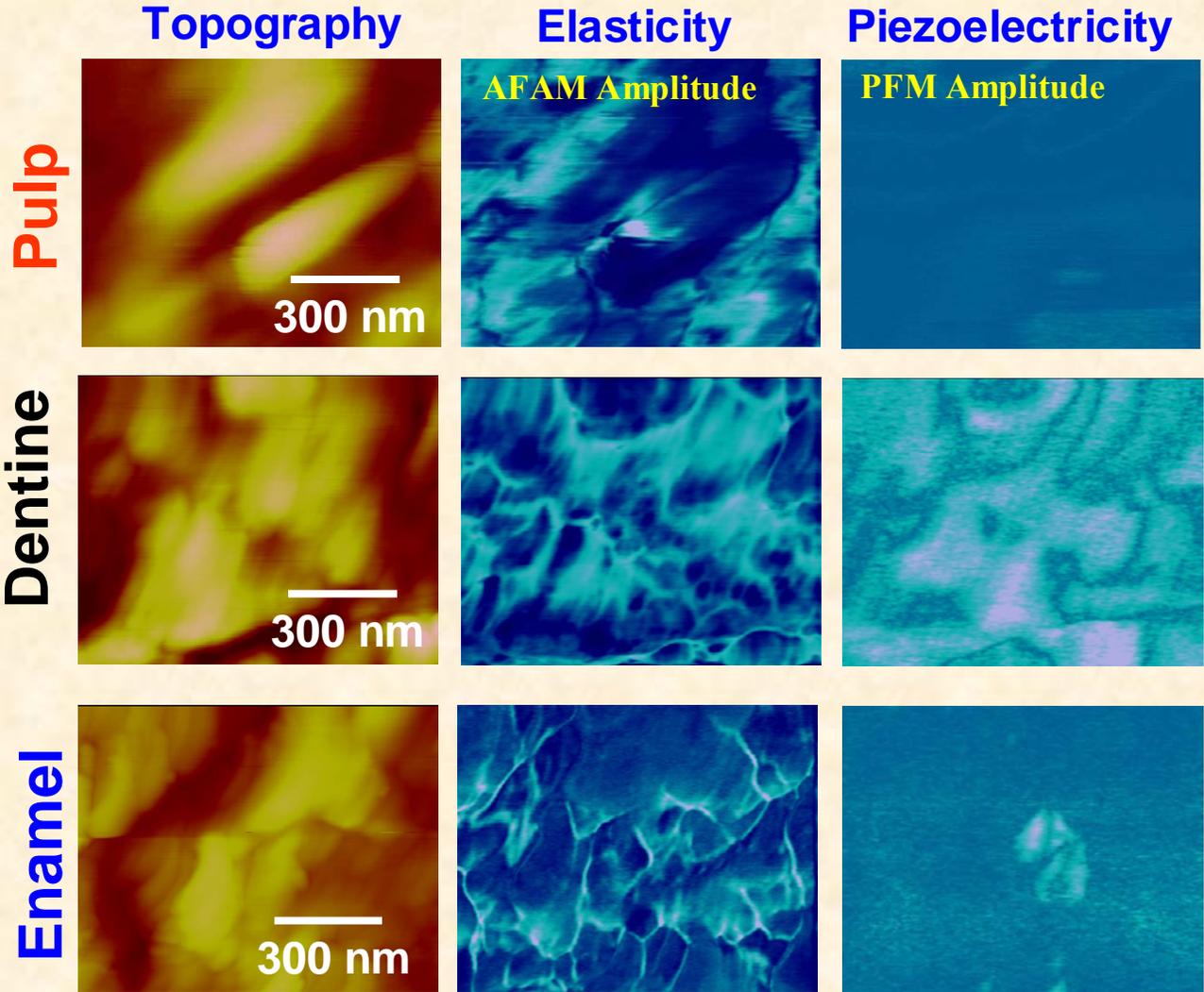
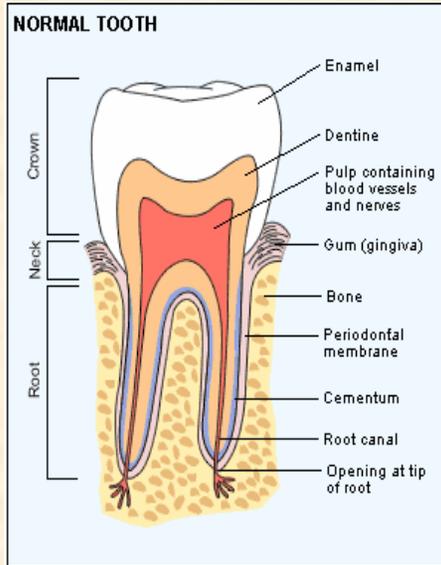
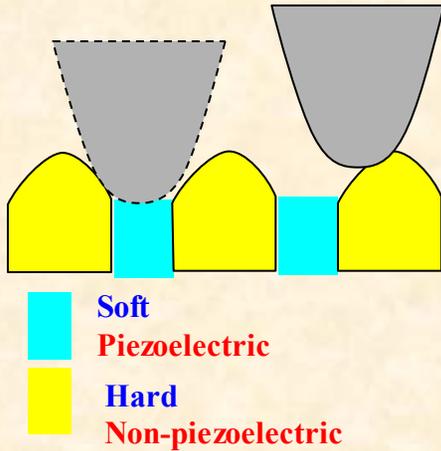


Clear contrast for both modes

What about higher resolution scans?

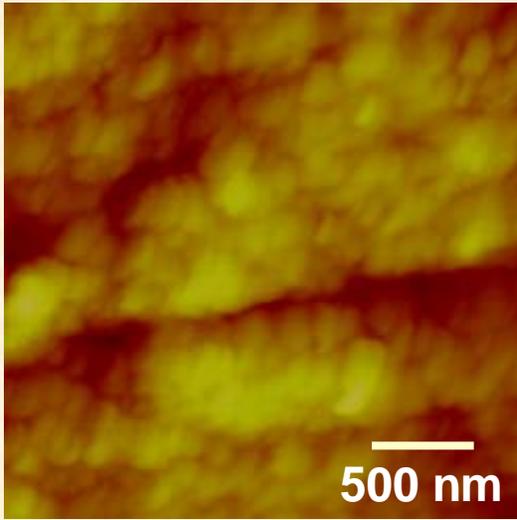
# Nanoelectromechanics of Human Tooth - I

Dentine and enamel are based on hydroxyapatite, but have different mechanical properties as they differ by the content of organic fibers.

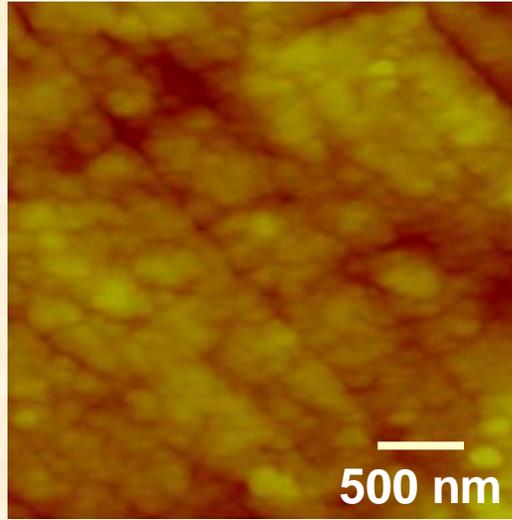


# Nanoelectromechanics of Human Tooth - II

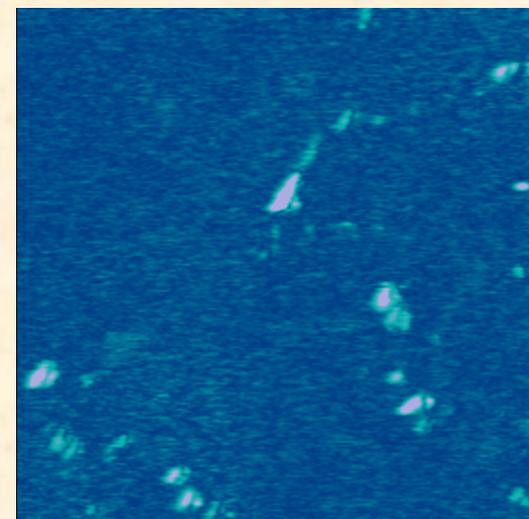
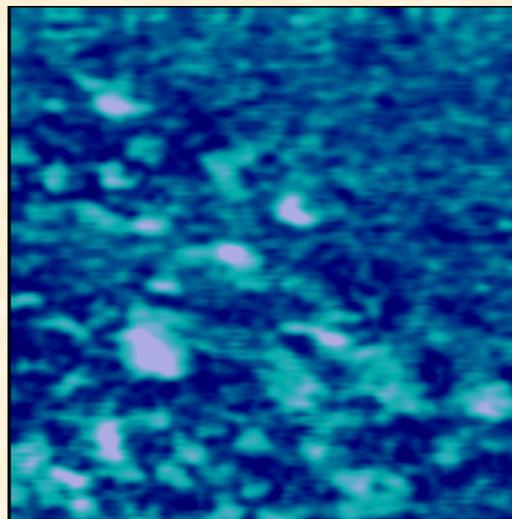
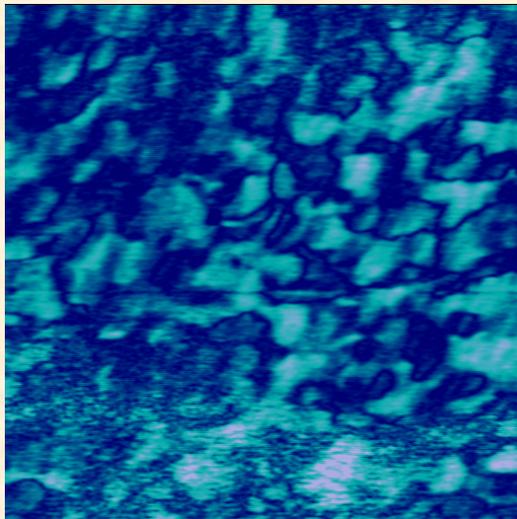
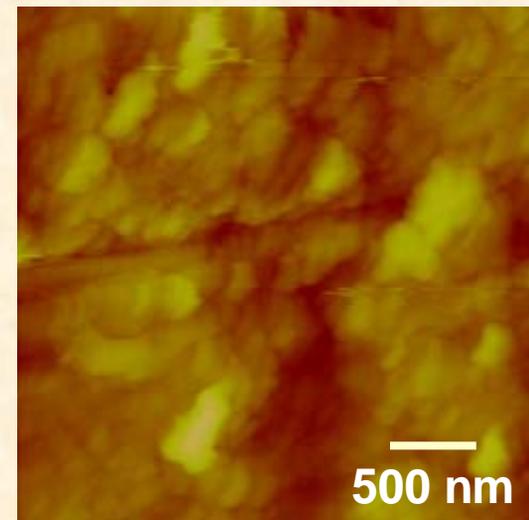
Dentin



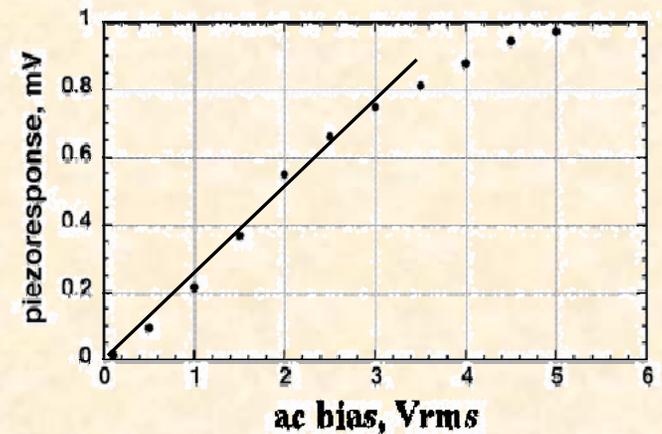
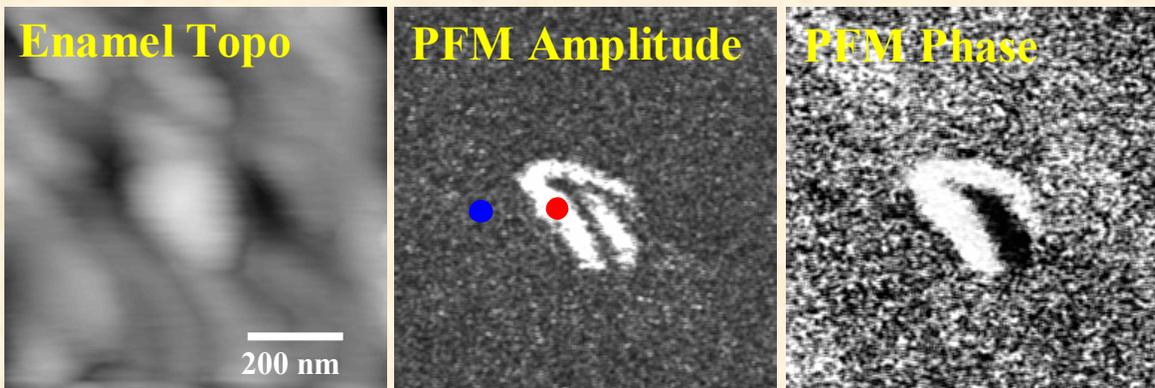
Dentin-Enamel Junction



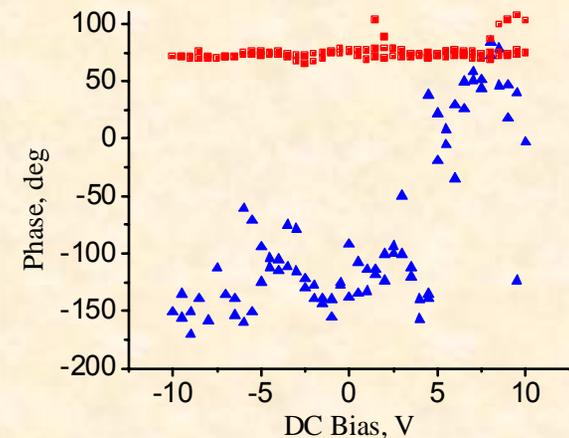
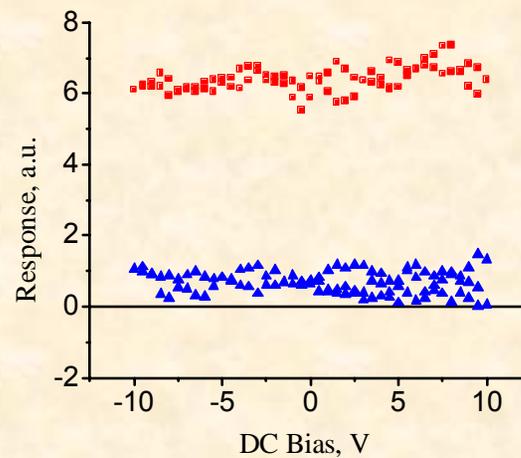
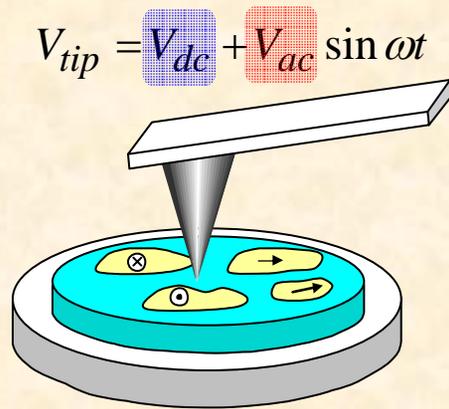
Enamel



# Piezoelectric Spectroscopy



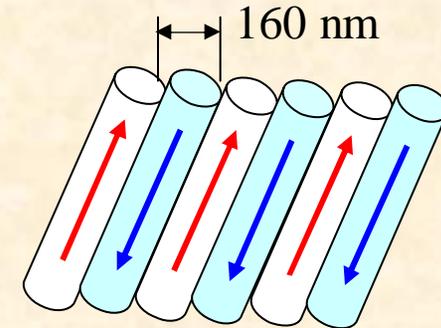
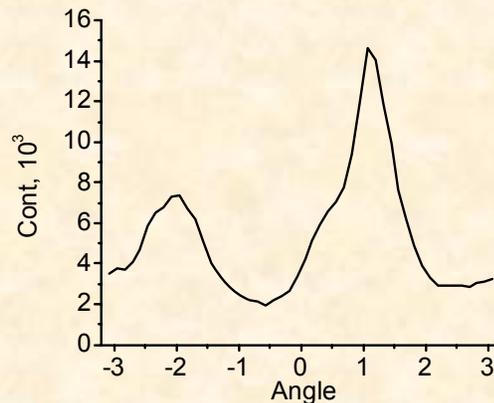
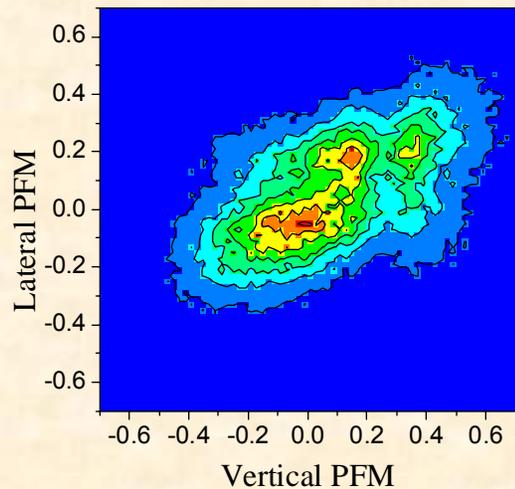
## Hysteresis loops of a single protein fibril in tooth enamel



- Collagen is piezoelectric, but not ferroelectric.
- Response is almost linear in bias,  $d_{local} = 0.5 - 2 \text{ pm/V}$

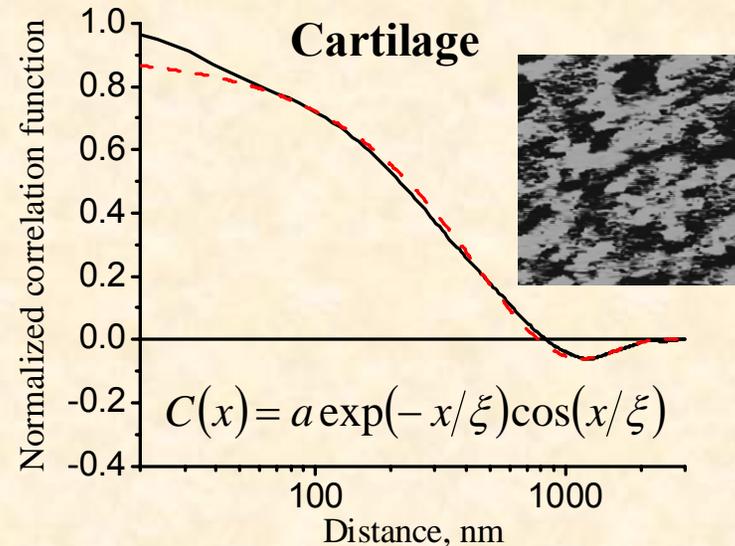
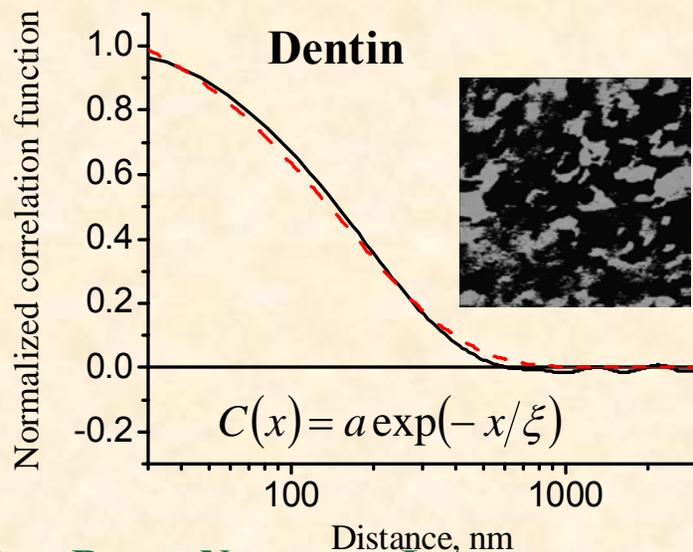
# Microstructure of Dentin vs. Cartilage

Doubly histogram of vertical and lateral PFM shows that there are two dominant fiber orientations (significant disorder)

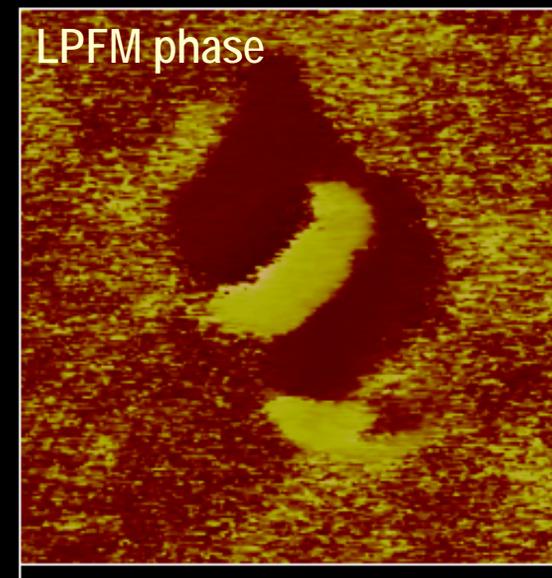
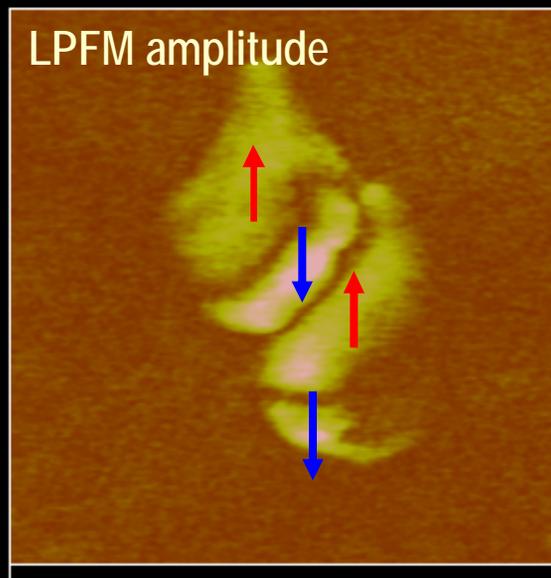
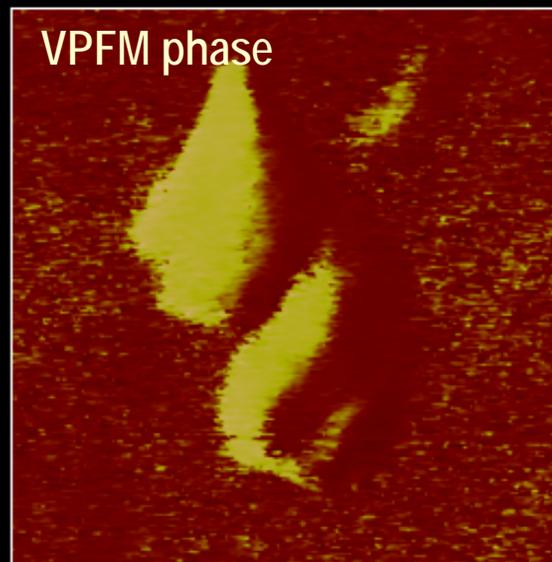
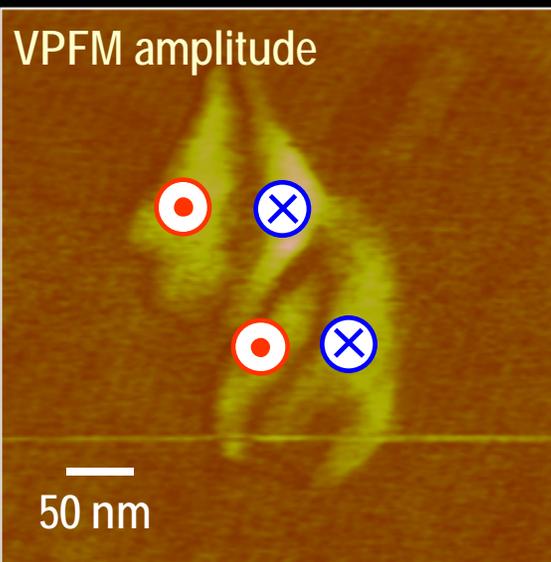


*Microstructural model*

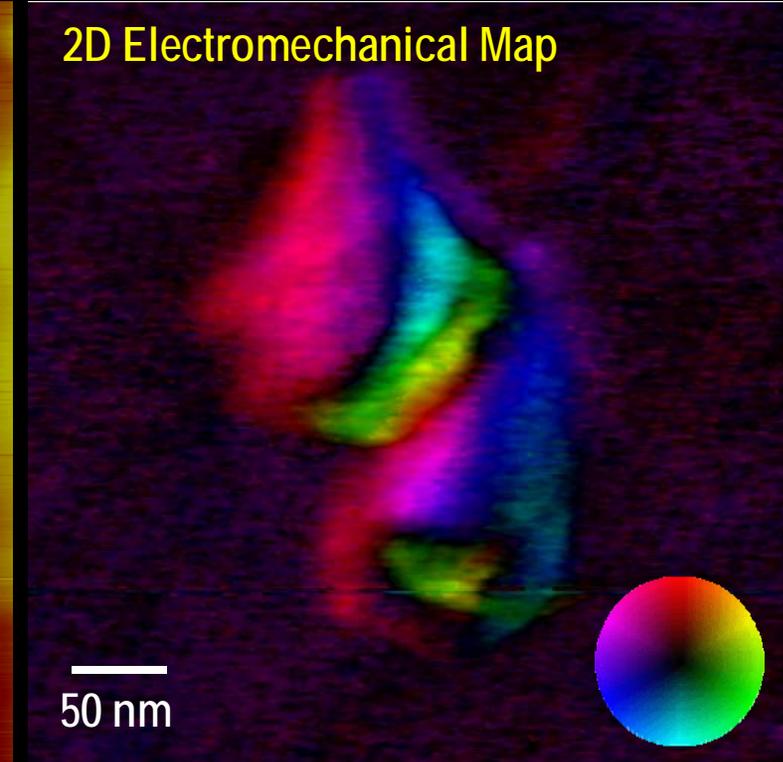
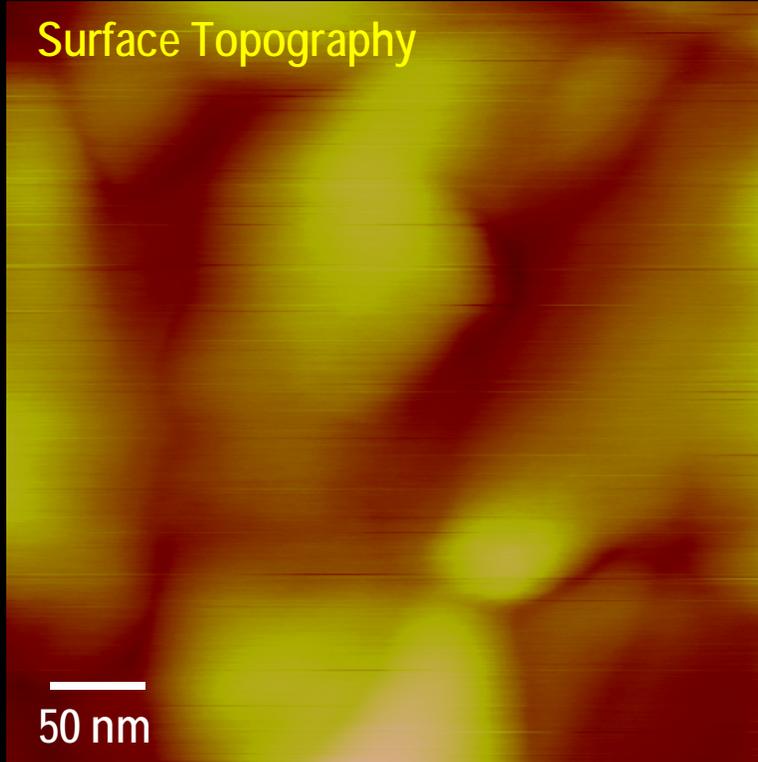
## Correlation function analysis



# Toward Single Molecule Imaging



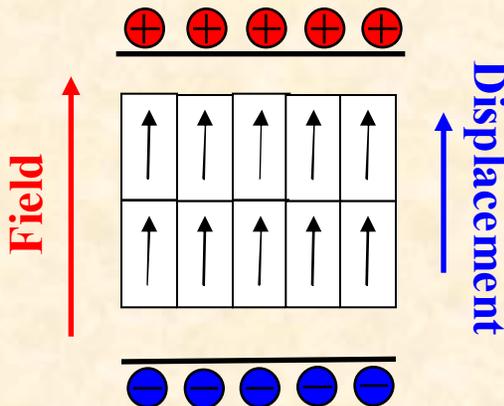
# Toward Single Molecule Imaging



SPM opens a possibility to observe how proteins interact with hydroxyapatite crystals to form these tissues. We are moving to an exciting and completely unexplored area!

