Development of Liquid-Environment Frequency-Modulation Atomic Force Microscope for Biological Applications

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Subnanometre-resolution of frequency-modulation atomic force microscopy (FM-AFM) has been demonstrated on various surfaces. Until recently, however, the high spatial resolution of FM-AFM was available only in ultrahigh vacuum environments. This limitation has prevented a wide range of applications in air and liquids. Recently, Fukuma *et al.* presented a way to overcome this limitation. They demonstrated true-molecular resolution of FM-AFM in air [1] and liquid [2] using a low noise cantilever deflection sensor [3]. One of the attractive applications is subnanometre-scale imaging of biological materials in their physiological environments. In this study, we have developed a liquid-environment FM-AFM dedicated to biological applications.

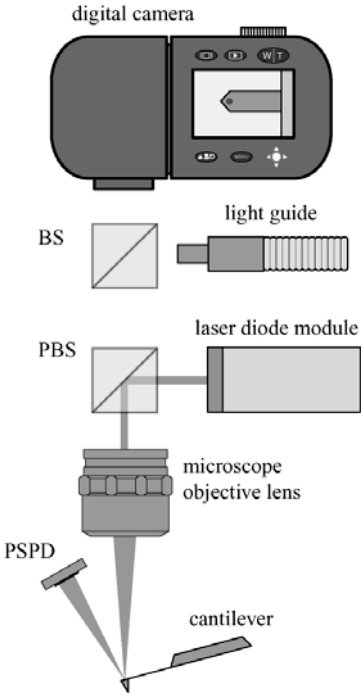

Fig. 1 Schematic structure of the optical part of our FM-AFM.

Imaging of biological materials often requires a large scan range: typically over 50 μm in *xy* and 10 μm in *z* directions. On the other hand, a rigid and compact scanner with a small scan range is desirable for high-resolution imaging. Another problem is the viscous damping of the cantilever vibration in liquids. This reduces the frequency and *Q* factor of the cantilever resonance, resulting in low force sensitivity and slow time response. In our AFM (Fig. 1), a microscope objective lens is used for focusing the laser beam as well as for providing a high magnification optical view through a digital camera. The high-resolution optical view provides the ability to control tip position on a large biological material so that we can deliberately choose the imaging area even with a narrow range scanner. The large numerical aperture (NA) of the objective lens reduces the laser spot size on the cantilever backside. This allows us to use small cantilevers with a high cantilever resonance frequency and high *Q* factor in liquids. The short cantilevers also provide higher deflection sensitivity in optical beam deflection method. The objective lens can be easily replaced with the one having a smaller NA and the same parfocal length. The small NA reduces the divergence of bounced laser beam. Thus, we can obtain high deflection sensitivity even with a relatively long cantilever. The laser diode module can be easily replaced with the one having a different wavelength. This is useful for combining a fluorescence microscope with the FM-AFM using an infrared laser diode.

[1] T. Fukuma, T. Ichii, K. Kobayashi, *et al.*, Appl. Phys. Lett. **86**, 034103 (2005)
[2] T. Fukuma, K. Kobayashi, K. Matsushige, *et al.*, Appl. Phys. Lett. **86**, (2005), in press
[3] T. Fukuma, M. Kimura, K. Kobayashi, *et al.*, Rev. Sci. Instrum. **76**, 053704 (2005)