Lecture 26
AFM in liquids

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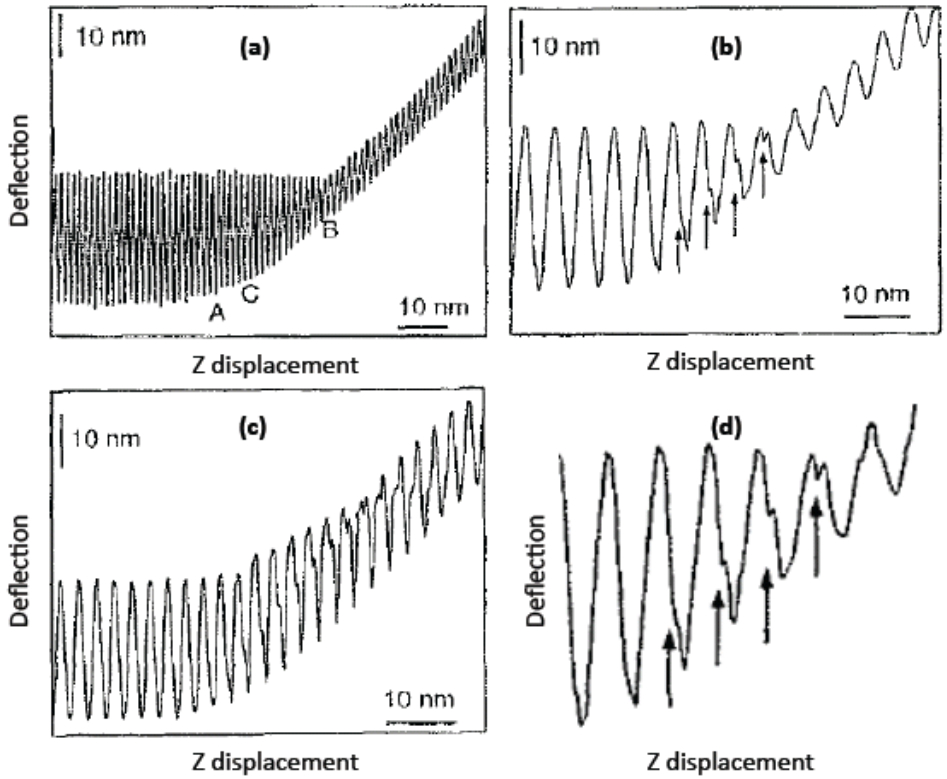
How does a tip tap in liquids?



Many difference in approach curves between air and liquids

C. A. J. Putman, et al., Applied Physics Letters, 1994.

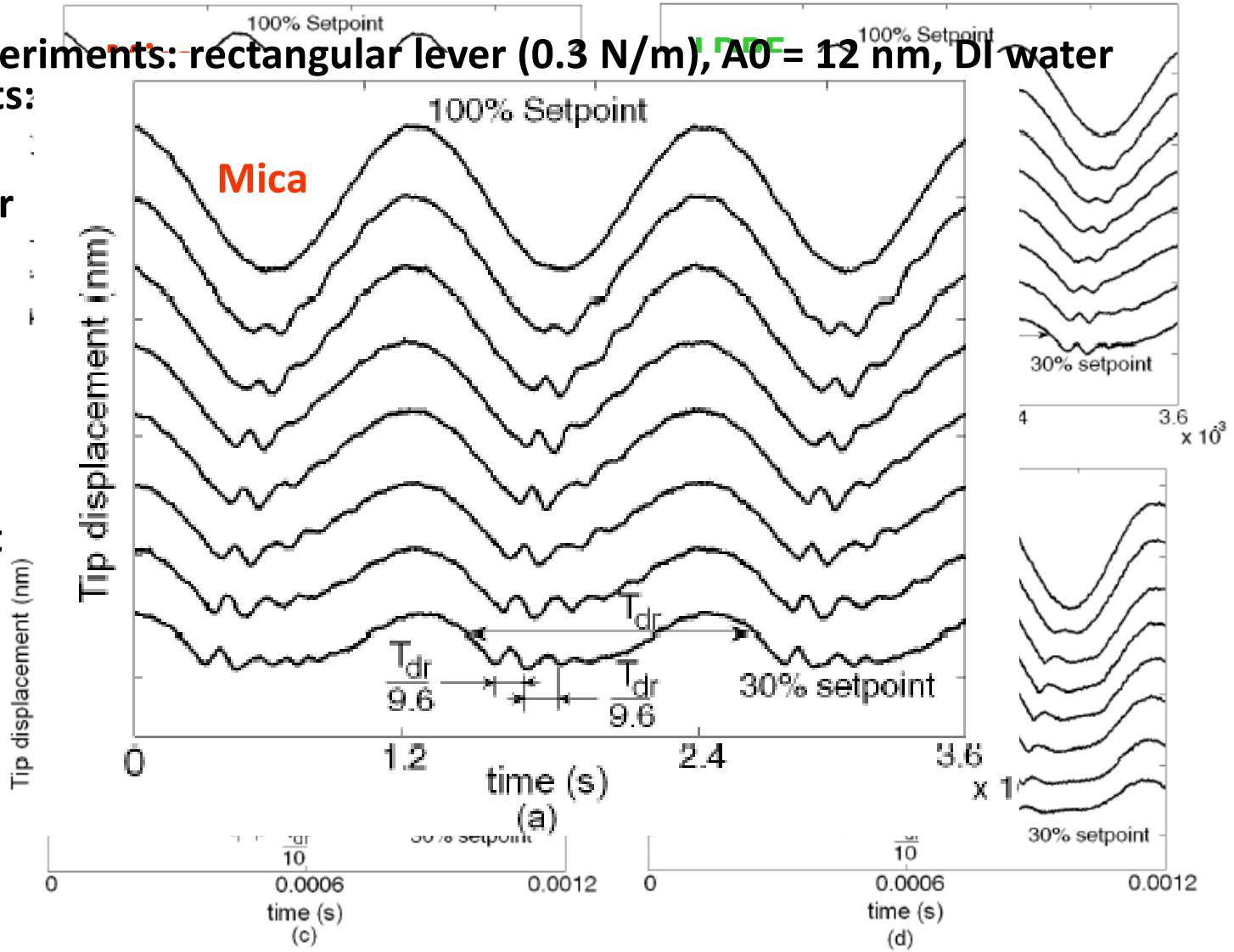
How does a tip tap in liquids?

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

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

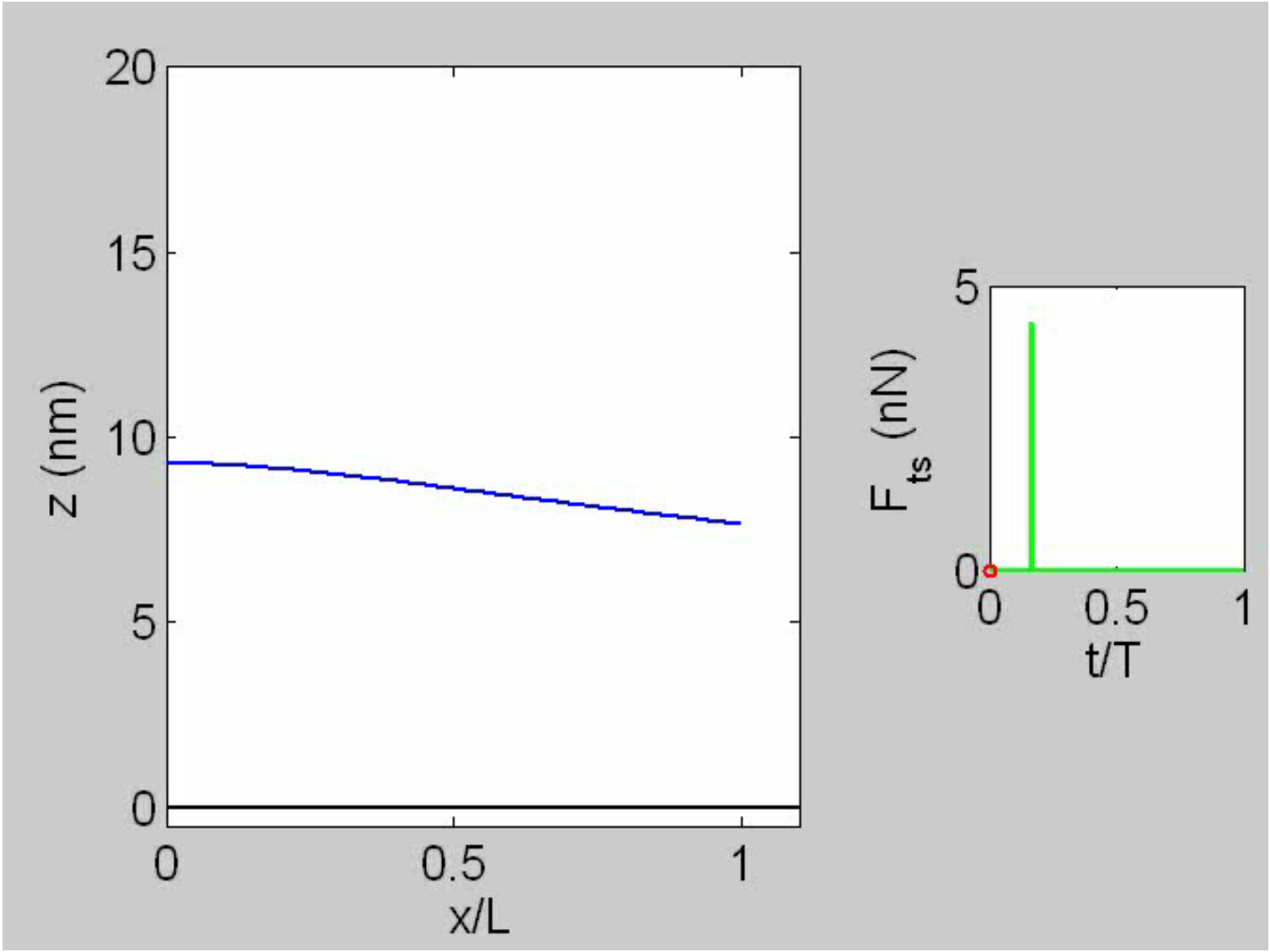
Experiments: 0.3 N/m rectangular lever

Experiments: 0.1 N/m triangular lever



How does a tip tap in liquids?