# Principles of Orientation Imaging

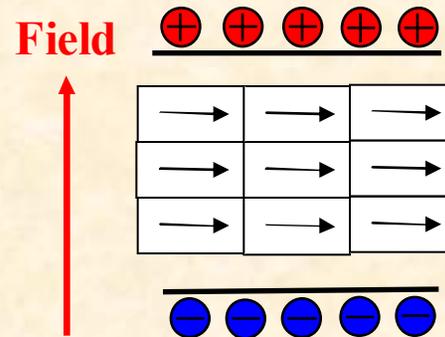
**Experiment:** measure the mechanical displacement in the applied electric field direction



For tetragonal ferroelectric such as BaTiO<sub>3</sub>, if the field is **parallel** to the c-axis, there is strong deformation of the crystal in the field direction

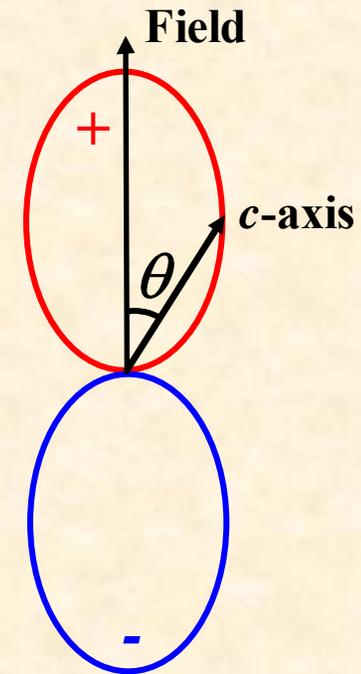
$$\delta z = d_{33}E_3h \quad \text{or} \quad \delta z = 80V \text{ pm}$$

If the field is **perpendicular** to the c-axis, the deformation in the field direction is zero



**In the general case:**

$$\delta z = (d_{15} + d_{31})\sin^2 \theta \cos \theta + d_{33} \cos^3 \theta$$

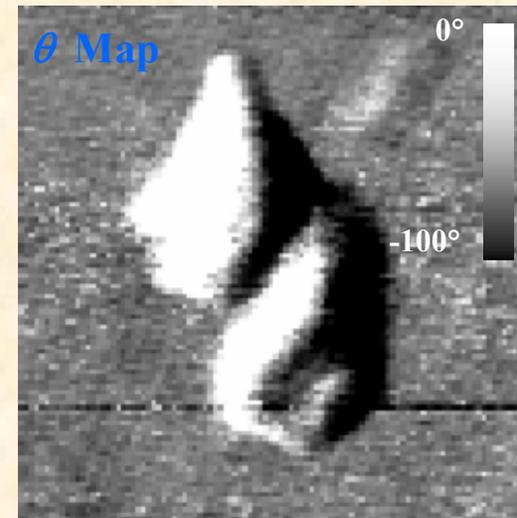
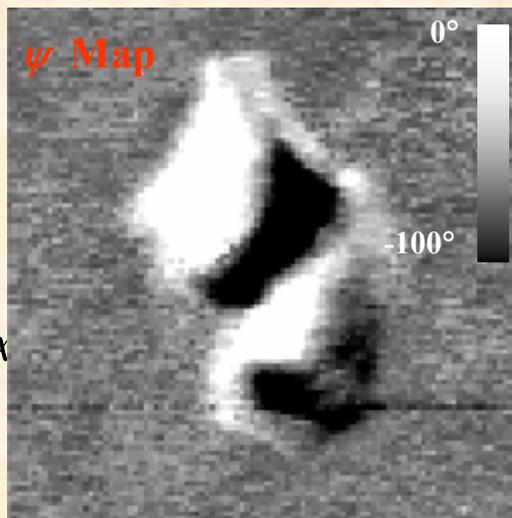
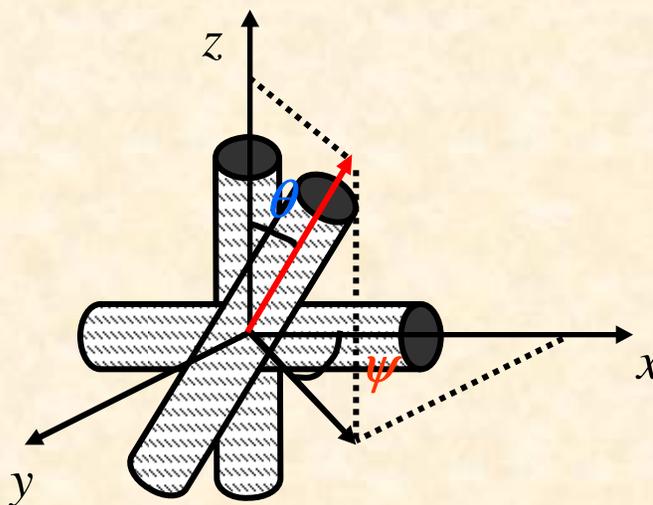


**From known electromechanical response, we can determine crystal orientation!**

- small displacements (10-100 pm)
- spatially localized (< 1 μm)

# Molecular Orientation from PFM Data

## Semiquantitative Orientation Map for Collagen Fibril



### Tetragonal material (4mm):

$$\begin{pmatrix} 0 & 0 & 0 & 0 & d_{15} & d_{15} \\ d_{31} & d_{33} & d_{31} & d_{15} & 0 & 0 \\ d_{31} & d_{31} & d_{33} & d_{15} & 0 & 0 \end{pmatrix}$$

Vertical PFM

Lateral PFM (x)

Lateral PFM (y)

$$d_{ij} = A_{ik}(\phi, \theta, \psi) d_{kl}^o N_{lj}(\phi, \theta, \psi)$$

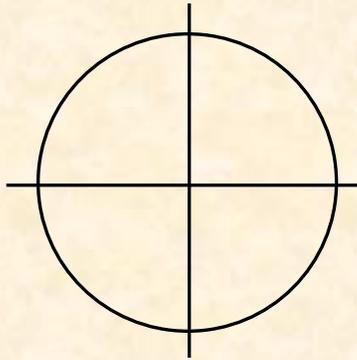
$d_{ij}$  Piezotensor – lab coordinate system

$d_{kl}^o$  Piezotensor – crystal coordinate system

$N_{ij}(\phi, \theta, \psi)$   $A_{ij}(\phi, \theta, \psi)$  Rotation matrices

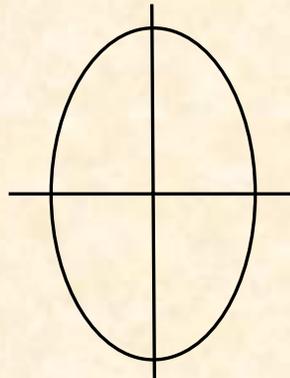
# Can we do it any other way?

*Scalar*



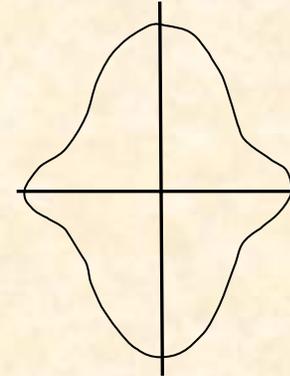
*Temperature,  
Potential, etc.*

*2<sup>nd</sup> order tensor*



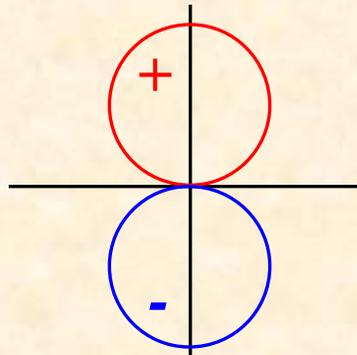
*Dielectric constant  
Thermal conductivity*

*4<sup>th</sup> order tensor*



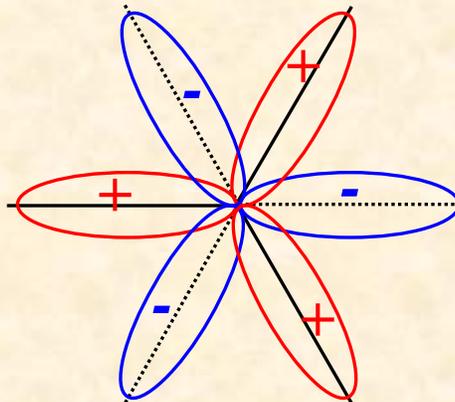
*Elasticity, Kerr effect  
Electrostriction*

*Vector*



*Polarization  
Magnetization  
Pyroelectricity*

*3<sup>d</sup> order tensor*

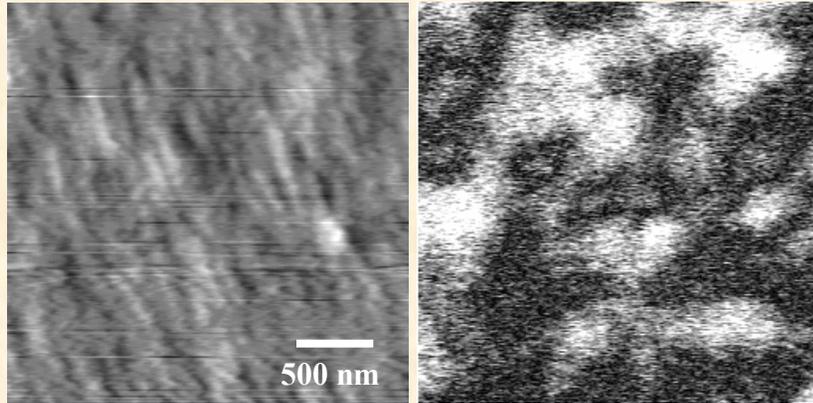


*Piezoelectricity  
Electrooptics, SHG*

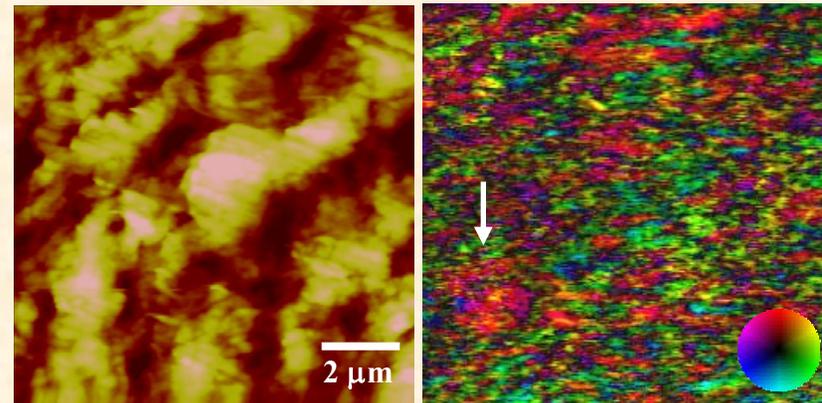
**Electromechanical** coupling coefficient can be measured by SPM with sub 10 nanometer resolution. Measurements are not sensitive to tip geometry, unlike **elastic** and **dielectric** measurements

# Piezoelectricity in Biosystem

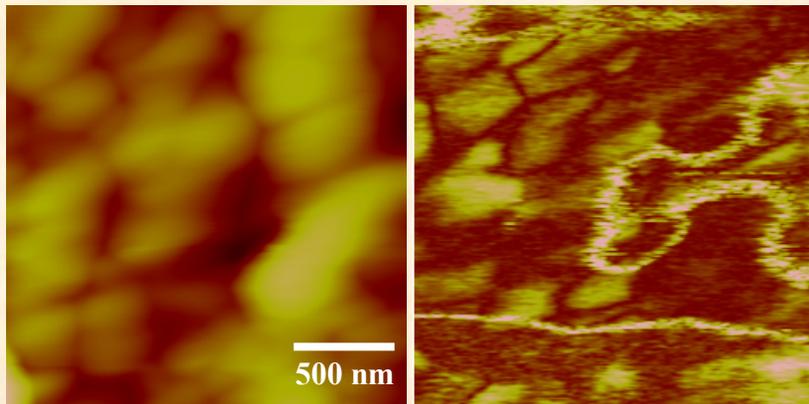
*Artificial collagen film*



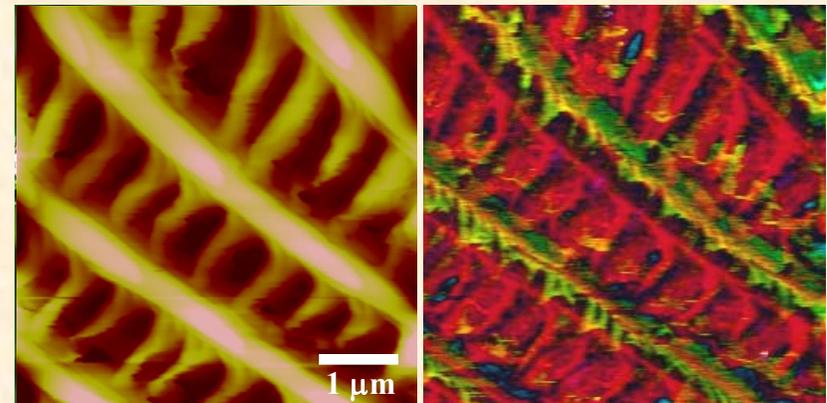
*Cross-sectioned deer antler*



*Canine femur cartilage*



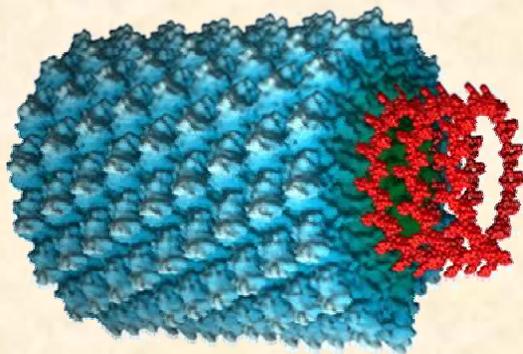
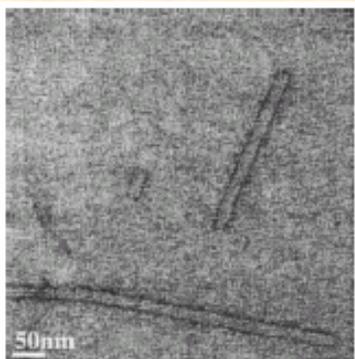
*Vanessa Virginiensis wing*



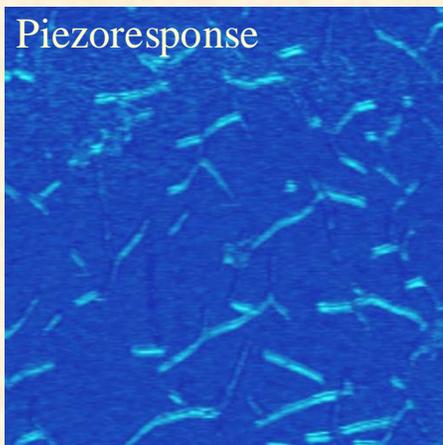
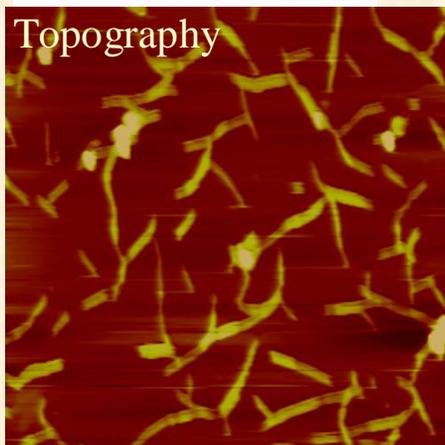
*Nanoscale piezoelectricity is ubiquitous in biosystems, stemming from the combination of polar bonds and optical activity in biopolymers*

# Flexoelectricity in Tobacco Mosaic Virus

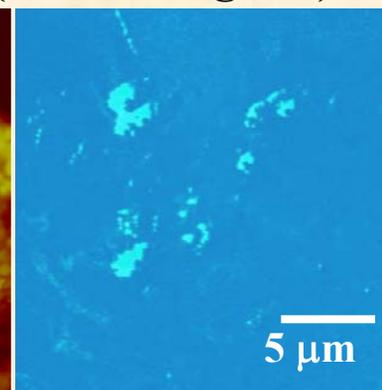
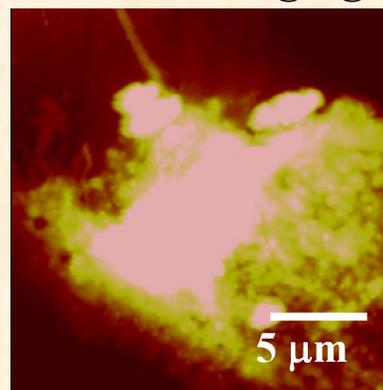
More complex steps: tobacco mosaic virus and cells



*Electrical modification of TMV*



Cellular imaging (mouse lung cell):



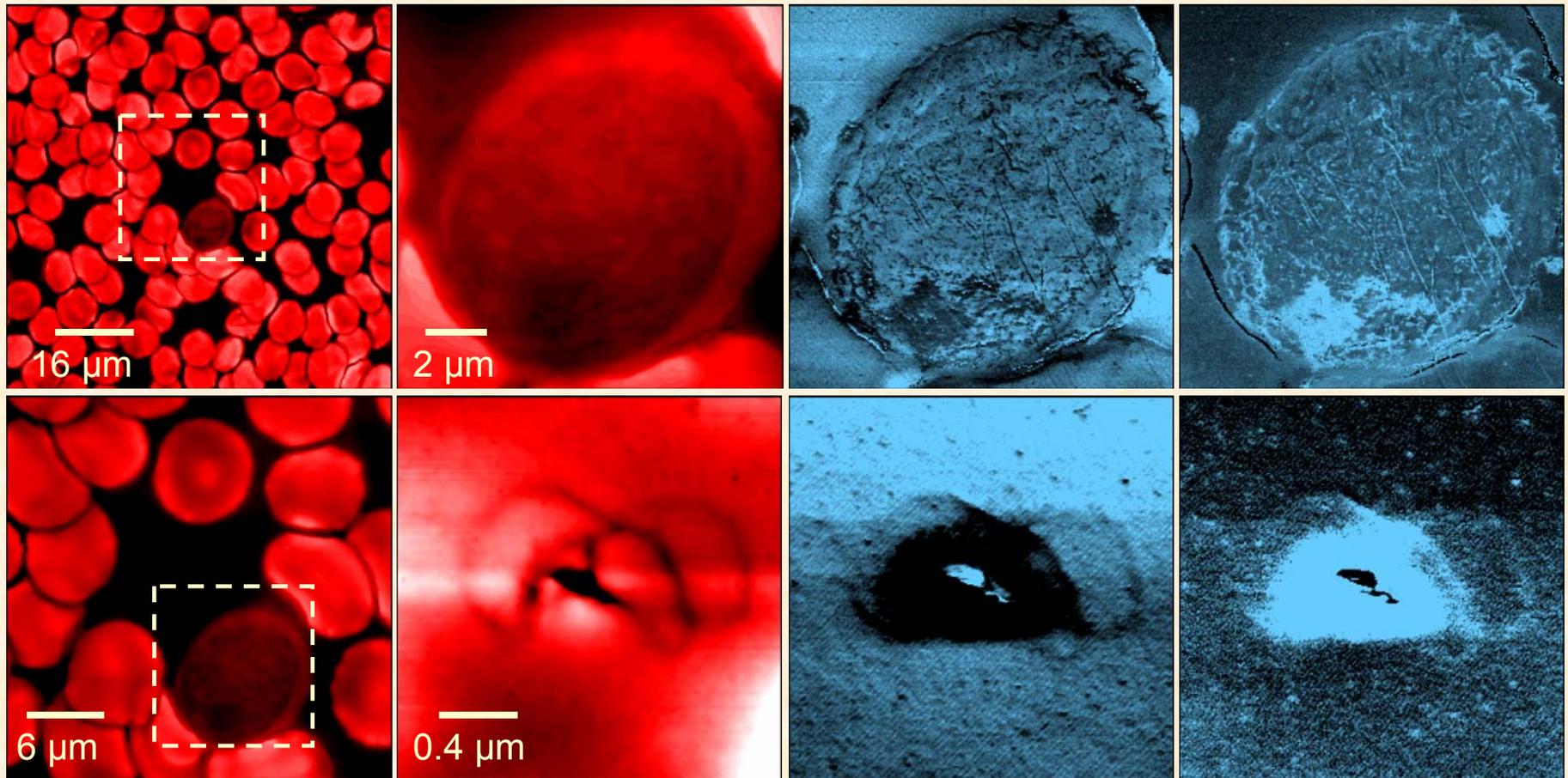
Collaboration with A. Balandin (UCR)

*Electromechanical coupling in viruses is presumably flexoelectric due to symmetry limitations*

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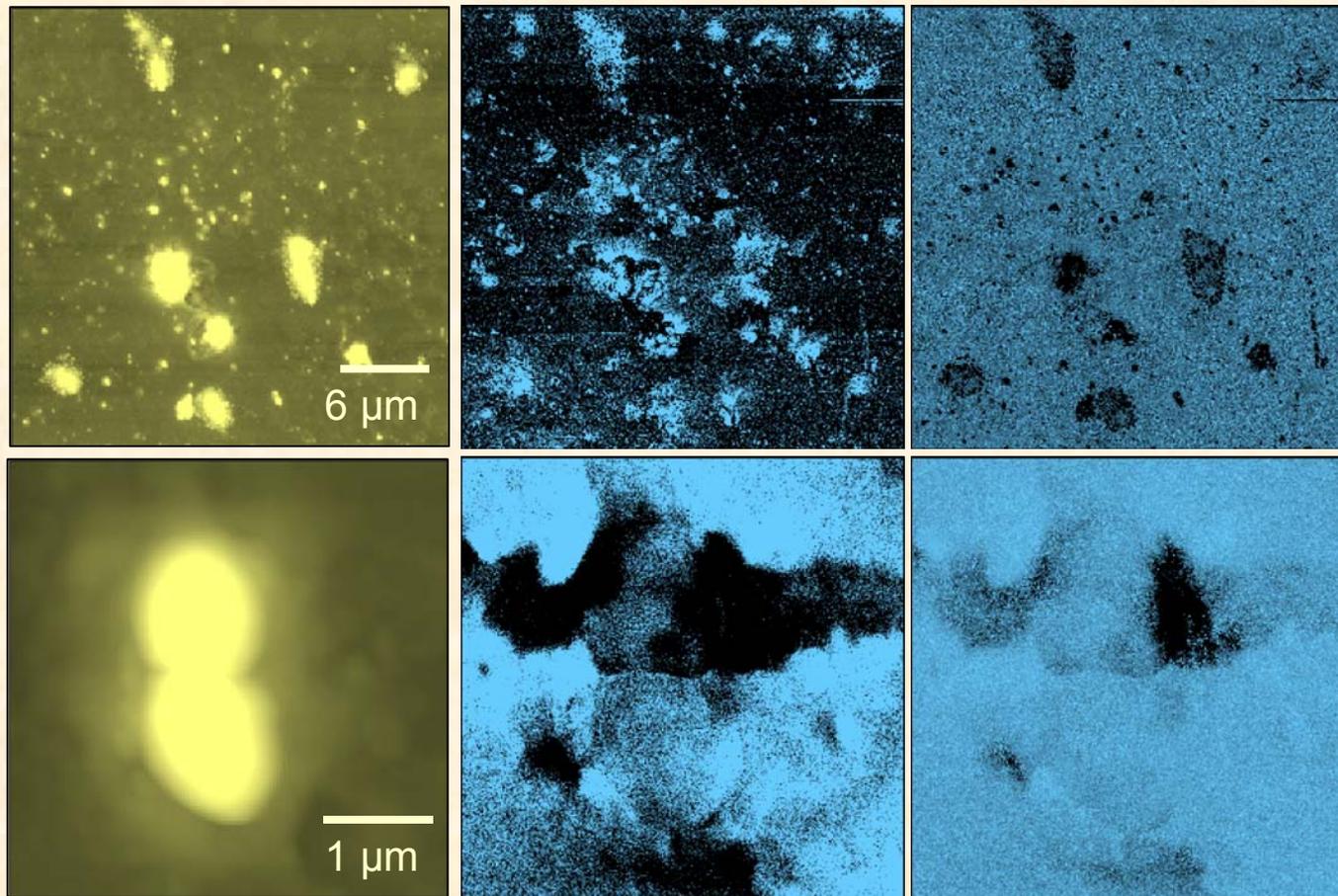


