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Switching surface polarization of atomic force microscopy probe utilizing photoisomerization of photochromic molecules

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An attempt to develop an atomic force microscopy (AFM) probe with optically switchable polarization is described. Modification with a single molecular layer of photochromic molecules was attempted onto a Si substrate that is a prototype for a probe surface. Polarization switching caused by alternate irradiation of UV and visible lights were detected using the electrostatic force spectroscopy (EFS) technique. Si substrates modified with spiropyran and azobenzene exhibited reversible polarization switching that caused changes in CPD of about 100 and 50 mV, respectively. Modification with spiropyran was also attempted onto a Si probe and resulted in a CPD change of about 100 mV. It was confirmed that modification of an AFM probe or substrate with a single molecular layer of photochromic molecules can generate surface polarization switching of a mechanically detectable level. © *2011 American Institute of Physics*.
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I. INTRODUCTION

An electrostatic interaction is the dominant driving force for the majority of intermolecular and intramolecular processes. This is also the case for molecular-scale biological phenomena taking place in liquid or aqueous environment. Besides directly acting between isolated charges or ions, the electrostatic interaction is transformed into various types of secondary interactions and acts between microscopic entities like molecules, clusters, membranes, etc. Although the dispersion force, the major component of so-called van der Waals force, is essentially originated from an electronic interaction, it is a ubiquitous interaction and cannot generate chemical specificity; more interest is oriented toward interactions between the charges or dipoles that accounts for localization and selectivity of the interaction. It is remarkable that even the complicated proposition of protein folding might be accounted from rather simple amphiphilicity of each amino acid residue arising from its electronic polarity.[1]

Experimental approaches to detect such a weak and localized force field have shown remarkable progress during the recent decades. Among them, the atomic force microscopy (AFM) has drawn attention due to its high force sensitivity and resolution. Recent progress in time resolution of AFM for biological samples provided, for example, a real-time movie of individual protein molecules moving under a physiological condition.[2] The spatial resolution has also been so improved as to give molecularly resolved images of ionic species adsorbed onto a mica surface under aqueous environment.[3] These breakthroughs will orient the research of this field toward the next stage: to reveal the underlying mechanism for molecular-scale motion of biological systems under physiological conditions. The goal of the present research is to present a simple potential method to discriminate forces arising from local polarization on the molecule out of intermolecular interaction by means of AFM and to demonstrate the possibility of its application to biological phenomena.

Imaging of local polarization using the AFM is not unprecedented. Techniques called Kelvin probe force microscopy (KPFM)[4–6] or electrostatic force spectroscopy (EFS)[7–9] have shown their high potential in revealing local contact potential difference of the solid surfaces in which polarization properties of the surfaces play a dominant role. For biological application, however, these are not always ideal ones for the following reasons. These approaches involve use of electrodes and bias voltages to cancel out the electrostatic force between the two surfaces. Here it is implicitly assumed that both surfaces are conductive so that the bias voltage can modulate the relative position of the chemical potential of the both surfaces. This is not always the case for biological systems where insulating substrates like glass, mica, etc. are mostly preferred to metals or semiconductors. Even conducting surfaces adsorb solute ions in aqueous solutions, and their polarization state is not as straightforward as they were in the vacuum condition. Thus an alternative approach to directly regulate the polarization state of the surface of the AFM probe and measure its interaction with the sample is required. This requirement can be fulfilled via an optical method if the probe surface is covered with a material the polarization of which is optically switchable. An optical regulation is simple and free of electrochemical perturbation. The present article is to describe our first attempt to regulate the polarization of an AFM probe using photochromism[10]

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and to detect the resultant polarization variation via mechanical detection method.

II. IMPLEMENTATION OF OPTICALLY SWITCHABLE POLARIZATION

The optimum choice of material with optically switchable polarization is a key issue for the present study. Before describing the photochromic AFM probes, the trace of seeking for appropriate materials with optically switchable polarization, which eventually led to photochromism, is shortly described in the following text.

