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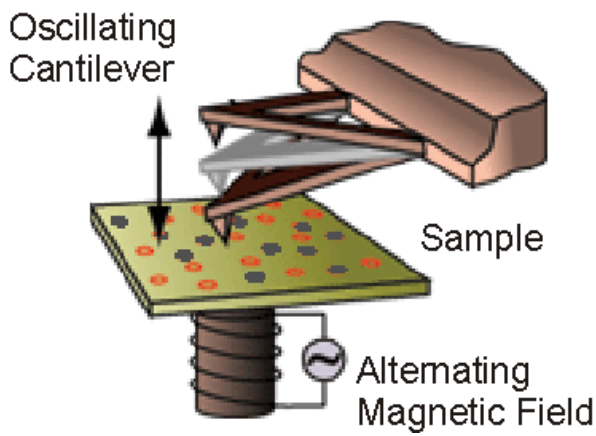
Johannes Kepler University of Linz
Altenbergerstr. 69
A-4040 Linz, Austria

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



Peter Hinterdorfer
Johannes Preiner

Agilent eSeminar,
May 28th, 2008

Model: 1-DIM Harmonic Oscillator

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

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

Contact Mechanics: Derjaguin-Muller-Toporov (DMT)-model for a sphere-flat geometry:

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

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

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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

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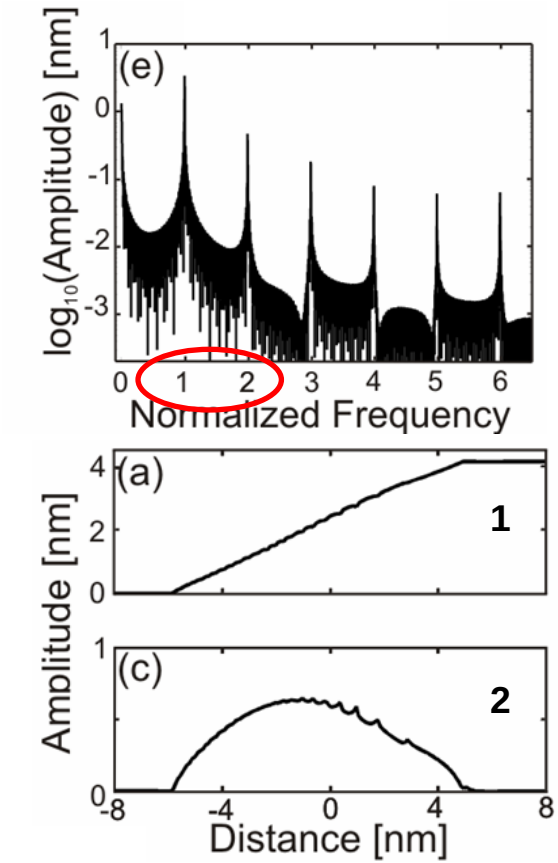
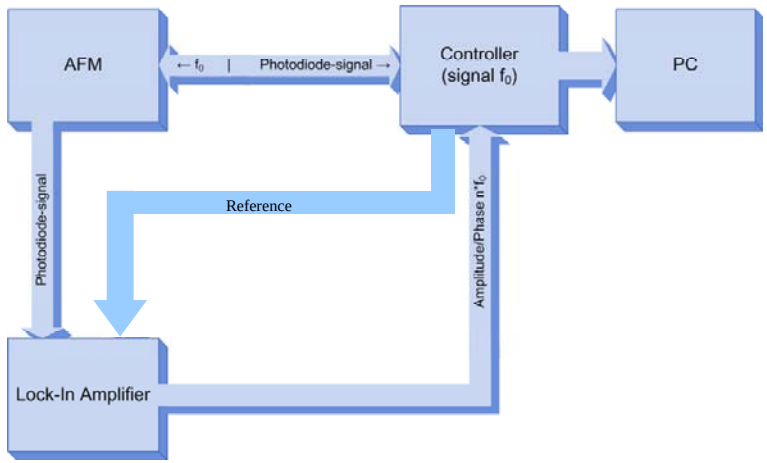
Simulation of amplitude vs. distance curves

Simulation



Experimental Setup:

