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Height anomalies in tapping mode atomic force microscopy in air caused by adhesion

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Abstract

Height anomalies in tapping mode atomic force microscopy (AFM) in air are shown to be caused by adhesion. Depending on the damping of the oscillation the height of a sticking surface is reduced compared to less sticking surfaces. It is shown that the height artefacts result from a modulation of oscillatory movement of the cantilever. Damping and excitation of the cantilever by the driver continuously compete. As a consequence a severe modulation of the cantilever oscillation occurs, depending on the phase mismatch between the driver and the cantilever. Phase images of tapping mode AFM show contrast which correlates with adhesion. Examples of a partially removed gold layer on mica, a Langmuir–Blodgett film and DNA show height artefacts ranging up to 10 nm.

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1. Introduction

In recent years the use of the tapping mode in Atomic Force Microscopy (AFM) has gained increasing popularity [1]. In tapping mode the cantilever, on which the tip is mounted, is oscillated at resonance. As the tip approaches the surface the oscillation is damped and the reduced amplitude is kept constant in a feed back loop. Because the tip only touches the sample during the impacts, the lateral forces on the sample, which are liable for the destructive nature of the constant force mode, are significantly reduced. Furthermore, the penetration of the tip into the surface will be less because of the viscoelastic properties of the sample. Thus both damaging and indentation of the sample by the tip are minimised.

Because of this gentleness tapping mode AFM has become favourable in most biological AFM studies. The resulting height measurements, however, have

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been the subject of discussion in a number of papers [2–4]. In order to explain the discrepancies in the height measurements a detailed analysis of the dynamics of the oscillating tip is necessary. In this paper a study of one aspect of the contrast mechanism of tapping mode in air is presented: the way in which adhesion between the tip and the sample affects the height information in the acquired tapping mode image. In the samples we have studied, large adhesion differences are caused by differences in the thickness of the adsorbed water layer, which is a function of the hydrophobicity. In order to rule out indentation of the sample, which affects the height measurement, we use only hard samples.

2. Analysis

In order to estimate the interactions between tip and sample, calculations on a driven damped harmonic oscillator as a model system have shown quite useful [5]. The movement of the cantilever z can be described by Eq. (1), using an effective mass m, spring constant k and quality factor Q.

$$m\frac{\partial^2 z}{\partial t^2} + m\frac{\omega}{Q}\frac{\partial z}{\partial t} + kz(t) = KA\sin(\omega t) + F(z(t)) . \quad (1)$$

When the driver oscillates at the resonance frequency ω_0 of the cantilever, with an amplitude A and the tip does not impact on the sample, the position dependent force F is zero. This results in a free oscillation z of the cantilever:

$$z(t) = QA\sin(\omega_0 t + \varphi) , \quad (2)$$

and a phase shift φ between the driver and the cantilever, being equal to 90° at resonance.

The impact of the tip on the sample causes a nonlinearity in the movement of the cantilever which can be modelled as a height dependent force F. This force depends on the (visco-)elastic properties of the sample when the tip is in contact, but equals zero if not. At low frequencies this force-distance curve is experimentally accessible and has shown useful in a number of applications. The influence of the elasticity of the sample on the tip penetration for example, can be accounted for, both in theory using numerical methods, and in experiment [6, 7].

When, for delicate samples, the repulsive forces are minimised, i.e. a small damping of the oscillation is used, attractive adhesion forces, which can range up to 100 nN, become dominant. The force $F(z(t))$ is not only discontinuous, the hysteresis of the adhesion force should also be included, making it non-trivial to solve Eq. (1) numerically. Only a few papers deal with these attractive forces between the tip and the sample in the tapping mode [8, 9].

In ambient conditions the adhesion between the tip and the sample can be attributed mainly to the water contamination layer on the sample. In force distance curves one can observe the tip snapping in the water layer a few nanometers above the surface. The tip will be decelerated by the viscous damping of this water layer. When the tip retracts the water layer has to be disrupted. Using environmental SEM Amrein [10] has visualised nicely the water layer covering both tip and sample. Both for the disruption of the water layer and for the viscous damping in the water layer, energy will be dissipated at the expense of energy of the oscillating cantilever. So after the impact the cantilever will not reach the same amplitude as before the impact, and the tip may not hit the sample surface during the next swing. Next to this effect, the sticking of the tip will increase the interaction time, which results in an extra phase lag. Because of this additional phase lag with the driver, the excitation is less efficient, also affecting the cantilever amplitude.