# Towards Cell Imaging

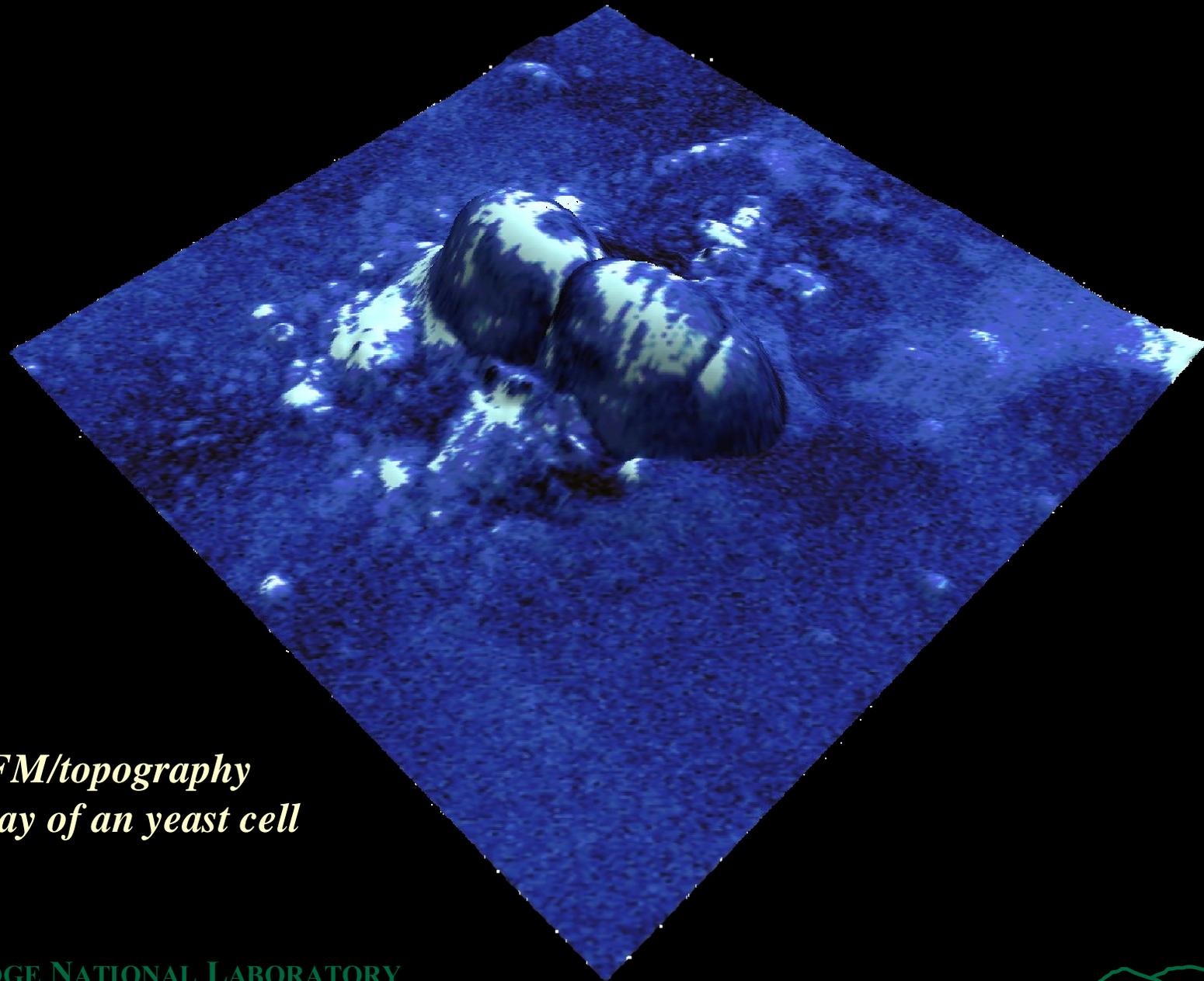


*PFM of cells in ambient usually shows additional details of structure, beyond topographic contrast*

# Yeast in Action



*PFM of cells in ambient usually shows additional details of structure, beyond topographic contrast*



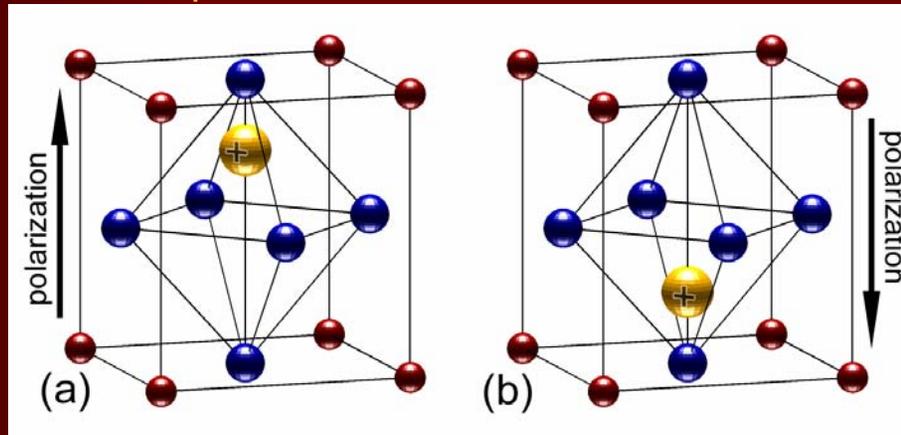
*PFM/topography  
overlay of an yeast cell*

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# Motivation: Why PVDF?

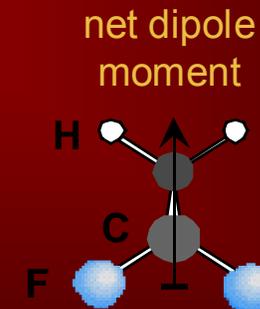
Stable polarization states of traditional FE



Inorganic FE: Subject to lattice phenomena

Which lead to imperfect behavior:  
interface phenomena  
chemical compatibility  
size effect

**PVDF is a polymer that has polar bonds, is piezoelectric, pyroelectric and ferroelectric on the *molecular* scale**



# Imaging and Domain Structure

Topography

PFM Phase

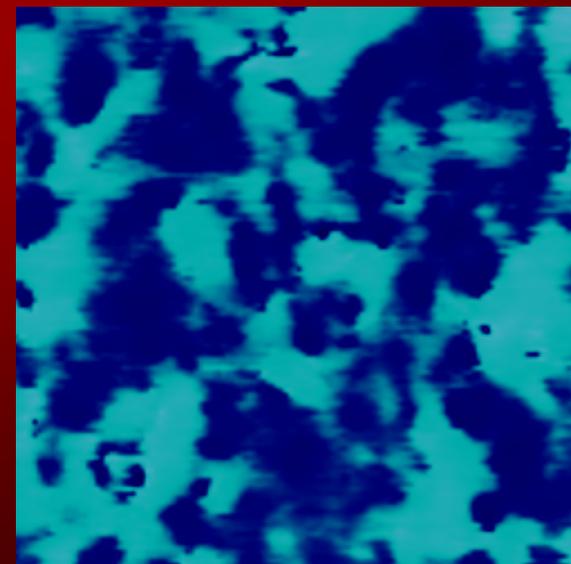
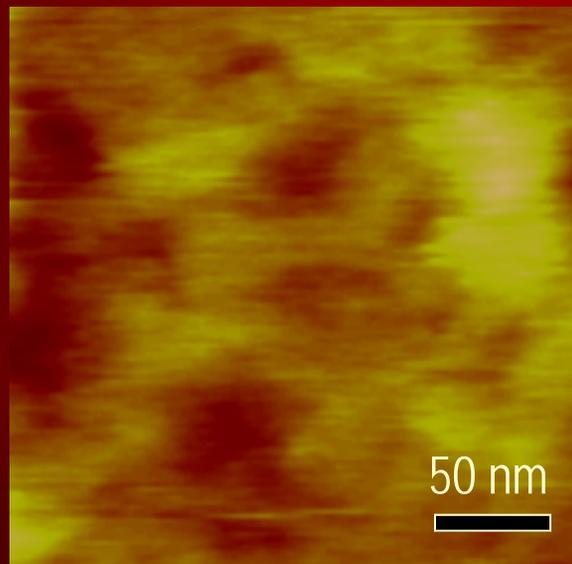
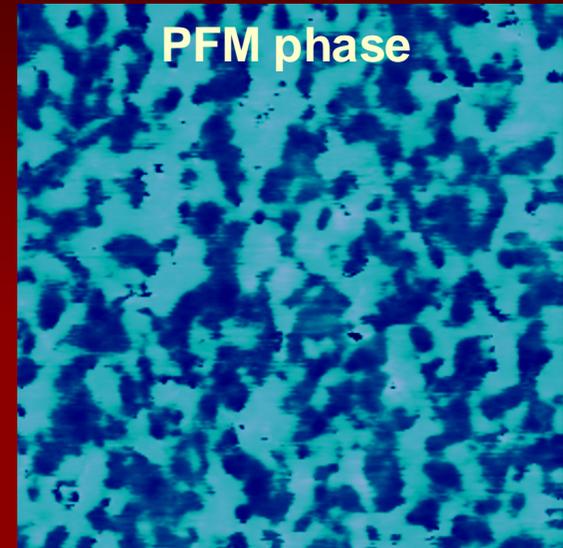
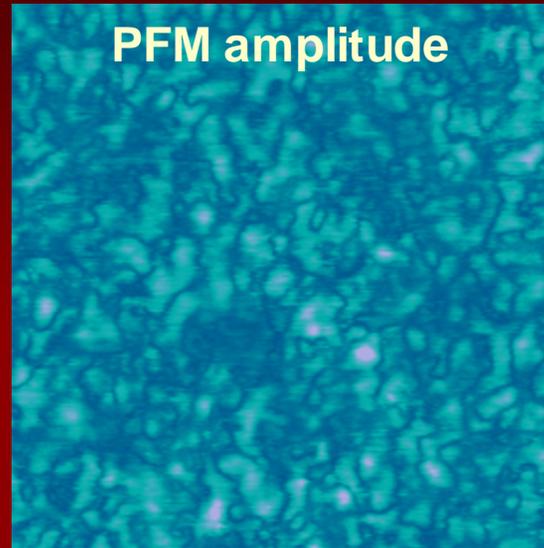
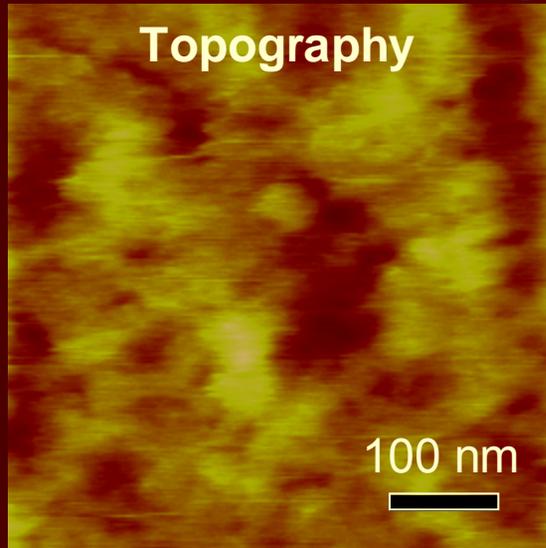
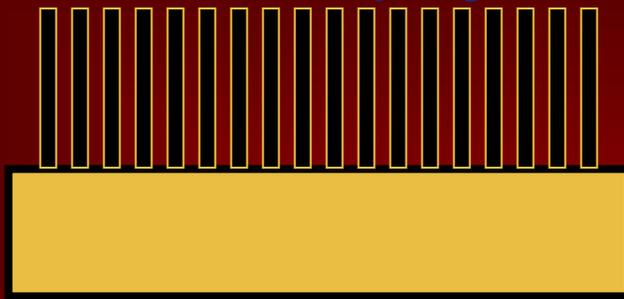
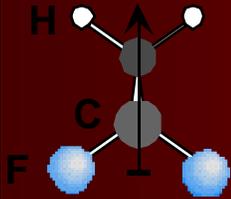


Image analysis reveals domains are 20-40 nm in size. PFM reveals 25 nm characteristic length

# Domain Structure - Lateral PFM of PVDF

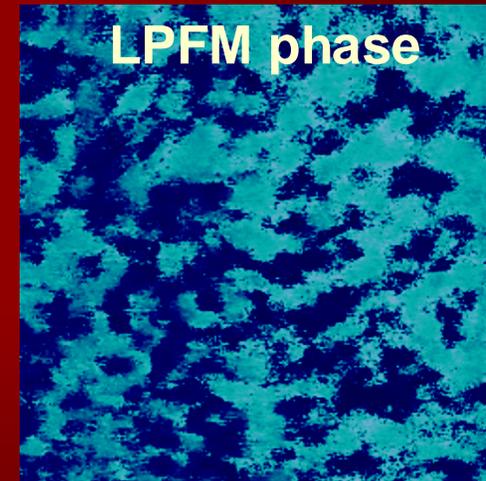
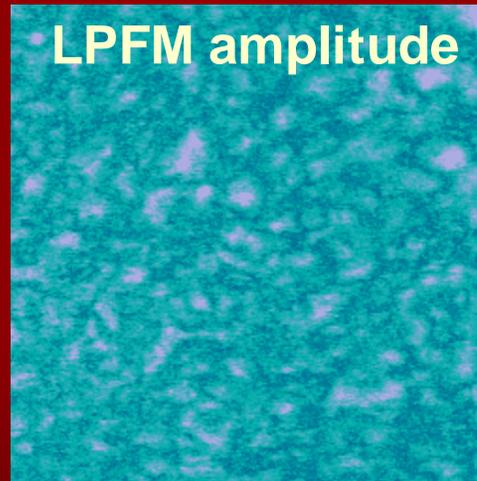
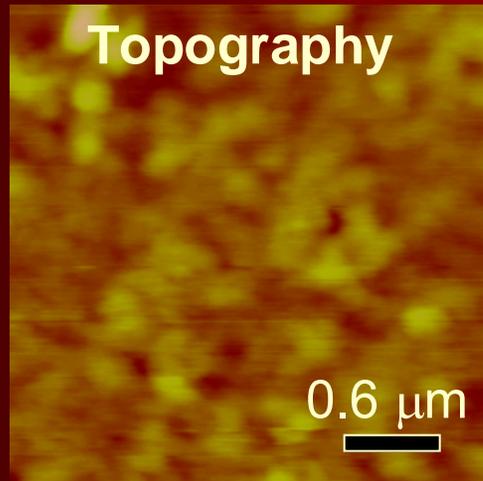
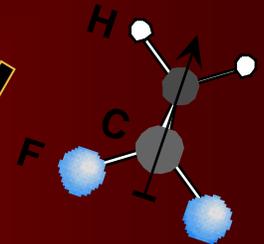
*How are polymer chains aligned?*



*vertical alignment*



*lateral component*

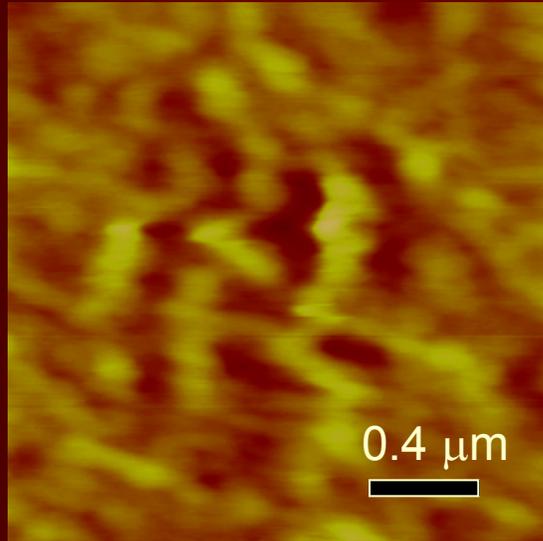


There is a lateral component to the PFM signal which implies that there is some angle between the polar axis of the polymer with respect to the substrate. The possible origins include:

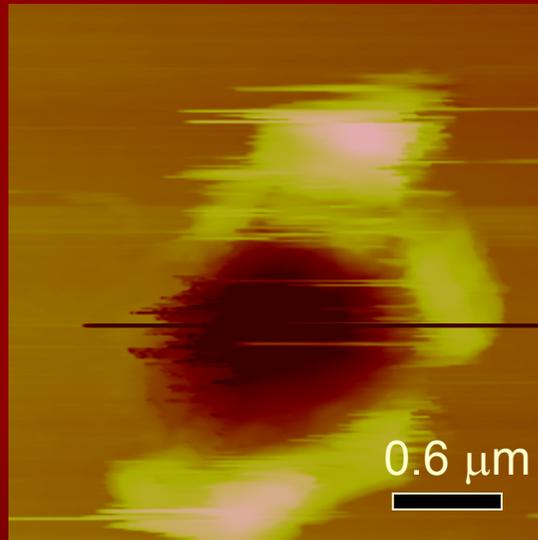
- tip-induced damage and subsequent molecular rotation
- intrinsic molecular orientation

# Modification

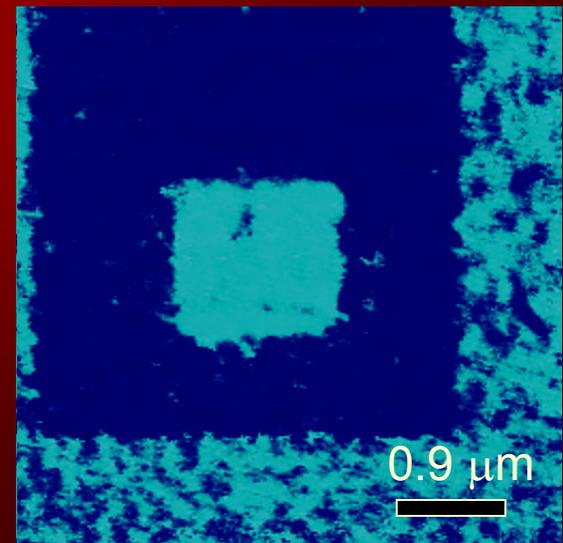
*Alignment*



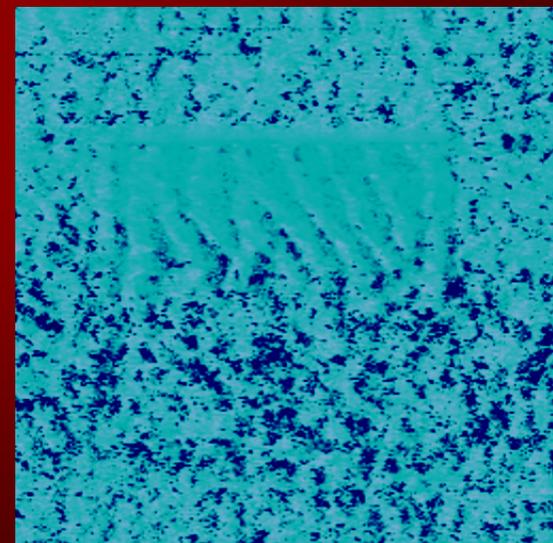
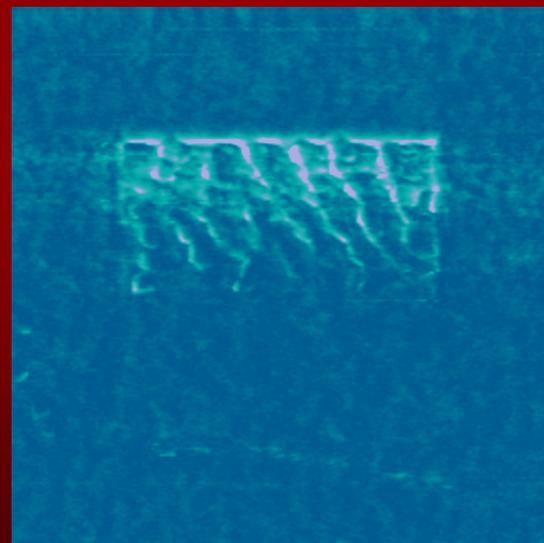
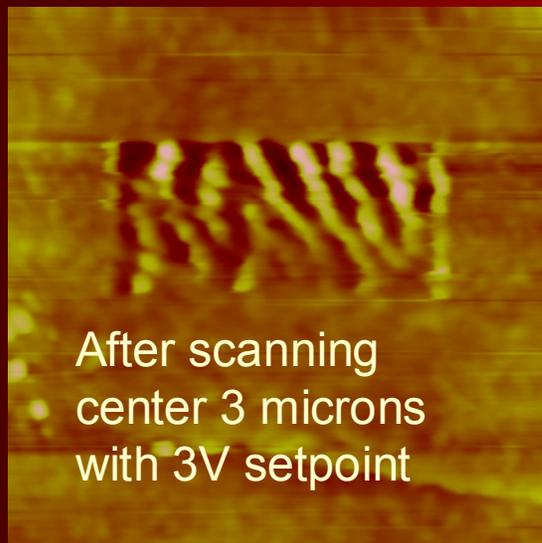
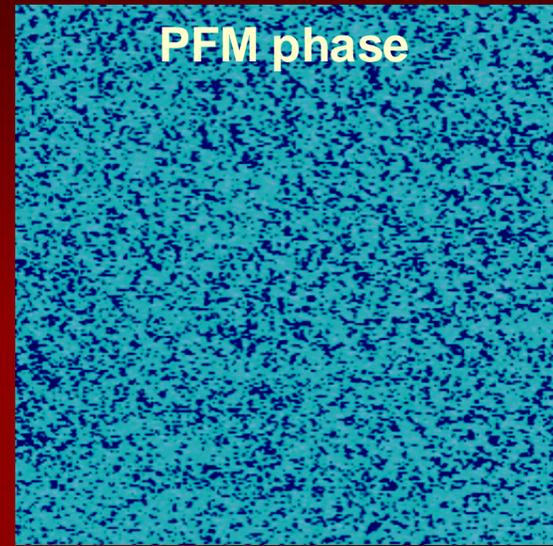
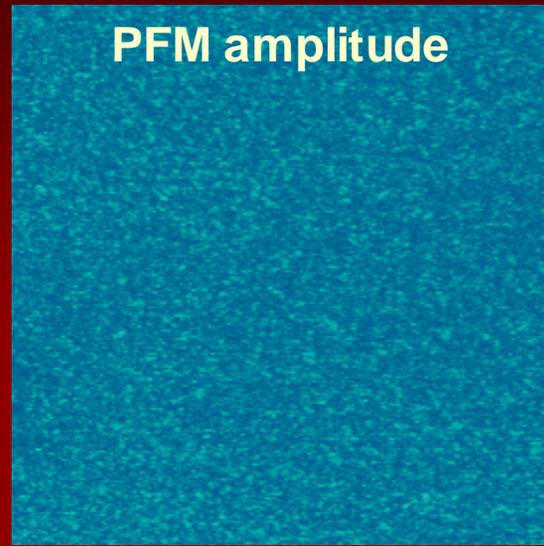
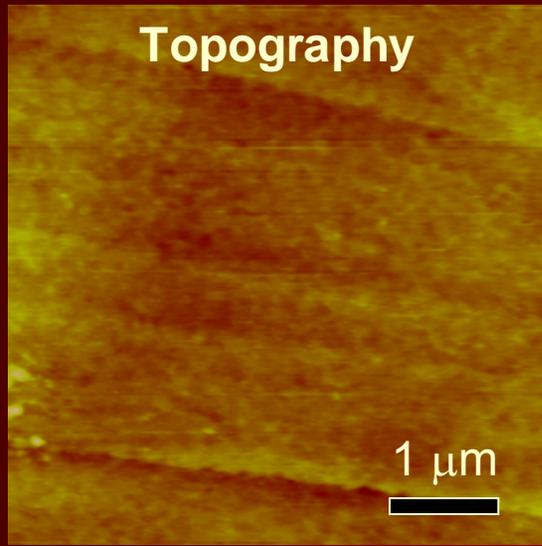
*Breakdown*



*Patterning*

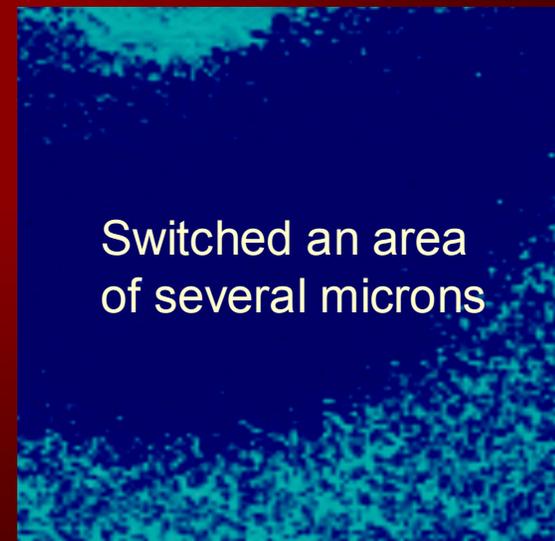
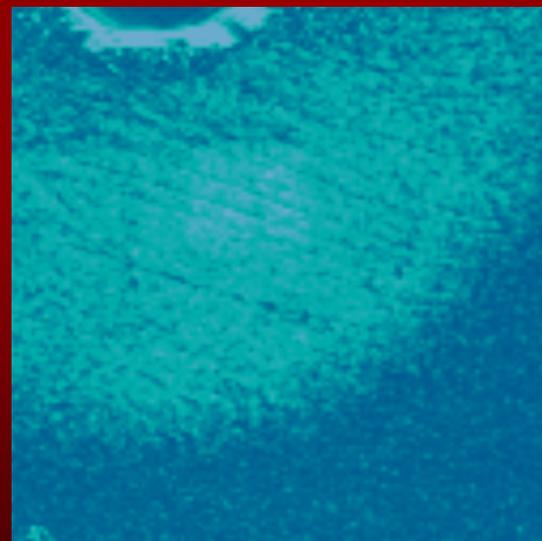
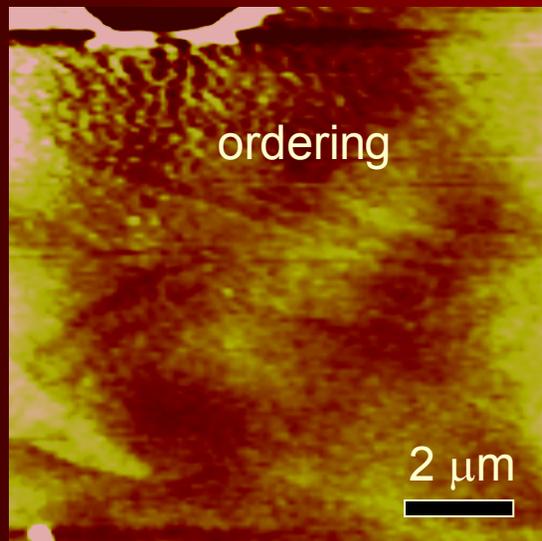
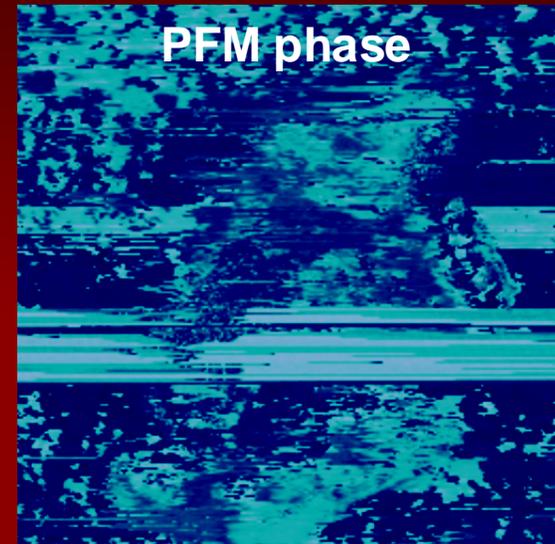
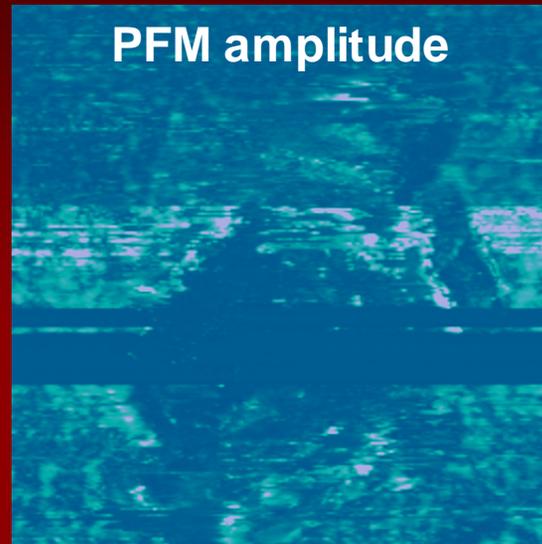
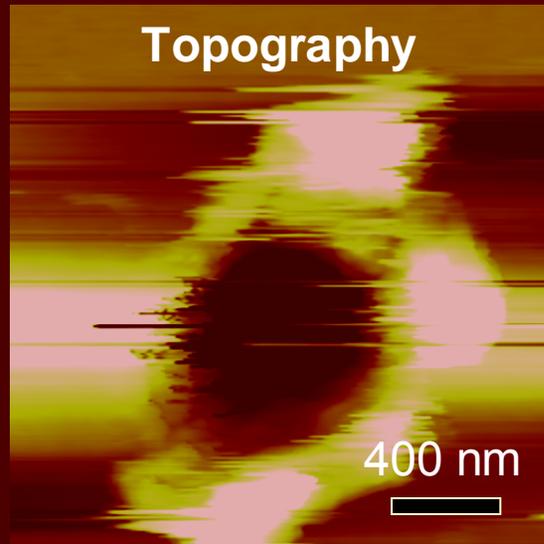