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



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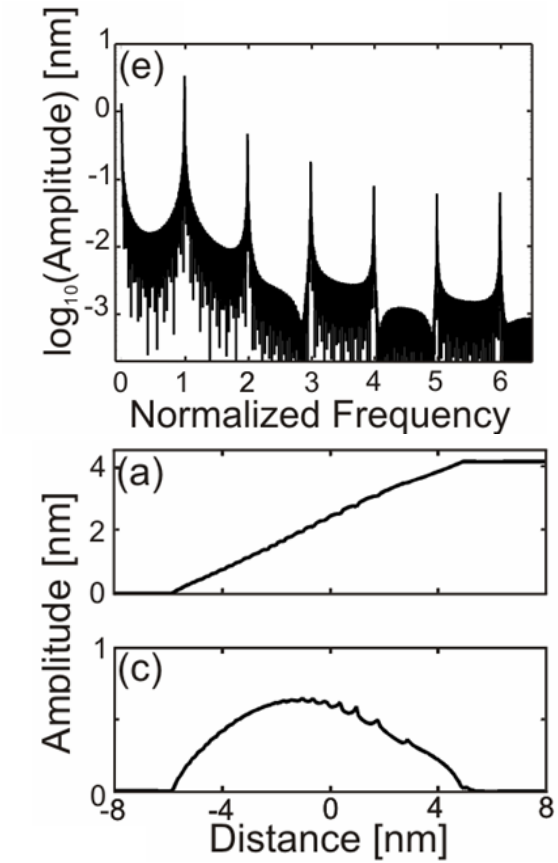
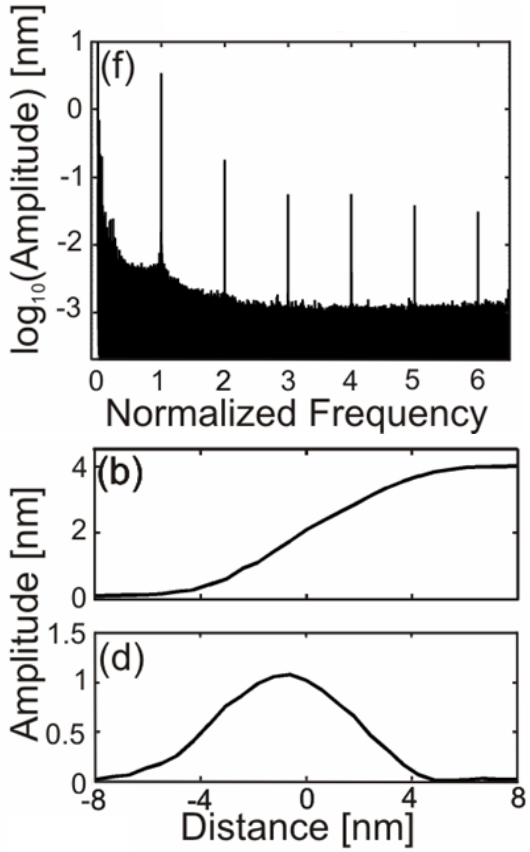
Comparison between simulation and experimental data

Simulation



Experiment



$A_0 = 4$ nm;
$A_{sp} = 3.5$ nm

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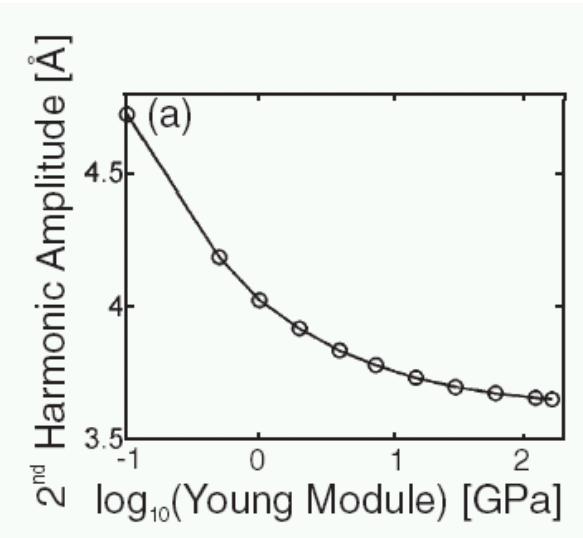
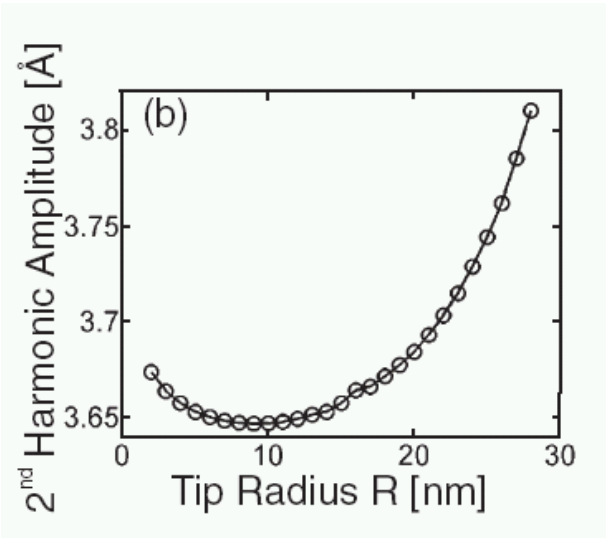
Dependence of the 2nd harmonic amplitude on interaction parameters