After the impact the cantilever, which has less energy than before, will gain amplitude again. The response of the oscillating system can be expressed with the time constant τ [11]:

$$\tau = 2Q/\omega_0 , \quad (3)$$

Thus when the driver starts oscillating, the amplitude of the cantilever grows exponentially with a rise time τ.

Depending on the quality factor Q and the amount of damping, it may take longer than one oscillation period before the amplitude is sufficient to impact on the sample again. Thus a periodic modulation of the oscillation will occur, resulting in a decrease of the average amplitude as detected by the AFM set-up. The smaller average amplitude will enter the feedback mechanism which is used to keep the oscillation amplitude constant. The AFM system will then respond to it like it responds to a decrease of amplitude caused by an increase in height of the sample: it will retract. In this way surfaces with a large adhesion force are expected to appear higher in topography than surfaces with a small adhesion force. Though under ambient conditions the main constituent in the adhesion force is capillary force of the contamination layer, other interaction forces are expected to result in similar effects.

The phase of the oscillating cantilever is very sensitive for the damping of the cantilever oscillation. Recently the phase was introduced [12] as a new contrast parameter in tapping mode AFM, claiming it to be sensitive for both the visco-elasticity and elasticity of the surface [9]. Magonov et al. [13] have shown how viscous damping of the cantilever tapping on polymer blends can be monitored using phase imaging. Next to (visco-)elasticity, tip sample adhesion is suspected to cause phase contrast. In the present paper adhesion induced phase contrast in tapping mode AFM is also studied. In order to separate elasticity effects from effects caused by adhesion, only rigid samples are used. This excludes many biological samples. The conclusions, however, are general and can be applied to all samples.

3. Experimental set-up

For the measurements a home built stand alone AFM was used, offering a great flexibility in parameter settings and tip choice [14]. In tapping mode the AFM measures, while keeping the oscillation amplitude constant, both topography and phase of the oscillation. In this article the set-point of the amplitude will be referred to as the damping and will be expressed in percentage of the free oscillation amplitude. The phase of the deflection is measured relative to the driver. Because the mechanical construction between the driver and the cantilever introduces an extra phase shift only relative values of the phase are measured.

When the phase of the oscillating signal varies, a proper amplitude detection scheme is necessary in order to measure the correct amplitude, which consists of a real and an imaginary part. If a lock-in amplifier is used for amplitude detection only the real part of the oscillating signal is measured. A change of phase will reduce the apparent amplitude to $A\cos(\Delta\varphi)$, which would affect the height measurement even further. In order to separate phase and amplitude information we used a true RMS detector, bandwidth 5 kHz, to measure the modulus of the amplitude.

Next to the constant force and tapping mode, our AFM system can also acquire real time adhesion force images in the *adhesion mode*. In this mode a force–distance curve is generated for each pixel, out of which both topography and adhesion force are extracted on-line. A detailed description of this mode is presented elsewhere [21].

For the experiments reported in this study a 100 μm triangular silicon nitride cantilever (Park Scientific) was used. According to the manufacturer's specifications this cantilever has a spring constant of 0.58 N/m, a resonance frequency of about 95 kHz, a tip radius of 20 nm and a quality factor of about 80. Due to the design of the AFM head, large oscillation amplitudes up to 300 nm can be used, which are necessary to overcome the adhesion forces exerted on this relatively weak cantilever. The properties of this cantilever enable one to acquire tapping mode, constant force mode and adhesion mode images without changing the cantilever. Thus the adhesion force, which depends on both the tip and the sample, is equal in experiments using different modes.

In order to follow the cantilever deflection more closely, deflection traces were recorded using a digital oscilloscope (LeCroy 9360, 600 MHz). The scope was triggered by the driver in order to be able to compare the phase of the different traces.