# *Effect of Mechanical Modification*



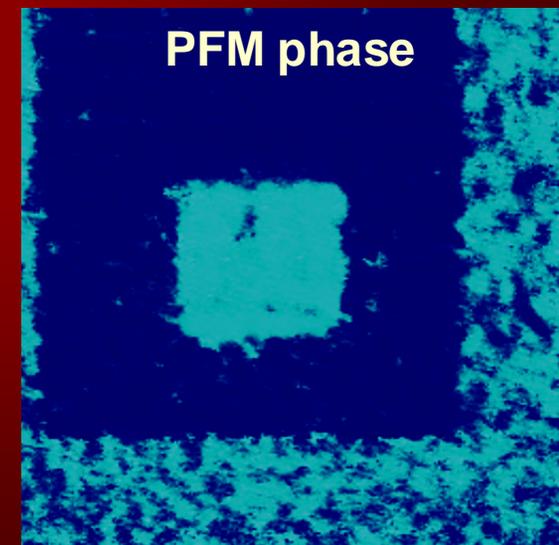
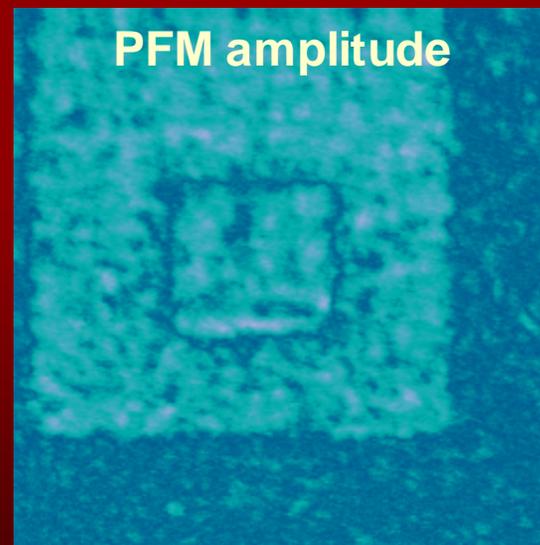
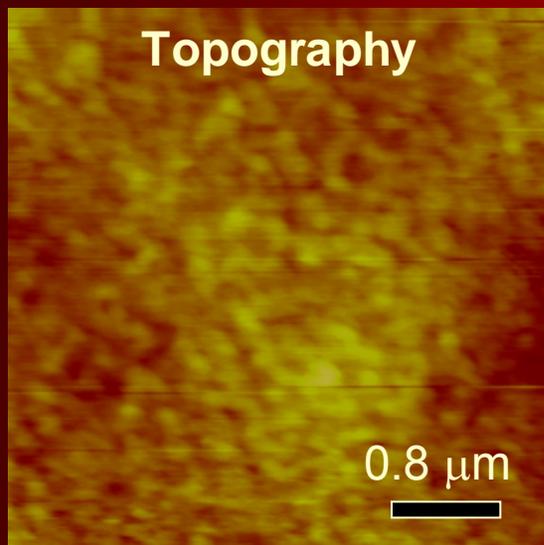
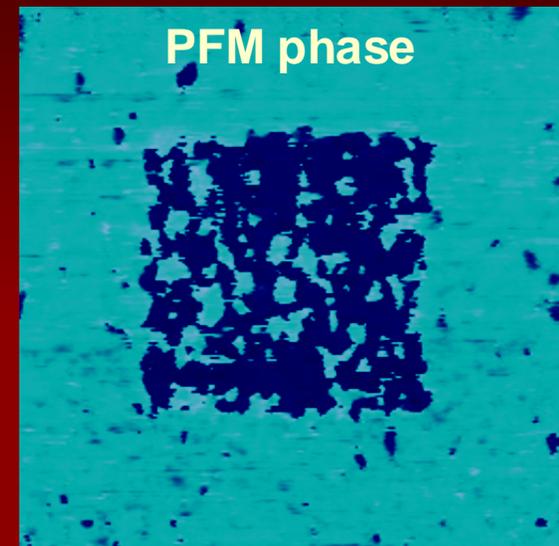
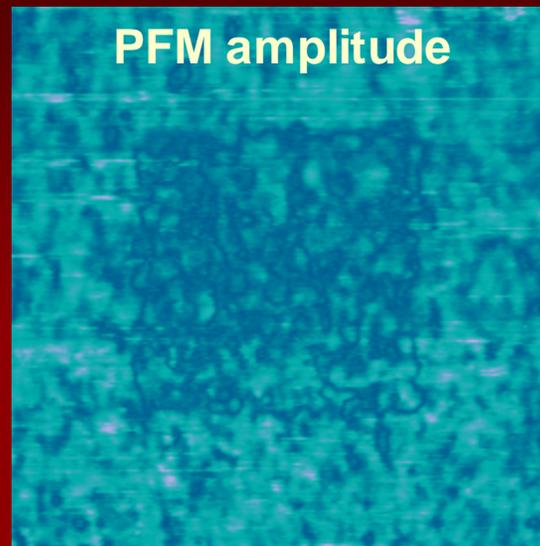
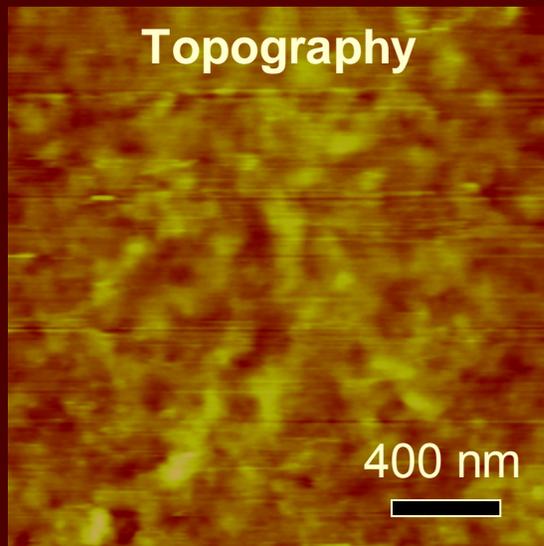
Continuous scanning with large indentation forces results in ordering of the film that can also be observed in PFM amplitude and phase images.

# Modification – Breakdown



The PVDF film can be affected by dielectric breakdown, associated with partial poling of material over large length scales and local ordering. - Examples of what not to do!

# Modification – Switching



Inhomogeneous properties – some regions switch at low bias, others at high.  
Also, note the roughness of the domain wall – it has an irregular shape

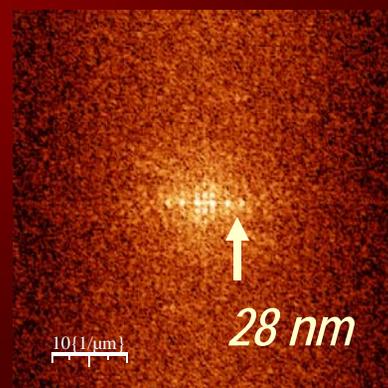
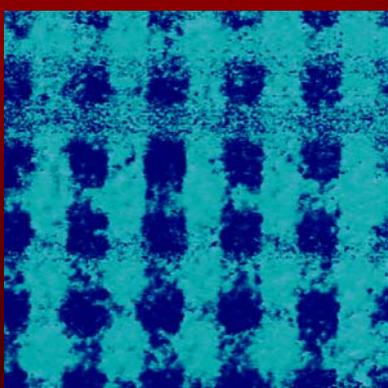
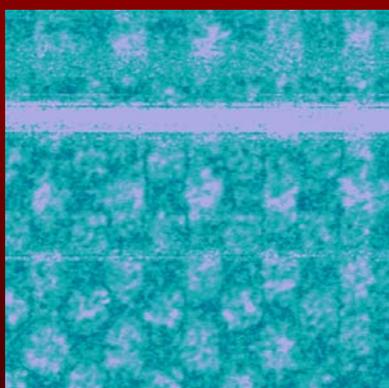
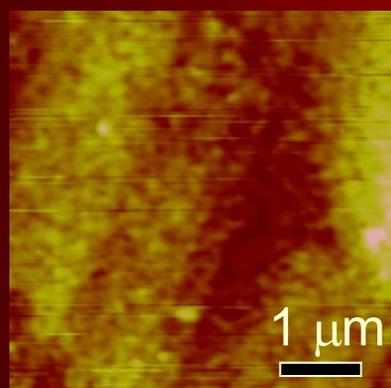
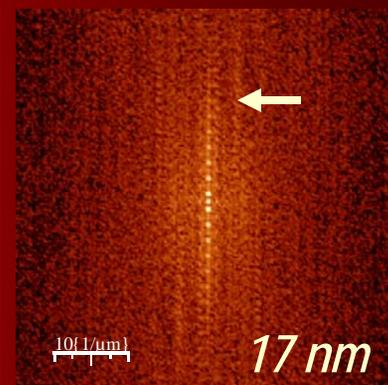
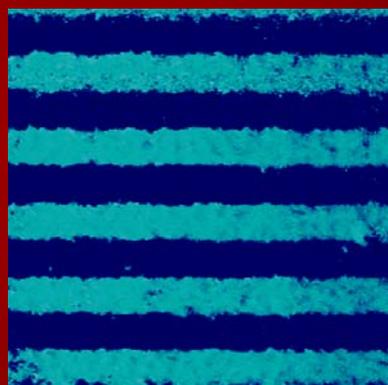
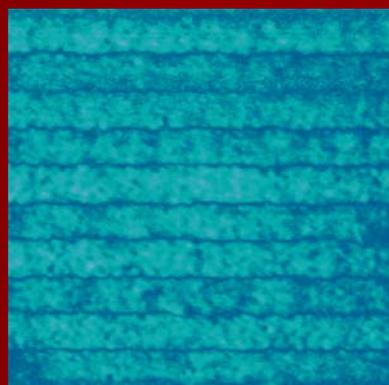
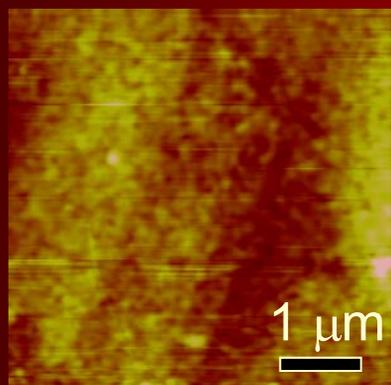
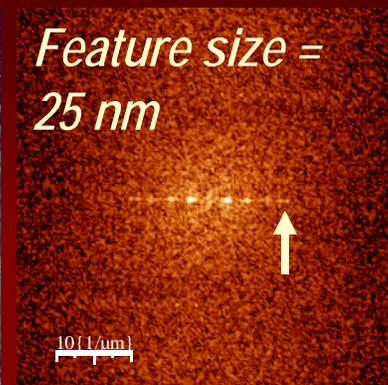
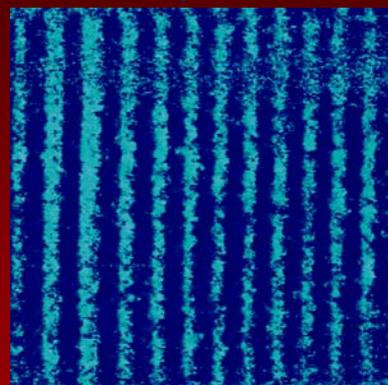
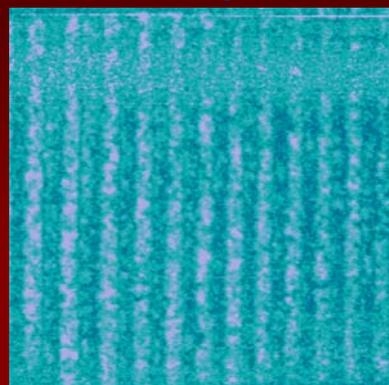
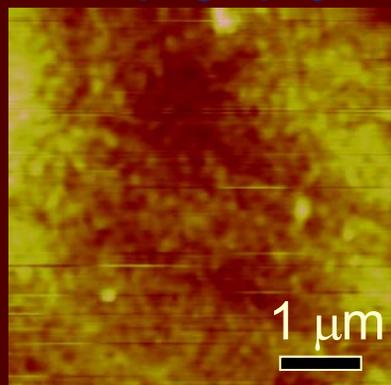
# Patterning in 10 mL PVDF Thin Films

Topography

PFM amplitude

PFM phase

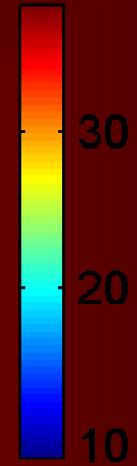
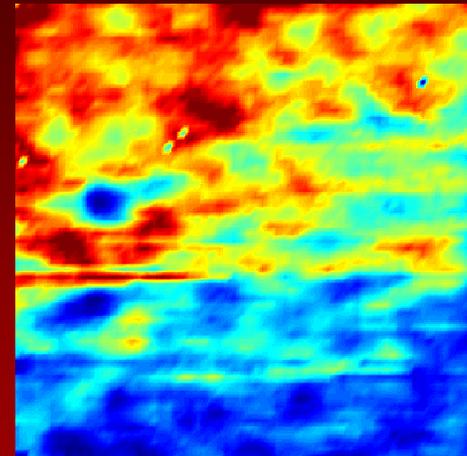
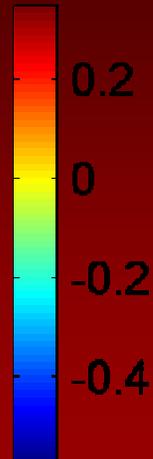
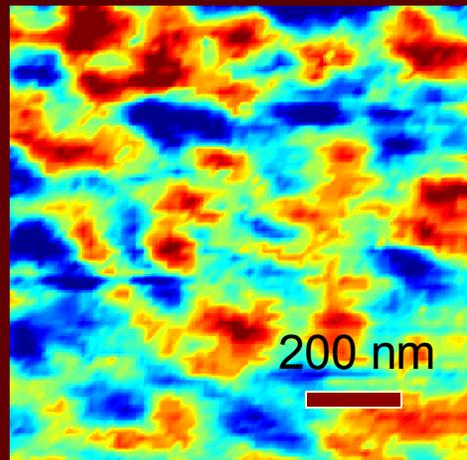
2D FFT



# Ongoing: SS-PFM of PVDF Thin Films

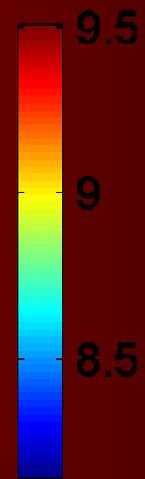
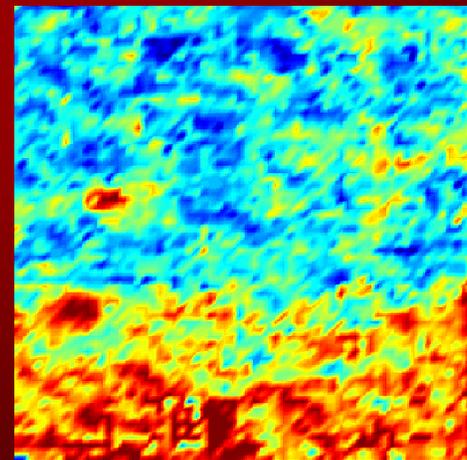
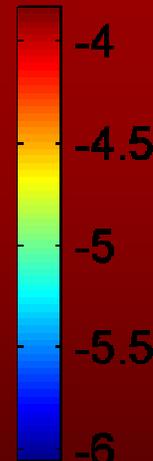
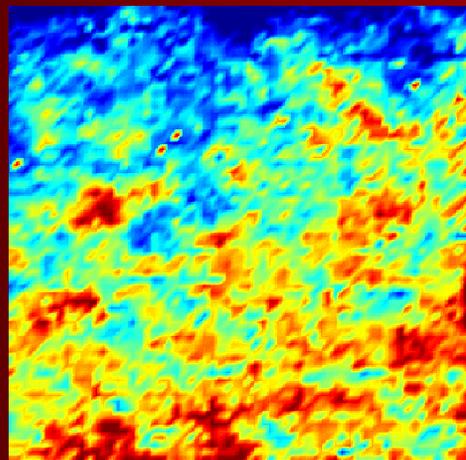
*PFM*

*Work of Switching*



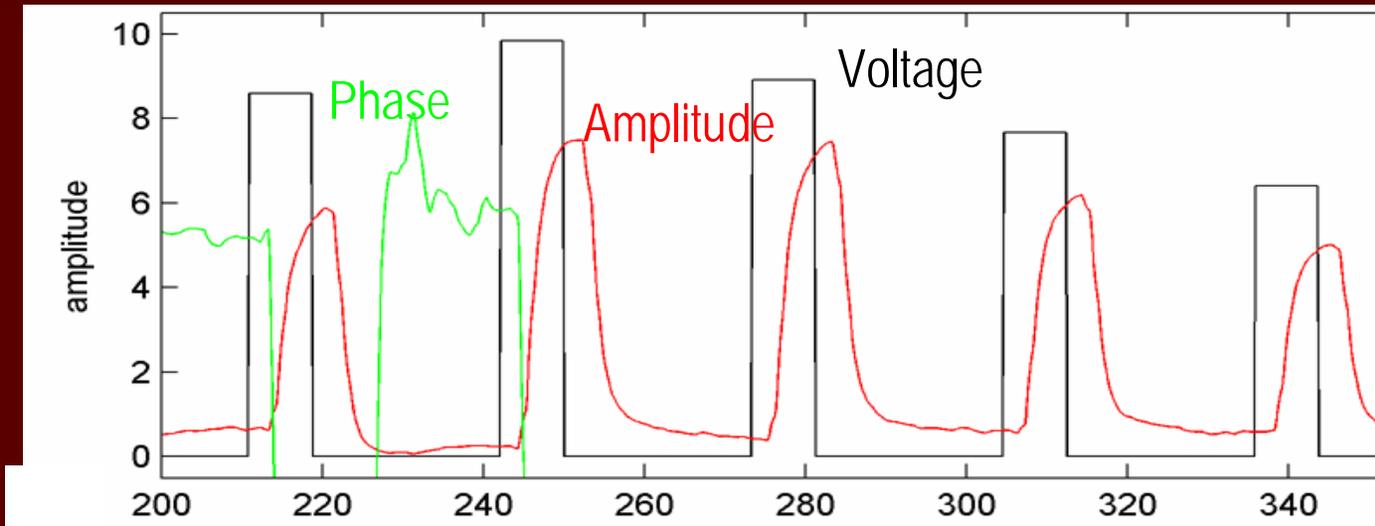
$V^-$

$V^+$

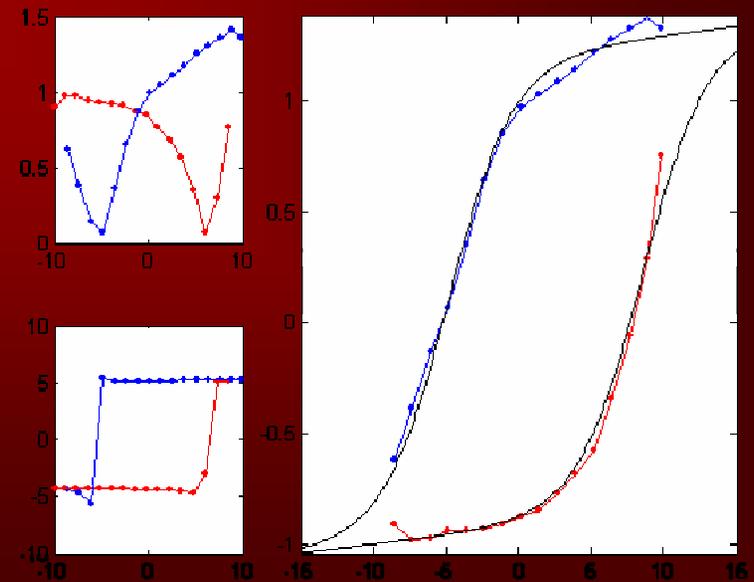


SS-PFM images of a 10 mL thick (18 nm) LB thin film of copolymer P(VDF-TrFE 70:30)  
Variations of work of switching and imprint biases throughout the film are observed

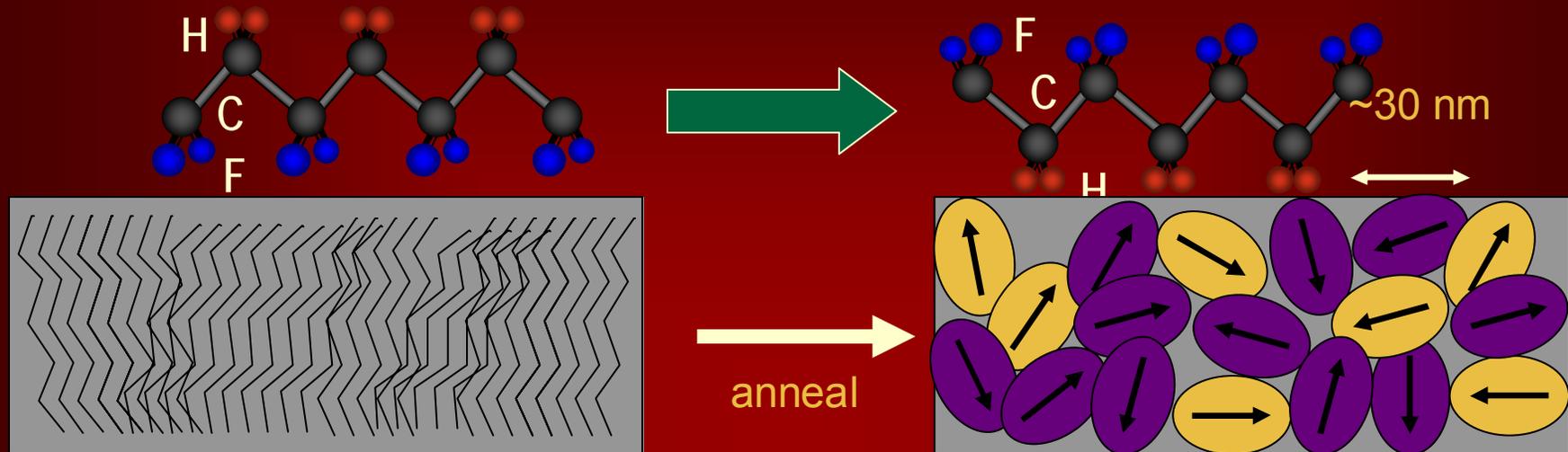
# Ongoing Work - Relaxation Dynamics



The piezoresponse amplitude (red) to the applied bias is delayed. Additionally, once the bias is removed, the response appears to relax to a stable state. This behavior has not been observed on other ferroelectric ceramics, thin films, or nanoparticles.



# Switching Dynamics



boundaries of switched areas are irregular and repeat the grain-pattern of the film

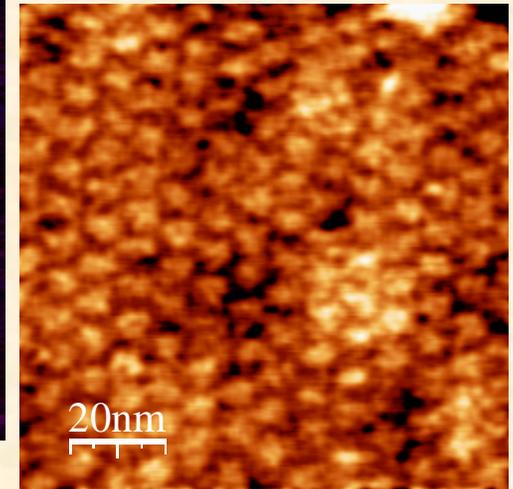
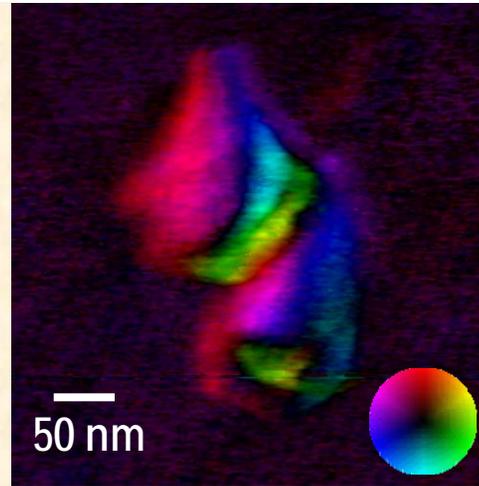
switching in the films occurs on a grain-by-grain basis

the switching was observed to be relatively slow and only prolonged applications of dc bias to the tip ( $> 1-100$  ms) yield switched ferroelectric domains.