Contact Mechanics (DMT-model):

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





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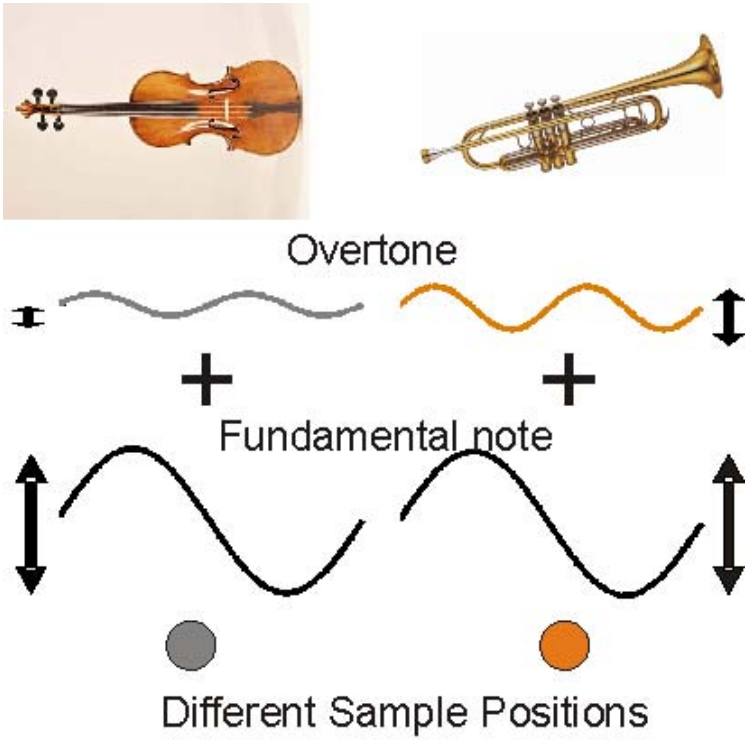
Dependence of the 2nd harmonic amplitude on interaction parameters



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

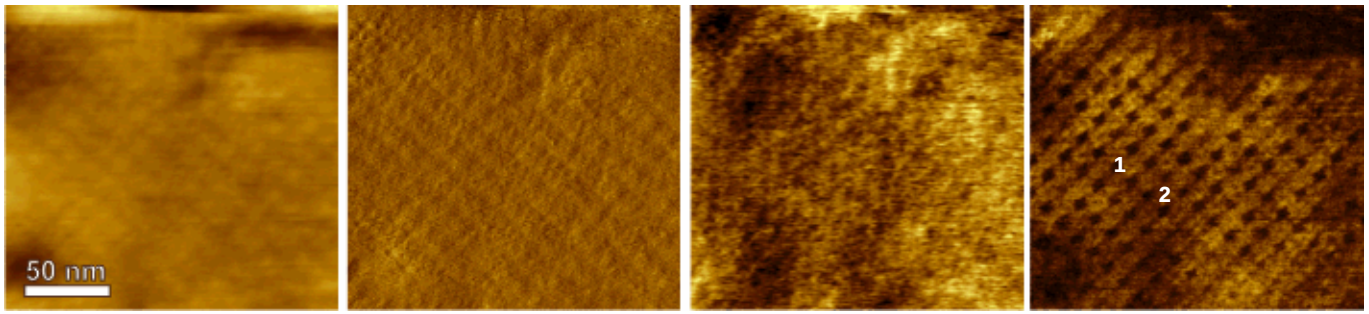
Reason: it‘s not only the fundamental note what defines the timbre (color of sound) but the sum of the fundamental note and all the harmonics

Analogy to AFM: recording harmonics in the cantilever movement (timbre) enables to distinguish different interactions

