Dynamic force microscopy in liquids

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We applied dynamic force microscopy in a liquid environment to silanized and derivatized glass surfaces, InGaAs, as well as to biological materials such as hexagonally packed intermediate layers of deinococcus radiodurans. The vertical and lateral resolution were estimated to be <1 Å and 7–10 nm, respectively. Upon immersing the cantilever into water, the resonance frequency was found to be reduced by a factor of two and the $Q$ factor was lowered to 20–30. The experimental working distance between sensor and sample was determined with approach curves indicating that the range of interaction in water is much shorter compared to air.

I. INTRODUCTION

Scanning probe methods (SPM) have the potential to become important techniques for structural analysis of biological samples in direct space. The biological specimen can be probed in situ without intense sample preparation. Scanning tunneling microscopy (STM) and scanning force microscopy in the contact mode (SFM) have shown the successful application of these SPM techniques for investigating biological objects such as DNA and two-dimensional (2D) protein array membranes. The extension of SFM to liquids allows investigation of soft biological materials in their natural environment. Additionally, the strong repulsive interaction forces always present during SPM measurements in air can be minimized by choosing adequate liquids. In situ investigations of adsorbed molecules should allow observation of their biochemical functions leading to a more complete and general understanding of biochemical structures and processes. The key for such a successful experiment is the minimization of the normal and lateral interaction force components in order to probe the biological specimen at highest resolution. Thus, dynamic force microscopy was introduced for nondestructive and reliable operation on soft biological materials.

In contrast to conventional SFM where the sample is probed by short-range repulsive interaction forces, noncontact scanning force microscopy (DFM) allow the investigation of many types of samples nondestructively by long-range attractive forces. The operation of the DFM in a liquid environment is prerequisite for the investigation of very soft biological materials. Scanning probe methods (SPM) have the potential to become important techniques for structural analysis of biological samples in direct space. The biological specimen can be probed in situ without intense sample preparation. Scanning tunneling microscopy (STM) and scanning force microscopy in the contact mode (SFM) have shown the successful application of these SPM techniques for investigating biological objects such as DNA and two-dimensional (2D) protein array membranes. The extension of SFM to liquids allows investigation of soft biological materials in their natural environment. Additionally, the strong repulsive interaction forces always present during SPM measurements in air can be minimized by choosing adequate liquids. In situ investigations of adsorbed molecules should allow observation of their biochemical functions leading to a more complete and general understanding of biochemical structures and processes. The key for such a successful experiment is the minimization of the normal and lateral interaction force components in order to probe the biological specimen at highest resolution. Thus, dynamic force microscopy was introduced for nondestructive and reliable operation on soft biological materials.

In contrast to conventional SFM where the sample is probed by short-range repulsive interaction forces, noncontact scanning force microscopy or dynamic force microscopy (DFM) allow the investigation of many types of samples nondestructively by long-range attractive forces. The operation of the DFM in a liquid environment is prerequisite for the investigation of very soft biological matter in its natural conformation, i.e., in aqueous or in buffer solution.

In this article we report on DFM investigations in a water environment. Results on InGaAs, silanized and derivatized glass surfaces, and hexagonally packed intermediate protein layers (HPI) are presented. Furthermore, we analyze and discuss cantilever resonance curves and “force versus distance” curves measured under ambient and liquid conditions.

II. EXPERIMENT

In contrast to conventional SFM where quasi-static deflections of a soft cantilever are measured, in DFM a stiffer cantilever is dynamically driven close to its mechanical resonance frequency by 1–3 nm. The cantilever motion is commonly detected either by cantilever deflection or interferometry readouts. Force gradients resulting from various interaction forces between tip and sample, such as van der Waals, electrostatic, or magnetic forces, detune the mechanical resonance system, according to

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{c - F'}{m_{\text{eff}}}}$$

where $F'$, $c$, and $m_{\text{eff}}$ denote force gradient, cantilever spring constant, and effective mass of cantilever, respectively. Depending on the $Q$ value of the resonance, frequency detection is mostly applied in vacuum ($Q \sim 50000$) whereas amplitude detection is used in DFM experiments under ambient conditions ($Q \sim 1000$) and under liquids ($Q \sim 20$). For our measurements in an aqueous environment we either use lock-in technique or a rectifier (rms-to-dc converter) for amplitude detection. In contrast to the lock-in technique where amplitudes are measured at a certain reference frequency, a rectifier converts all amplitude contributions in a large frequency window (in our setup: 30 kHz–2 MHz). One qualification is that not only force gradients affect the cantilever amplitude; damping effects resulting from, e.g., hydrodynamic interaction have to be taken into account as well.

All data presented here were taken using microfabricated rectangular Si cantilevers with integrated tips ($f_0 \sim 400$ kHz, $c = 60–100$ N/m) that have been modified in some cases with contamination tips.

For measurements in liquid environment the cantilever was immersed into nanopure water. However, we also worked in buffer solution, but no significant difference was perceptible. All data presented in this work is uncorrected.