TiO_2 (Refs. 11–13) is one of the most well-known materials with optically switchable polarization. It changes from hydrophobic to hydrophilic upon irradiation with UV light, showing a change in contact angle as large as 40°.[12] However, the reported transition time is as long as several hours, and, in addition, the backward transition cannot be optically induced and has to rely on thermal relaxation process that requires even longer time.[13] A pair of thymine molecules dimerize into a pyrimidine upon irradiation with 280 nm UV light and lose their strong polarization, showing contact angle change of 25°.[14] This reaction takes place in fairly short time of about 10 min, and similarly does the reverse transition with 240 nm UV light.[14] Although this system seems to be a good candidate, the required two wave lengths are too close to each other, and therefore an appropriate choice of light sources is not realistic. In addition, UV lights with such short wave lengths may cause damage to biological samples. Thus the preceding systems do not meet the requirement of the present research.

Besides these materials, however, there are a number of molecules undergoing photoisomerization the properties of which have been studied systematically. Reversible isomerization between two stable (or metastable) conformations of the molecule accompanying change in its optical properties, known as photochromism,[10] has been intensively studied since the 19th century. Fortunately the change in polarization accompanying the photoisomerization is also reported for some of the photochromic molecules.[15] It should be noted that because photochromism is a purely intramolecular process, its application to surface modification is little restricted by the electronic or geometrical properties of the substrate or other environmental factors.

The present approach, however, confronts additional difficulties or restrictions when its application to an AFM probe is considered. Because molecular resolution is required for the goal of the present study, the asperity of the probe must retained so that it is sharp enough. Modification of the probe with photochromic molecules may spoil the sharpness unless its amount and configuration are well-characterized and optimized. On the other hand, because an AFM probe is subject to unexpected strong mechanical interaction with the sample, the adsorbed molecule must be bonded tight so that it is not damaged during the course of imaging. In addition, density of the adsorbed molecule must be high enough to secure a sufficient polarization change at the probe apex. This requirement is in a sense contrary to the first requirement of probe sharpness.

Considering the above requirements, guidelines for the probe modification were set as follows. (1) To secure robust modification, the photochromic molecules must be covalently bound onto the probe surface. This means that a derivative of the photochromic molecule rather than the molecule itself must be used. An appropriate coupler molecule to interconnect the derivative and the probe surface is also required. (2) To secure the sharpness of probe asperity, the photochromic layer with a single-molecule thickness is desired. Note that this is possible if the photochromic molecule is covalently bonded to the probe surface. (3) The first attempt should be focused on the most popular probe materials, that is, Si or Si-based materials. To verify the modification method, polarization of a modified Si substrate should be measured with a metallic AFM probe before the method is applied to modify the probe.

One potential photochromic molecule is spiropyran, one of the most well-known photochromic materials. As shown in shown in Fig. 1, the spiropyran molecule exhibits a strong polarization upon irradiation of 365 nm UV light as one C–O bond in it dissociates.[15–17] With this reaction, the polarization is reported to increase from 6.4 to 14.2 D,[15] where 1 D (Debye) is 10^{-18} esu·cm $= 3.33564 \times 10^{-30}$ C·m. The open bond is restored with irradiation of a visible light of 550 nm. Recently it was demonstrated that wettability, that is, surface polarization, of a microfluidics channel coated with single spiropyran layer is optically switchable.[17]

Another potential photochromic molecule is azobenzene, which undergoes a trans-to-cis isomerization with 365 nm UV light and transforms back with 440 nm visible light.[15,18] Its polarization was reported to change from 8.6 to 6.3 D upon the trans-to-cis isomerization.[15] Although this polarization change is smaller than that of spiropyran, azobenzene is still one of the candidates for the present research because of its relatively short reaction time. It should be noted that optical switching of geometry of AFM probe apex functionalized with azobenzene-derivative was also demonstrated recently.[19] Photoisomerization of azobenzene was measured in single polymer chain stretching experiments in which a photo-induced change in the tensile force or stiffness of a single polymer chain of azobenzene peptide tethered between the AFM probe and the substrate was measured.[20–22] A photoinduced change in surface potential was also measured with KPFM. These works are indicative

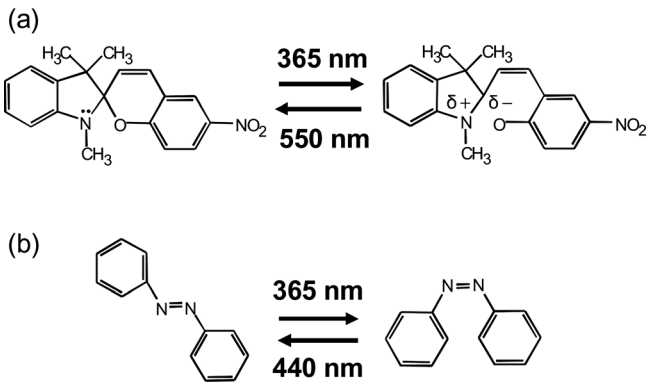

FIG. 1. Photoisomerization of (a) spiropyran and (b) azobenzene.

of the fact that the photoisomerization of azobenzene is spatially, mechanically, and electrically significant enough to be detected with the sensitivity of AFM.

