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Scanning Probe Microscopy for Biological Samples

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Introduction

The importance of scanning-probe microscopy (SPM) is comparable to that of electron microscopy (EM) and optical microscopy (OM). SPM measures the near-field interaction between the scanning-probe tip and the sample surface that lies beneath it. A member of the SPM family, atomic force microscopy (AFM), has been of interest to various physical, chemical, material, biological, and electrochemical researchers, and highly functional instruments have been developed.[1-3] This interest may be because of its applicability for samples in *in situ* environments. In biological studies, the major advantage of AFM is that it can produce high-resolution topographic images in aqueous and physiologically relevant environments without the need to stain the specimen. This means that AFM overcomes the two major problems with both OM and EM. The heart of AFM is the probe tip, since it interacts directly with the sample. The tip is normally made of silicon or silicon nitride, using micro fabrication techniques. The material and shape of the tip cause a few problems in the use of AFM techniques.

One of the traditional problems in the imaging of hydrophilic polymer, biomolecule and even pure metal surfaces is the adhesive force between the probe tip and the surface. For example, the existence of the adhesive force causes a tip-induced broadening of the image, so that it does not measure a precise topographic image. To solve this problem, hydrophobic coatings have been used;[4] however, the coating is easily removed by the tip interaction with the surface, especially during dynamic force mode (DFM) imaging. In our experience, the coating of the entire tip area is difficult using normal self-assembling methods because of tip surface contamination. This includes the existence of air- or water-filled regions on the tip surface structure.

Another problem is the shortcoming in the tip design caused by limitations in the current silicon-based micro fabrication process. For new tip designs, focused ion beam (FIB) techniques are widely used, but these techniques are limited in fabrication size, and require an initial tip height to be fabricated. Two-photon adsorbed photopolymerization methods (TPAP) offer a technique to transform 3-dimensional computer aided design (CAD) data to 3-dimensional micro structures using the layer-by-layer accumulation of sliced multiple CAD data.[5, 6] TPAP uses a nonlinear light source to enable the polymerization of photo-reactive chemicals. The earlier technique, photon adsorbed photopolymerization (PAP)is only used for prototyping models of a few millimeter up to sub-meter in size. However, it has merit in the flexible design of 3-dimensional microstructures. The TPAP method has merit in high-resolution fabrication. A number of photo-reactive resins have also been developed to enable various functions, and additional types of resins can be fabricated by mixing the resin with other functional materials.

In the current presentation, first, we will show sharp photo-polymerized tips fabricated using TPAP techniques, and show their potential as AFM tips by comparing them with commercial AFM tips in an effort to solve adhesive force related problems. Second, we will also present some solution for biological imaging-related problems.

Experiment

Figure 1 (a) shows a schematic illustration of the instruments used for polymeric tip fabrication in our study. The instruments include a femtosecond pulsed laser (wavelength, 750 nm; pump power, 5 W; output power, 1 W; model, MaiTai, SpectraPhysics, USA), two galvanic mirrors, and a piezoscanner for optical control of the lateral and perpendicular directions, respectively. In the resin tank, tipless cantilevers (μmasch NSC12-tipless (Si) for the DFM cantilever, and Digital Instruments ULNC-NTNM (Si_3N_4) for the contact mode cantilever) were mounted. Tip fabrication was performed by focusing laser light onto the cantilever surface. We used a commercially available photo-reactive resin (TSR 820, CMET, Japan) to fabricate the polymeric tips.

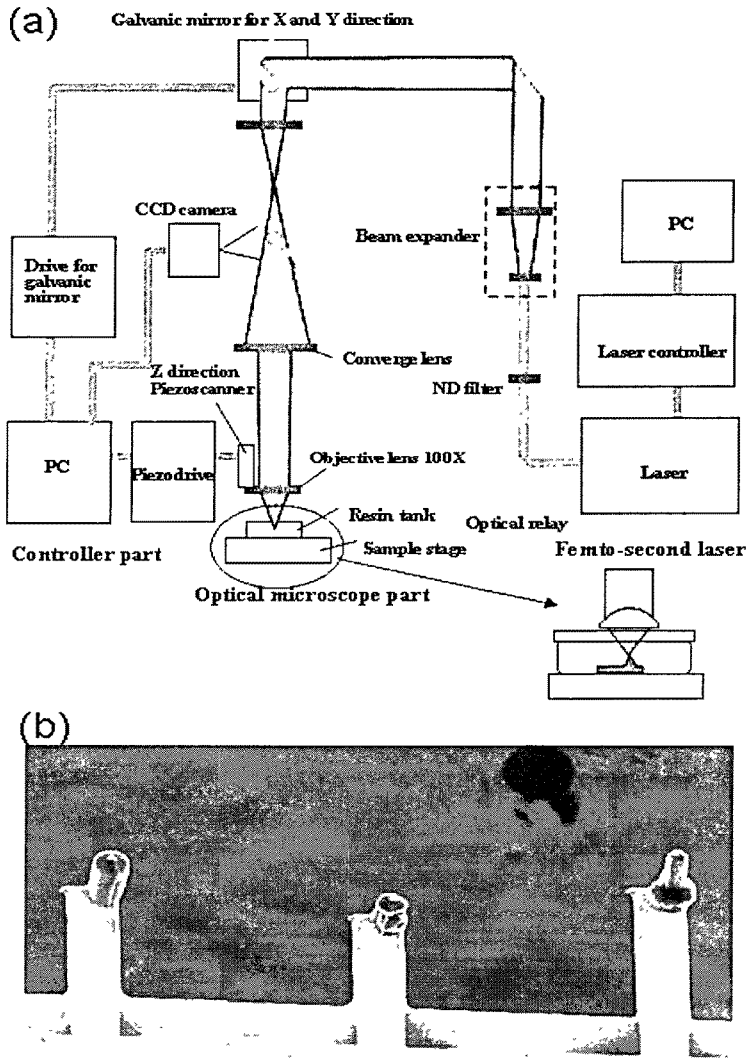

Fig. 1. A schematic illustration of the instrumentation for the polymeric tip fabrication (a), a SEM image of a few tip shapes (b).

