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## Understanding Cantilever Dynamics in Amplitude Modulation AFM: A Path to High Resolution Imaging and Compositional Mapping of Complex Biological Systems

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Amplitude modulation AFM (AM AFM) has long been used as the method of choice for imaging biological samples. This is due to two main reasons: firstly, elimination of damaging shearing interactions between the AFM tip and the surface, and secondly, the requirement to image in ambient conditions or liquid has tended to restrict cantilevers to those with relatively low quality factors cantilevers, as compared to UHV operation where frequency modulation (FM) is usually used. Since the amplitude response of the cantilever to changing interactions between the tip and sample is slow, the cantilever is rarely in a steady-state either during imaging or during approach/retract curves. This means that to understand image formation and extract the maximum amount of information from AM AFM images, requires an in depth insight into cantilever dynamics for given situations, i.e. regions of AFM operational parameter space and/or different environmental conditions.

This presentation will focus on two application areas as exemplars of how understanding the cantilever dynamics can dramatically improve the use of AM AFM:

- 1) Imaging of single molecule DNA samples in air
- 2) Higher harmonic imaging of complex surfaces in aqueous liquids.

### 1) Single molecule DNA imaged in ambient conditions

A decade or more of theory and modeling of cantilever dynamics has now yielded a fundamental basis for understanding image contrast formation in AM AFM in air [1]. Despite these efforts, experimental studies are few and far between and the connection between experiment and theory has not often been made. It is important to emphasize that the models may over-simplify the complexity of the experiments but they largely reproduce the general behaviour. I will present recent work using double-stranded DNA on mica as a test system to show the connection between modeling and experiments [2].

This work highlights the effect on resolution of the cantilever oscillating in the High or Low amplitude states (Figure 1). Relationships between amplitude states, phase-shift, force regimes and tip-sample contact times will be discussed (Figure 2). Counter-intuitive results, such as the emergence of noise in certain regions of parameter space can be rationalized through the stochastic nature of the cantilever bi-stability. Understanding of the occurrence of bi-stability

in AM AFM and control of the cantilever dynamics leads to higher resolution imaging on a consistent basis including the helical pitch of DNA.

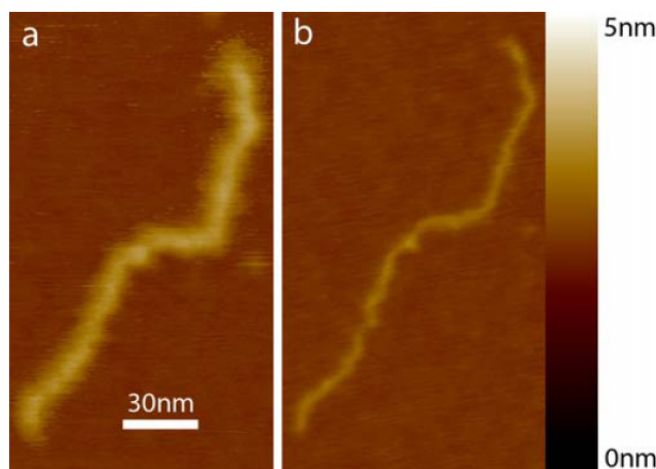


Figure 1: Imaging of double-stranded DNA using AM AFM in air. When imaging in the Low amplitude state (a), the half-width of the DNA is 14.7 nm and in the High state (b) it is 5.9nm, in this particular case.

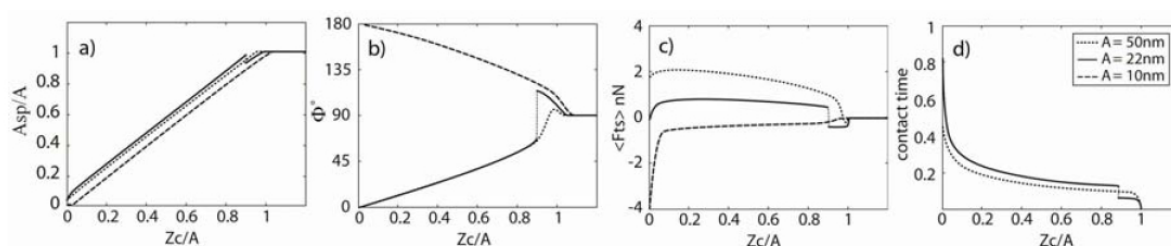


Figure 2: Numerical simulations of tip-sample distance relationships for a) amplitude, b) phase, c) average force and d) normalized tip-sample contact time per cycle obtained at resonance for three values of cantilever free amplitude. The cantilever parameters are  $f_0=300$  kHz,  $Q=500$ ,  $k=40$ N/m and the sample parameters are those typical of an intermediately stiff sample ( $E=1.5$ GPa).

## 2) Higher harmonic imaging in aqueous liquids

Recent developments in AM AFM include multi-frequency methods where higher eigenmodes or higher harmonics of the cantilever are excited, either through transient interaction with the sample during every cycle of intermittent contact or by direct external excitation. In liquids, higher harmonics of the driving frequency are transiently excited in the AFM cantilever, where the quality factor is typically close to unity. Higher harmonic imaging under liquids has the potential to reveal information about surface properties more directly than the fundamental amplitude and phase signals, while topographical coupling appears to be reduced. To date, very few studies have been carried out on biological samples while the tip-sample interactions affecting excitation of these higher frequencies could be better understood.

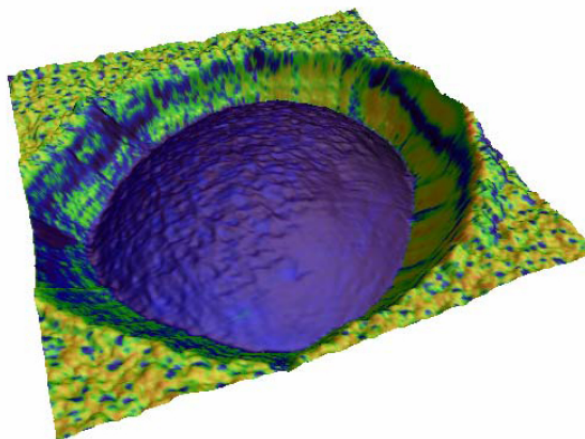


Figure 3: Individual *Staph. aureus* bacterium trapped in an specifically etched filter membrane. 3D-rendering of the second harmonic amplitude (false colours) painted onto the Z-piezo signal. Image size: 1.4 x 1.4  $\mu\text{m}$ .

Here we used collagen fibrils physisorbed on silicon and bacteria (*Staphylococcus aureus*, NCTC 8532) trapped individually in polycarbonate track-etched filter membranes [3] as test systems to elucidate factors that influence the second harmonic signal [Figure 3]. Higher harmonic excitation was reduced as the AFM tip moved from the hard background supports onto the softer biological structures. Sufficient signal-to-noise was available in the second harmonic to produce images of the bacterial cell surfaces and collagen fibrils. There was a broad correlation between second harmonic amplitude and the elasticity of the surfaces, and there appeared to be more detailed contrast on the biological structures. Analysis of correlations between signals indicated that the second harmonic signal is not strongly correlated with topography (Z-piezo) [4]. Comparison of the second harmonic signal on collagen fibrils with mechanical measurements, taken using force-volume imaging, indicated that there is a negative correlation between the plastic work done on the fibrils during indentation in contact mode and the second harmonic signal in amplitude modulation. This suggests that work done in deforming the sample is one of the mechanisms by which energy is dissipated from the AFM tip to produce contrast in a higher harmonic images.

#### References

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