4. Results

4.1. Gold and mica

For the first set of experiments an evaporated gold layer on a mica substrate was used. The gold layer was partially removed by cleavage, resulting in patches of freshly cleaved mica. In air the capillary force of the thin contamination layer that covers the sample is the main constituent of the tip-sample adhesion [15]. Thus differences in adhesion in this sample are correlated with the hydrophobicity. The hydrophobicity can be quantified by measurement of the contact angle of a water drop on the surface. The contact angle measurement yielded $\pm 55^\circ$ for the gold layer and $\pm 15^\circ$ for the freshly cleaved mica.

In Fig. 1 the topography of the partially removed, evaporated gold layer on mica, as measured by constant force AFM, is shown. The adhesion force was measured using the adhesion mode and yielded an average of 60 nN at the hydrophilic mica surface and 21 nN at the gold surface. Fig. 2a shows a histogram of the height distribution of the image shown in Fig. 1. In the same plot the height distribution is compared with a tapping mode image of the same scan area. The latter was acquired with an oscillation amplitude of 150 nm. The height step at a mica gold fracture as measured by tapping mode AFM appears 9 nm less than measured with constant force mode. The stiffness of both surfaces ensures that elasticity does not affect the measurement. In order to make sure that the anomalous height measurement is indeed solely caused by adhesion the experiment was repeated with a similar, but silanized, AFM tip. The silanization took place by incubating the tip for 5 minutes in a solution of dichlorodimethylsilan (DCMS), which results in a covalent binding between the Si_3N_4 and the DCMS. This procedure reduced the adhesion forces down to 10 nN at the mica surface and 7 nN at the gold surface, while all other properties of the tip were not noticeably affected. The resulting height distributions, which were measured at another area of the sample, correspond quite well, as is shown in Fig. 2b. The difference in the average measured height step is reduced to less than 1 nm. This result

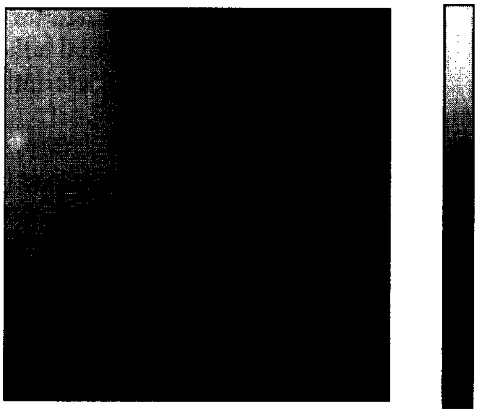

Fig. 1. Constant force mode AFM image of a partially removed, evaporated gold (left) layer on mica (right). Scan area 5 × 5 μm, scale bar 200 nm. Acquisition rate: 200 ms/line.

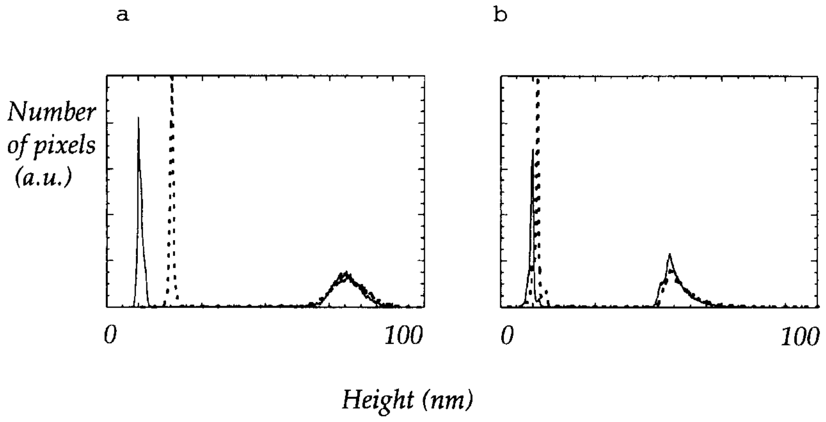

Fig. 2. Height distributions of tapping mode image (dashed line) compared with constant force mode image (solid line) of the sample shown in Fig. 1. The histograms were shifted to a coincidence of the peak corresponding to the gold surface. The tapping amplitude was 200 nm. In (a) the sample was scanned with a fresh silicon nitride tip. In (b) the experiment was repeated at a different part of the sample with a hydrophobic tip, which reduced the adhesion force. The solid line represents constant force mode data, the dotted line tapping mode.

proves that the height artefact is indeed exclusively caused by adhesion between the tip and the sample.

