Other emerging dAFM techniques

- Multi-frequency AFM
- Sub-surface imaging
- High-speed/video rate AFM
Multi-frequency AFM

- Generic term applied to all methods where either cantilever is excited and or measured at more than one frequency
  - Kelvin force microscopy in tapping mode (discussed in class)
  - Higher harmonic imaging
  - Internally resonant or "harmonic" cantilevers
  - Momentary excitation in liquids
  - Bimodal or dual AC mode
  - Band excitation (Oakridge, S. Jesse, S. Kalinin)
Multi-frequency AFM

- Higher harmonic dAFM
- Insight:

M. Stark et al, PNAS, 99, 2002
Crittenden et al, PRB, 72, 2006

mechanical properties & loss mechanisms
Hamaker constants, electro-statics & -dynamics
Multi-frequency AFM

Higher harmonic dAFM

FIG. 1. Experimental setup for the detection of anharmonic signals. A commercial AFM is equipped with a second lock-in amplifier for the detection of anharmonic signals.

FIG. 2. (a) Topographic (b) control error, and (c)–(e) higher order harmonic images of a 4-nm-thick Pt–C test structure on a fused silica cover slip. The driving frequency was $f = 52.2$ kHz, the detection frequencies were (c) $3f = 156.6$ kHz, (d) $5f = 261.0$ kHz, and (e) $8f = 417.6$ kHz.

FIG. 4. Detail of a silicon test structure imaged in tapping mode (scan direction right to left). (a) Topography, (b) control error, and (c) eighth harmonic. The instabilities due to the bistable behavior of the system are difficult to be seen in the conventional images (a) and (b). However, in the harmonic image (c) a strong contrast prevails.

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Heckl and Stark, RSI, 74, 2003
Amplifying higher harmonics

![SEM image of a harmonic cantilever. Width, length and thickness of the cantilever are 50, 300, and 2.2 μm, respectively. The rectangular opening is 22 μm x 18 μm and centered 190 μm away from the cantilever base.]

Tune second eigenmode frequency to an integer multiple of the fundamental

Sahin et al, Sensors and Actuators, 114, 2003, also PRB (69), 2004
Using tuned cantilevers

- In attractive regime, vibration spectrum depends on local vdW and electrostatic forces
- Experiments performed using 47 kHz microcantilever on wild and mutant bacteriorhodopsin membrane
- 2nd bending mode freq $\sim 7 \times 1^{st}$

- Thermal vibration
- Driven in air
- On mica (50 % setpoint)

Crittenden et al, PRB, 72, 2006
Using tuned cantilevers

3500 nm x 3500 nm scans

- Clear distinction between lipids and proteins
- Presence of internal resonance critical in the method
- The method shows promise for the measurement of local Hamaker constants of soft biomolecules
- Can be extended to electrostatic force microscopy
Momentary excitation in liquids

Compositional contrast in liquids

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


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

Experiments:
0.3 N/m
rectangular lever

Experiment s:
0.1 N/m
triangular lever
Momentary excitation - theory

Decomposed cantilever motion $A/A_0 = 0.85$

First eigenmode

Second eigenmode

- Momentary excitation is greater on stiffer samples

Cantilevers:
- Olympus Biolever ($k_1 = 0.036$, $k_2 = 1.4$, $Q_1 = 1.2$, $Q_2 = 2$, $\omega_1 = 9.3 \text{ kHz}$, $\omega_2 = 71 \text{ kHz}$).
- MAClever: $k_1 = 0.11 \text{ N/m}$, $k_2 = 8 \text{ N/m}$, $Q_1 = 1.6$, $Q_2 = 4.3$, $\omega_1 = 3.5 \text{ kHz}$, $\omega_2 = 30 \text{ kHz}$.
Momentarily Excited (ME) Harmonics

Simulations: MAClever, \( A_0 = 15 \text{nm}, \ A/A_0 = 0.92 \)

![Graph showing 1st and 2nd mode motion over time, with a total spectrum and highlighted ME harmonics.](image)

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Application to elasticity mapping
Higher Harmonic Imaging