The base monomers of TSR 820 are acrylate and epoxy; these maintain small grain sizesafter polymerization. In the tip fabrication, the lateral and perpendicular direction optical movements required to manipulate the focused beam in a 3-dimensional space during the laser scanning

are controlled using self-made software. The scanning is first performed in the lateral direction, and then in the perpendicular direction. With the layer-by-layer procedure, all sliced 3D CAD data can be transformed to the 3-dimensional shape. The polymerization is controlled by two variables:exposure time and optical intensity. In our experiment, the intensity was adjusted first to give a laser output power of 50 mW, which was focused in the resin tank using an objective lens (100×, 1.30 NA). The exposure time was controlled by fixing the scan speed at about 500 μm/s. For these conditions, we found the thickness of the polymerized layer reached about 300 nm for a single line scan without any overheating effect.

Figure 1b shows a scanning electron microscopy (SEM) image of a few polymeric tips. In the TPAP technique, as shown in Fig. 1b, the final tip shape is only limited by the design itself; therefore, fabrication of any tip shape is possible on the same probe substrate. Regarding the optical limitation to the fabrication resolution, a recent report shows that sub-wavelength fabrication of about 100 nm can be achieved by the so-called "threshold effect" using the TPAP method; that is, the confinement of the optical limitation can be overcome by the threshold effect. A detailed explanation for tip fabrication will be discussed in the presentation.

Results and discussion

Fig. 2 shows λ-DNA topographic images obtained using the normal tip (a) and the polymeric tip (b). Although the two images are not for the same DNA molecule, the two DNA images were obtained from the same sample surface. As shown in Fig. 2, both of the tips can successfully producetopographic images for the long DNA molecule over a large area. The height of the DNA molecules is 0.3 nm ± 0.1 nm in both images. This variation in height of ± 0.1 nm results from changing the measured position. Such a deviation is normal for large-area images of long DNA molecules. The DNA widths are similar in both images (20 nm for the normal tip, 15 nm for the polymeric tip at full-width-half-maximum (FMWH) in the line-profile analyses of Fig. 2c). The height and the width are consistent with DNA images obtained by AFM in air. In the line-profile analyses (Fig. 2d) for the mica surfaces, the surface roughness is about 0.25 nm for both images, and this is similar to the surface roughness of the fleshly cleaved mica surface (will be shown in the presentation).

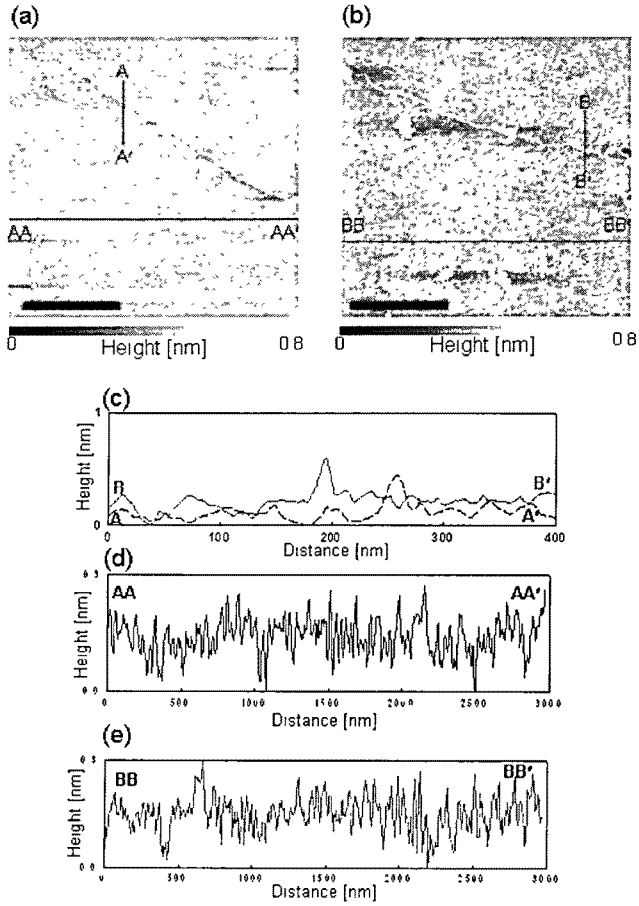

Fig. 2. A comparison of DNA topographic images obtained using the normal tip (a) and the polymeric tip (b). (c) shows line profile analyses for the DNA molecules noted "A-A" and "B-B". (d) and (e) show line profile analyses for the mica surfaces noted "AA-AA" and "BB-BB", respectively. The bars in the images indicate 1 m.

As for the potential of the technique, the polymeric tip can be easily functionalized by the addition of other materials in the resin, or by processing chemical reaction after the polymerization. For example, we have already obtained a hard tip by the addition of inorganic particles and whiskers in the polymer tip. Relative to the merit of the hydrophobic tip, the degree of the hydrophobicity can be easily controlled by the addition of hydrophilic materials. In addition, because the TPAP technique allows a flexible tip design, it can be useful for interesting applications such as large scale applicable dip-pen techniques and molecular stamp techniques. We are currently investigating the tip function in liquid

environments for imaging a single molecule, and the possibilities for high-speed imaging (because the phase lag is weak).

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