Overall cantilever motion A/A_0 = 0.95



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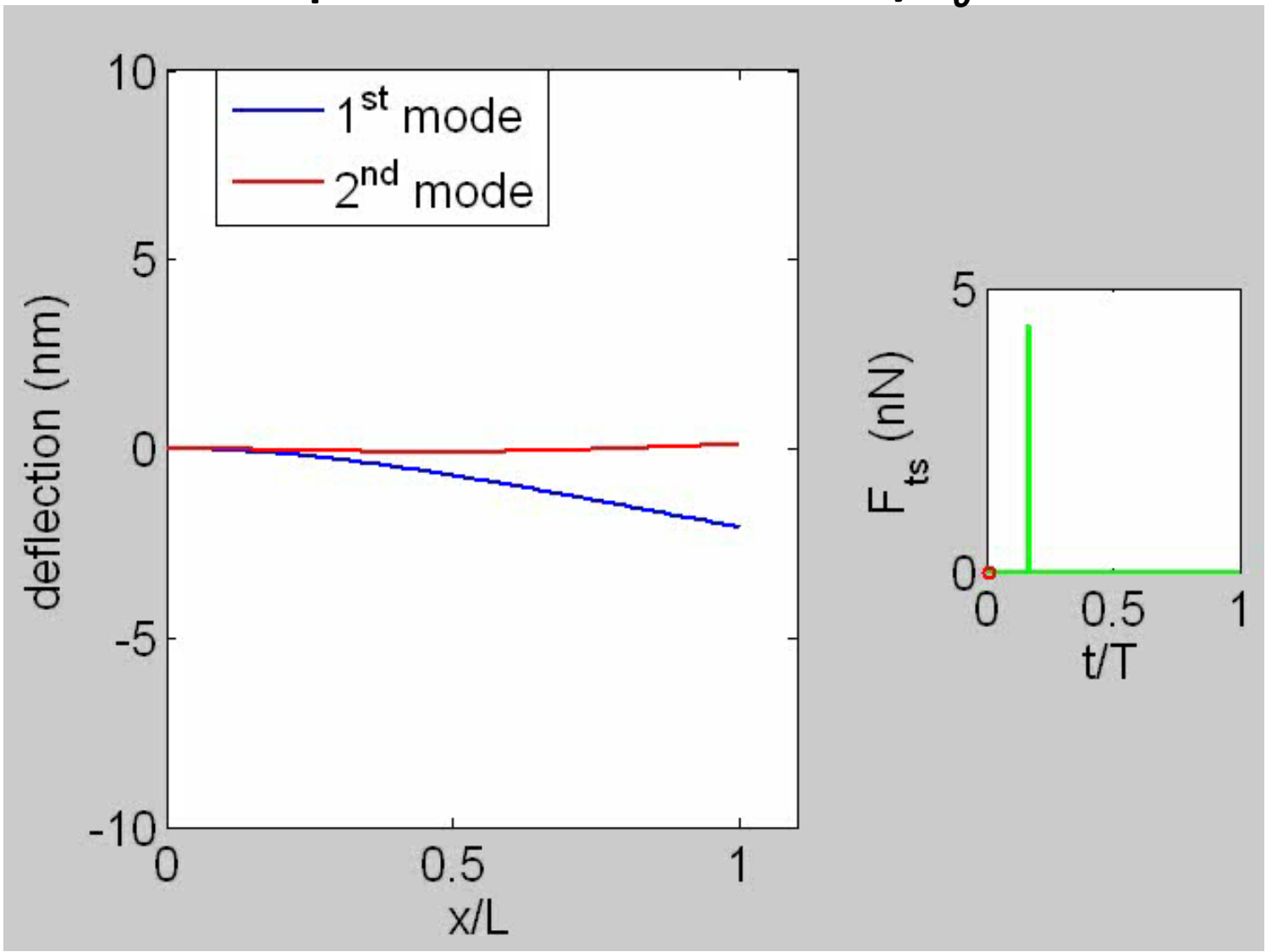
How does a tip tap in liquids?

Decomposed cantilever motion A/A_0 = 0.95



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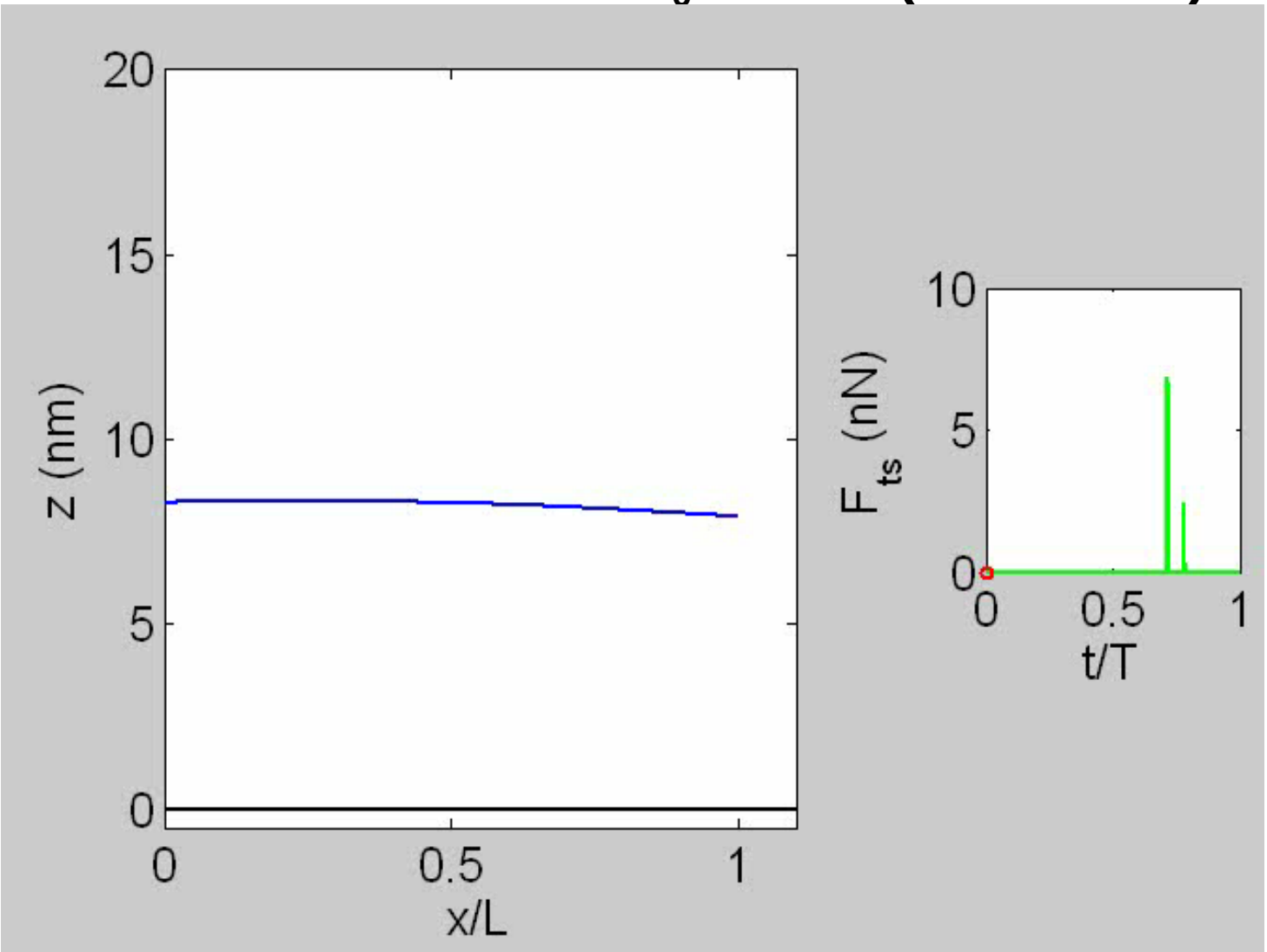
Multiple impact regimes in liquids

(Melcher et al. *Appl. Phys. Lett.* 2008)

Overall cantilever motion A/A_0 = 0.88 (Z =8.3 nm)



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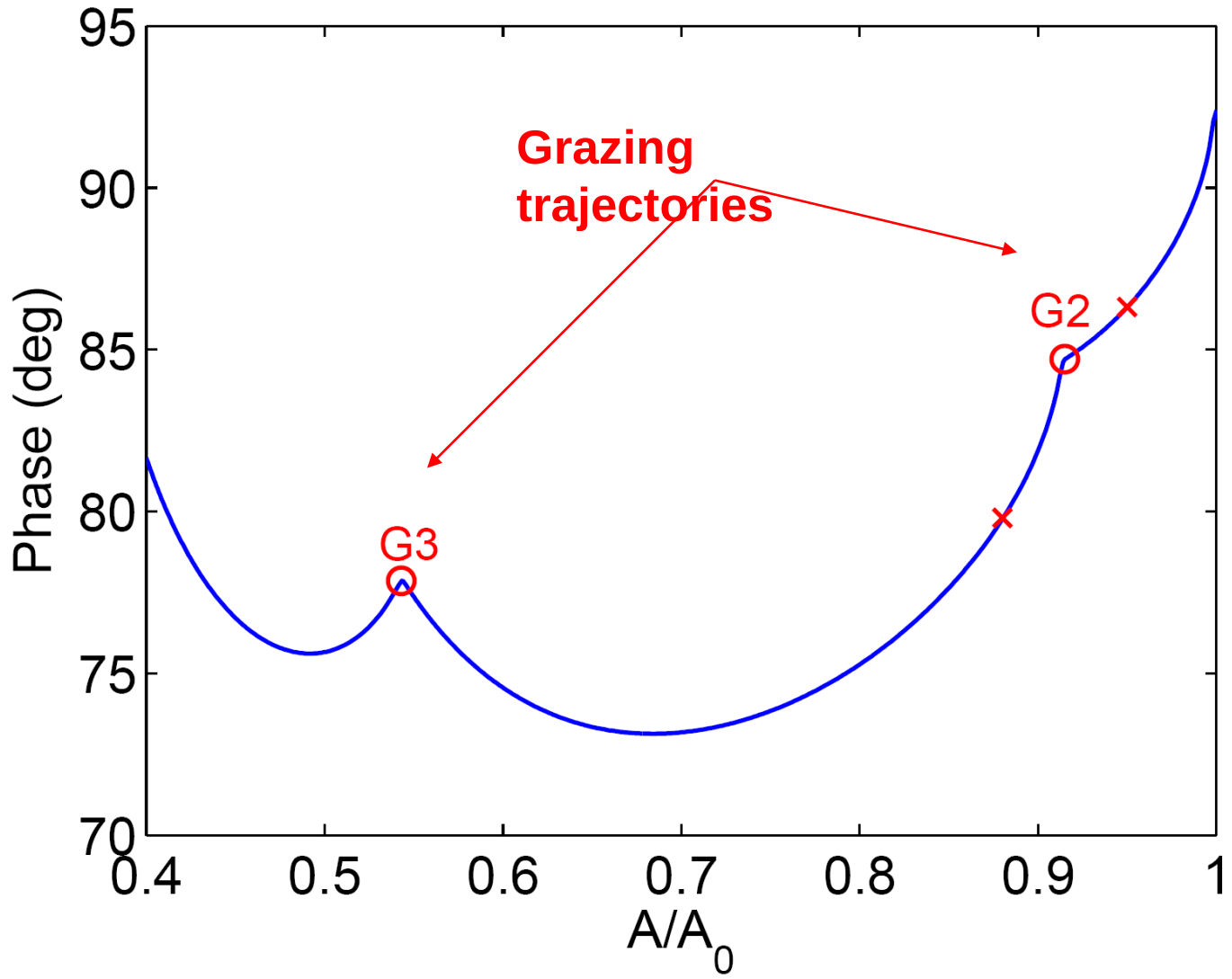
Identification of multiple impacts