Among various commercially available derivatives of these molecules with versatile reactive groups, careful choice was made so that the attached groups do not affect isomerization or the polarization change. Because one of the most appropriate methods to ensure covalent bonding of the molecule with the Si substrate is to use silane coupling agents, matching of the derivatives with the agents was taken into account. Because the polished surface of a Si wafer is usually covered with oxide and mechanically damaged layer, the substrate was heated up to about 1200 °C in ultrahigh vacuum to remove them; this is a technique routinely used for cleaning Si surface and was exposed to ambient air so that an oxide layer with a flatness of nearly-atomic level. Detailed procedure for medication of a Si substrate or AFM Si probe with each molecule is described in the following text.

1-(2-Hydroxyethyl)-3,3-dimethylindolino-6′-nitrobenzopyrylospiran (abbreviated as hydroxy-spiropyran), shown in Fig. 2(a), was chosen as spiropyran derivative. This molecule has a hydroxyl group at the end of an alkyl chain extending from a N atom, which can form a covalent bond with an isocyanate group via a urethane bonding. Such a long alkyl chain is expected to relieve steric hindrance against isomerization.[16] 3-isocyanatopropyl triethoxysilane [Fig. 2(b)], a silane coupling agent, was added to 14 mM solution of hydroxyl- spiropyran in acetonitryl so that the final agent concentration was 3–8%. The hydroxy-spiropyran molecule is supposed to form a chemical bond with this agent. The cleaned substrate (or AFM cantilever) was immersed into this solution for 20 min. The excess hydroxy-spiropyran physisorbed onto the surface was removed by subsequent ultrasonic cleaning in pure water. Figure 2(c) shows the expected final bonding configuration of the hydroxyl-spiropyran onto the SiO_2 substrate via the silane coupling agent.

4-(phenylazo)benzoic acid (azobenzene carbonic acid), shown in Fig. 3(a), was chosen as an azobenzene derivative. Because an azobenzene molecule largely transforms its shape upon isomerization, it is desirable to leave a space between neighboring molecules for efficient isomerization.

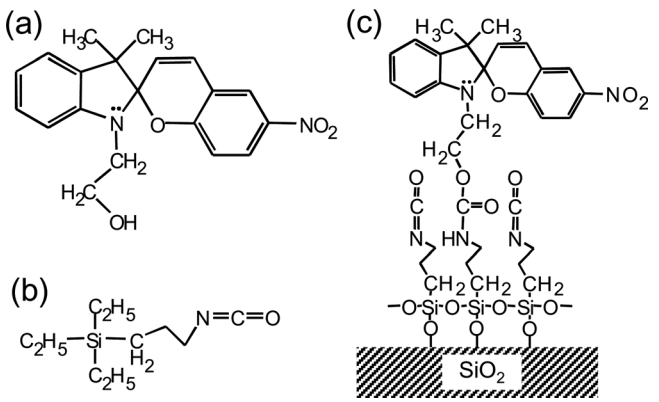

FIG. 2. (a) 1-(2-Hydroxyethyl)-3,3-dimethylindolino-6′-nitrobenzopyrylospiran (hydroxy-spiropyran), a derivative of spiropyran, (b) 3-isocyanatopropyl triethoxysilane, a silane couping agent, and (c) modification of an oxidized Si surface with these molecules.

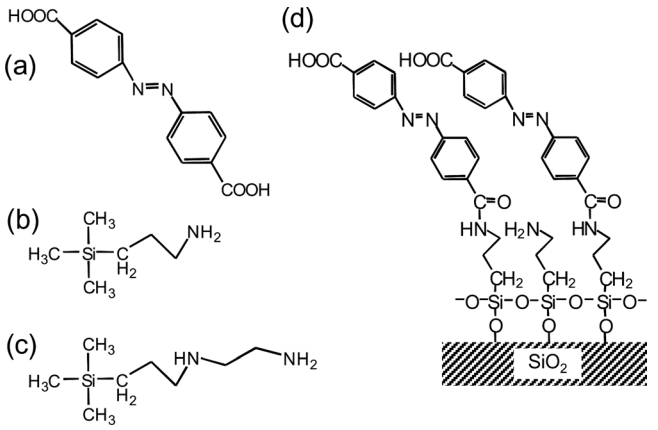

FIG. 3. (a) 4-(phenylazo)benzoic acid (azobenzene carbonic acid), a derivative of azobenzene, (b) 3-aminopropyl triethoxysilane, and (c) N-2(aminoethyl)3-aminopropyl triethoxysilane, silane couping agents, and (d) modification of an oxidized Si surface with these molecules.