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

ME harmonics show high contrast!
Elasticity contrast for soft samples


$k_1 = 0.11 \text{ N/m}, A_0 = 12.5 \text{ nm}, A_{\text{setpoint}} = 92\%, \text{ buffer: } 300 \text{ mM KCl, 20 mM Tris-H}$
Origin of contrast in ME harmonic images

- One order of magnitude improvement in elasticity contrast using ME harmonics compared to 2\textsuperscript{nd} harmonic for soft materials.
- ME harmonics are closely correlated to contact time (which varies inversely with sample elasticity).
- Image contrast seen is entirely local elasticity contrast.
Phase contrast imaging in liquids

Melcher et al. PNAS, 2009

- Phase contrast is a measure of energy lost during interaction with the sample.
- Momentary excitation is a form 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)*

Purple Membrane on mica substrate

\( \phi 29 \) virus capsid on a glass substrate

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

Levitation: \( K_1 = 0.58, 0.09 \) N/m, acoustic excitation

Cryo-EM reconstruction

35 nm

Capsid

Collar

Tail knob

Phase lag

EC

Phase lag

3.5 nm

4.9 nm

\( \phi_1 (\text{deg}) \)
Multi-frequency AFM

- **Bimodal or dual AC**
  - Key insight is that the second mode $A_2$, $\phi_2$ varies in time
  - Thus $\phi_2$ not only measures dissipation but also conservative tip-sample interactions!
  - It becomes possible to see material contrast in the attractive regime!

Rodriguez and Garcia, APL, 84(3), 2004
Lozano and Garcia, PRL, 100(7), 2008
Lozano, Garcia, PRB, 79(1), 2009
R. Proksch, APL, 89(11), 2006
Multi-frequency AFM

Bimodal or dual AC

Figure 1. Comparison between amplitude modulation and bimodal AFM. (a) AM-AFM (monomodal excitation). (b) Bimodal AFM. (c) Schematics of the bimodal AFM instrument. The bimodal excitation/detection unit performs the multifrequency excitation and the multicomponent signal processing while the control unit runs the feedback.
Multi-frequency AFM

Bimodal or dual AC

Figure 3. Comparison between AM-AFM and bimodal AFM images of IgG antibodies. (a) Topography and (b) phase images of an IgG obtained in AM-AFM. (c) Tip oscillation in AM-AFM (top) and bimodal AFM (bottom). (d) Topography in bimodal AFM. (e) Phase shift image of the first mode in bimodal AFM. (f) Bimodal AFM phase image (second mode) of the same antibody. The image shows a Y-shaped object.

Figure 7. (a) Bimodal AFM phase images (second mode) of IgM antibodies in water. The objects that show a pentagonal shape are marked by circles. The inset shows the frequency spectrum of a commercial cantilever in water. The dashed lines indicate the frequencies of the first and second flexural modes of the cantilever. They were determined by measuring the thermal noise spectrum. (b) Topography of an isolated antibody. (c) First mode phase image and (d) bimodal AFM phase image (second mode) of the same antibody.

Martinez et al, Nanotechnology, 19, 2008
FIG. 2. (Color online) HOPG graphite surface, 30 μm scan. The cantilever was driven at its fundamental (~69.5 kHz) and second eigenfrequency (~405 kHz). (a) shows the topography and (b) is the fundamental amplitude channel, used for the feedback error signal. The fundamental phase image (c) shows an average phase lag of ~34° indicating that the cantilever was in repulsive mode for the entire image. The second mode amplitude is shown in (d). The three dimensional rendered topography colored with the second mode amplitude is shown in (e). This method of display allows easy spatial correlation of the two channels.

FIG. 3. (Color online) Dense mat of DNA imaged in buffer, 750 nm scan. The 60 μm Bio-Lever was driven at its fundamental resonance (~8.5 kHz) and at its second mode (~55 kHz). The topography (a), fundamental amplitude (b), and fundamental phase (c) all show very little differentiated contrast. The second mode amplitude (d) shows clear, high contrast images of what appear to be strands of DNA molecules. The second mode amplitude was painted onto the three dimensional rendered topography (e) to allow spatial correlation of the two data channels.
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Slide switch at 6:30
Start ~ 50:30