(Melcher et al. *Appl. Phys. Lett.* 2008)

Grazing trajectory (G2)



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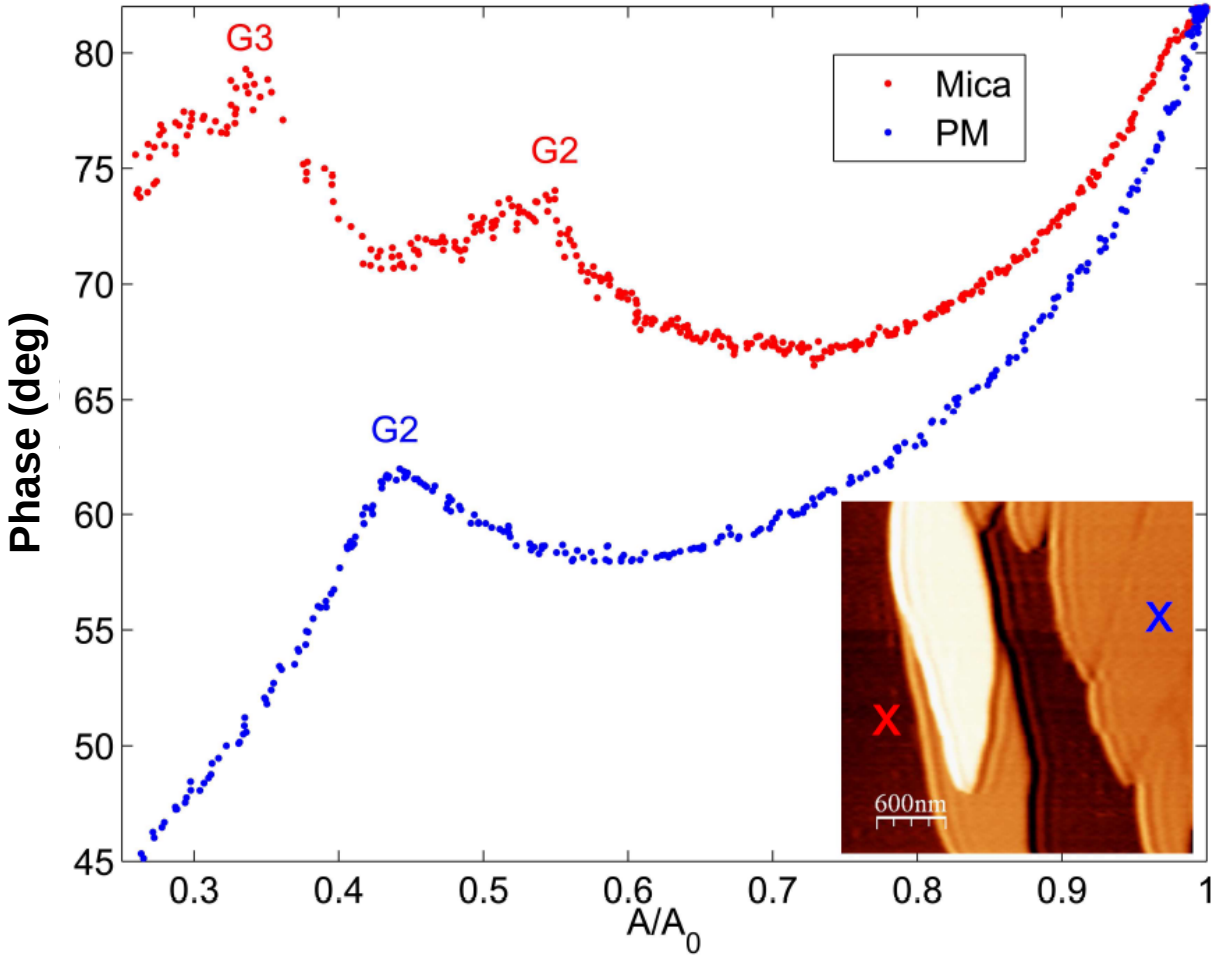
Experimental verification

(Melcher et al. *Appl. Phys. Lett.* 2008)

Sample (inset)

Wild type purple membrane deposited on mica in buffer solution



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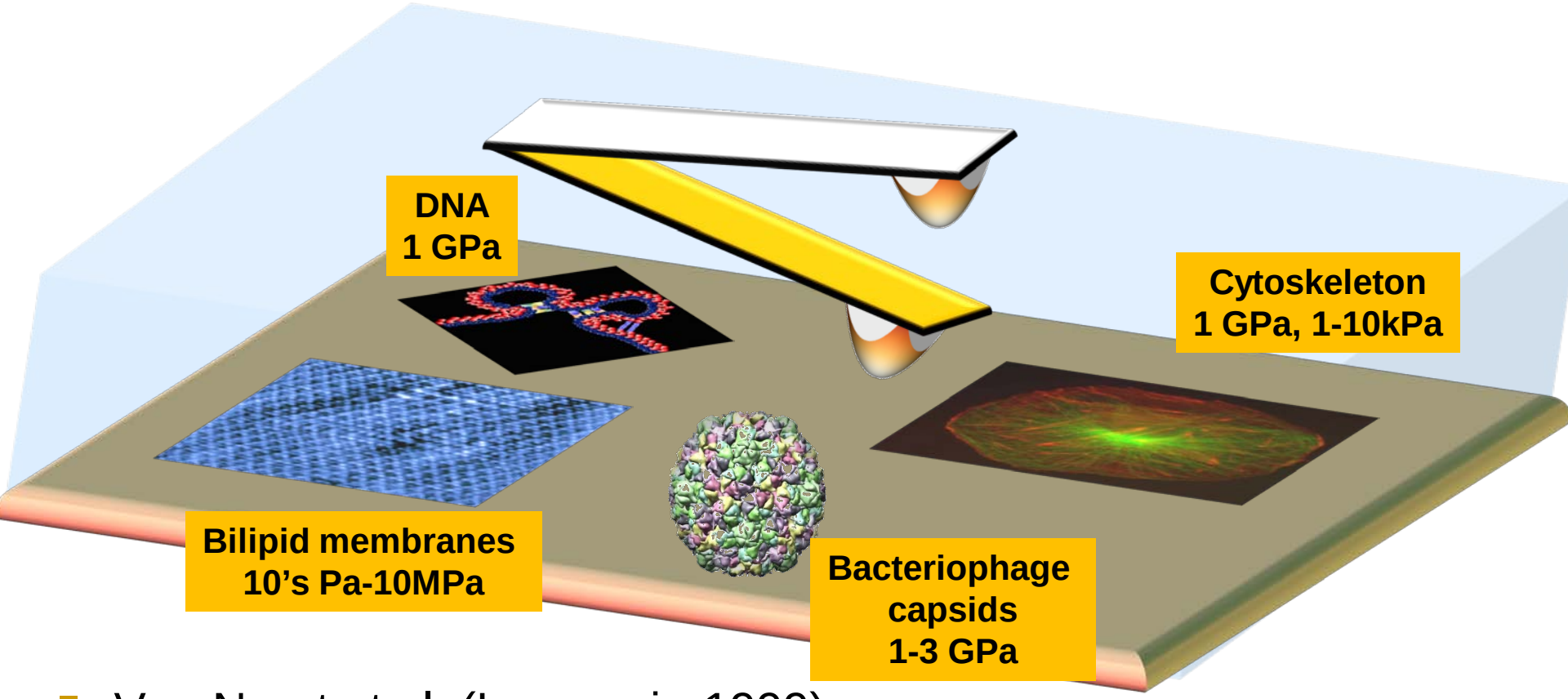
Momentary excitation in liquids



- Van Noort et al, (Langmuir, 1999)
- Preiner, Hinterdorfer et al (PRL, 2007) Second harmonic
- Xu, Melcher, Raman, Reifenberger (PRL, 2009) Momentary Excitation

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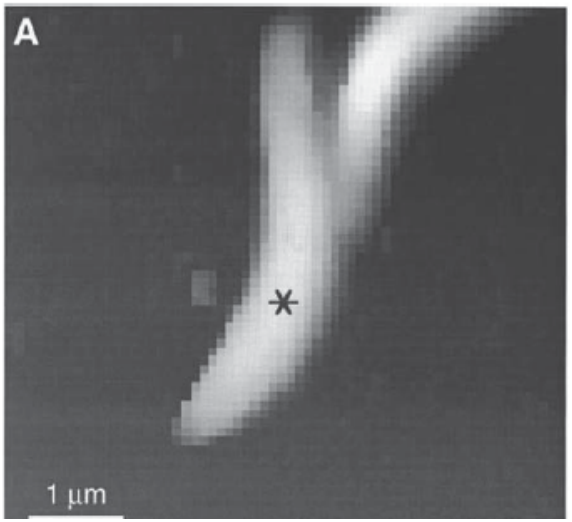
Mapping properties of live cells