Images of bacterial S-Layer

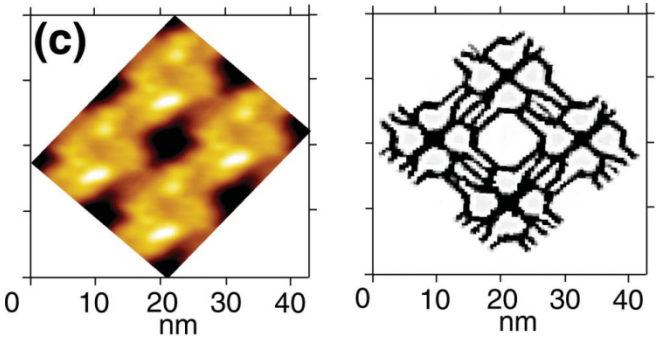
(sbpA, Bacillus sphaericus CCM 2177)

Topography **Amplitude** **Phase** **2nd Harmonic Amplitude**

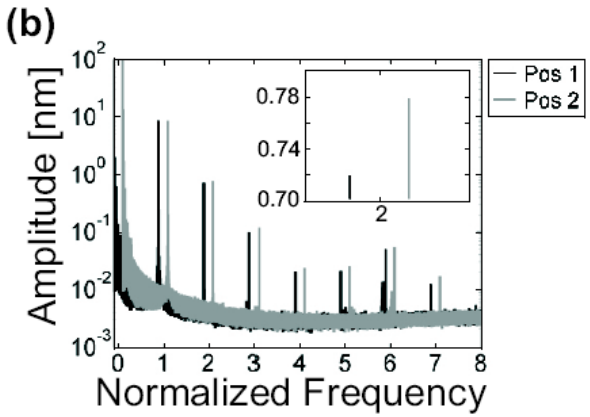


(A_0=10 nm,
A_{sp}/A_0=0.85,
f_d 7.3kHz,
k=0.1 N/m,
PBS Buffer)

color code: 0-5 nm



Periodicity
~14 nm



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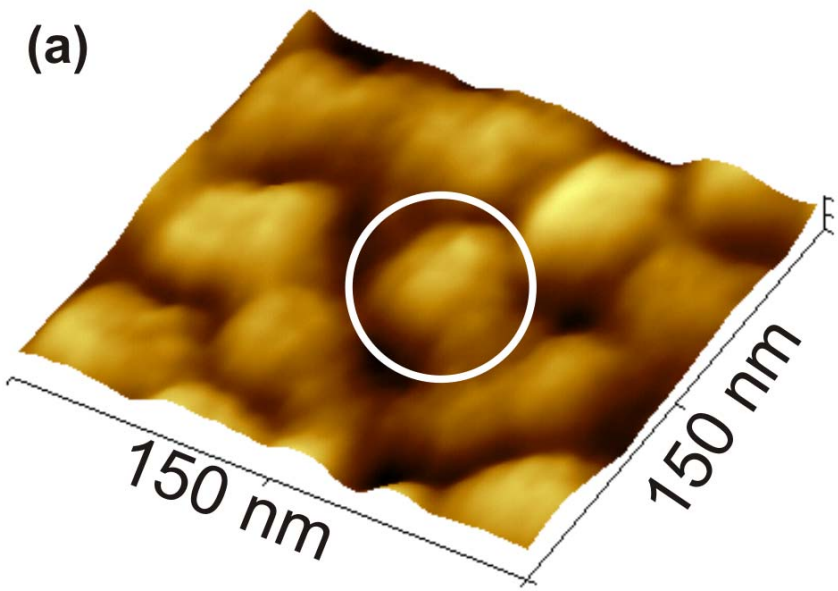
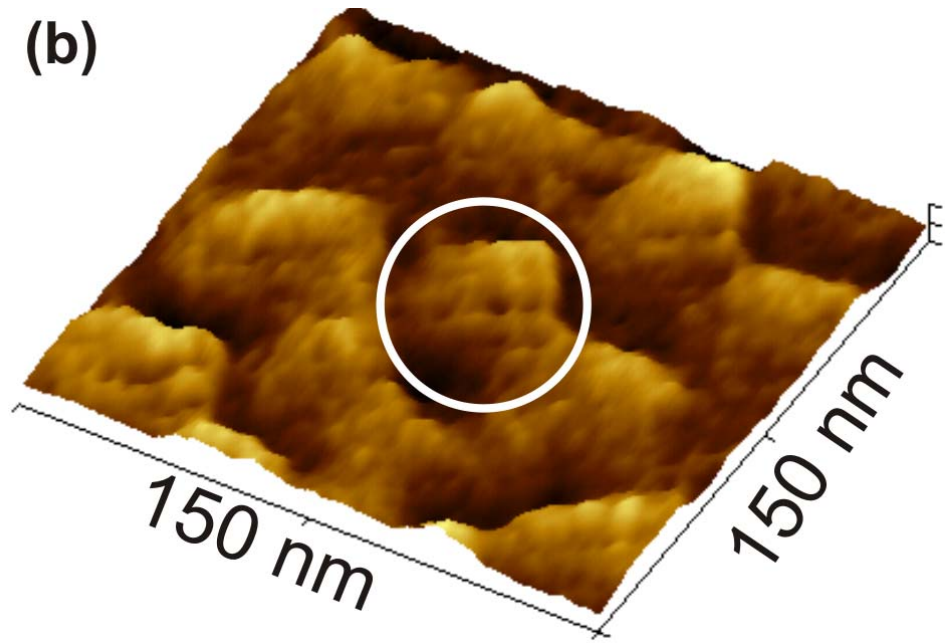
Rhino Virus (HRV2)