Thus two types of silane coupling agent molecules with different chain length, 3-aminopropyl triethoxysilane [Fig. 3(b)] and *N*-2(aminoethyl)3-aminopropyl triethoxysilane [Fig. 3(c)], were mixed so that the resultant two types of bonding configurations have an offset to each other. A 3-8% aqueous solution of these agents mixed at a ratio of 1:1 was dispensed onto the substrate (or cantilever). After removing unreacted molecules by ultrasonic cleaning, it was immersed into 18 mM solution of azobenzene carbonic acid in pyridine at about 100 °C for 20 min and finally ultrasonic-cleaned. Figure 3(d) shows the expected bonding configuration of the azobenzene carbonic acid onto the SiO_2 substrate via the silane coupling agent.

Prior to modifying the Si substrate or tip with these photochromic materials, the absorption spectrum of the both derivatives dissolved in acetonitryl was measured with a spectrophotometer (Shimadzu UV-1600). The hydroxy-spiropyran solution exhibited a major peak at around 350 nm and a faint broad one around 560 nm. These peaks are attributed to the absorption associated with C–O bond dissociation and its restoration, respectively. On the other hand, azobenzene carbonic acid solution exhibited a major peak also at around 350 nm and a minor broad one at 440 nm, which are attributed to trans-to-cis and cis-to-trans isomerizations, respectively. These results guarantee that addition of hydroxyl or carboxyl groups does not essentially affect the photoisomerization. It was also confirmed that the wavelength of the laser 670 nm used for detection of cantilever deflection is not absorbed in the either molecules and hence does not affect isomerization.

The potential change expected for each photochromic molecule is roughly evaluated as follows. For simplicity, the polarization of the molecule is assumed to be normal to the substrate. The expected CPD between the probe and the sample with presence of a charge dipole is given as

$$\Delta\phi = -\frac{1}{4\pi\varepsilon}\int_{-\infty}^{\infty} z\{\rho_-(z) - \rho_+(z)\}dz, \qquad (1)$$

where $\rho_-(z)$ and $\rho_+(z)$ are distribution of negative and positive charge in the dipole, respectively, and ε is the dielectric

constant of the medium.[23] Assuming that the molecule has a dipole composed of point charges spaced with a distance a, that is, $\rho_-(z) = (q/S)\delta(z-a)$ and $\rho_+(z) = (q/S)\delta(z)$, where S is the area on the substrate occupied by one molecule and q the charge amount, the CPD is expressed as

$$\Delta\phi = -\frac{qa}{4\pi\varepsilon S}. \tag{2}$$

Assuming a vacuum environment and taking a rough estimate of S to be 3 nm^2, the CPD values expected from the preceding values of polarization change are 0.7 and 0.35 V for spiropyran and azobenzene, respectively.

III. DETECTION OF PHOTO-INDUCED POLARIZATION VIA ELECTROSTATIC FORCE SPECTROSCOPY

To confirm that the surface polarization is optically switchable and that the resultant polarization change is mechanically detectable, the change in the contact potential difference (CPD) between the tip and the substrate was measured with EFS[7–9] at RT in ambient air. The fundamental mechanism of detecting surface polarization using EFS is essentially same with that of KPFM[4–6] as explained in the following text using Fig. 4. Here an insulating polar adlayer is introduced onto the substrate, and a metallic character is assumed for both the tip and the substrate for simplicity. Surface potentials of the tip, the substrate and the adlayer are represented as ϕ_t, ϕ_s, and ϕ_a, respectively. As an effect the polar adlayer the apparent potential of the sample drops (in the case of positive polarization) by $\Delta = \phi_s - \phi_a$. When the tip and the substrate are electrically connected, their Fermi levels E_{Ft} and E_{Fs} equal each other, and consequently the vacuum energy level has a slope across the gap, hence an electrostatic force F_{ES} between the tip and the sample. The dc bias voltage V_{dc} required to compensate this slope gives the CPD between the tip and the sample, which is the difference in the surface potential of the two surfaces. Because the surface polarization essentially dominates the surface potential, if the polarization is modulated, its change $\Delta_1 - \Delta_2$ can be directly measured as a shift in the measured CPD. In the usual setup of KPFM, the CPD value is reached via a negative feedback applied to V_{dc} to minimize variation in electrostatic force in response to ac modulation in the bias voltage. In EFS, the negative feedback is dispensed with, and the CPD is found by simply sweeping the bias voltage. It is advantageous over KPFM in detection speed.