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



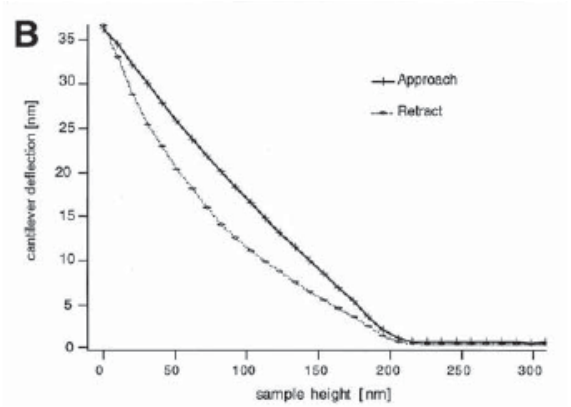


FIG. 7. Force mapping on intact bacteria. (a) Reconstructed height signal of intact bacteria. (b) Force versus distance curve during approach to bacterium at point marked by the asterisk. Note that the linear relationship agrees with the theoretical approach (Sec. II), predicting a linear force-indentation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

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

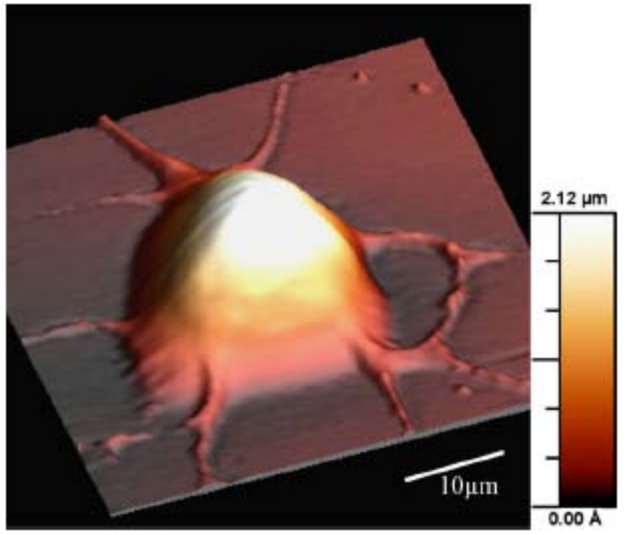
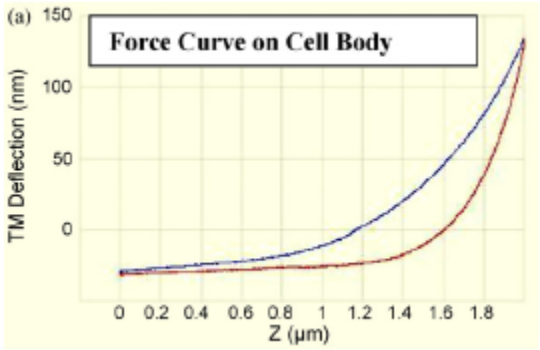
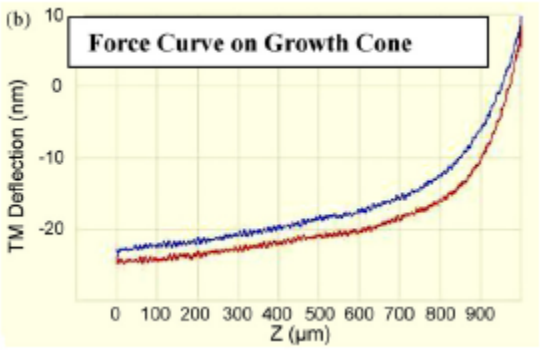
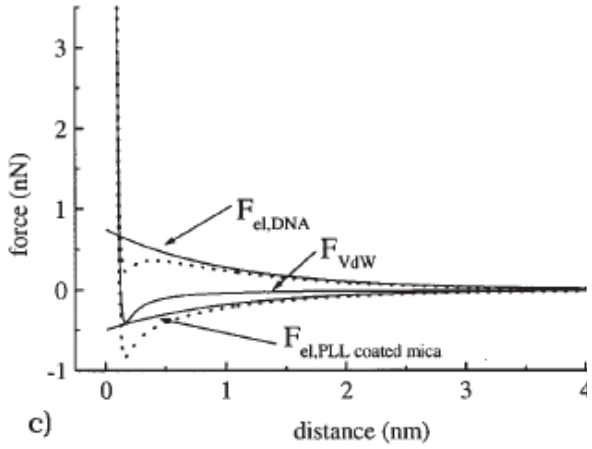


Fig. 3. Sympathetic neural cell body imaged in tapping mode with the Bioscope II.





Higher harmonic imaging in liquids



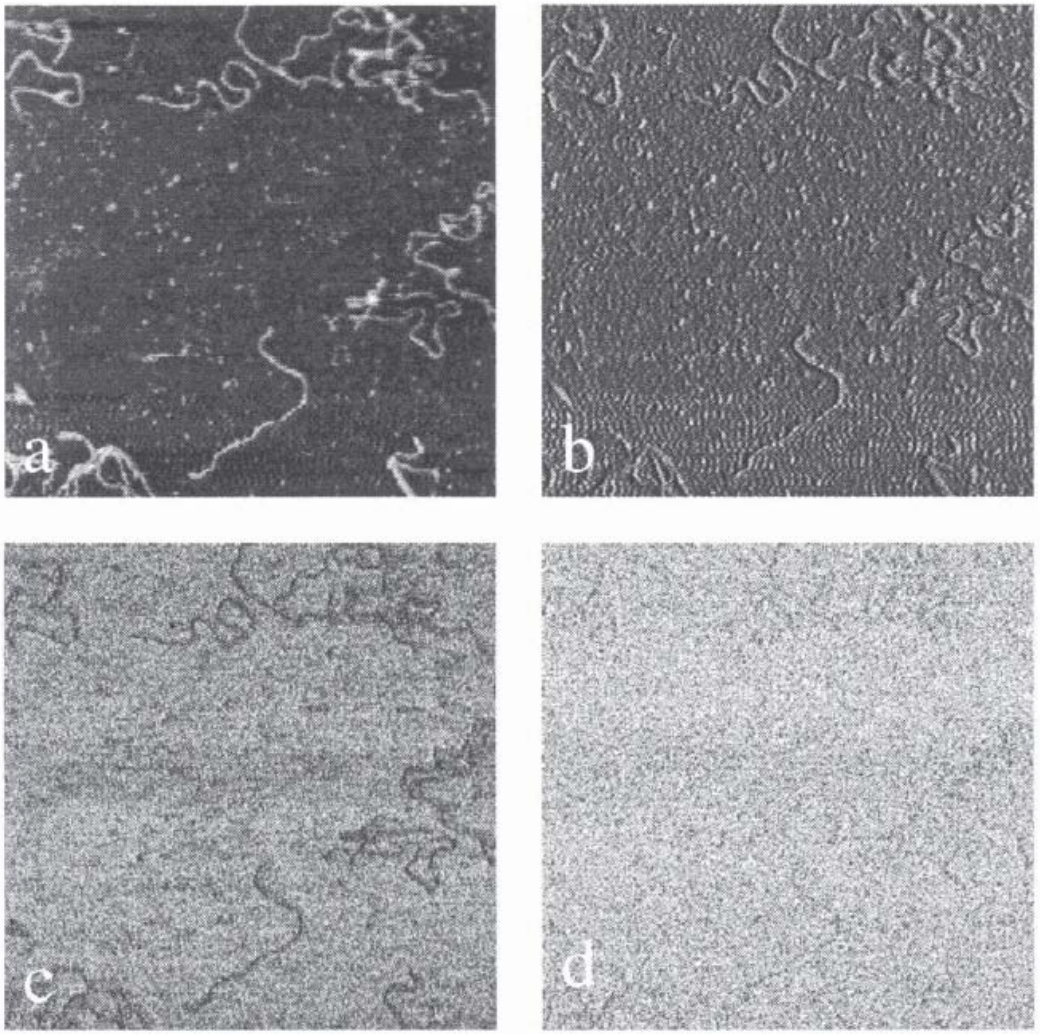


Figure 8. (a) Topography image of 700 bp DNA, scan area 250 × 250 nm², height range 4 nm. (b) Driving frequency, amplitude range 3.5–4.5 nm. (c) Second harmonic, amplitude range 0.5–0.75 nm. (d) Third harmonic, amplitude range 0–0.25 nm.

- Van Noort et al, 15, (Langmuir, 1999)

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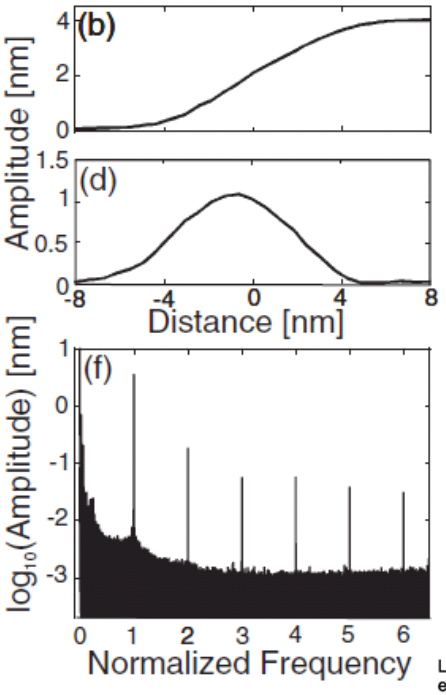
Higher harmonic imaging in liquids