Topography



color code: 0-13 nm

2nd Harmonic Amplitude



(A_0=15 nm, A_{sp}/A_0=0.9, f_d 7.9 kHz, k=0.1 N/m, Ni-Tris Buffer)

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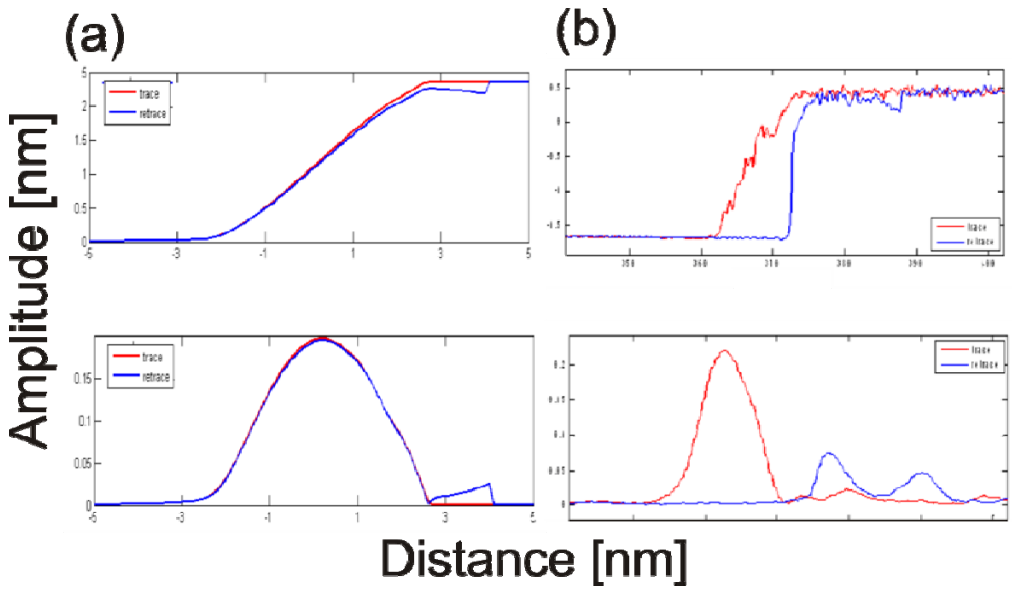
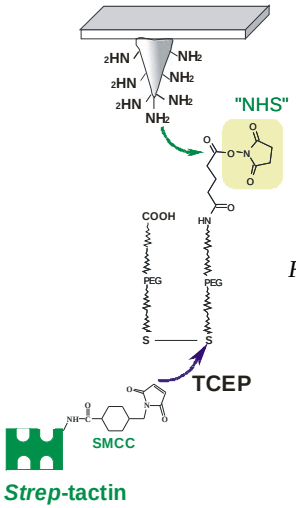
Imaging Recognition using 2nd Harmonic



Additional non-linear force due to PEG-linker stretching:



$$F_{WLC}(z) = \frac{k_B T}{L_P}\left[\frac{1}{4[2-(z/L_0)]^2} - \frac{1}{4} + \frac{z}{L_0}\right]$$

