Higher harmonic atomic force microscopy: Imaging of biological membranes in liquid

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Model: 1-DIM Harmonic Oscillator

Equation of Motion (EQM):
\[ m\ddot{x} + \frac{\omega_0 m}{Q} \dot{x} + kx = F_{\text{hyd}} + F_{\text{DMT}} + F_0 \cos \omega t \]

Hydrodynamic Damping:
\[ F_{\text{hyd}} = F_{\text{hyd}}(d, \dot{x}) \]  
C. Rankl et al. Ultramicroscopy 2004

Contact Mechanics: Derjaguin-Muller-Toporov (DMT)-model for a sphere-flat geometry:
\[
F_{\text{DMT}} = \begin{cases} 
-\frac{HR}{6(d + x)^2} & d + x \geq a_0 \\
-\frac{HR}{6a_0^2} + \frac{4}{3}E^*\sqrt{R}(a_0 - d - x)^{3/2} & d + x < a_0
\end{cases}
\]

Hamaker constant for silicon nitride/Water/Mica: \( H = 3.1 \times 10^{-21} \text{ J}; \)
\( f_0 = 8.46 \text{ kHz}; \) \( Q = 2.078; \) \( m = 35 \text{ ng}; \) (obtained from thermal spectra), \( k = 0.1 \text{ N/m}; \) \( f_d = 7.184 \text{ kHz} \)

Simulation

• Numerically solving EQM

• Stepwise approach to the surface in 0.1 nm steps

• FFT analysis of the solution (timetrace) at every step => frequency spectra

Simulation of amplitude vs. distance curves

Experimental Setup:

Data Acquisition:
NI PCI-6013
200 kS/s, 16 bit

Comparison between simulation and experimental data

Simulation

Experiment

$A_0 = 4 \text{ nm}$;

$A_{sp} = 3.5 \text{ nm}$

Dependence of the 2nd harmonic amplitude on interaction parameters

Contact Mechanics (DMT-model):

\[
F_{DMT} = \begin{cases} 
-\frac{HR}{6(d+x)^2} & d + x \geq a_0 \\
-\frac{HR}{6a_0^2} + \frac{4}{3}E\sqrt{R}(a_0 - d - x)^{3/2} & d + x < a_0
\end{cases}
\]

J. Preiner et al. PRL 99 2007 (046102)
Dependence of the 2\textsuperscript{nd} harmonic amplitude on interaction parameters

**Music:** different instruments can be distinguished even when they are playing the same note

**Reason:** it’s not only the fundamental note what defines the timbre (color of sound) but the sum of the fundamental note and all the harmonics

**Analogy to AFM:** recording harmonics in the cantilever movement (timbre) enables to distinguish different interactions
Images of bacterial S-Layer
(sbpA, Bacillus sphaericus CCM 2177)

Periodicity ~14 nm

Amplitude color code: 0-5 nm

Phase

2nd Harmonic Amplitude

(A₀=10 nm, Aₛᵣ/A₀=0.85, fᵣ=7.3kHz, k=0.1 N/m, PBS Buffer)

Rhino Virus (HRV2)

Topography

2nd Harmonic Amplitude

(A₀=15 nm, Aₚ/ₐ₀=0.9, f_d 7.9 kHz, k=0.1 N/m, Ni-Tris Buffer)

Imaging Recognition using 2nd Harmonic

Simulation and experimental verification of dynamic approaching curves revealed characteristic signature of molecular recognition in 2nd harmonic amplitude.
Imaging Recognition using 2\textsuperscript{nd} Harmonic

Comparison of TREC and 2\textsuperscript{nd} harmonic imaging of mol. recognition

- Bacterial S-Layer SbpA
- native:Strep tagged=7:1
- Streptactin on tip
- Block with free Strep tag II
Summary

• Simulations of dynamic AFM in liquid: Amplitudes of higher harmonics are sensitive to variations in the nonlinear tip-sample interactions

• 2\textsuperscript{nd} harmonic images of various samples in liquid (S-layer, HRV2,) showed detailed substructures not visible in topographical images

• 2\textsuperscript{nd} harmonic images of molecular recognition
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