To gain more insight in the dynamics of the oscillating cantilever a few oscillations of the deflection of the cantilever have been plotted in Fig. 3. The deflection was measured with a fresh Si_3N_4 tip, at the gold and the mica surface while the system was in the feedback loop. This ensured the average amplitude to be 208 nm during both experiments, corresponding to a damping of the free oscillation amplitude of 31%. The deflection of the cantilever tapping on the mica surface, which has the largest adhesion force, shows a phase shift of about 34° compared to the curve that was acquired while

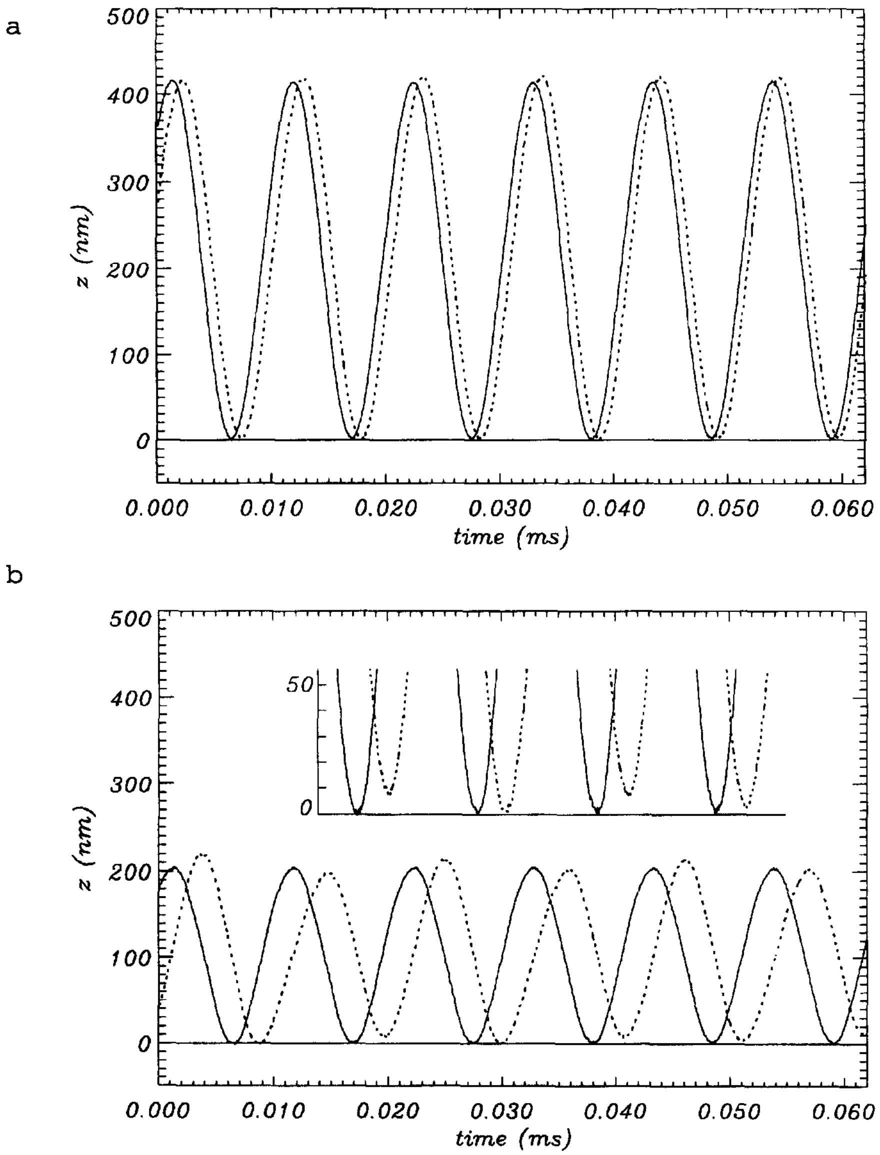

Fig. 3. Deflection of the cantilever tapping on a hydrophobic gold surface (solid line) and a hydrophilic mica surface (dashed line); (a) corresponds to a damping of 31%, (b) to 65%. Data acquisition was triggered by the driver oscillation in order to match the phase of the curves.

tapping on the gold surface. Furthermore, detailed examination of this curve reveals a small deviation of it's sinusoidal shape.