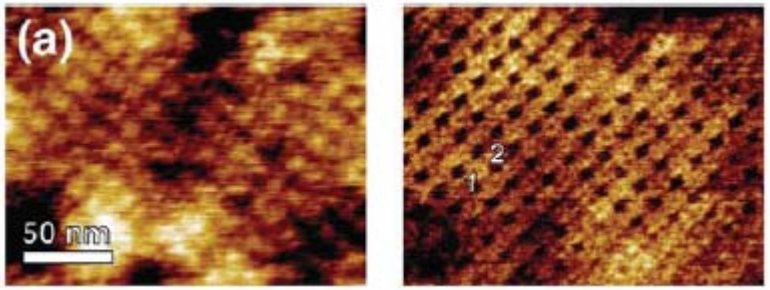
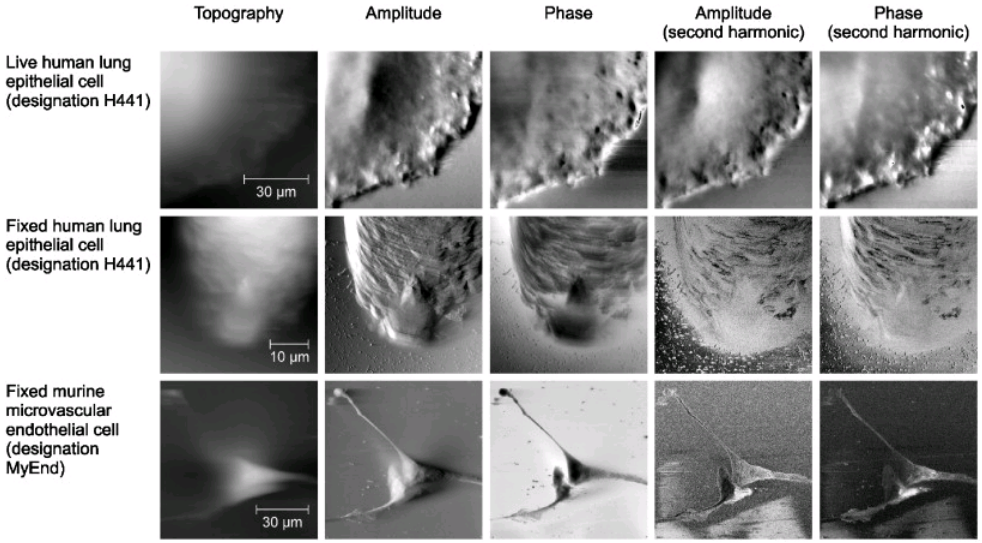


FIG. 3 (color). High resolution on a bacterial *S*-layer. Second harmonic images were recorded using an external Lock-in amplifier (SR 830 DSP, Stanford Research Systems, Sunnyvale Cal., USA). (a) Simultaneous recorded topography (left panel) and second harmonic image (right panel). Substructures within the unit cell can be clearly observed (resolution ~0.5 nm). Scansize: 210 × 175 nm^2; color code: 0–0.9 nm, 0–0.5 V.



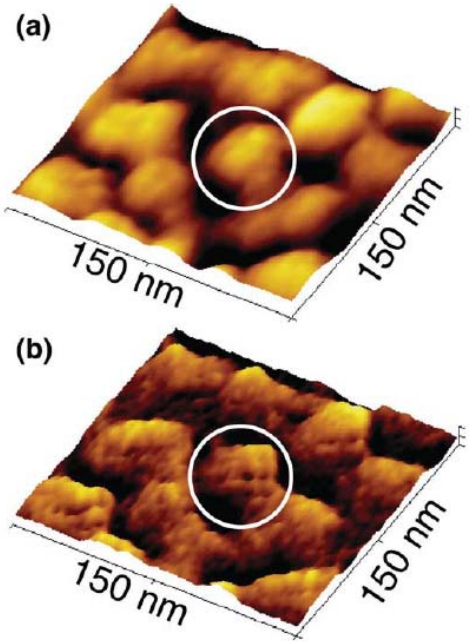


FIG. 4 (color). Measurements on human rhinovirus serotype 2 (HRV2). (a) Topography image of HRV2 layer on mica. (b) Simultaneously recorded second harmonic amplitude image clearly revealing substructures of the viral capsids (circle). Scansize: 350 × 350 nm^2; color code: 0–13 nm, 0–0.35 V.

- Preiner, Hinterdorfer et al, PRL, 99, 2007, also Ultramicroscopy, 2009

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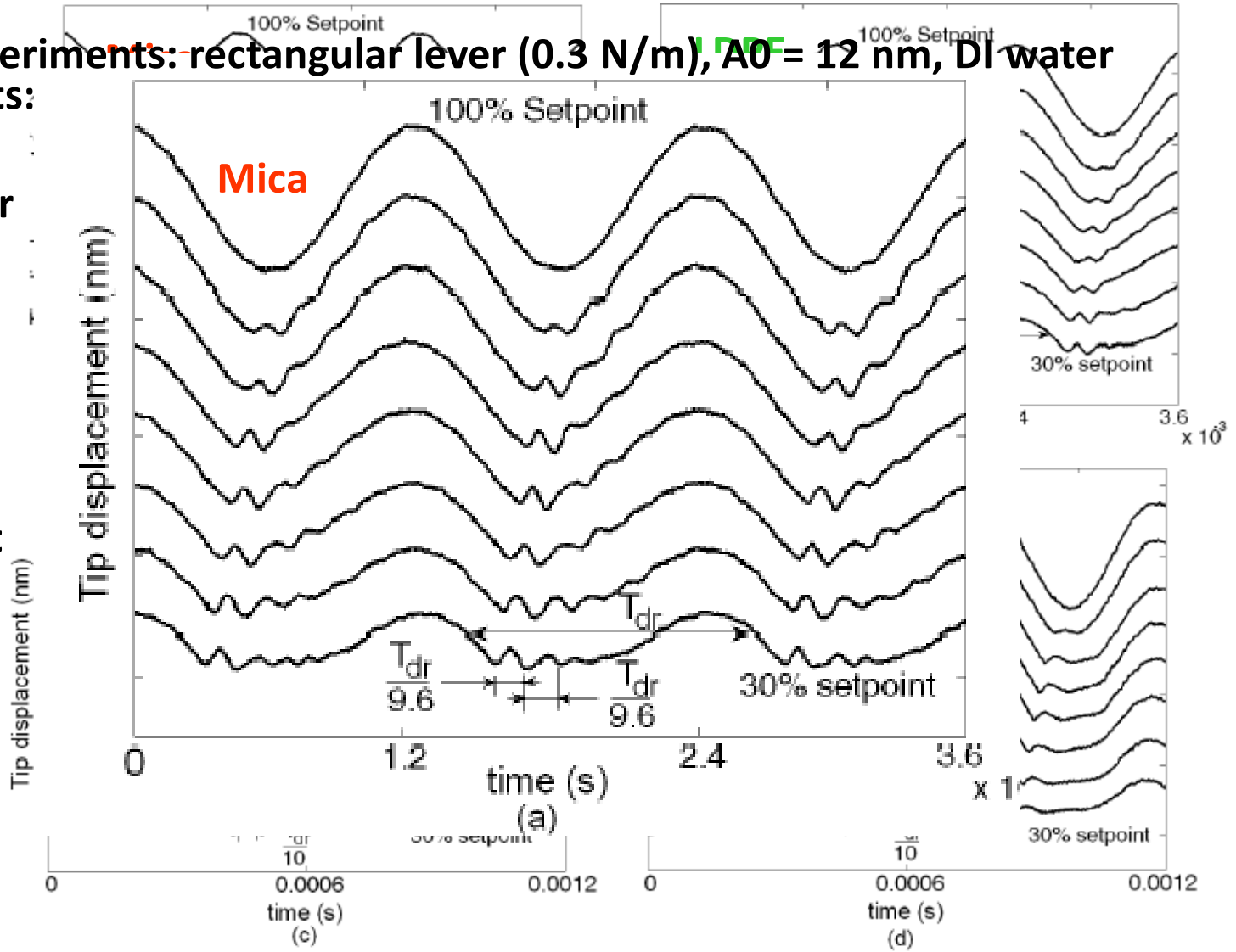
Momentary excitation - experiments