We attribute the observed behavior to the molecular nature of PVDF, in which a single dipole can not be rotated without changing the macromolecular conformation.

# Motivation: from Ambient to Liquid

- Does the nanometer scale bring new aspects to ferroelectric behavior and can we see it?
- Can the electromechanical response of a single molecule be probed?
- Can electromechanical response of biosystems be imaged in a physiological environment?

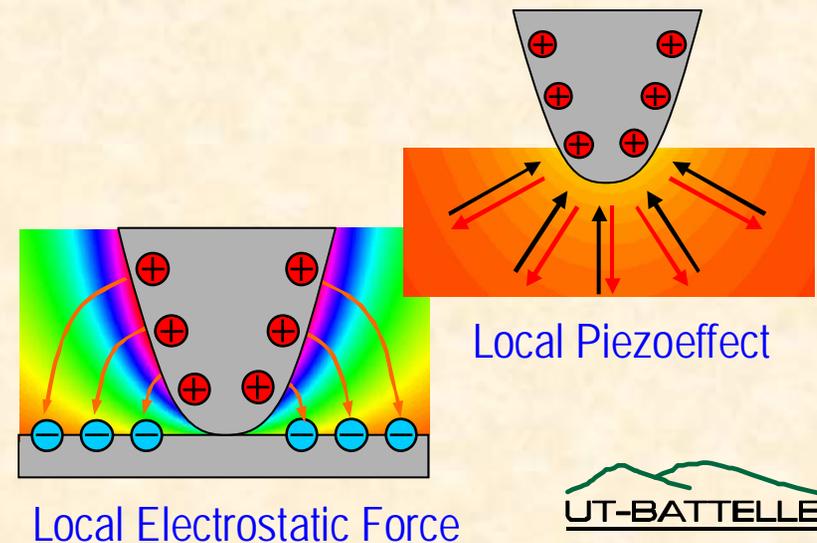


We need improved resolution and sensitivity for nanoferroelectrics, novel electromechanical phenomena, soft materials and biosystems

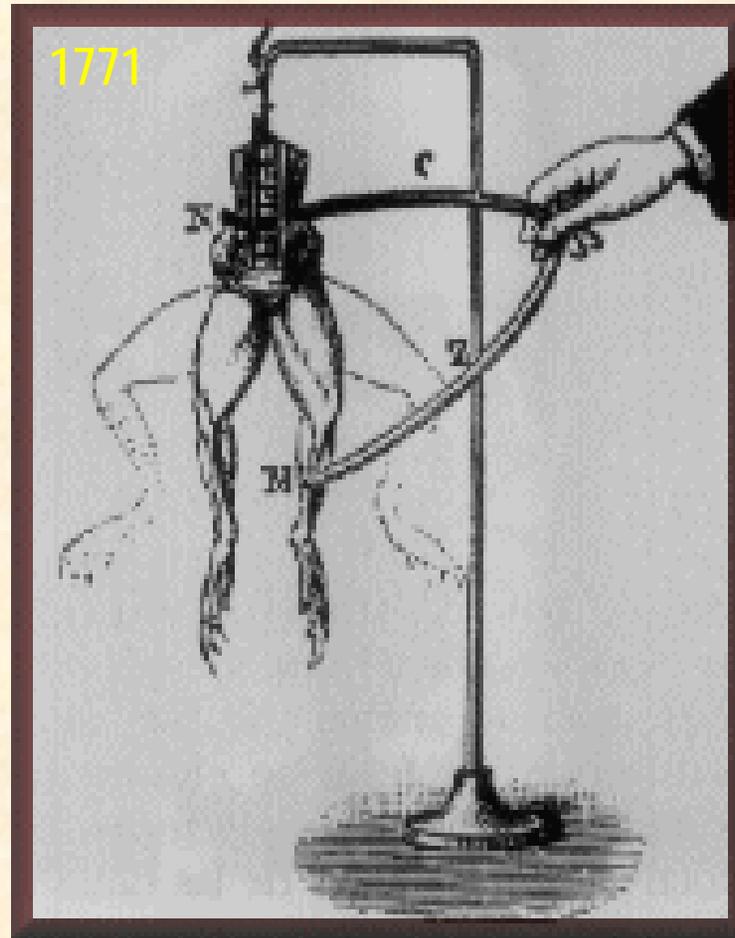
## PFM Limitations: Contact Mode technique

- Electrostatic tip-surface interactions
- Formation of liquid necks at the tip-surface junction
- Large contact force – large contact area

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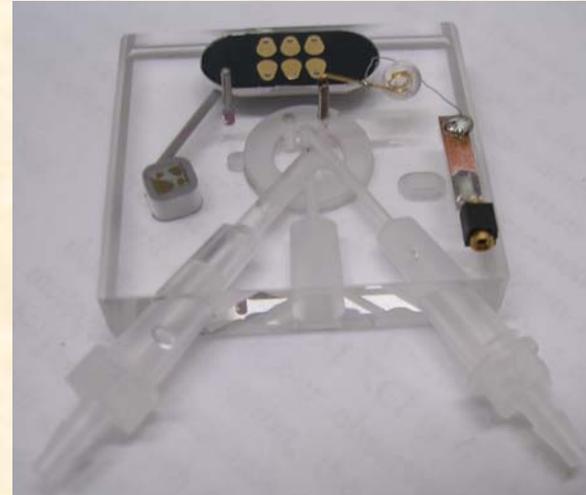
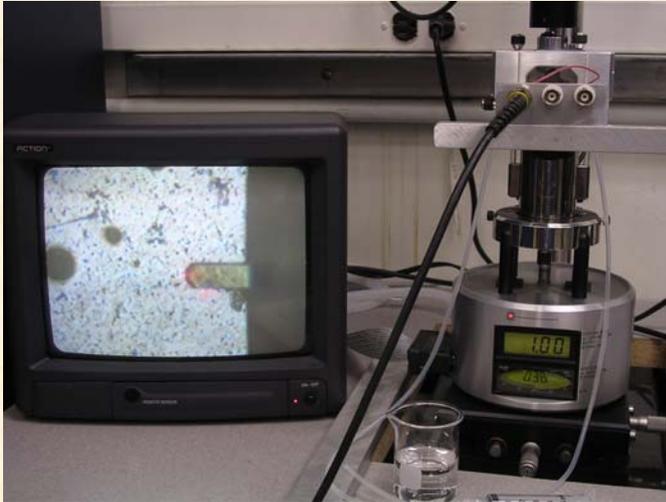
# Electromechanics and the Origins of Physics



Electromechanics in biology: outer hair cell stereocilia, ion channels, electromotors, etc...

- Can we image electromechanics on a cellular level?
- Can we image activity in voltage-controlled ion channel?
- Can we image electromechanics on a single molecule level?

## Experimental Setup for Liquid PFM



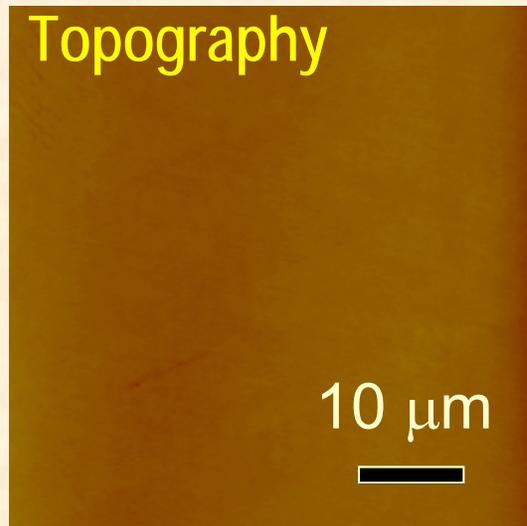
### Advantages

- Sign and magnitude of the Van der Waals interactions can be controlled
- Precise control of tip-surface interactions due to the lack of capillary forces
- Higher dielectric constant of liquid minimizes electrostatic contributions to the signal (force is reduced for  $\kappa > 80$ , force is repulsive for  $\kappa < 80$ )

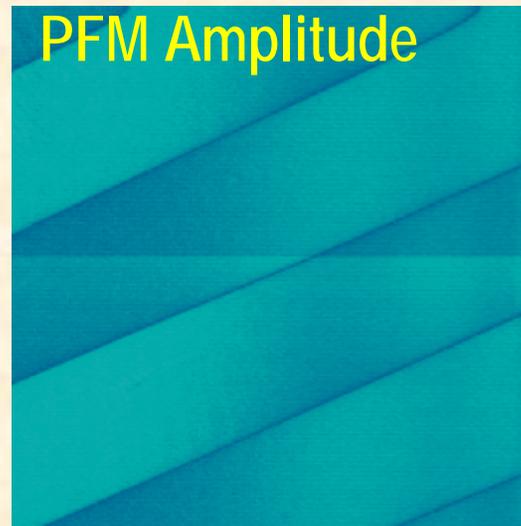
### Disadvantages

- Liquids are not a natural environment for ferroelectric materials
- Conductivity can result in stray currents and potential drops
- Viscous and added mass damping of the cantilever motion
- Formation of electric double layers and other surface effects such as hydrophobic interactions can complicate or preclude imaging

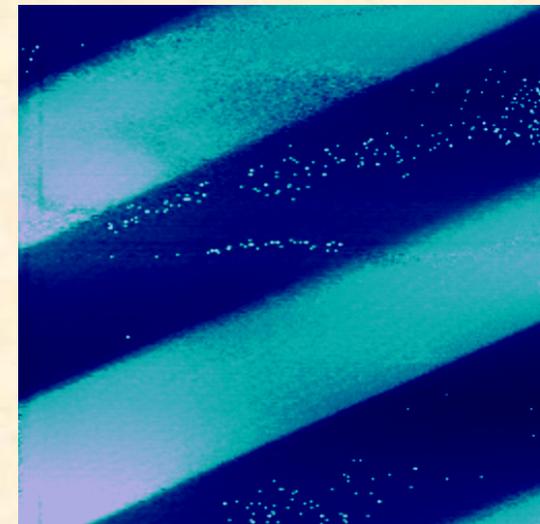
# *First Attempts - PPLN*



5V 200kHz ambient

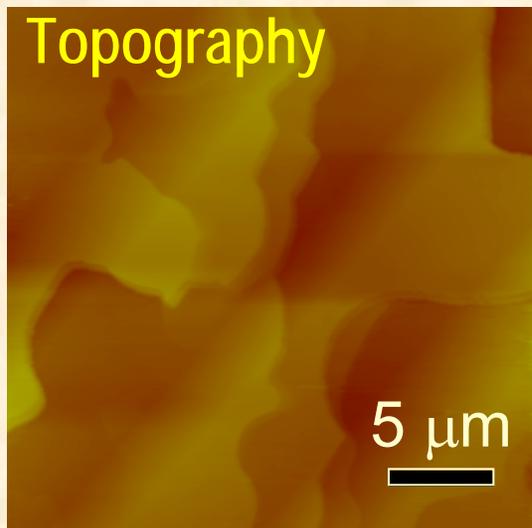


Promising initial result:  
Contrast associated with  
domains

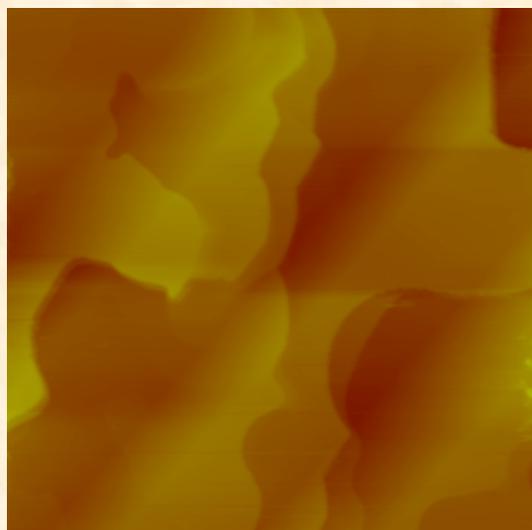
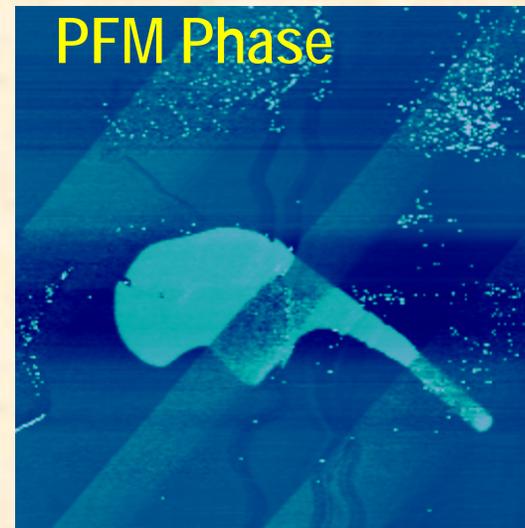
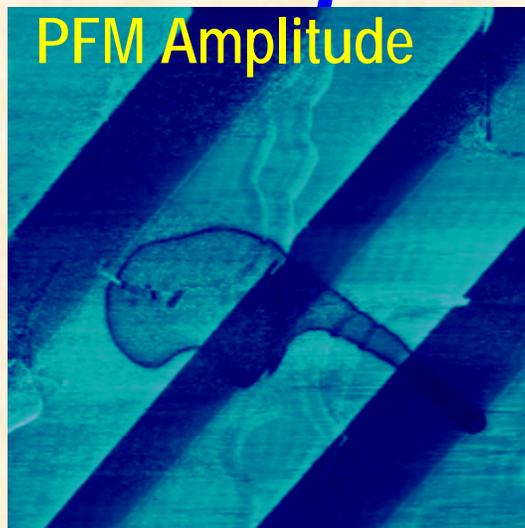


10V 790kHz water

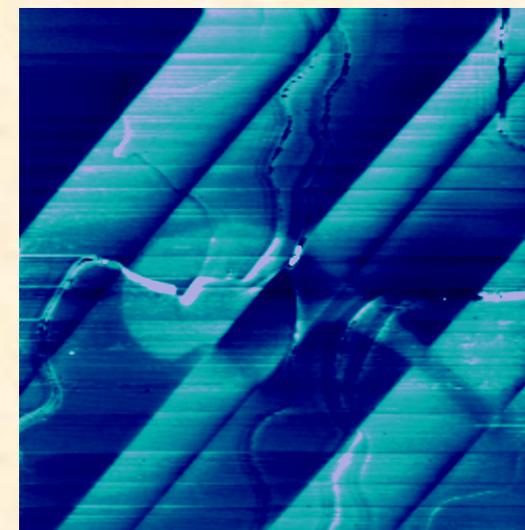
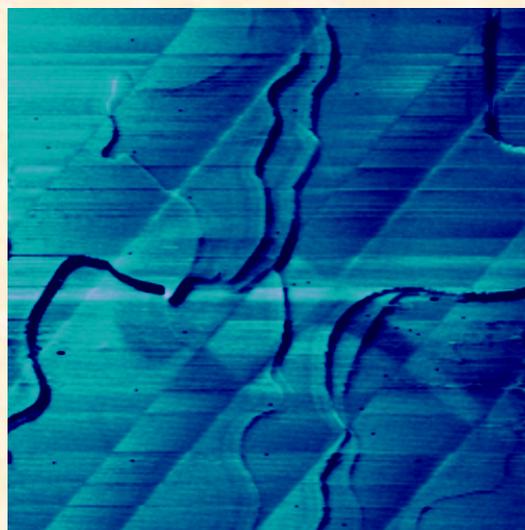
# First Attempts - BTO



10V 630kHz ambient

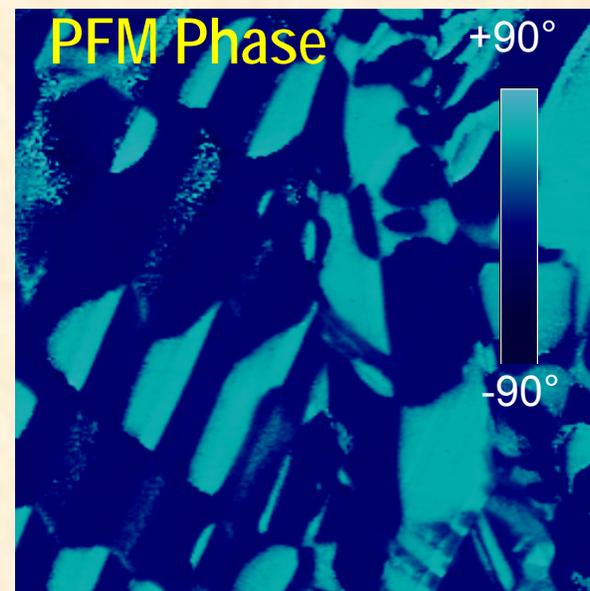
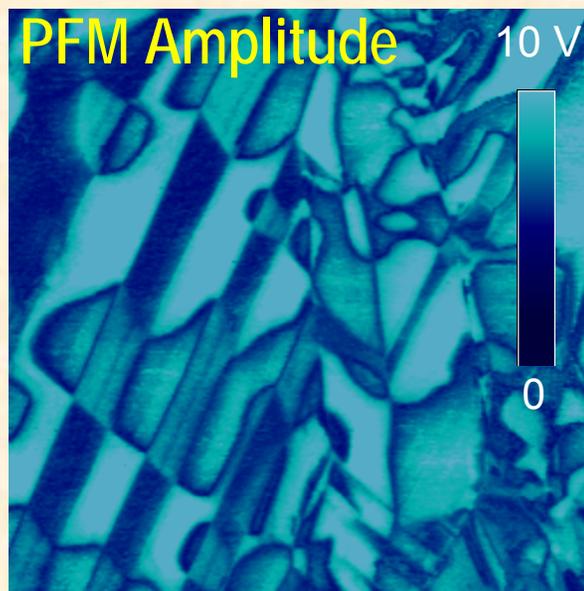
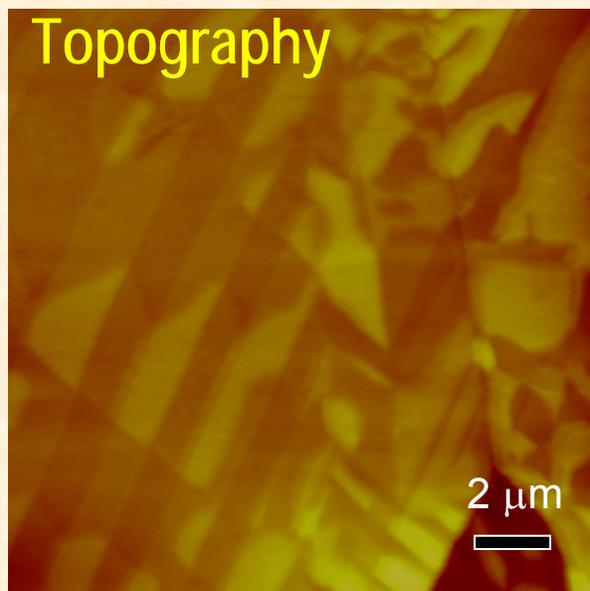


10V 1.8MHz water



# *PFM in Liquid - PZT*

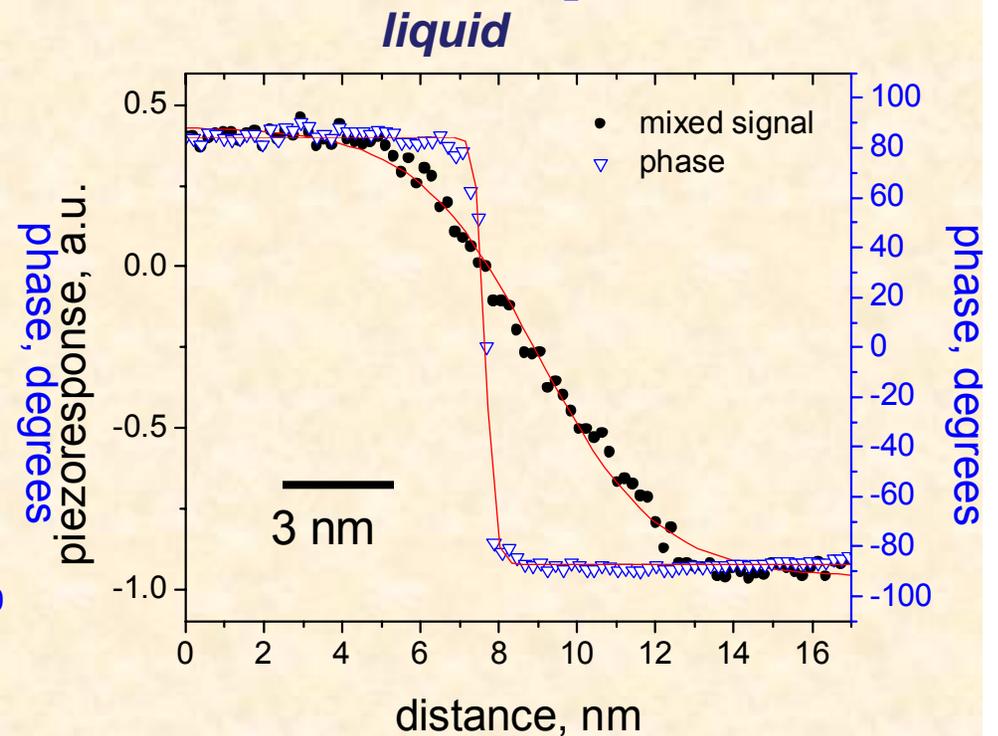
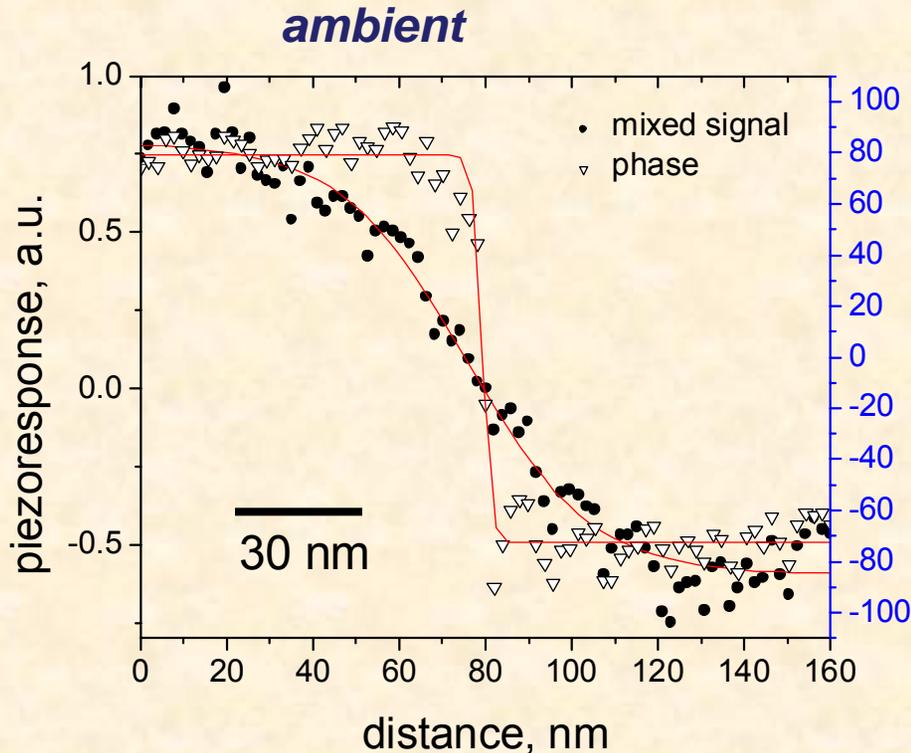
PZT Ceramic



Liquid PFM imaging of PZT shows high-quality contrast.

Interestingly, some of the domain walls in liquid demonstrate extremely small widths

# Resolution: Ambient vs. Liquid



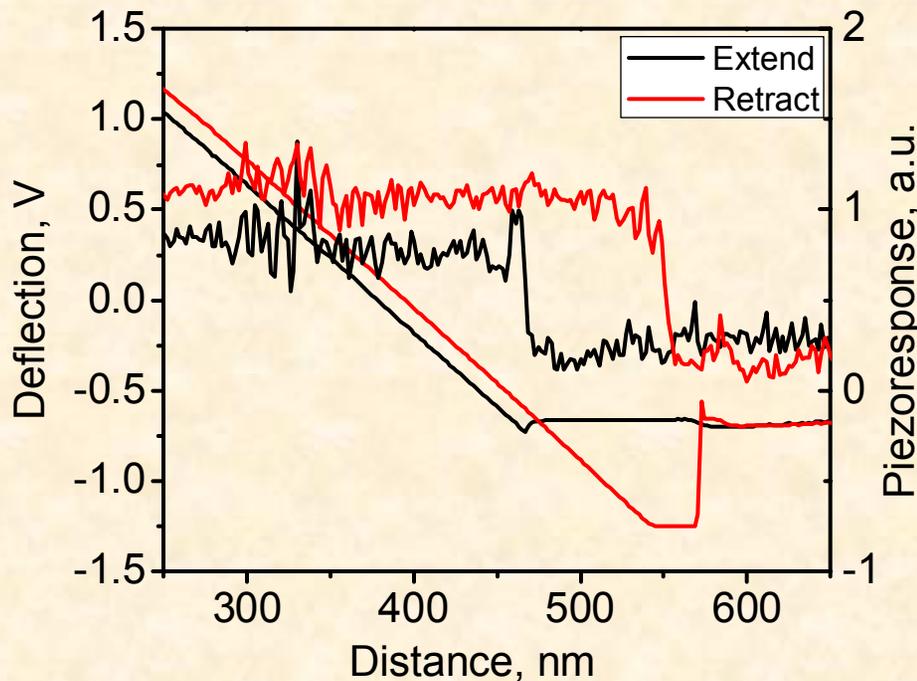
**3 nm for the amplitude signal and 0.2 nm for phase, as compared to ~30 nm and 3 nm, respectively, typically observed in ambient!**

## Why does it work?

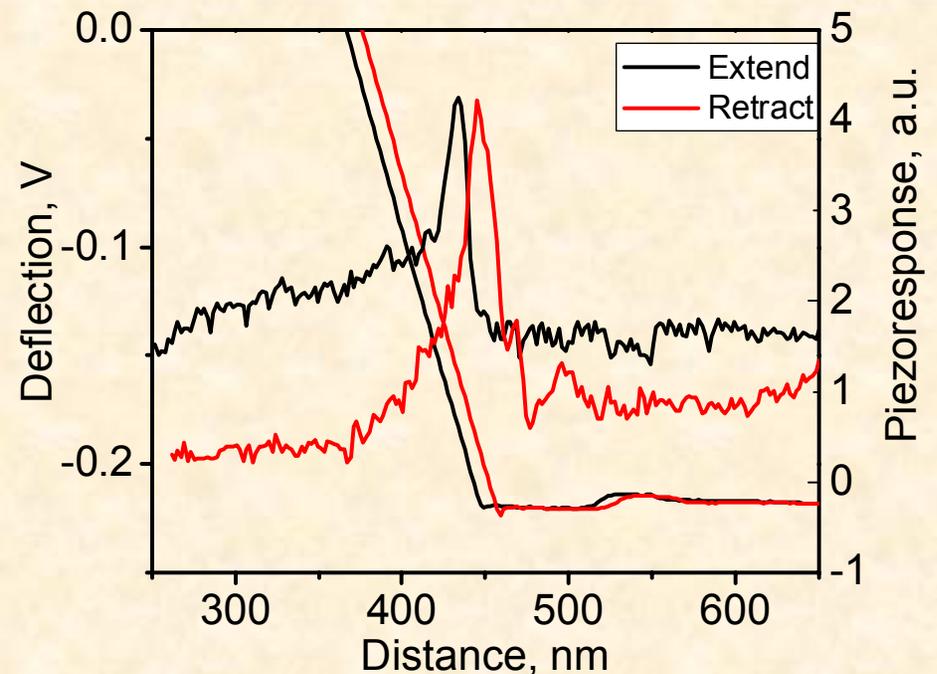
- The absence of long-range electrostatic forces due to screening by mobile ions in liquid
- The absence of capillary interactions.