The deflection at the gold surface still looks nicely sinusoidal when the damping of the free oscillation amplitude is increased to 65% in Fig. 3b. The oscillation of the cantilever tapping on the mica surface however, is severely modulated. The tip only touches the surface at the very bottom of the deflection curve. It is clear that in the case of large adhesion forces and severe damping the tip does not impact on the surface every time it moves downward. Instead, after each impact on the surface the oscillation is severely damped. Due to the Q and the large damping it takes a few oscillations before the amplitude is recovered and the tip impacts on the surface again. This sequence is repeated continuously, and in order to keep a constant average amplitude, the tip has to be retracted from the sample, which accounts for the anomalous height measurement.

Next to the modulation of the sinusoidal movement of the cantilever, the phase of the oscillation is shifted backward by an average of 91° compared to tapping on the gold surface. When the damping of the free oscillation amplitude is increased from 31 to 65% the phase is hardly affected when tapping at the gold surface. In the inset of Fig. 3b it is shown that the tip touches the gold surface every oscillation period. On the mica during only one of the four shown oscillations the tip impacts on the surface. During the intermediate periods the tip approaches the surface, but it retracts at a distance of 3–10 nm from it. Furthermore, on mica, a subtle variation of the phase shift of the cantilever occurs, decreasing as amplitude is gained. In the intermediate regime of damping the number of oscillation periods between impacts grows and the phase shift decreases as the damping increases (data not shown). Remarkably, when sufficient damping is applied, damping of the oscillation can also occur when the tip does not impact on the sample as can be seen in the last period of the cantilever tapping on mica. Because of the large phase shift the cantilever is driven out of phase. Depending on the exact phase of the cantilever compared to the driver, damping and excitation continuously compete, resulting in a large modulation of the oscillatory movement.

4.2. Langmuir–Blodgett film

To study the adhesion related distortions on tapping mode AFM further a Langmuir–Blodgett (LB) film of lignoceric acid deposited on a silicon substrate was used. Lignoceric acid is a 24 carbon saturated fatty acid naturally occurring in animal tissue. The layer was deposited at such a surface pressure that only half the surface is covered. Fig. 4a shows a topographic image of the LB film,

Fig. 4. Adhesion mode AFM image of a lignoceric acid Langmuir–Blodgett film. The dark areas in the topography image represent the holes in the LB film. Scan area 2 × 2 μm; (a) represents height (range 2 nm), (b) the adhesion force (range 30–60 nN). Acquisition rate: 1400 ms/line.