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

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

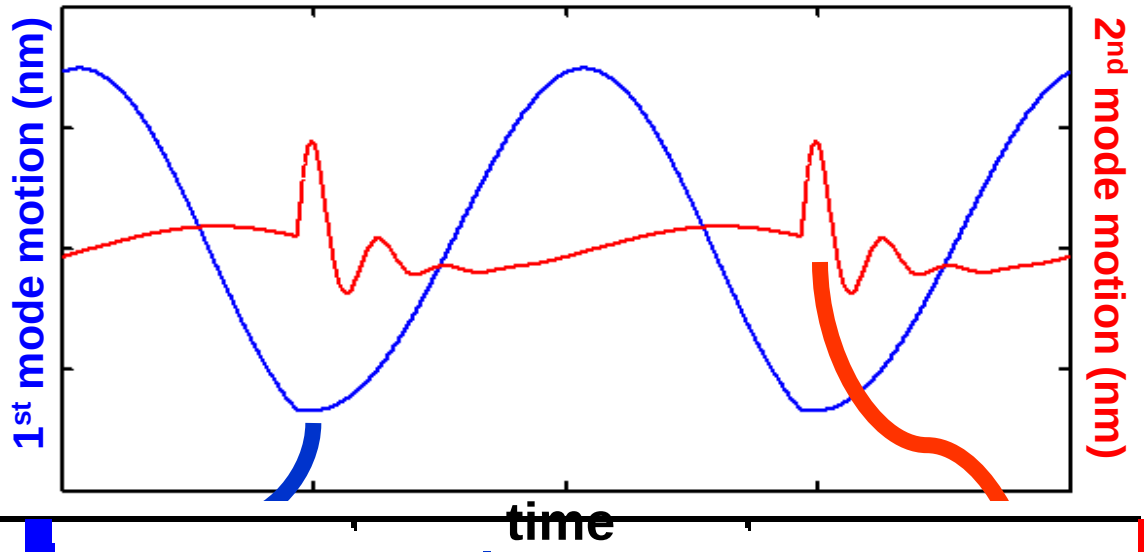
Experiments: 0.3 N/m rectangular lever

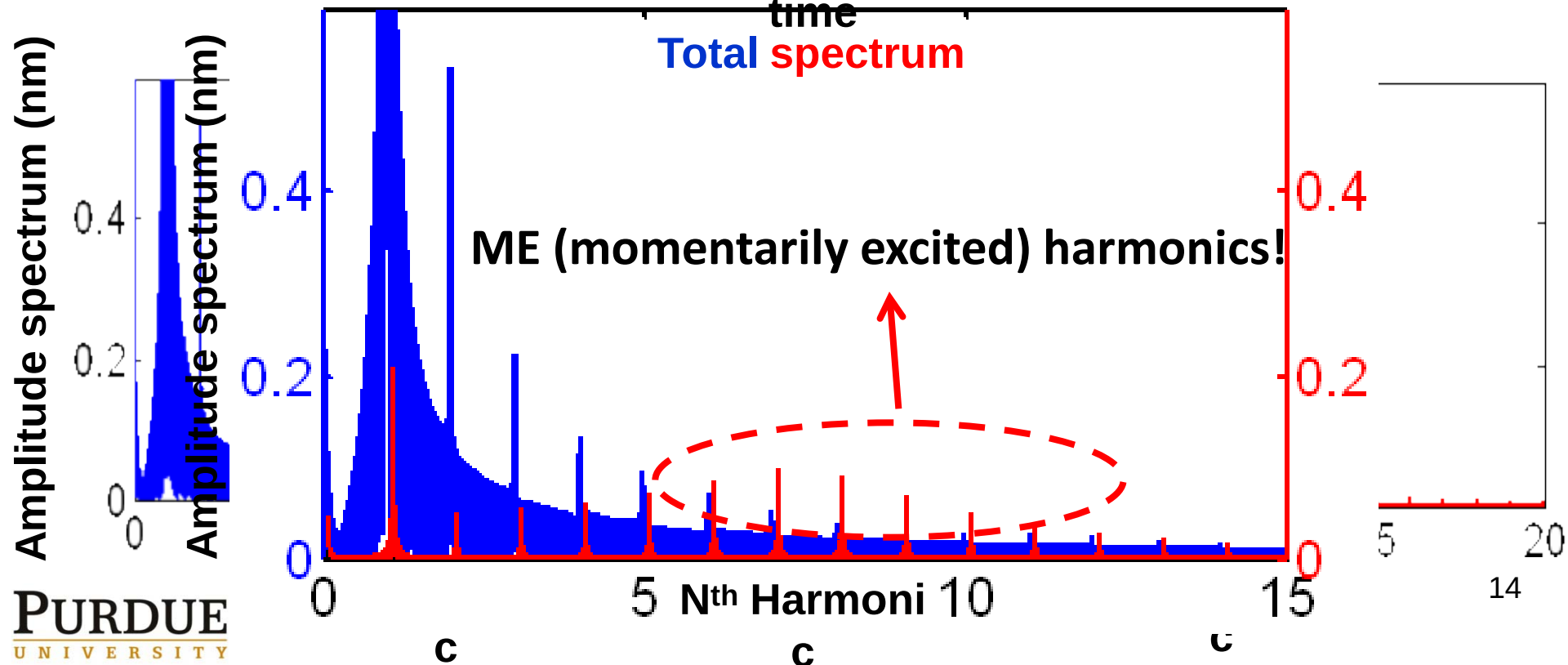
Experiments: 0.1 N/m triangular lever



Momentarily Excited (ME) Harmonics

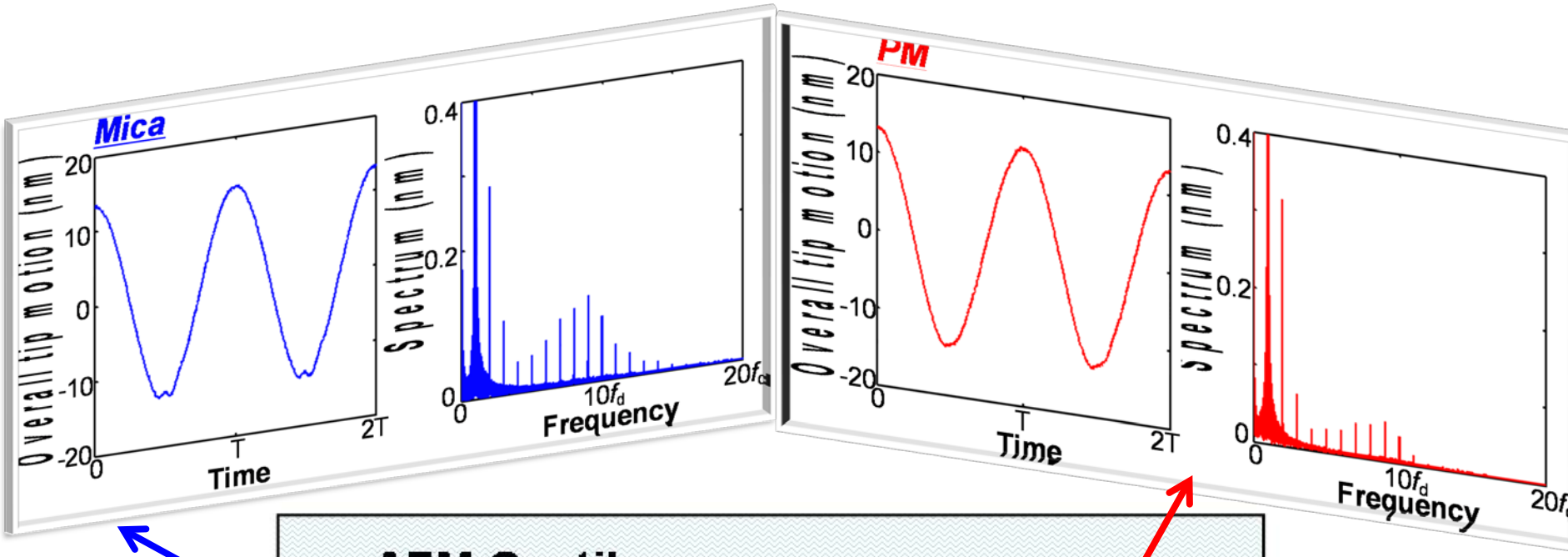
Simulations:
MAClever,
A_0=15nm,
A/A_0=0.92

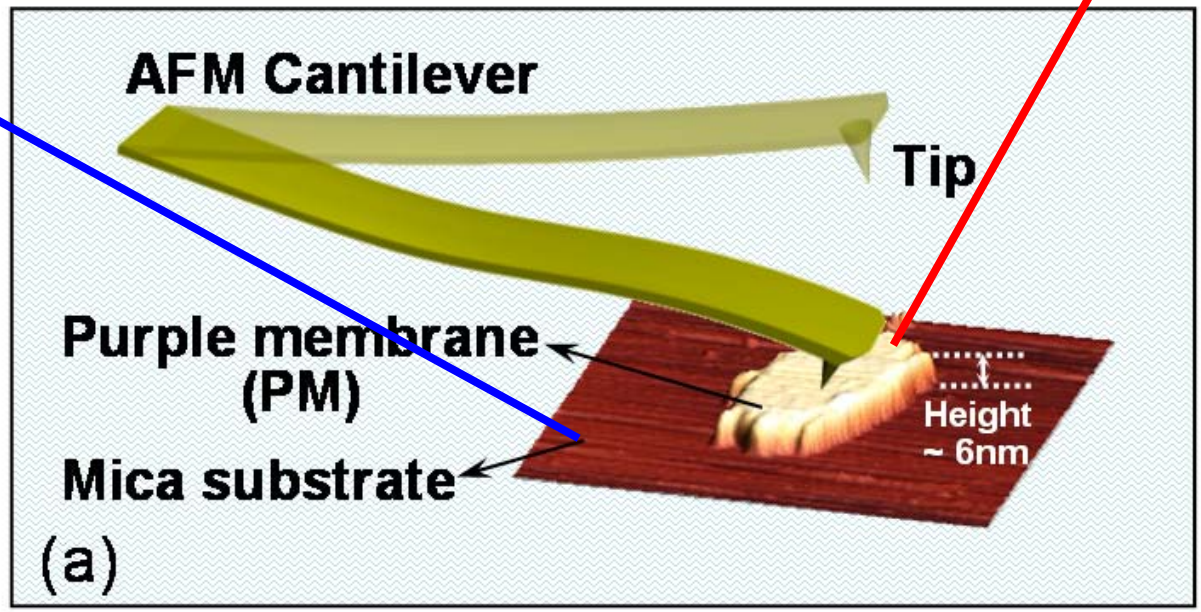




Application to elasticity mapping

Xu et al. Phys. Rev. Lett. 2009





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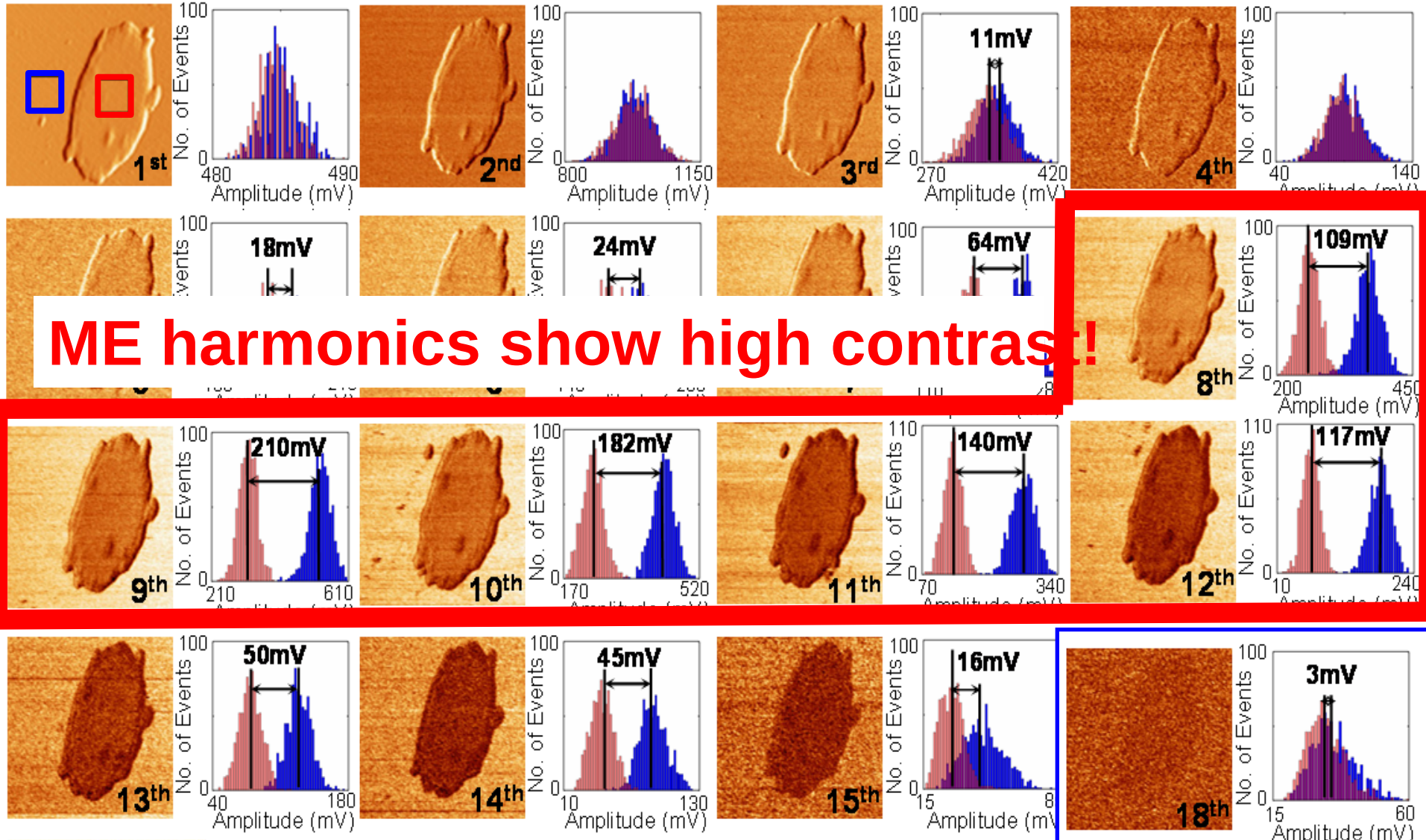
Higher Harmonic Imaging