# Force-Distance Curves

## Ambient



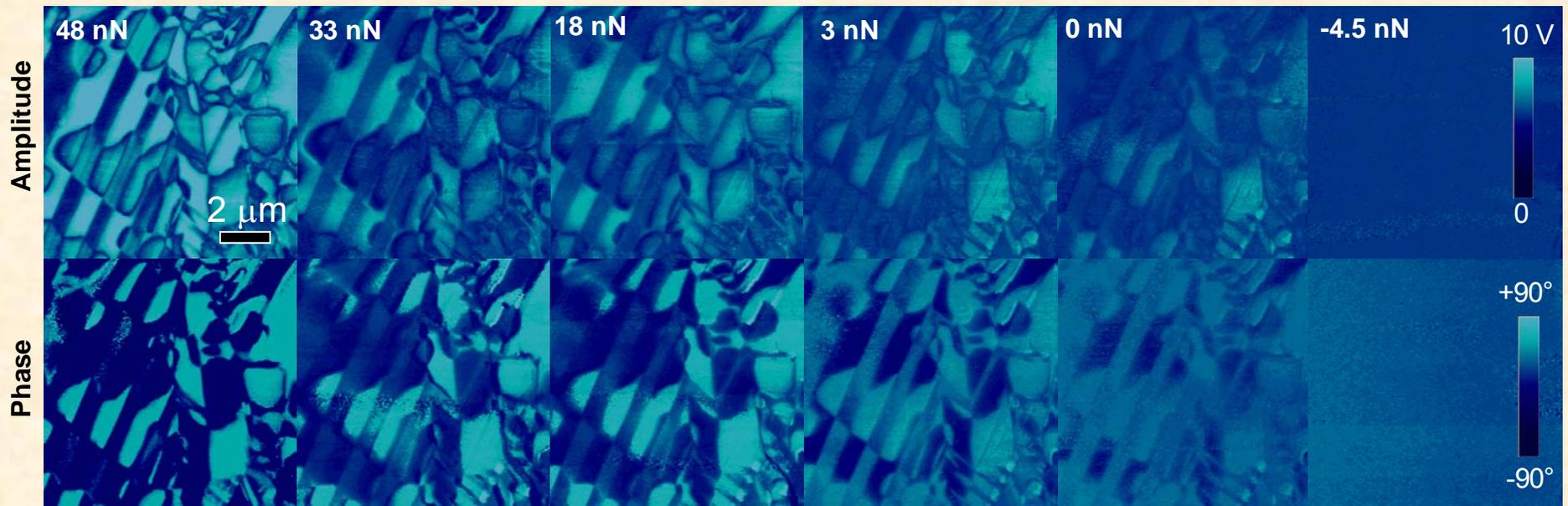
## Liquid



**There is no capillary hysteresis in liquid.**

- In air, hysteresis of  $\sim 100$  nm is observed, corresponding to 30 nN of capillary forces.
- This corresponds to  $\sim 3$  nm contact area even under optimal conditions.
- The resolution is further reduced by liquid bridge formation.

# *Effect of Loading Force*



A series of PFM amplitude (top) and phase (bottom) images obtained at different loading forces.

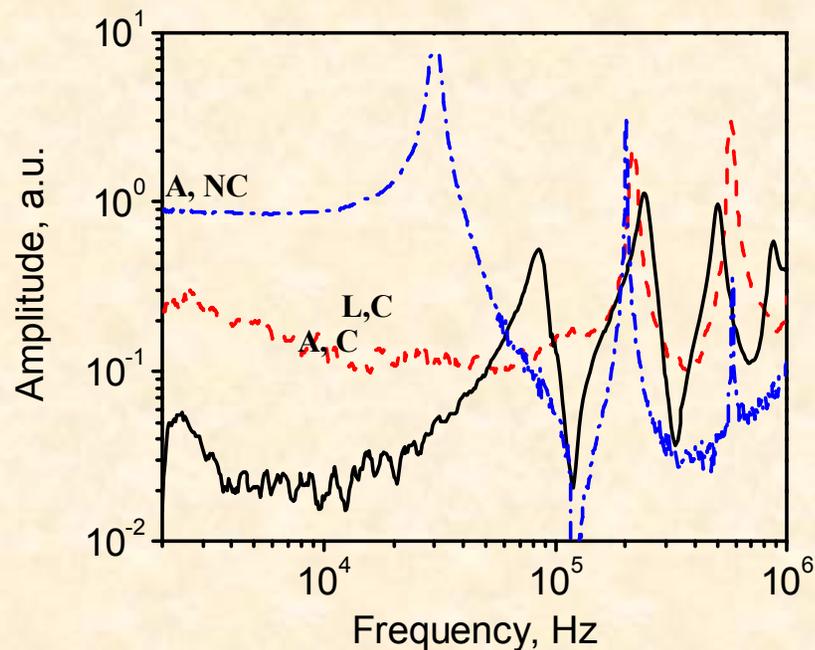
The contrast disappears above the surface

In ambient – contrast remains (EFM & SKPM)

# Cantilever Dynamics

PFM signal amplitude-frequency curves in ambient contact and non-contact regimes and in liquid.

## Ambient vs. liquid

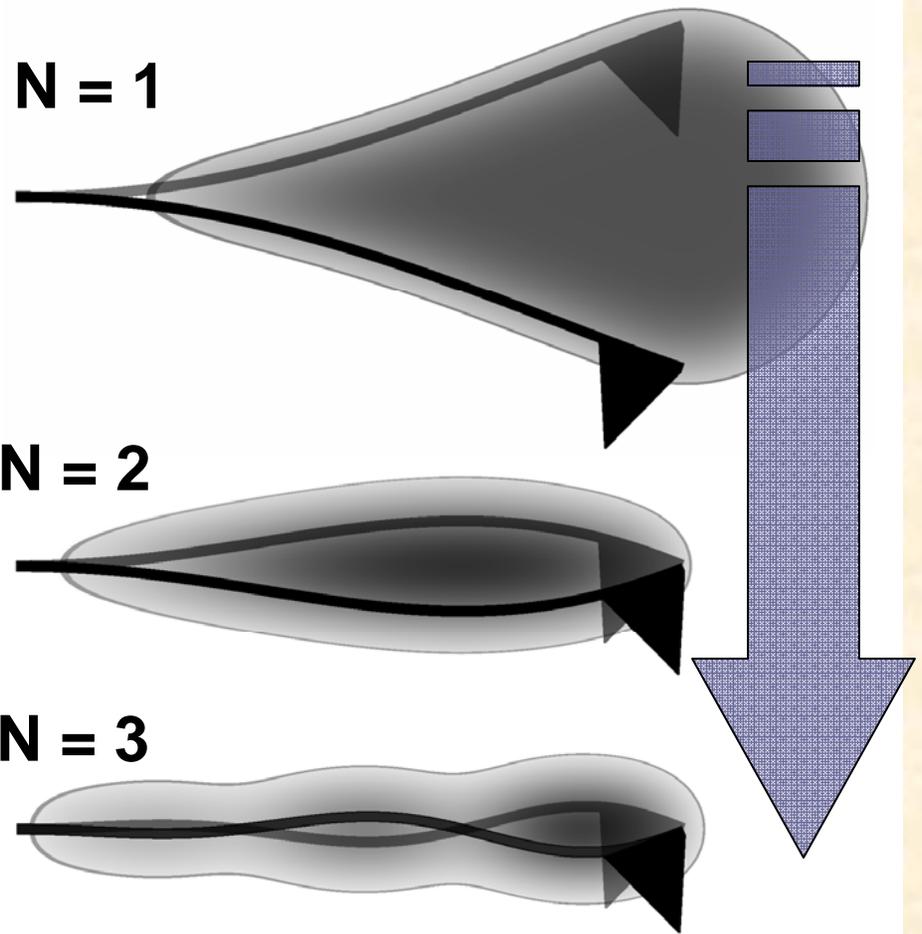


Note that response at resonances is comparable to ambient.

**N = 1**

**N = 2**

**N = 3**



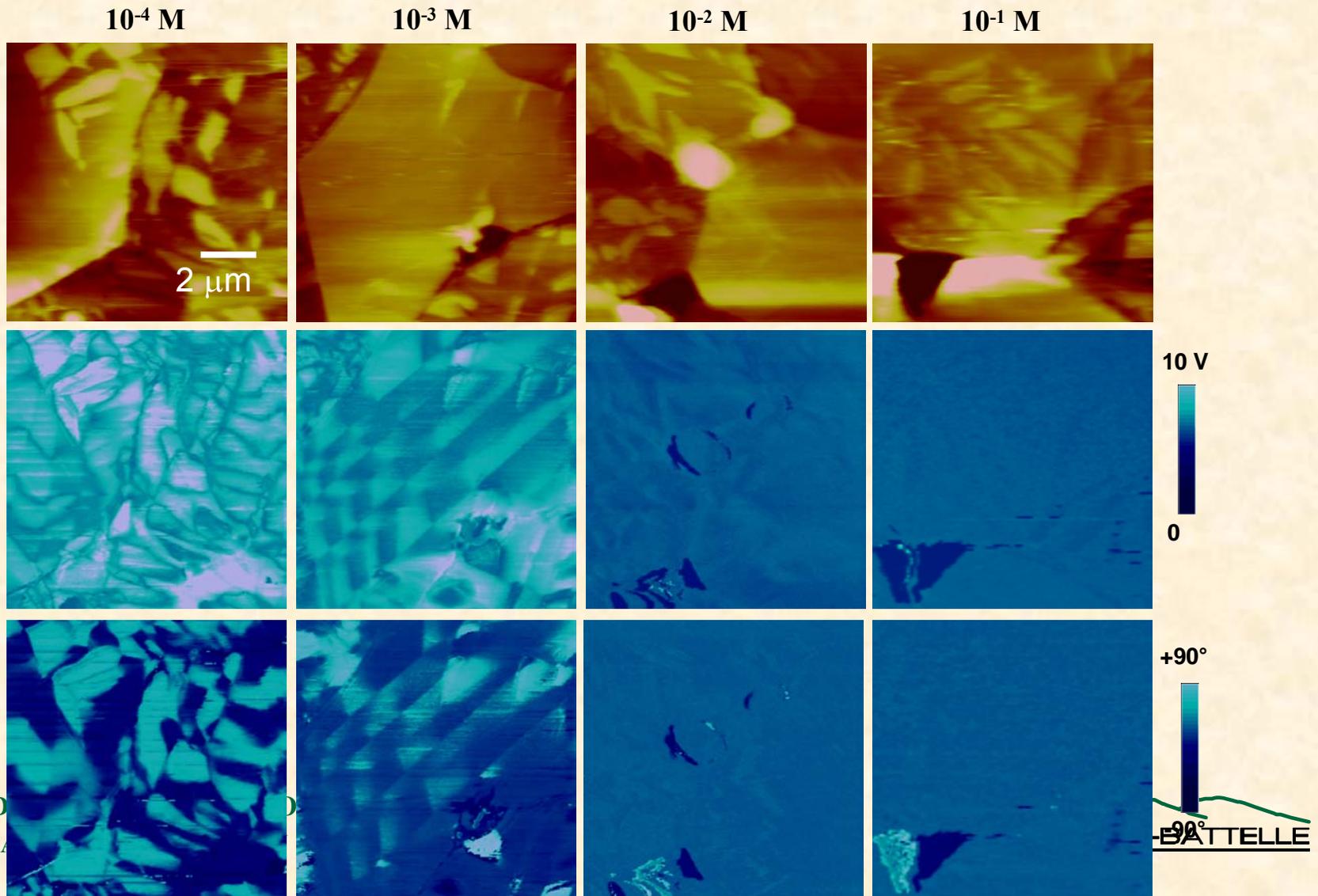
Viscous damping and added mass effects on cantilever dynamics are reduced at high frequencies

Excited volume is reduced at higher modes

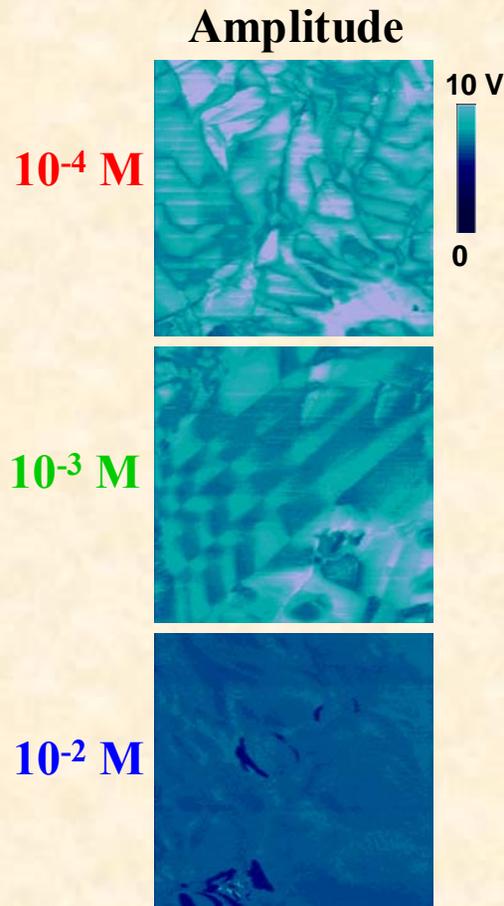
# Effect of Salt Concentration

Concentration dependence of PFM images in NaCl solutions. The images were taken in different locations on the sample.

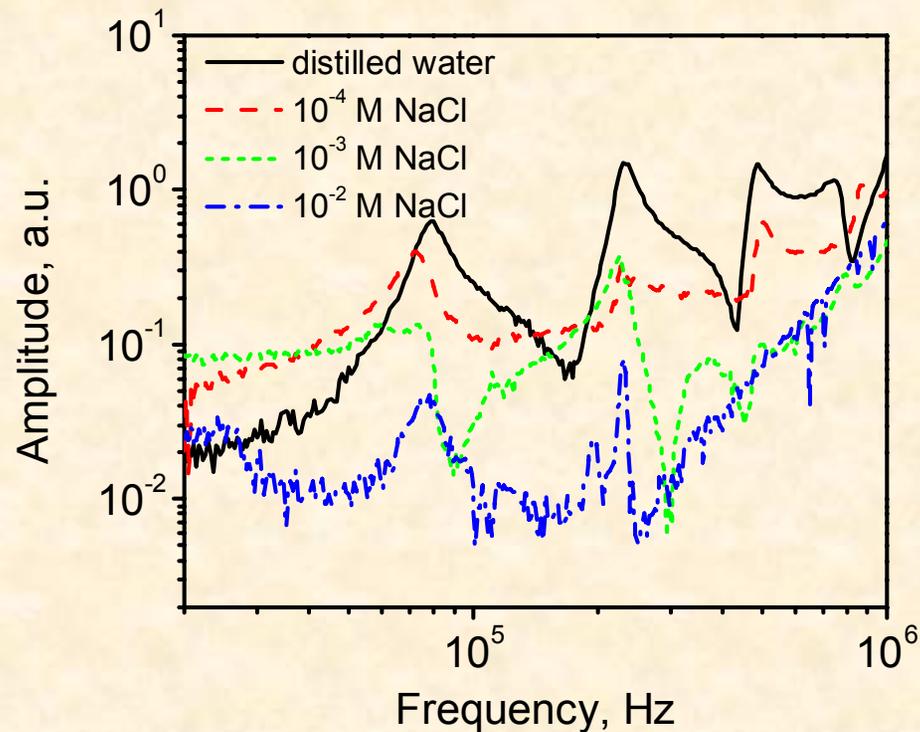
The signal disappears for high ionic strength of solution.



# Effect of Salt Concentration

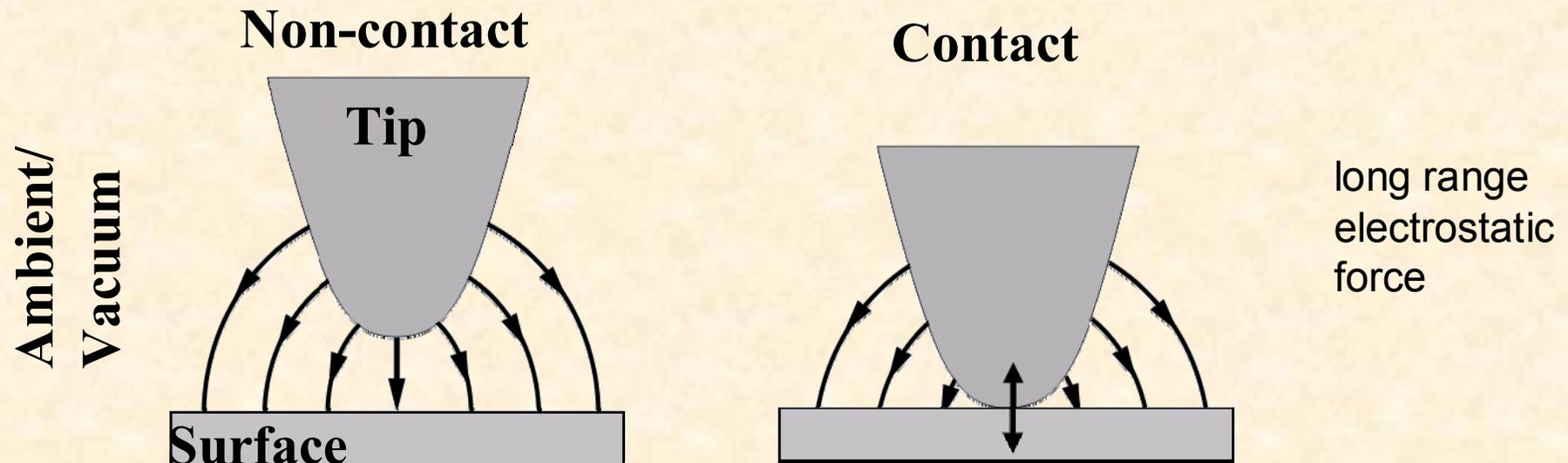


PFM signal amplitude-frequency curves in liquid contact



PFM signal is reduced with increasing salt concentration

# Theory: Ambient Model



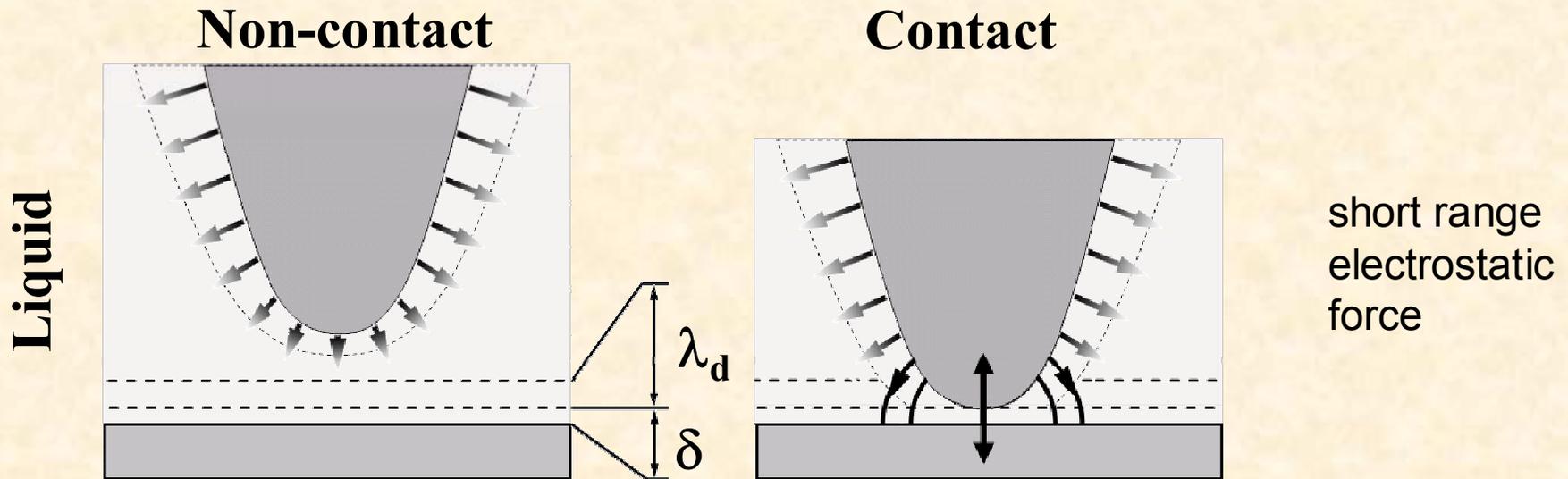
$$PR_{amb} = \alpha_a(h)d_{33} + F_{sphere}/k_1 + F_{cone}/k_1 + PR_{nl}$$

$\alpha_a(h)$  is the ratio of ac tip potential to the ac surface potential of the ferroelectric in ambient (i.e., the potential drop in the tip-surface gap)

$k_1$  is the spring constant of the tip-surface junction

**Both electrostatic and electromechanical interactions are present in ambient**

# Theory: Liquid Model



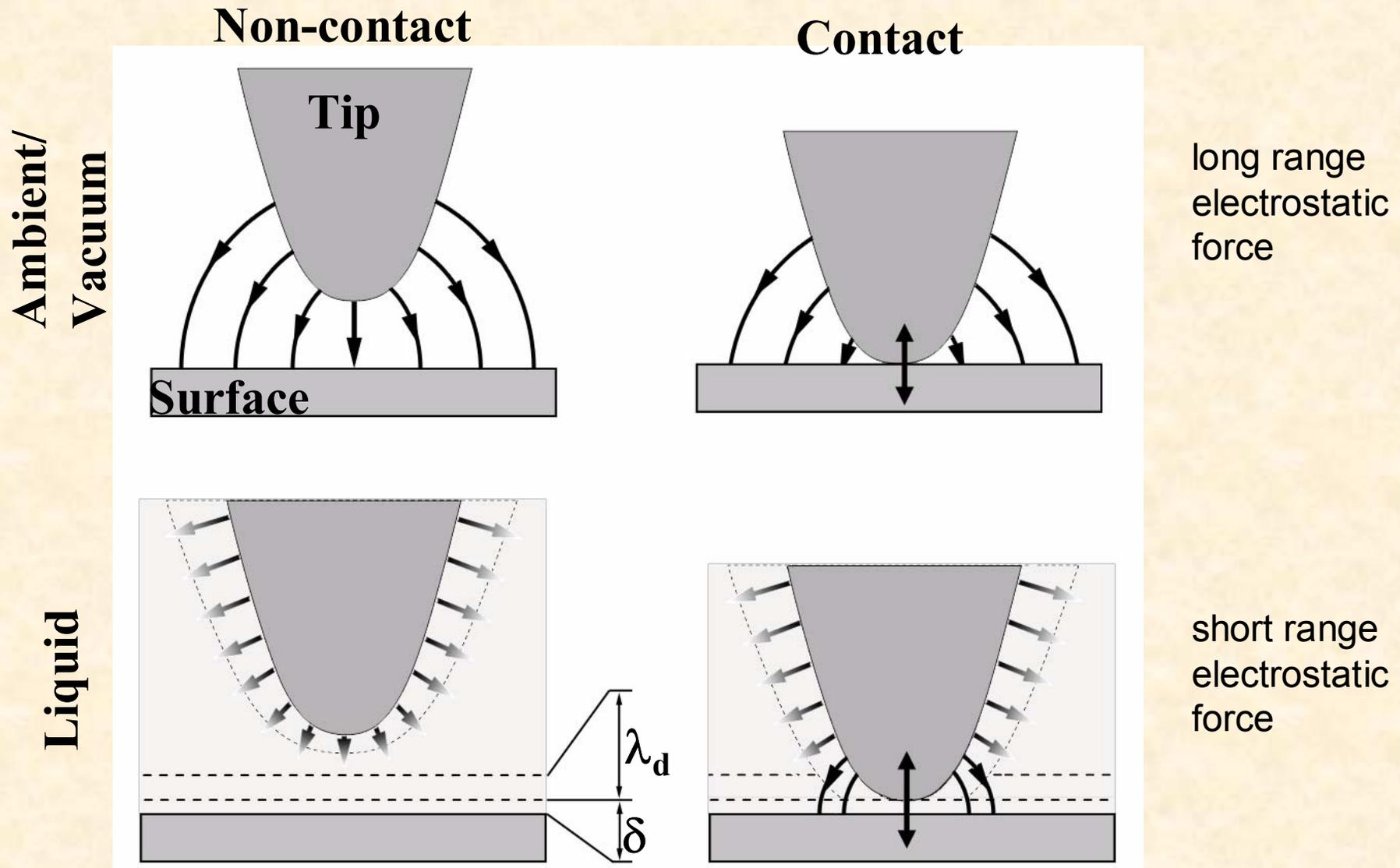
$$PR_l = \alpha(h)d_{33} + \frac{\epsilon\epsilon_0 R}{k_1 \lambda_D} \frac{2V_s \exp(h/\lambda_D)}{\exp(2h/\lambda_D) - 1}$$

electrostatic coupling in the double layer

contribution from the conical part of the tip and cantilever are absent  
 response decays on distances on the order of the Debye length of the solution, and the electrostatic contribution is significantly minimized compared to ambient or vacuum

**electrostatic contributions are minimized in liquid**

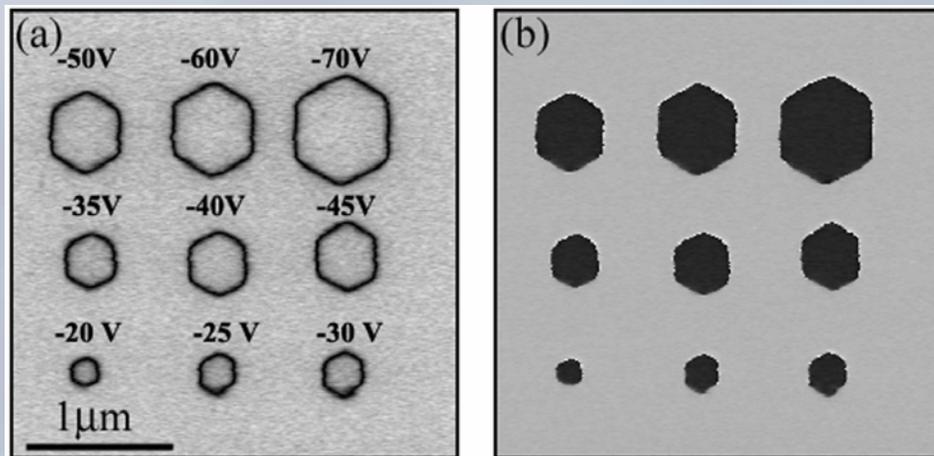
# Theory: Electrostatics



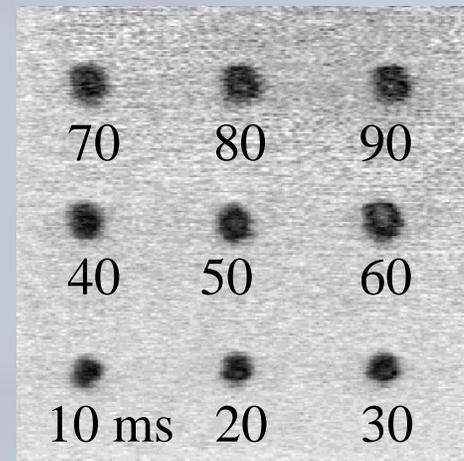
Schematic mechanism of electrostatic and electromechanical interactions in ambient and liquid environments and mechanism for PFM contrast enhancement.