III. RESULTS AND DISCUSSION

InGaAs surfaces were investigated with the DFM in air and in liquids. The cantilever was thereby driven just above its lowest resonance frequency (368 kHz) and in some cases at its second harmonic (1.19 MHz). The measurements at the second harmonic oscillation did not reveal any difference. Figure 1(a) shows a topography image taken under ambient conditions of a 900-nm-thick InGaAs layer epoxially grown on a InP wafer. In Fig. 1(b) the same surface was measured in a water environment. The flat areas exhibit uni-
Fig. 1. (a) Topograph of a 900-nm-thick InGaAs layer epitaxially grown on an InP wafer obtained by DFM in air. The image reveals a very flat surface with deep trenches resulting from the cleaving procedure. The flat area exhibits uniformly spaced surface steps with a measured step height of 2–3 Å, which agree with assumed monatomic surface steps of 2.5 Å. For better visualization of the steps a derivative image is shown as an insert. (b) Derivative image of an InGaAs surface investigated with the DFM in water. The cantilever was driven near its second harmonic at 1.19 MHz. The noise level along DFM traces crossing the monatomic steps was estimated to be ∼1 Å. (c) Etched glass coverslips, commonly used as substrates for biological materials, exhibit a characteristic hole pattern. (c) represents a DFM image in air where holes with a diameter of 20–25 nm are clearly visible. (d) Corresponding measurements taken under water exhibit holes with the same spatial distribution and a depth of 3–6 nm.

Formally spaced surface steps with a terrace width of 140–200 nm. For better visualization a derivative map is shown as an insert in Fig. 1(a). The measured step heights of 2–3 Å agree well with assumed monatomic surface steps of 2.5 Å. From the noise level along DFM traces, the vertical resolution was estimated to be <1 Å in both cases.

Etched glass coverslips commonly used as substrates for the immobilization of biological materials exhibit a characteristic hole pattern, presumably due to surface etching during substrate cleaning. Figure 1(c) represents a DFM image taken under ambient conditions where holes with an average diameter of 20–25 nm are clearly visible. The corresponding measurements taken under water [Fig. 1(d)] also show holes with the same spatial distribution and a depth of 5–6 nm. Nevertheless, we found that these chemically treated glass substrates provide a very flat and smooth surface well suited for the adsorption of biological specimens.

As a last example, we investigated HPI layers, a hexagonal packed intermediate layer of Detococcus radiodurans forming two-dimensional protein sheets. For immobilization, the HPI layers have been covalently bound by a photoactive crosslinker on treated glass coverslips. Figure 2 shows a DFM image of these protein sheets taken in air (a), and in water (b). The hexagonal structure of the protein array with a periodicity of 18 nm is clearly visible. At present, measurements under ambient conditions allow the resolution and identification of the hollow core structure as well as the bridges interconnecting the protein rings of the HPI, suggesting a lateral resolution of ∼2 nm. In water, a lateral resolution of 7–10 nm, estimated from spatially resolved surface adsorbents, was achieved. In both cases the measured layer thickness of ∼5 nm is in good agreement with theory.

In addition, approach experiments were performed to determine the apparent interaction and the working distance between cantilever and sample surface. In contrast to the tapping mode, where the cantilever is oscillated at an amplitude of 50–100 nm and where the tip interacts repulsively, hitting the sample surface, we work with much lower amplitudes of 0.5–3 nm. These experiments therefore allow differentiation between tapping mode and noncontact mode. To determine this interaction distance we performed approach experiments where the cantilever was driven a little above its resonance frequency with an amplitude of ∼1 nm and used the rectifier circuit to control and stabilize the tip with respect to the sample surface. In Figs. 3(a) and 3(b) we compare two approach curves taken in air and in water opposite to a glass substrate, respectively. The average deflection of the vibrating cantilever corresponding to the force acting between tip and sample is plotted in curve F. The rectified amplitude components from the oscillating cantilever are plotted in curve A. The center vertical line (dotted) in the diagram represents the working distance. By comparing curve A in Figs. 3(a) and 3(b) we found that the slope of the rectified amplitude signal in water is much steeper than in air. Obviously the interaction range between tip and sample in water is drastically reduced. It seems that long-range attractive (van der Waals) force components, determining measurements in air up to a tip-sample separation of 70 nm, are no longer dominant in water. Other forces responsible for strong damping effects are now relevant, reducing the interaction range in liquids down to 2–5 nm. Since larger amplitudes lead to strong repulsive interaction components, increasing the probability of damaging the surface, very small
The dynamical properties of the sensors were determined by driving the cantilever with a white noise signal and by Fourier analyzing the cantilever photodetector signal. The resonance frequency and the $Q$ factor were determined by fitting the experimental data with a calculated resonance curve. In Fig. 3(c) we present the frequency spectrum of a typical Si cantilever (60 N/m) in air with a resonance frequency of 368 kHz and its second harmonic at 2.25 MHz. (d) The frequency spectrum of the same cantilever immersed in water. A remarkable decrease of the resonance frequency (195 kHz) and its second harmonic (1.19 MHz) by a factor of 1.9, as well as a 20-times-lower $Q$ factor, is measured. The second and third harmonics measured in our experiments agree well with theoretical calculation on rectangular oscillating Si bars (Ref. 18).

In summary, we operated the dynamic force microscope in a water environment and applied it to glass and InGaAs samples. The in situ DFM investigation of these materials at a very high resolution with minimized lateral force components. The in situ DFM investigation of HP1 layers under water demonstrates the possibility of applying this noncontact technique to surfaces in their realistic native biological environment. Ongoing work will show the power and reliability of in situ noncontact DFM with respect to investigation of the structure and function of biomaterials.

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21 Type I reagent-grade water, Millipore Alpha-Q, resistivity > 18.2 MΩ cm, 
toc < 10 ppb.

23 The white noise signal was generated with a function generator from 
Stanford Research System.
24 In a liquid environment, contribution to the effective mass of the cantile-
ver from the liquid which is partly moved with the cantilever has also to 
be taken into account.