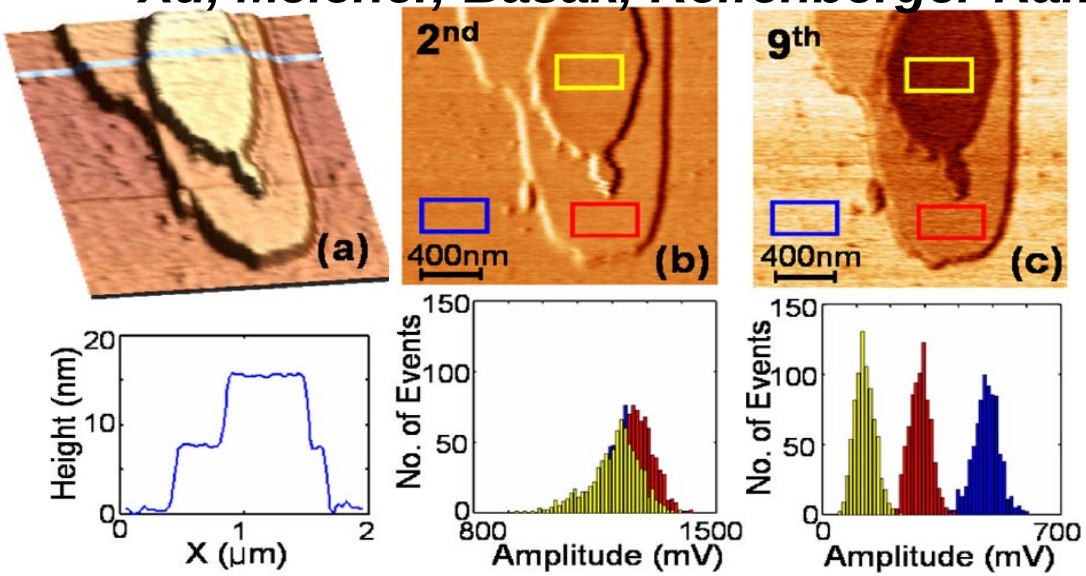
Xu et al Phys. Rev. Lett. 2009

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



Elasticity contrast for soft samples

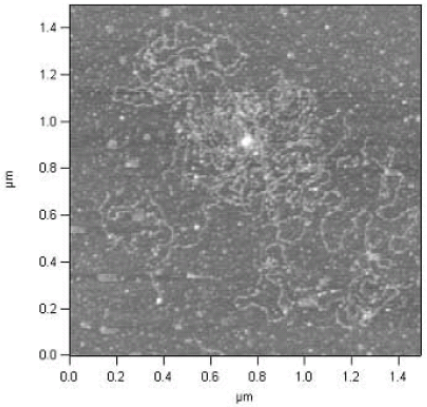
Xu, Melcher, Basak, Reifenberger Raman, Phys. Rev. Lett. 2009



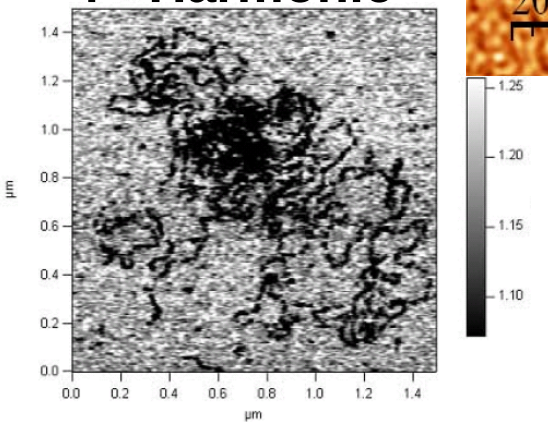
k_1=0.11 N/m, A_0=12.5nm, $A_{setpoint}$ =92%,
buffer: 300 mM KCl, 20 mM Tris -HCl, pH 7.8

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

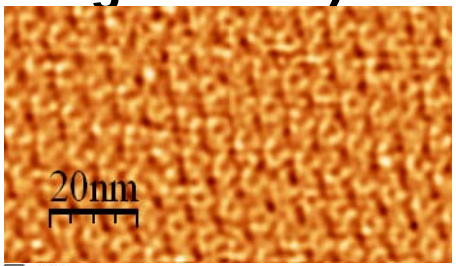
Topography



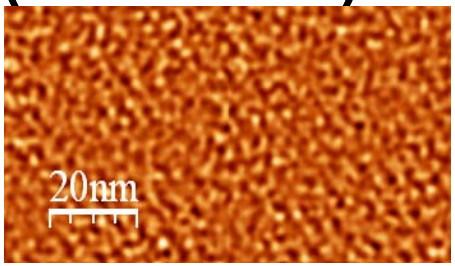
4th Harmonic



Topography (2nd eigenmode)

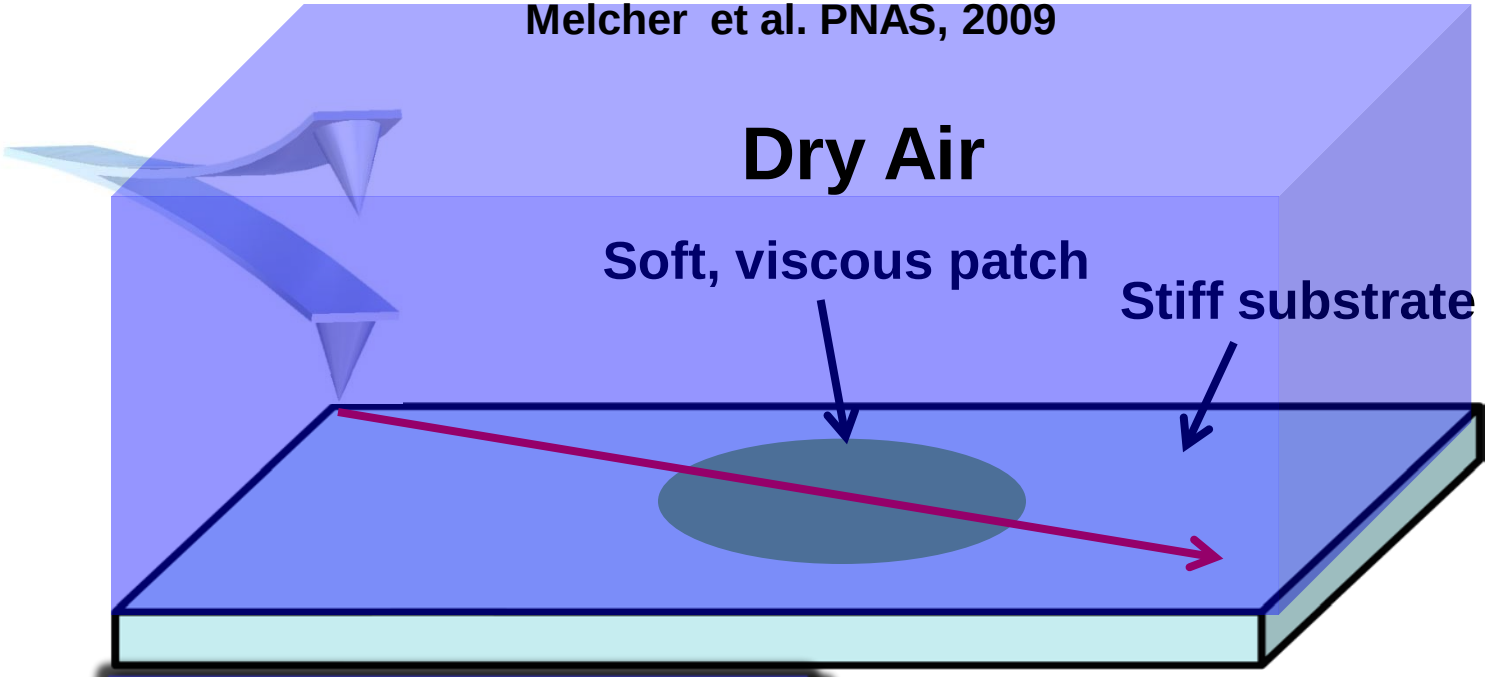


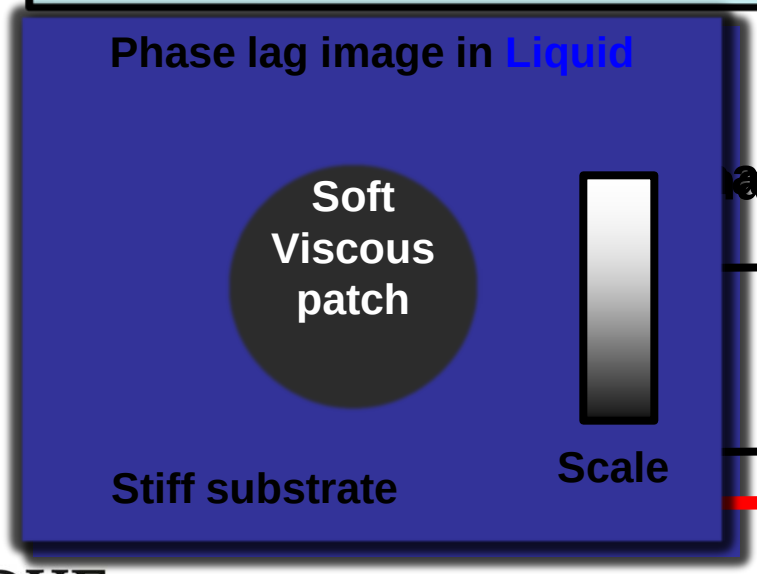
Material property (3rd harmonic)