# Switching in solutions?

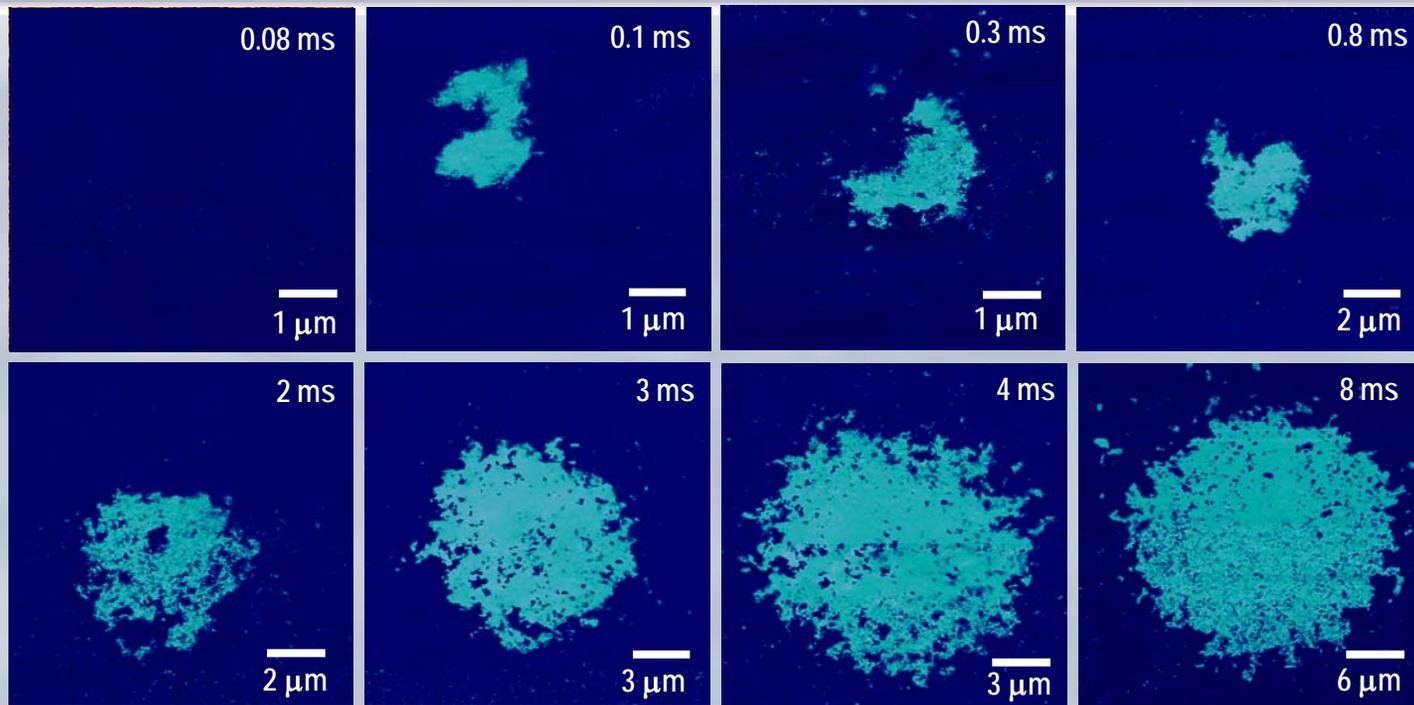
Bias dependence of domain size:



Time dependence of domain size

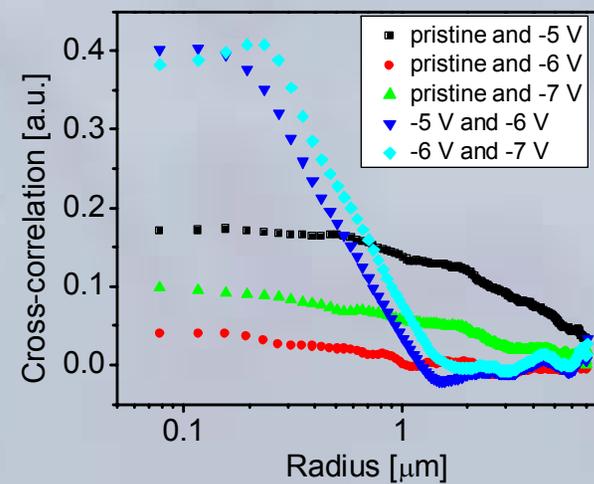
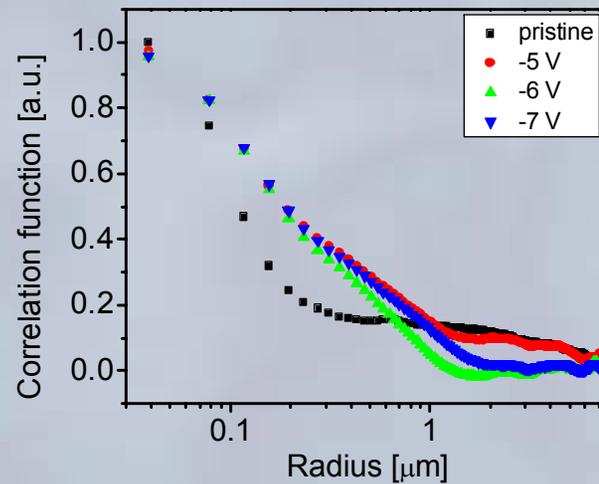
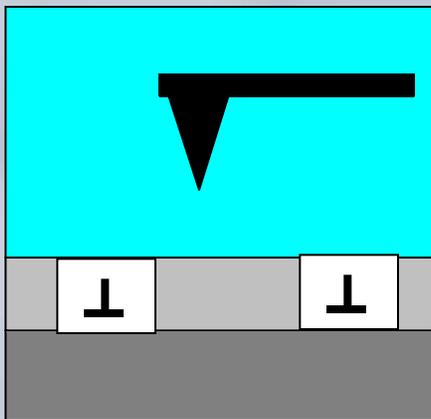
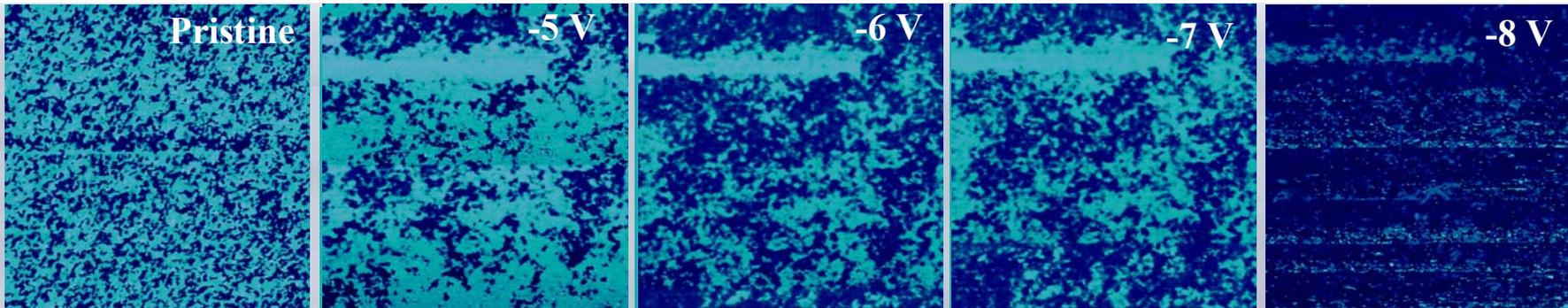


## Localized Switching in Liquids



- Local switching is possible only in non-polar solvents.
- Imaging is possible even in polar solvents
- Localized switching (isopropanol) results in fractal clusters
- **We cannot concentrate dc field!**

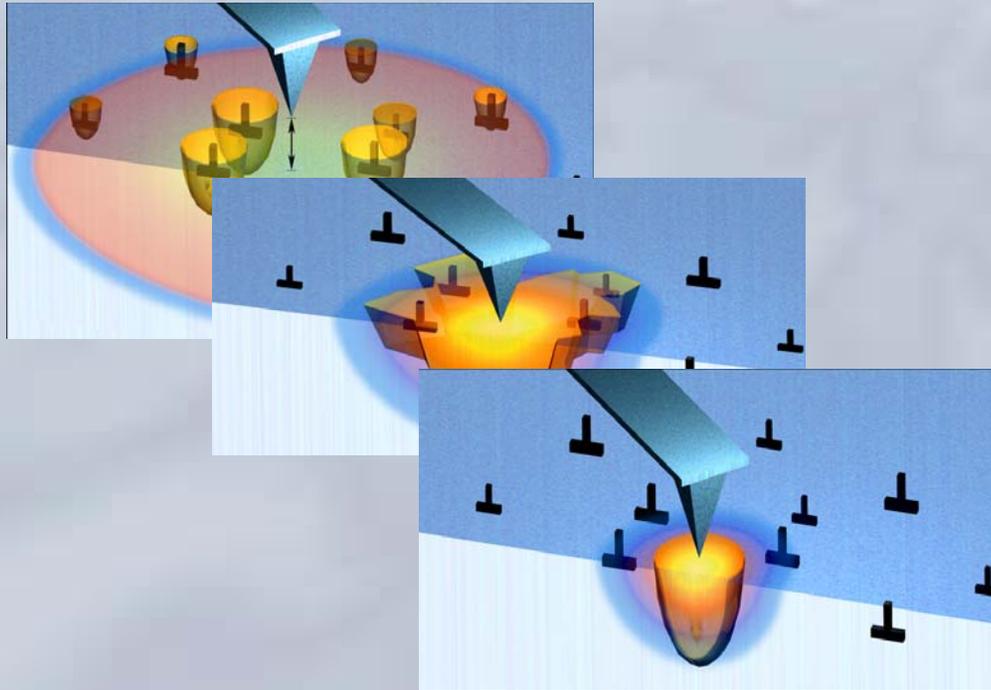
# Non-Local Switching in Liquids



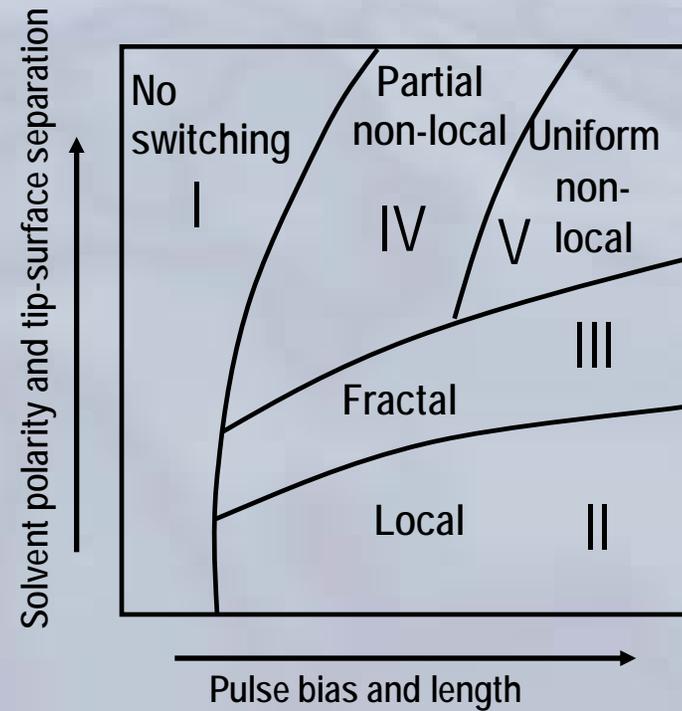
- Uniform switching in liquid allows mapping of switching centers
- Long range interactions are important
- Disorder is frozen

# Solvent Polarity Control of Switching Mechanism

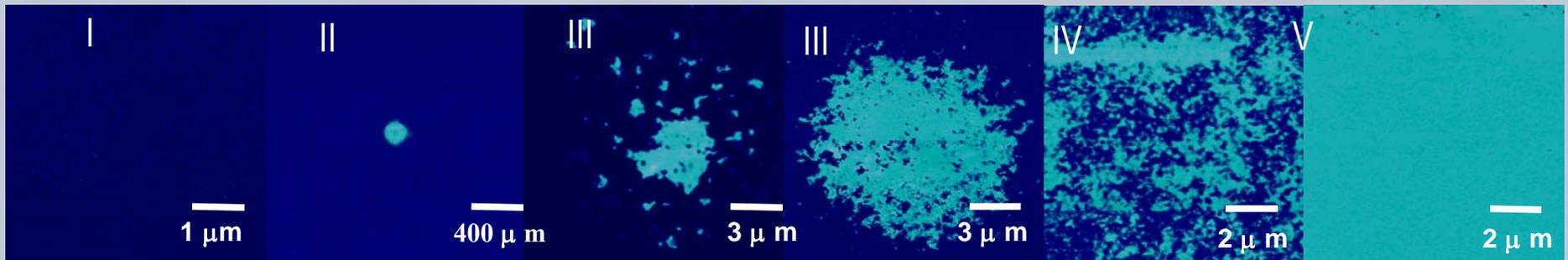
## Switching mechanism



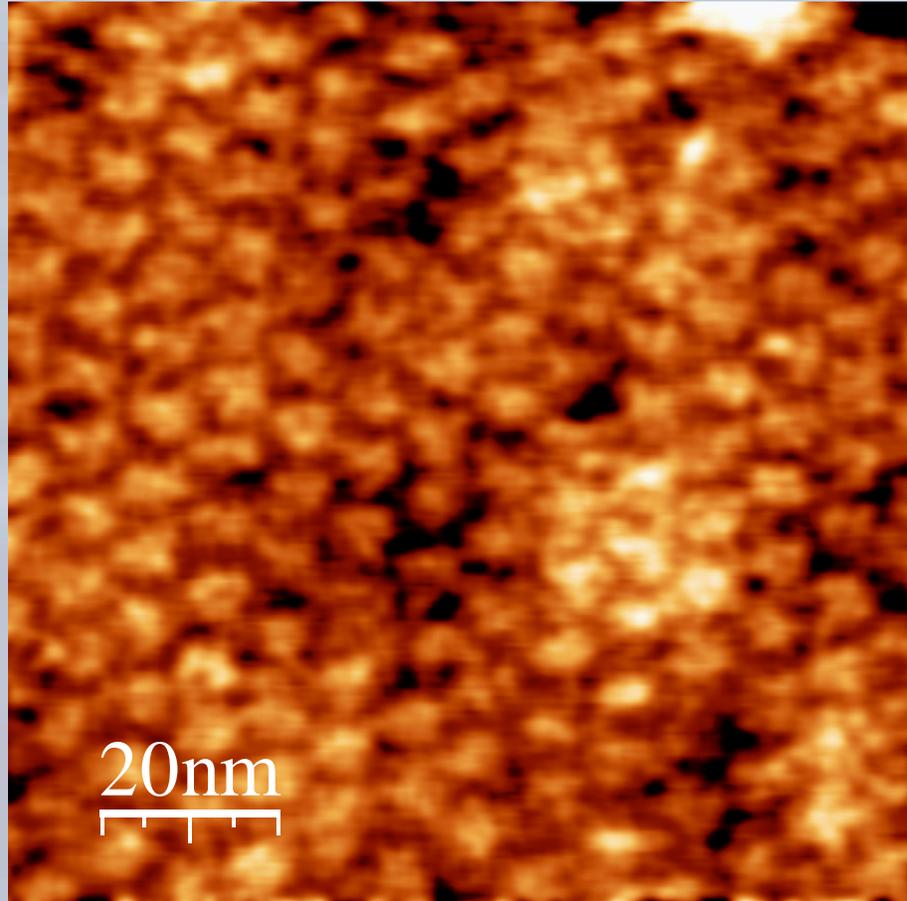
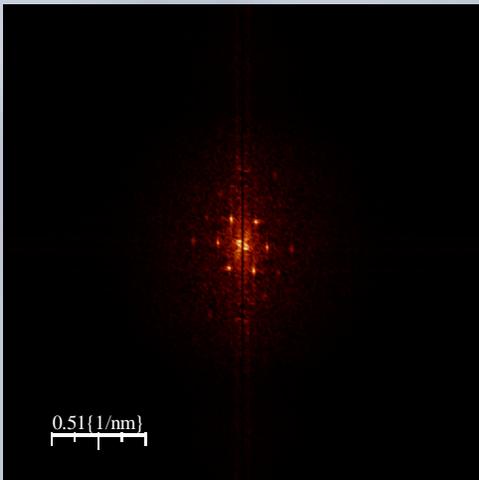
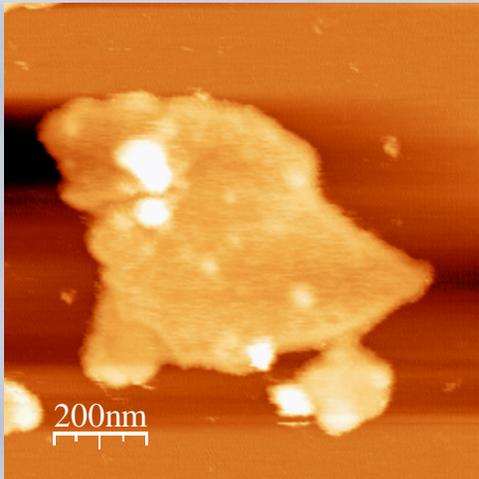
## Switching map



## Experimental domain morphologies

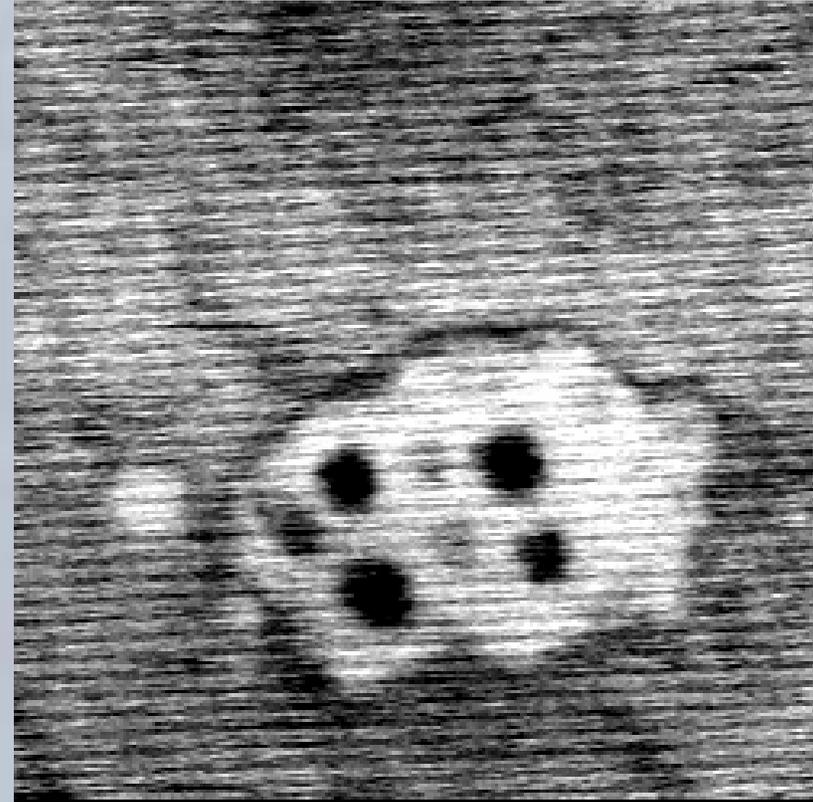
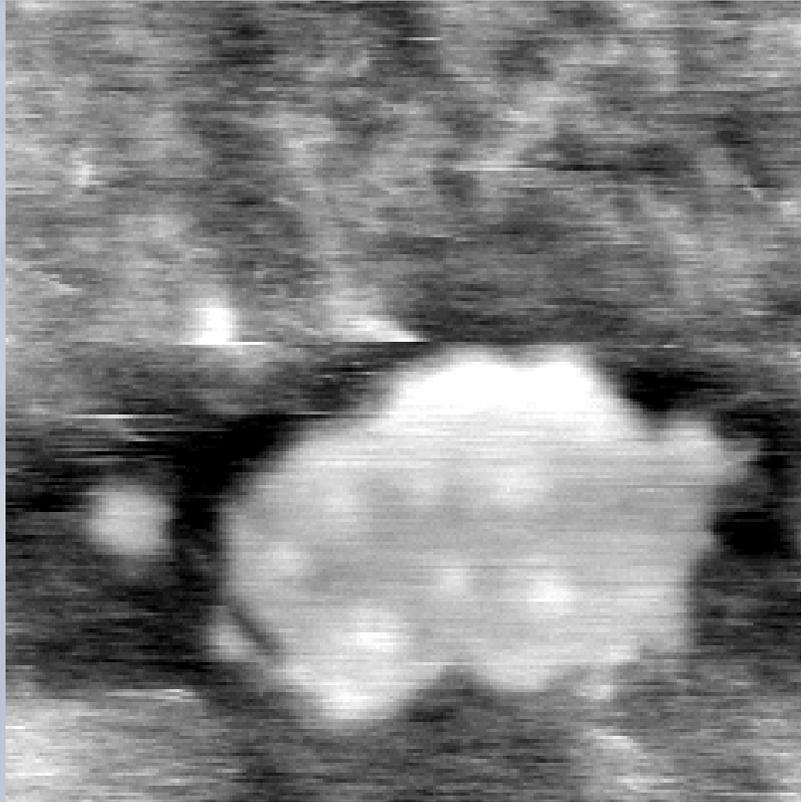


# *Imaging Membranes with Metallized Tips*



Tapping mode image

## *Liquid PFM of Protein Membrane*

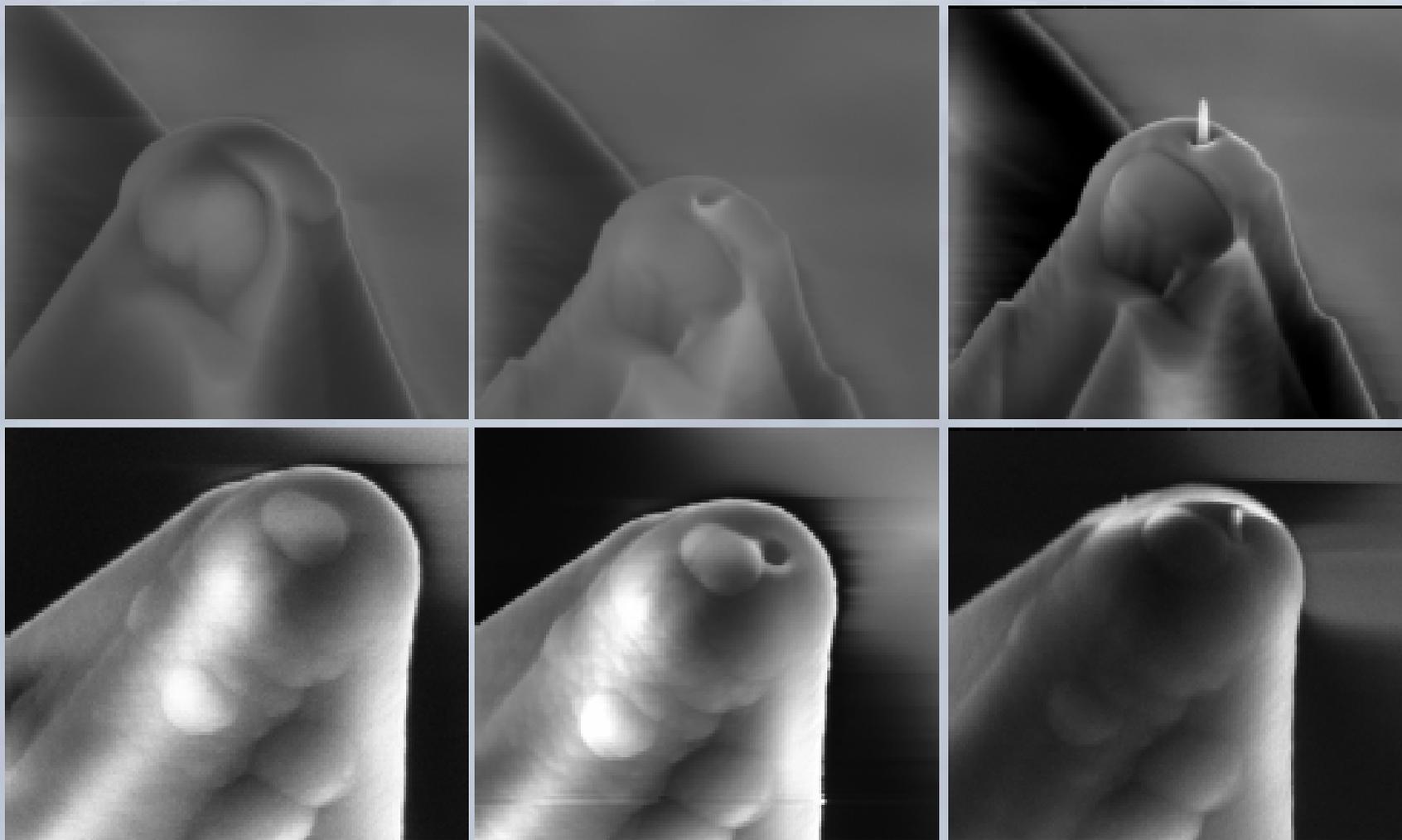


Imaging of the purple membrane, bacteriorhodopsin, with an ac-field in buffer solution reveals possible polarity induced contrast between extracellular and cytoplasmic regions.

