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Imaging soft matters in water with torsional mode atomic force microscopy

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ABSTRACT

We have developed a high-sensitivity atomic force microscopy (AFM) *mode* operated in aqueous environment based on the torsional resonance of the cantilever. It is found that the torsional mode can achieve a good spatial resolution even with a relatively large tip. We have used this mode to image different soft materials in water, including DNA molecules and purple membrane. High-resolution images of purple membrane can be obtained at a *relatively* low ion concentration under a long-range electrostatic force. Thus the torsional mode allows investigators to probe *surface structures and their properties under a wide range of solution conditions*.

1. Introduction

Operation of atomic force microscopy (AFM) [1] in aqueous environment is of great importance. Only this environment allows us to probe biological samples in their physiological conditions and to study many fundamental issues *at* water–solid interfaces. It has been proved that AFM can achieve a very high spatial resolution in vacuum as well as in air. However, operation in aqueous solutions is still very challenging because the force sensitivity of AFM in water is usually much reduced compared with the environment in air or in vacuum. If AFM can be operated with high force sensitivity in the aqueous environment, many puzzles in life science, electrochemistry, and liquid–solid interfaces may be unraveled.

One major problem for AFM imaging in water is the long-range electrostatic interactions resulting from the charges on sample surfaces. Müller et al. demonstrated that high-resolution images of biological samples can be obtained in aqueous solutions through carefully adjusting the ion concentrations in a buffer before AFM imaging [2]. The purpose is to balance the electrostatic interactions and van der Waals forces on the sample of interest, such that the tip loading force can be controlled to be smaller than 100 pN. At this “electrostatic balanced condition”, the ion concentration is typically high enough to screen the *surface charges.* In fact, it is very desirable to achieve high-resolution AFM imaging under solution of *relatively* low ion concentrations as well. This will not only simplify the AFM operation, but also allow us to probe different characteristics, such as surface charges and surface potentials, of the surfaces under a wide range of solution conditions.

Since electrostatic interactions are acting in the normal direction (the direction perpendicular to the surface), all flexural modes, including the contact and tapping modes, will be affected by them. On the other hand, in the torsional mode, the AFM tip is vibrating in the lateral direction (parallel to the sample surface) and the oscillation is affected by the lateral force gradient only. It has been shown that the normal forces have no effect on the torsional resonance (TR) [3]. The oscillation behavior starts to change only when the tip is getting in contact with the sample, which allows clear detection of the contact point and maintaining a soft contact between the tip apex and the sample. Thus one can use the amplitude, phase, or frequency shift of the torsional resonance as the feedback input signal for the torsional mode imaging.

The TR mode was initially developed to map in-plane mechanical properties on hard surfaces, such as friction, shear stiffness, and other tribologically relevant properties [4–7] and was typically operated in air with the amplitude-modulation (AM) detection scheme. Frequency-modulation (FM) TR mode was first demonstrated by Kawai et al. in vacuum [8]. Later, Yurtsever et al. used the FM-TR mode to image samples in air [9]. In very recent years, the TR mode has been applied to imaging in liquid [3,10]. Mullin and Hobbs reported their operation of the AM-TR mode in water [10]. Yang and Hwang have reported high-resolution imaging with the FM-TR mode in water [3]. In this work, we would like to show that TR mode allows high-resolution imaging of soft biological samples under a long-range electrostatic force. We also find that good spatial resolution can be achieved with the torsional mode even when a *relatively* blunt tip is used.

2. Experimental

Our experiments are carried out with a modified commercial beam-deflection AFM (DI NanoScope III(a) from Digital Instrument, Santa Barbara, CA). Fig. 1a shows a schematic of our

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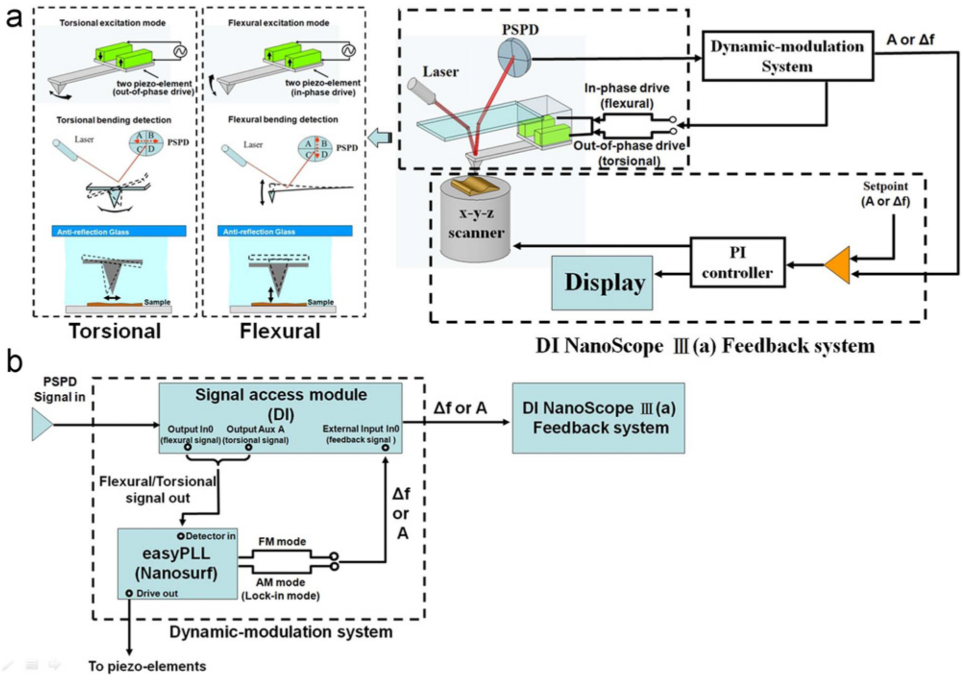

