Subnanometer-resolution Imaging in Liquid by Frequency Modulation Atomic Force Microscopy

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High-resolution imaging in liquid by FM-AFM is severely hindered by the extreme reduction of the Q-factor due to the hydrodynamic interaction between the cantilever and the liquid. We recently found that the use of the small amplitude mode and the large noise reduction in the cantilever deflection sensor brought a great progress in FM-AFM imaging in liquid. The force sensitivity is increased by FM detection with small amplitude oscillation because of the increase in the duration of the proximity interactions. Note that the small amplitude mode can be used only when the noise in the deflection sensor is sufficiently reduced down to a level of the thermal fluctuation of the cantilever. We found that the noise was effectively suppressed by decreasing the laser light coherence, which was experimentally performed by modulating the laser power with a high frequency signal (300-500 MHz).

In this presentation we describe subnanometer-resolution imaging of organic molecules including biomolecules in liquid using the improved FM-AFM. Figure 1 shows an FM-AFM image of a muscovite mica surface taken in pure water. The honeycomb structure of SiO4 tetrahedrons with a period of 0.52 nm is clearly seen. We also succeeded in obtaining high-resolution FM-AFM images of *bacteriorhodopsin* protein molecules hexagonally packed in a purple membrane as well as GroEL molecules, the chaperonin of *E. coli*, with a sevenfold-symmetric structure. The images were taken in buffer solution. In addition, hydration structures on a mica surface in water were investigated by FM-AFM with small amplitude oscillation. Figure 2 shows a frequency shift (corresponding to conservative force) vs. distance curve (Δf-d), where clear oscillation with a spacing of about 0.2nm due to the hydration structure was observed. The value is close to the size of water molecule (0.26nm). The success in high-resolution FM-AFM imaging in liquid has opened the new way to direct visualization of *in vivo* molecular-scale biological process.

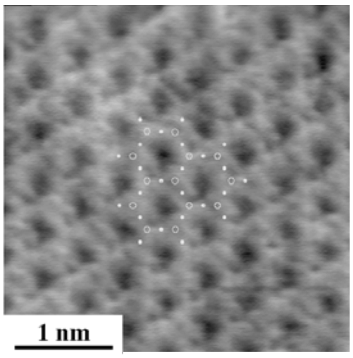

Fig. 1. FM-AFM image of a mica surface taken in pure water. (Δf = +20 Hz, A = 0.24 nm)

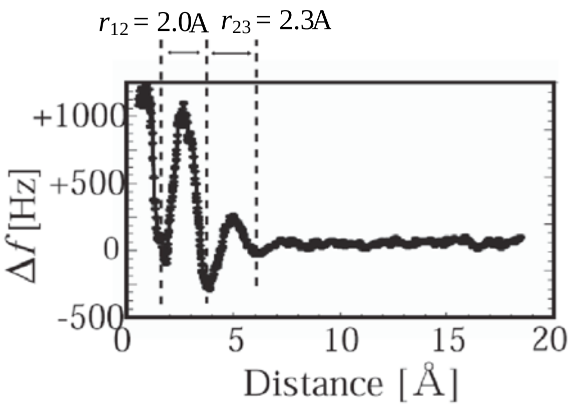

Fig. 2. Frequency shift vs. distance curve measured on a mica surface in water.