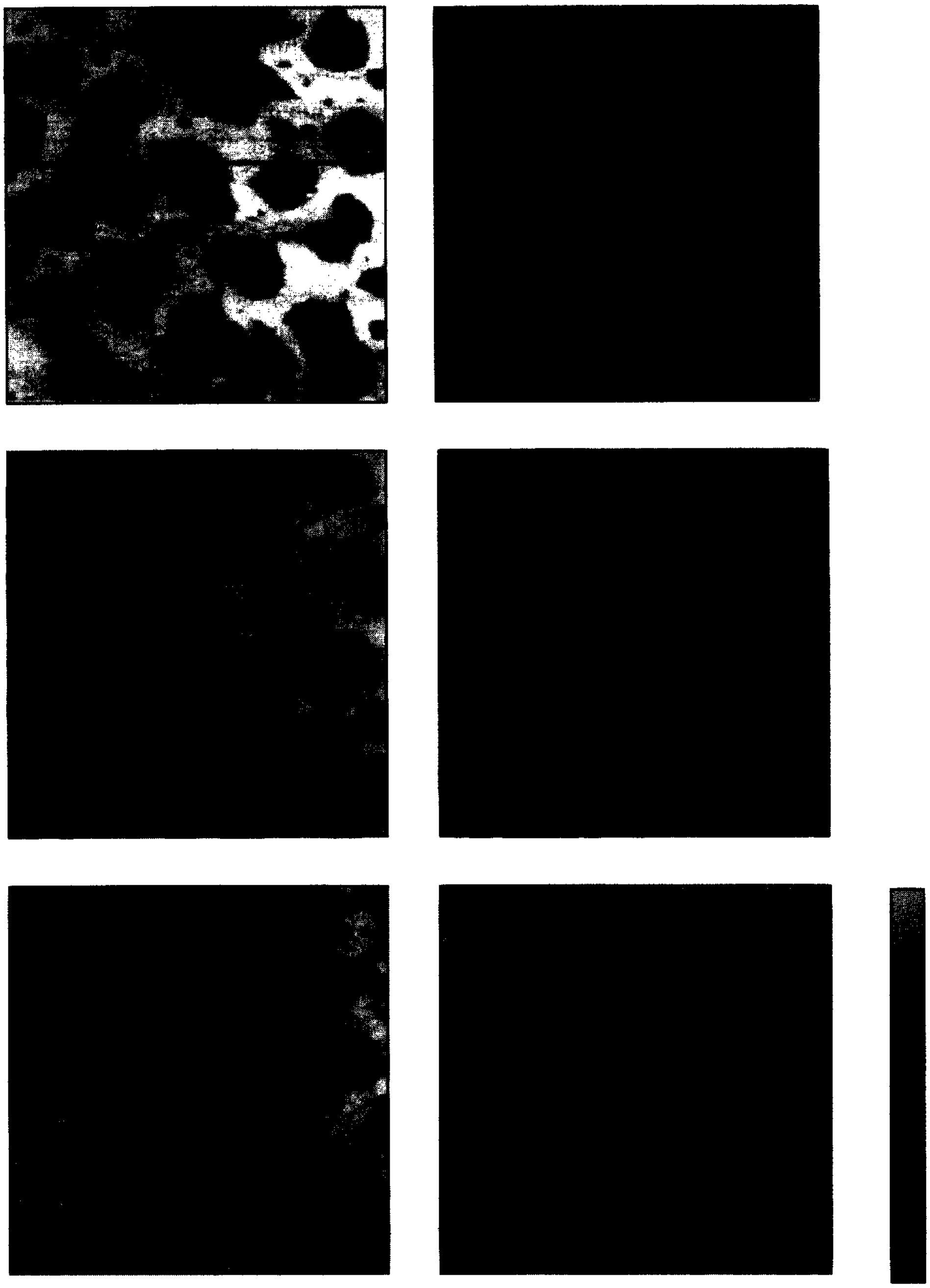

Fig. 5. Tapping mode AFM image of a lignoceric acid Langmuir–Blodgett film. Scan area 2 × 2 μm: (a), (c) and (e) represent height (scale bar 2 nm, (b), (d) and (f) the corresponding phase (scale bar 5°). The damping of the free oscillation (amplitude 135 nm) was increased from 8% in (a), 31% in (c) to 73% in (e). Acquisition rate: 200 ms/line.

measured with adhesion mode AFM. The layer thickness of the LB film yields 1.6 nm which equals the height as measured in constant force mode. The hydrophilic silicon substrate has a larger adhesion than the hydrophobic LB film as can be seen in Fig. 4b. The average adhesion force at the silicon substrate was 47 nN while at the LB film the adhesion force yielded 32 nN. The adhesion mode images were recorded at the end of the experiment, because the loading force using this mode was highest (100 nN). In Fig. 4 it can be seen that during a few lines the LB film was ruptured by the tip.

Fig. 5 shows the implications the extra damping due to adhesion can have on tapping mode topography and phase images. The images represent the same area as shown in Fig. 4 and were acquired with increased damping of the oscillation. As the damping increases the apparent height difference between the surfaces decreases and indeed in the last figure the topography contrast is inverted. In Fig. 6 cross sections shown in Fig. 5 images have been plotted. The average layer thickness deduced from the sequential tapping mode images was 1.2, 0.8 and − 0.3 nm. The measured height fitted the constant force mode data best when the damping of the oscillation was smallest, as shown in Fig. 5a. The height measured with the adhesion mode, where a loading force of 100 nN was used, matched the height measured with the constant force mode, using a loading force of 10 nN. Hence indentation is not likely to play an important role in the height anomalies.

Like the gold on mica sample the phase images of the sample show increasing contrast as the oscillation is damped. As the holes in the LB film have a higher adhesion the tip sticks longer to the surface, which results in a phase shift of the cantilever's oscillation. Indeed the phase correlates with differences in adhesion force when sufficient damping is used.