Image by B. Rodriguez, I. Revenko, and S. Holbauch

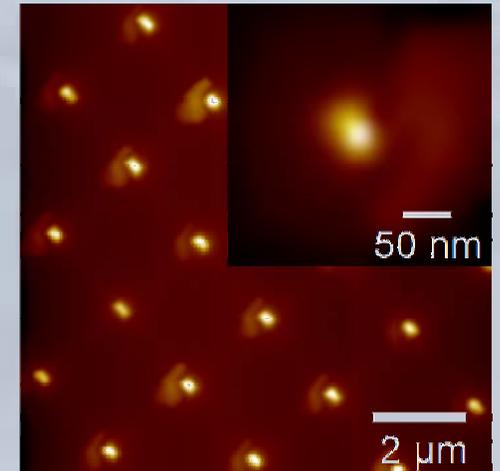
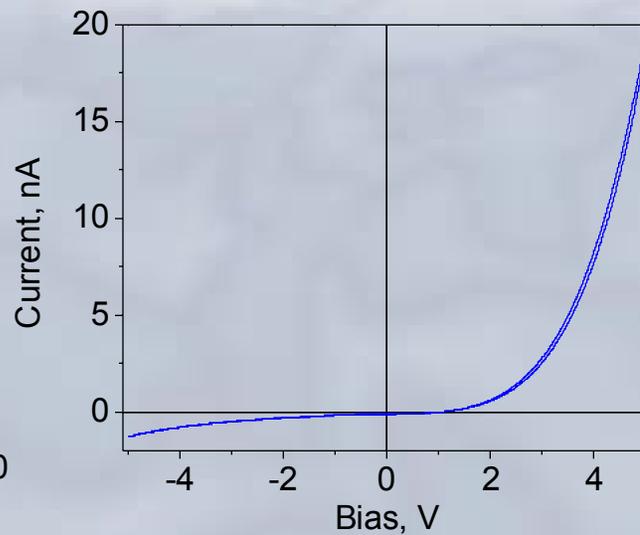
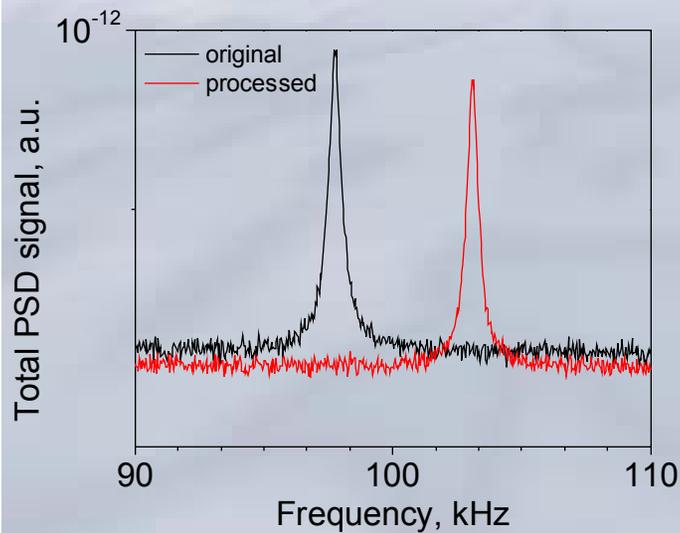
## *Development of Shielded Probes*

Electromechanical imaging in liquids requires insulated and shielded probes



Development by P.D. Rack, ORNL/UT

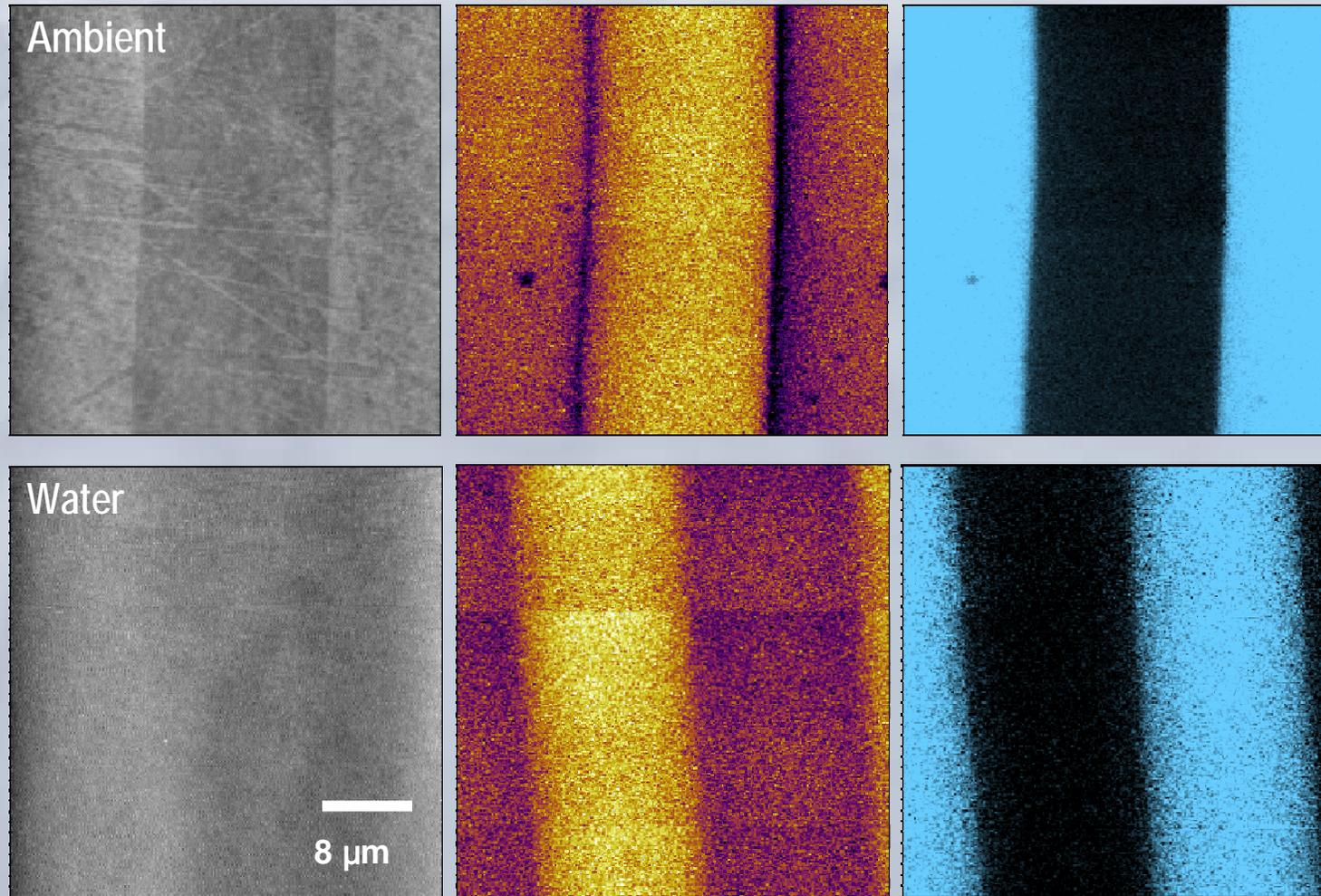
# Probe Characterization: Dynamics and Conductivity



Tip	Sum (V)	Resonance (kHz)	Q	k (N/m)	InvOLS (nm/V)
Tip 1 before	4.14	97.76	288	9.82	95.43
Tip 1 after	4.25	103.10	335.6	13.42	87.78
Tip 2 before	5.61	66.74	156	1.78	75.75
Tip 2 after	5.49	76.14	244.2	3.59	79.72

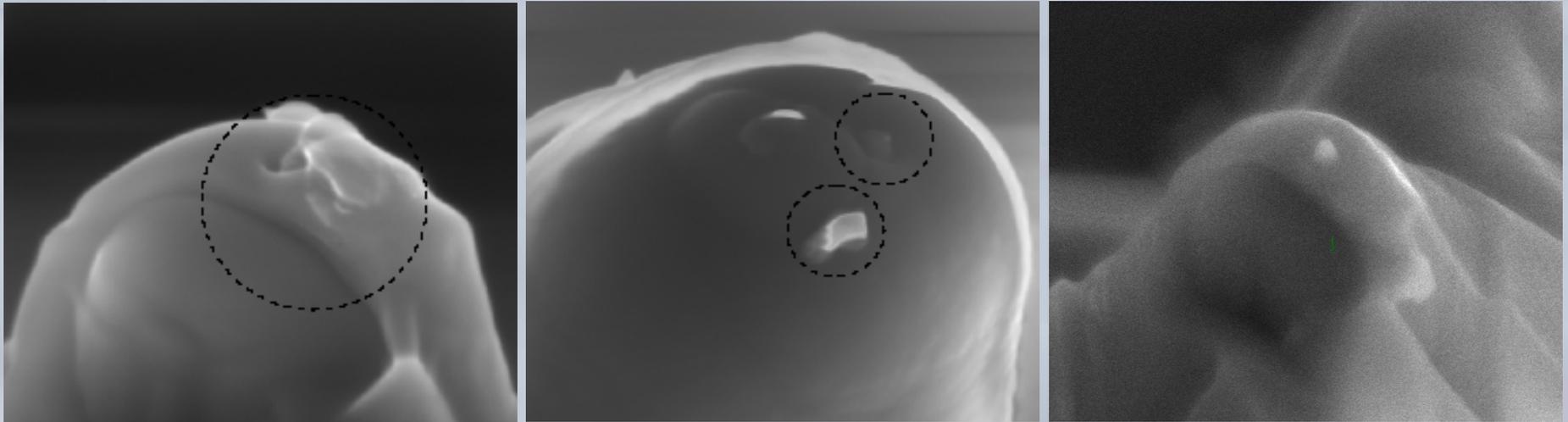
Stiffness increases. Conductance is non-ohmic.

# Probe Characterization: Dynamics and Conductivity



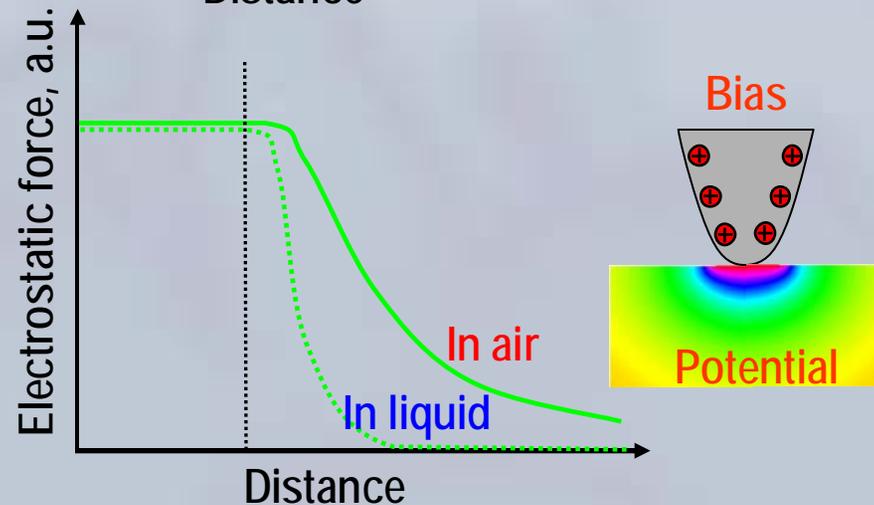
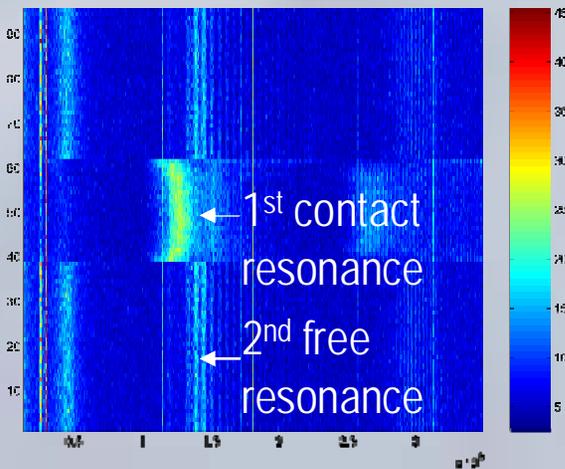
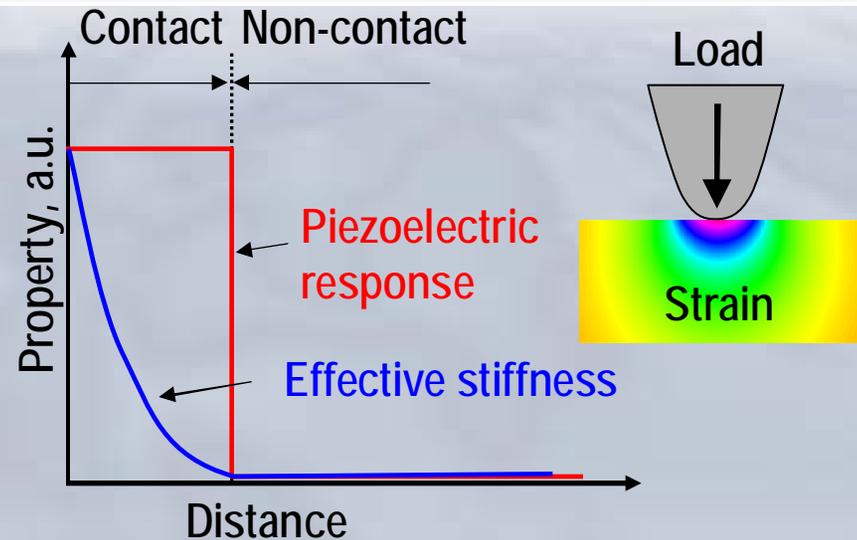
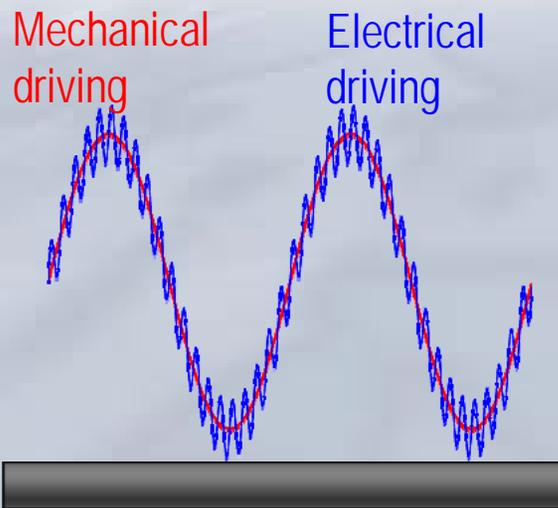
Tip allow PFM contrast both in ambient and in liquid environments.

## *Probe Modification and Repair*



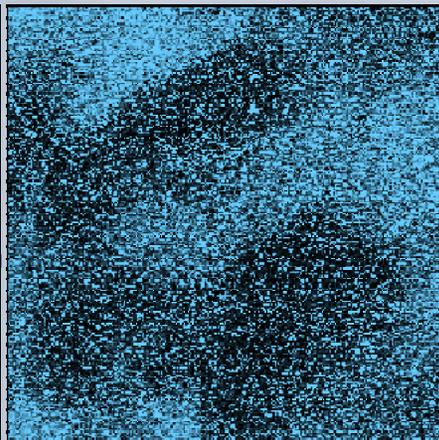
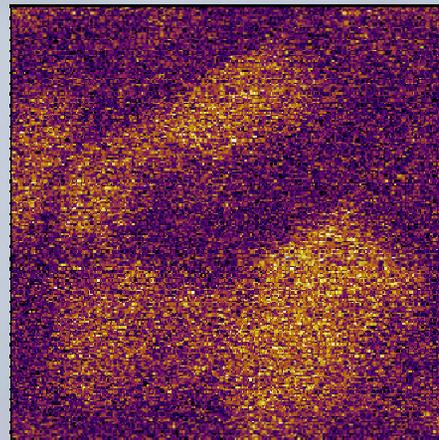
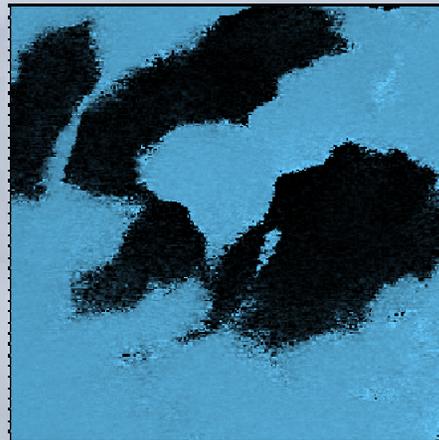
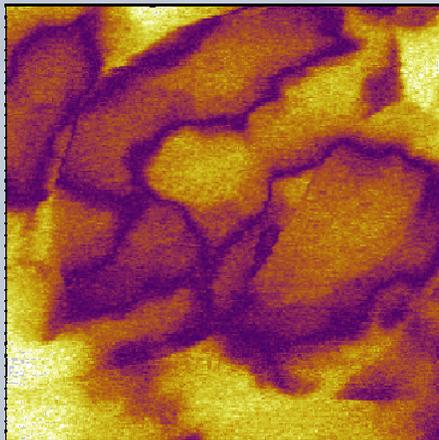
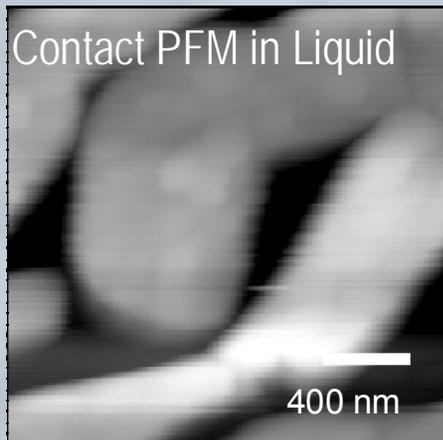
Tips can be repaired.

# Intermittent Contact Mode PFM?

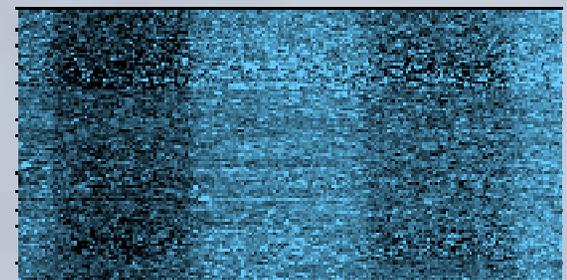
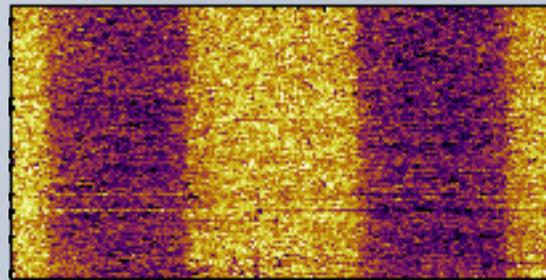
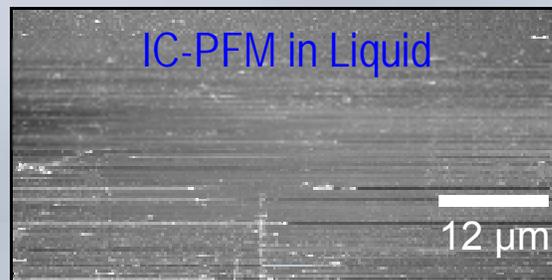
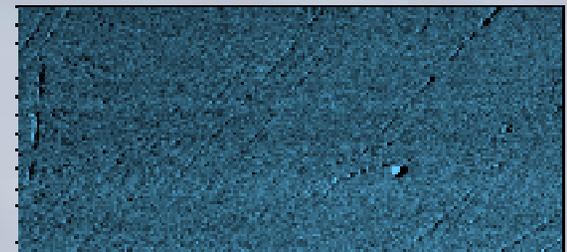
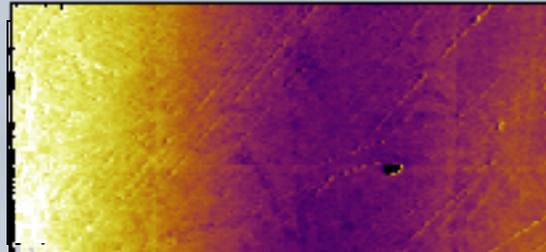
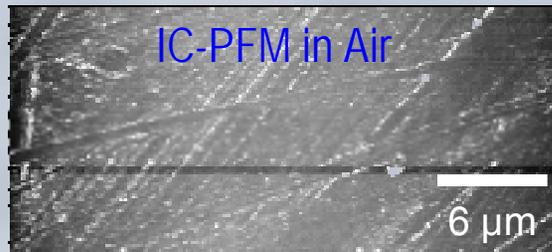
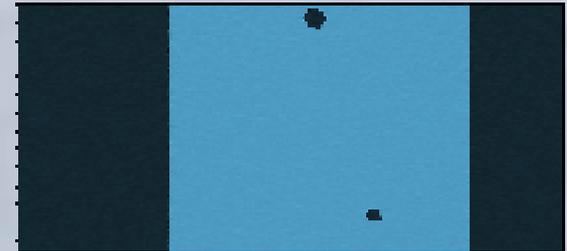
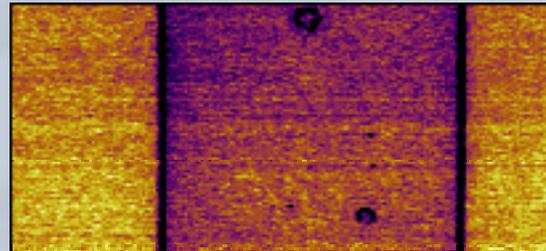


Intermittent contact PFM would be perfect for high resolution imaging. However, electrostatic forces are long range and are expected to dominate signal. ..

# First Results: PZT

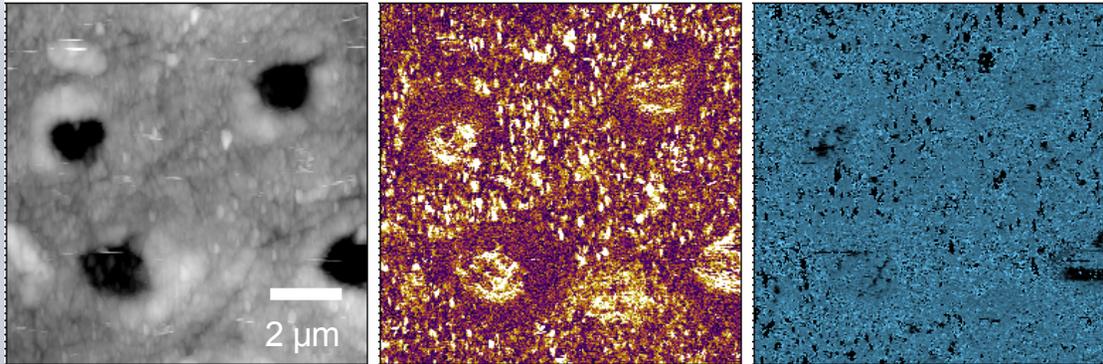


# First Results: PPLN

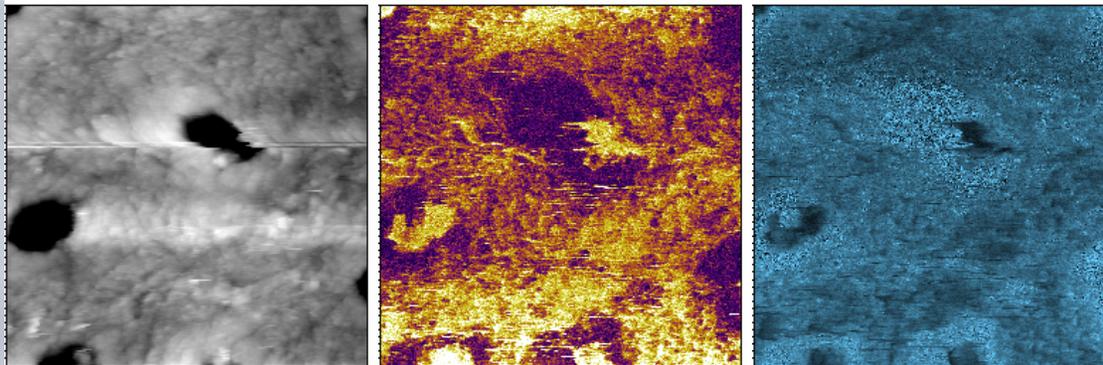


# First Results: Dentin

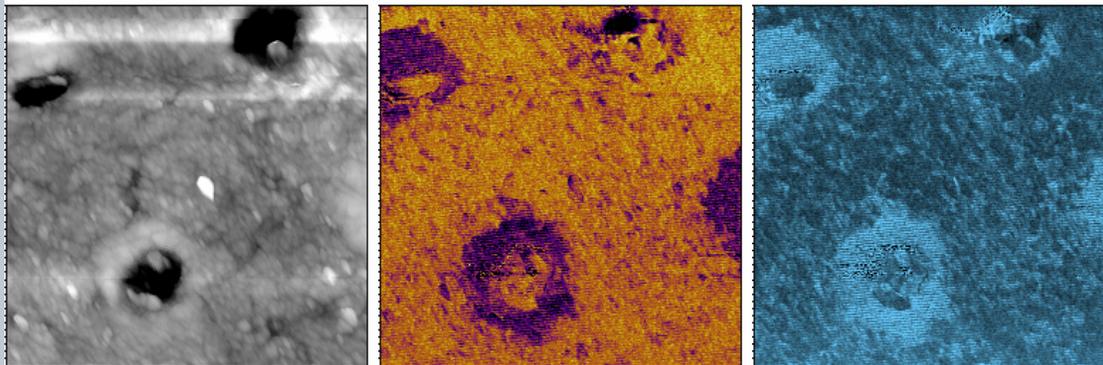
Contact PFM



PFM in Liquid



IC-PFM in Liquid



# Summary

- In the decade since invention, PFM has become a primary tool for imaging, spectroscopy, and modification of ferroelectric materials on the nanoscale.
- PFM is applicable for high resolution imaging of ultrastructure of connective and calcified tissues utilizing piezoelectricity of collagen and proteins
- PFM in liquids opens pathways to many interesting and unusual phenomena, including electromechanics of biological systems
- Kelvin Probe and Electrostatic Force Microscopies can be implemented in Liquid