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%

Phase contrast imaging in liquids





- Phase contrast is a measure of energy lost during interaction with the sample.
- Momentary excitation is a from of energy loss!
- Momentary excitation is larger on stiff samples

- For soft levers in liquids: Phase contrast images = local elasticity maps

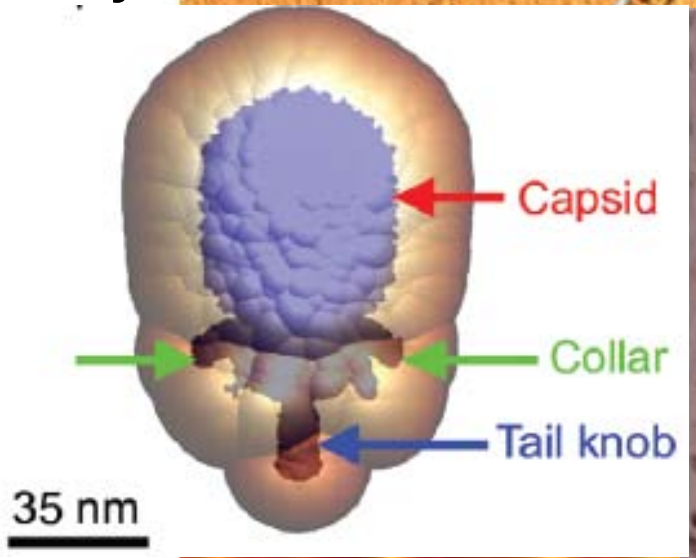
Experimental data

Melcher et al. (PNAS 2009)

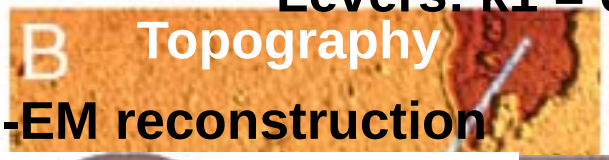
ϕ29 virus capsid on a glass substrate
buffer: TMS pH 7.8
Biolever: k1 = 0.03, acoustic excitation

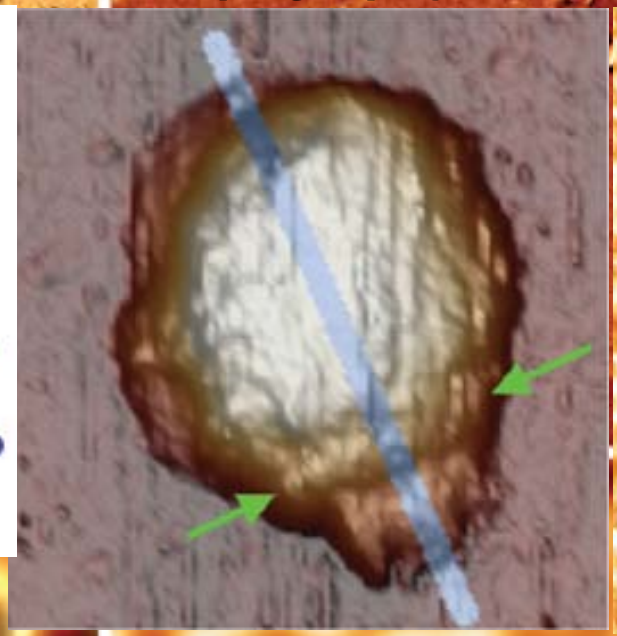
Purple Membrane on mica substrate
buffer solution: 300 mM KCl, 20 mM Tris-HCl, pH 7.8
Levers: k1 = 0.58, 0.09 N/m, acoustic excitation

Cryo-EM reconstruction

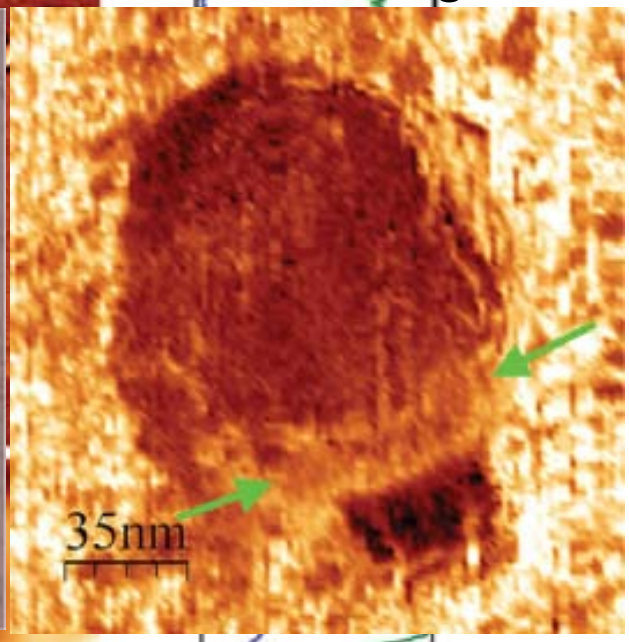


Topography

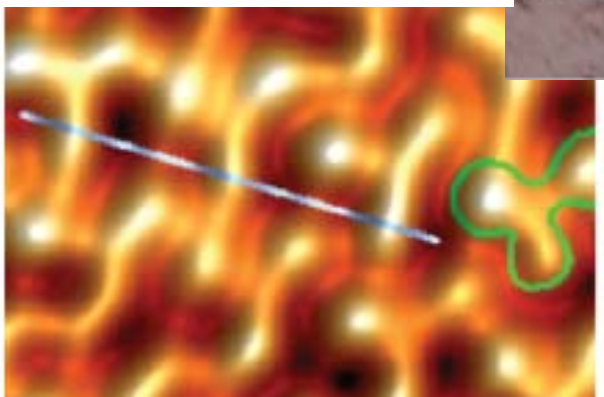




Phase lag



Topography



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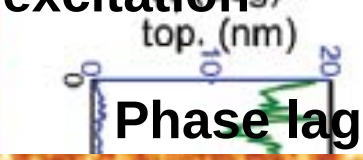

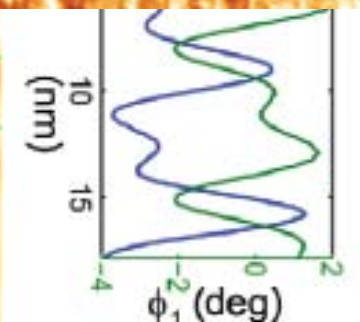

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Conclusions

- Many differences between air and liquid environment
- One of the most important growth areas in AFM

Experimental data

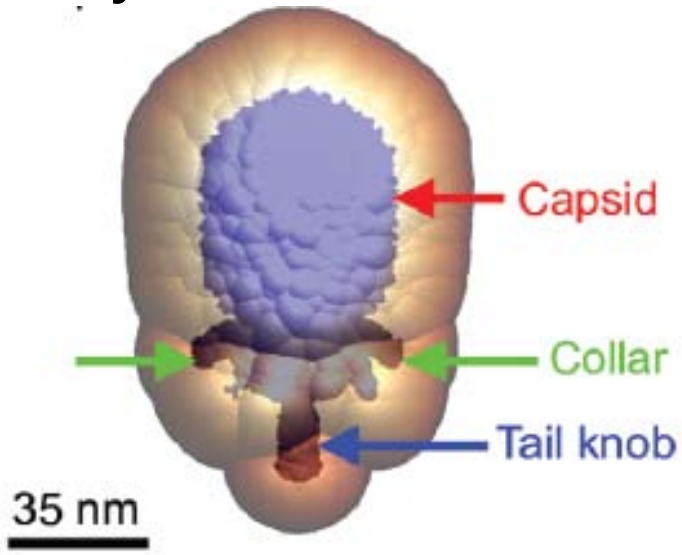
Melcher et al. (PNAS 2009)

ϕ29 virus capsid on a glass substrate

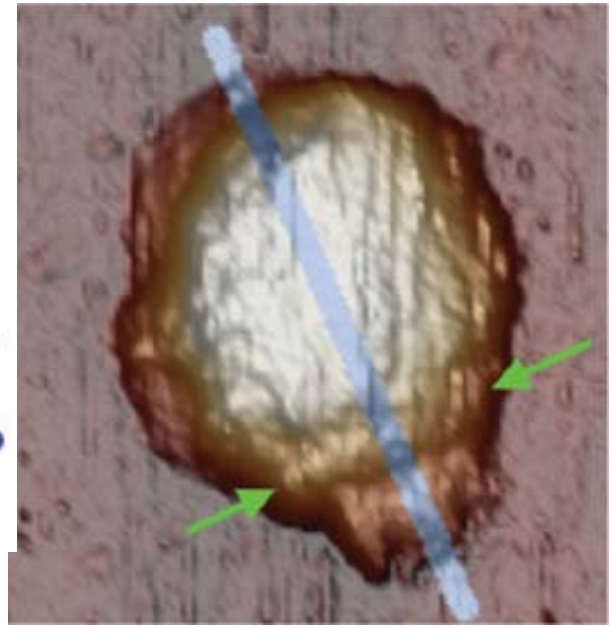
buffer: TMS pH 7.8

Biolever: k1 = 0.03, acoustic excitation

Cryo-EM reconstruction



Topography



Phase lag