Simulation and experimental verification of dynamic approaching curves revealed characteristic signature of molecular recognition in 2nd harmonic amplitude

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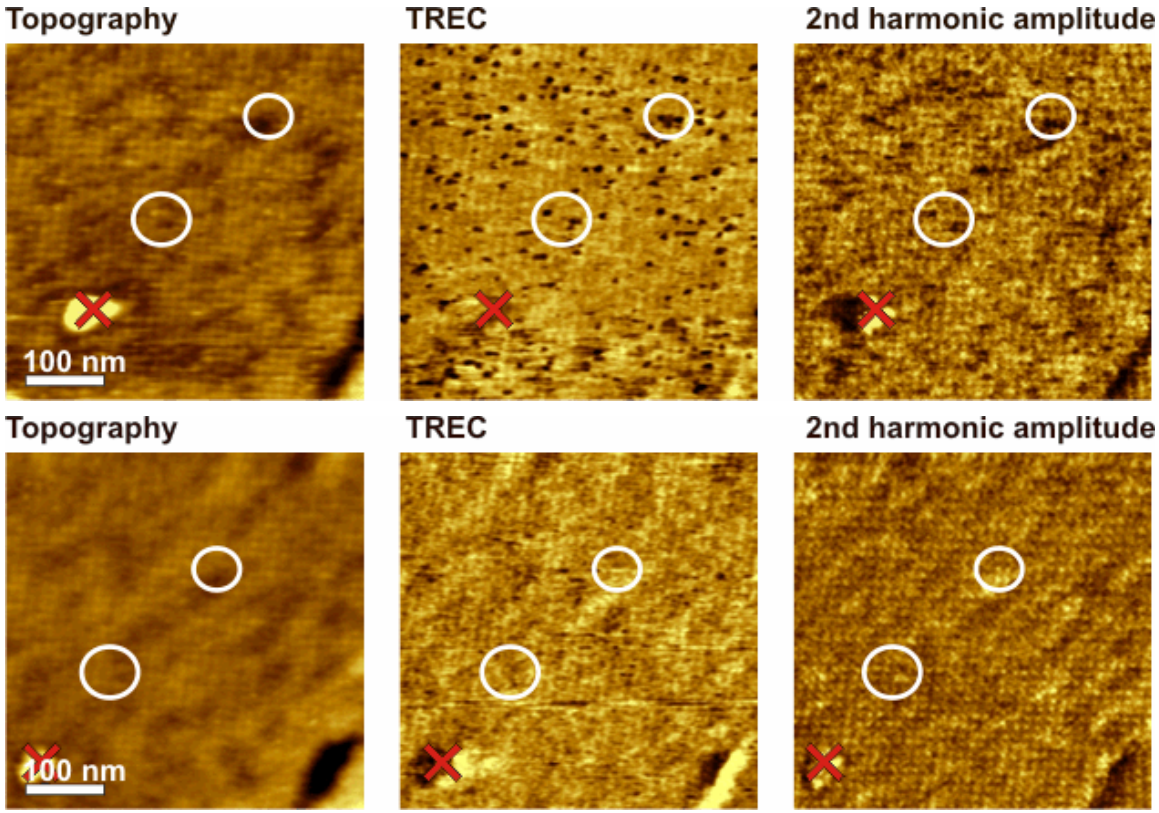
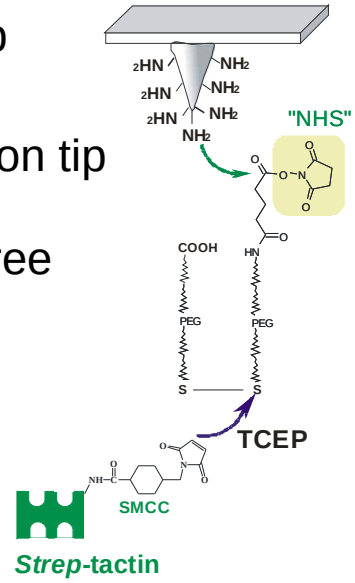
Imaging Recognition using 2nd Harmonic

Coparison of TREC and 2nd harmonic imaging of mol. recognition



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



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Summary

- Simulations of dynamic AFM in liquid: Amplitudes of higher harmonics are sensitive to variations in the nonlinear tip-sample interactions
- 2nd harmonic images of various samples in liquid (S-layer, HRV2,) showed detailed substructures not visible in topographical images
- 2nd harmonic images of molecular recognition

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