4.3. DNA

Finally, to show the relevance of the height artefact for biological samples a double stranded plasmid DNA (pSK31) sample is shown. The DNA solved in a $MgCl_2$ buffer was precipitated on a mica surface as described previously [16]. The height of DNA is usually a factor of 2 smaller than

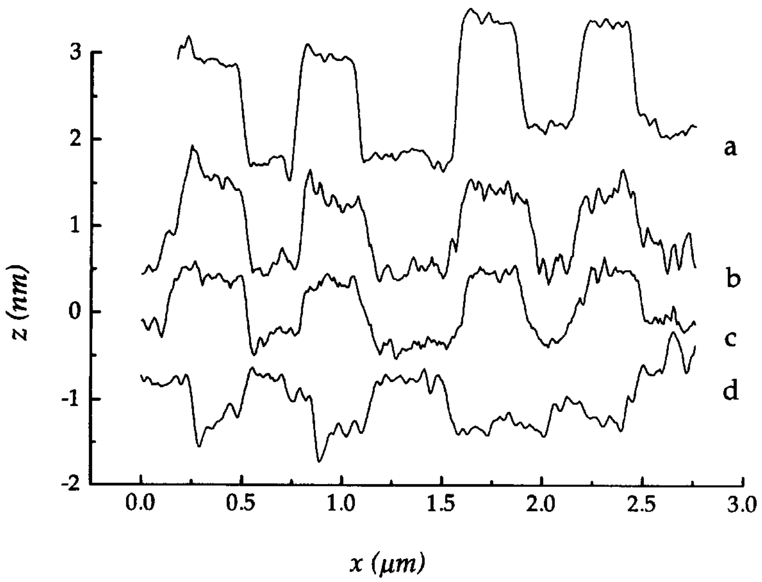

Fig. 6. Cross sections of the lignoceric acid LB film, taken from Fig. 5 at the line drawn in Fig. 5a. Line a is acquired in constant force mode, not suffering height anomalies, b, c, and d where taken from Fig. 5a, Fig. 5c and Fig. 5e.

Fig. 7. DNA strands on a mica surface. The damping of the oscillation was increased from top to bottom. Scan area 2×2 μm, height range 2 nm.

would be expected based known structure of double stranded DNA. This effect is commonly attributed to indentation of the DNA [3, 17]. Using non-contact AFM it was possible to measure the correct height of about 2 nm [18]. Fig. 7 shows an image of DNA in which the damping of the oscillation is increased from top to bottom. As a result of this the height of the DNA strands decreases from $+1.5$ to -0.5 nm. The negative height proves that indentation of the DNA cannot exclusively account for the discrepancy in these height measurements using tapping mode.

5. Detailed analysis of the cantilever oscillation

In the previous section it was shown that height measurements performed with tapping mode AFM can have errors of up to 10 nm if the differences in adhesion force are sufficiently large. This is caused by a severely affected cantilever oscillation due to tip-sample adhesion. When the tip sticks to the surface after an impact, extra damping of the oscillation occurs. Because of the high Q it takes time to gain amplitude again, and the AFM system responds to the reduced amplitude by retracting the tip. Increased damping results in a larger deviation from a sinusoidal movement and thus to a larger artefact in the topography images.

This behaviour can be explained by close analysis of the dynamics of the cantilever. When the oscillation is temporarily slowed down by adhesion, due to the high Q the amplitude will be gained slower as the oscillation is closer to free resonance. As a result of this the number of oscillations between each impact will increase as the damping is decreased, but what is more important for obtaining reliable topography data, the average deviation of amplitude is smaller. This is clearly demonstrated in Fig. 3, where little damping hardly causes a modulation of the oscillation while increased damping combined with adhesion causes a large modulation of the tip's movement. As the deviation of the sinusoidal movement is less when little damping is applied, the error in the measured average amplitude is less, resulting in a more reliable height measurement.