Fig. 1. Our experimental setup. (a) Schematic of flexural and torsional mode AFM. (b) Schematic for the dynamic-modulation system.

modified AFM set-up which allows selection of the flexural and torsional excitations and switching between the AM and FM detection schemes. The schematics for the torsional/flexural excitations, the optical detection, and the oscillation in liquid are illustrated in the left inset of Fig. 1a. For the torsional/flexural excitation, the two piezo-elements are out-of-phase/in-phase driven with a sinusoidal signal generated from a dynamic-modulation system. Under the torsional excitation, the cantilever executes a twisting oscillation about the long axis of the cantilever; under the flexural excitation, the cantilever oscillates in the normal direction, as illustrated in the upper part of the left inset of Fig. 1a. The middle part of the inset illustrates how the twisting and normal bending of the cantilever are detected with the beam-deflection method. The twisting/normal bending of the cantilever results in the lateral/normal movement of the laser spot on the quadrant position-sensitive photodetector (PSPD), and the related voltage output of the detector provides the amount of the torsional twisting/flexural bending of the cantilever. The lower part of the inset illustrates the torsional and flexural vibrations of the AFM tip in liquid. The tip vibrates laterally/normally for the torsional/flexural mode. An anti-reflection glass plate helps the trapping of a water droplet and also provides a stable interface for the detection of the laser beam. We usually wait for about 1 h before acquisition of AFM images is started because thermal drifts become less serious after that. Due to evaporation, each water droplet can last for ~3 h. If necessary, we may add water before the water droplet is about to disappear.

The oscillation of the cantilever is driven with a dynamic-modulation system, which is composed of a phase-lock-loop unit (Nanosurf® easyPLL plus system) and a Signal Access Module (Digital Instrument, Santa Barbara, CA). Schematic for the wire connections in the dynamic-modulation system is illustrated in Fig. 1b. We can select the FM/AM detection in the easyPLL unit. In the AM detection (*the tapping mode*), the lock-in mode is selected and the cantilever oscillation amplitude (A) is used as the input signal of the feedback system. In the FM detection, the easyPLL unit is used to track the resonance frequency of the vibrating cantilever and the resonance frequency shift of the cantilever (Δf) is used as the feedback signal of a PI controller, which outputs a z-axis signal to drive a piezo-scanner tube. We adopt the constant excitation mode, in which an oscillation signal with a constant amplitude is applied to the piezo-elements to drive the motion of the cantilever. The easyPLL also allows the operation with the phase-modulation (PM) detection schemes. In that case, the lock-in mode is selected in the easyPLL unit and the phase is used as the feedback signal of the AFM controller.

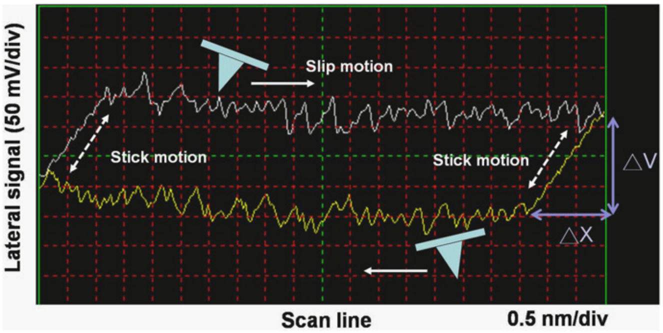

Fig. 2. Typical friction loop measurement. The white curve is the lateral signal corresponding to the twisting angle of the cantilever measured when the sample is displaced laterally from right to left. At a certain point, the scan direction is reversed and the measured lateral signal is shown in yellow. During the initial scan at a new direction, the tip sticks to the sample and the relation between the lateral signal (ΔV) from the PSPD and the lateral displacement (ΔX) of the sample can be determined. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In our AFM operation, we use a type of compliant silicon cantilevers with an Au-coated tip (MikroMasch, CSC38/Cr-Au, 350 μm × 35 μm × 1 μm, k=0.01–0.08 N/m, tip height ~15 μm, nominal tip radius ~50 nm), which is typically used for the contact mode operation. We have tested many AFM cantilevers, only this type of cantilevers can achieve nearly pure torsional resonance with little flexural oscillation at the torsional resonance frequency.

The torsional deflection can be calculated from the friction loop measurement [11], as shown in Fig. 2. In the sticking part of

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