The phase lag between the driving force and the oscillating cantilever can also affect the cantilever movement. In resonance the undamped cantilever is 90° out of phase compared to the driver. In the case of extreme damping and large adhesion it can occur that after an impact the cantilever is so far out of phase that the amplitude first decreases due to the out of phase excitation, and only later increases again. Phase mismatches of up to 181° are shown in Fig. 3b. Using a small damping the phase shift is less and the modulation of the oscillation will be less severe. It is expected that the use of a lock-in amplifier would only enlarge the anomaly in the apparent height, as out of phase signal does not contribute to the measured amplitude.

Distortions of the sinusoidal deflection may be expected when extra interaction of the tip with the sample affects the cantilever movement. If the tip sticks longer to the sample and the surface is not indented a flattening of the deflection is expected to occur. From the deflection curves in Fig. 3, however, one cannot reveal actual damping of the cantilever motion during contact. This is due to the limited bandwidth of our detection electronics, which can measure up to 500 kHz. Higher harmonics, which are necessary to describe the small distortions, are not detected by the electronics; the deflection signal does not represent details of the cantilever movement.

6. Discussion

The data presented in this study are typical for a series of deflection curves measured with different parameters like adhesion and damping. The modulation of the sinusoidal deflection curve is always present if the adhesion force is not negligible. The magnitude and period of the modulation however decrease with increasing amplitude, indicating a reduced influence of the adhesion. However, because of the large number of parameters that influence both the adhesion force and the dynamic behaviour of the cantilever, it was not feasible to obtain an exact relation between adhesion and height anomalies and phase of the oscillation in this experimental study. Theoretical studies of the dynamic behaviour of the cantilever in the case of grazing incidence may reveal more insight in this mechanism.

The results of a model developed by Nordmark [19] for the dynamics of grazing impact oscillators, which have been widely used both in theoretical and in experimental studies, show a variety of border collision bifurcations [19, 20]. The number of oscillations per impact very sensitively depends on the characteristics of the system like driving frequency, amplitude, etc. The periodic modulations of the sinusoidal movement can be stable, but truly chaotic behaviour can also occur. In macroscopic systems with a large spring constant the effect of adhesion forces between the oscillator and the border can usually be neglected. However, in AFM systems with very weak cantilevers the magnitude of the attractive adhesion force cannot be neglected and the hysteresis of the interaction forces shows to be quite important in the case of grazing incidence. Though these theoretical studies do not take adhesion in account, the modulations of the sinusoidal motion that are observed are extremely sensitive for the frequency of the driver. Exact control of this parameter is not feasible in our experimental set-up, but it may account for some variation in our experiments.

At a frequency far below the resonance frequency of the cantilever the mapping of adhesion force has proved very powerful to reveal information about the chemical properties of the sample [21]. Though it is hard to quantify the effect of adhesion on the phase, especially because the adhesion force may be very sensitive for the interaction time [17], qualitatively it is shown that phase contrast is related to adhesion. To reveal adhesion in the phase images of tapping mode not only reduces the acquisition time compared to mapping the adhesion at a low frequency, it also enables one to use this contrast parameter for very fragile samples which can only be scanned using this mode. On the other hand the contrast in the phase images can be minimised in order to obtain more reliable topography data in tapping mode AFM.

As a consequence of the complex dynamic behaviour of the cantilever the tip only exerts a force on the sample during the limited number of impacts on the sample. As the damping increases both the average number of impacts per oscillation and the force per impact increase as the amplitude grows faster. So small damping of the amplitude is not only preferable in order to obtain more reliable topography data, it also reduces the forces exerted on the sample and thus reducing damaging and indentation.

The mechanism described in this article not only applies to the relatively weak cantilevers shown in this study. Contrast inversion of DNA has also been measured using stiffer silicon cantilevers, more commonly used for tapping mode AFM. The larger Q factor, and thus larger rise times of the oscillation amplitude, and the higher resonance frequency make these cantilevers even more susceptible for adhesion induced height anomalies.

7. Conclusions

Differences in adhesion at the sample surface result in height anomalies in tapping mode AFM in air. When small features like, e.g., LB films, DNA and proteins are the subject of research these differences can even result in negative heights. In order to explain the discrepancies careful analysis of the involved forces and the dynamics of the cantilever is necessary. Less damping of the free oscillation reduces the adhesion induced height anomalies, but some margin in the amplitude set point is always necessary for a stable feedback loop. For more reliable topography data it might be preferable to reduce the adhesion for example by modification of the tip.

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