In the present study, the EFS measurement was combined with AFM operated in amplitude-modulation (AM) mode. The schematic diagram of the present system is shown in Fig. 5. The cantilever is excited with a voltage V_{ex} applied to the piezoelectric actuator installed in the cantilever holder. The excitation frequencyω_1 is set slightly higher than the cantilever's free resonance frequency so that frequency dependence of the amplitude is at its maximum. The feedback setpoint for regulation of tip-sample distance is set to about 90% of the free amplitude. Note that this condition is milder than that used in ordinary tapping-mode operation so that the tip does not enter the repulsive interaction regime and thus the dominant interactions are electrostatic or van der Waals forces. An ac bias voltage $V_{\mathrm{ac}}\cos\omega_2 t$ ($\omega_2 \ll \omega_1$) is applied between the cantilever and the substrate. This ac bias modulates the electrostatic force and results in modulation of oscillation amplitude of the cantilever δz_{tip}. Although the envelope of this oscillation A_{ω_1} detected via "lock-in amp. 1" reflects dependence of the resonance frequency on the electrostatic force and hence is not straightforward, its dominant component is a sinusoidal oscillation $A_{\omega_2}\cos(\omega_2 t + \delta)$, where $\delta = 0$ or π depending on the value of V_{dc}. For a small V_{ac}, the value of $A_{\omega_2}\cos\delta$ detected with "lock-in amp. 2" can be regarded as voltage derivative of the electrostatic force $\partial F_{\mathrm{ES}}/\partial V$. Consequently the V_{dc} at which $A_{\omega_2}\cos\delta$ crosses zero directly gives the CPD.

A 365 nm light-emitting diode (LED) with a nominal light power of 100 mW was used as the UV light source for

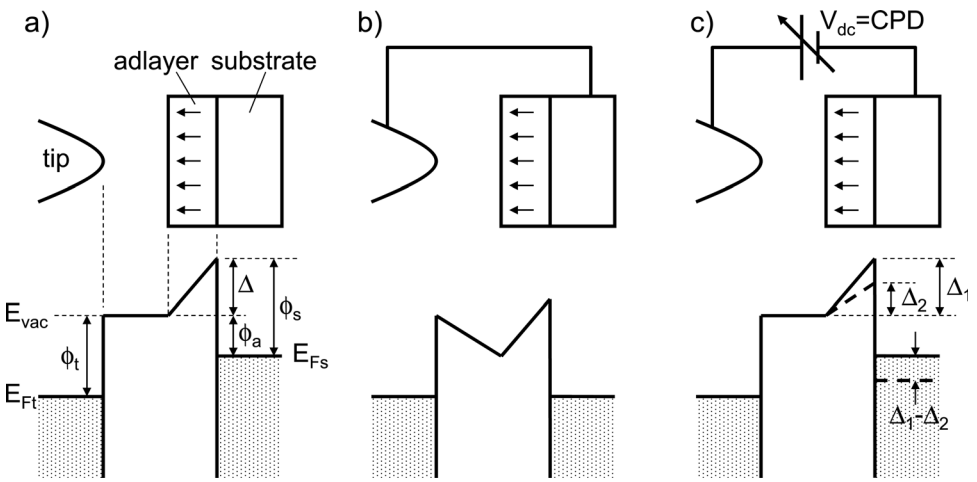

FIG. 4. Mechanism of polarization detection through electrostatic force measurement using KPFM or EFS. The surface potentials of the tip and the uncoated substrate are expressed as ϕ_t and ϕ_s, respectively. As a polar adlayer is introduced onto the substrate, it causes potential drop of Δ, leading to a net potential of the substrate $\phi_a = \phi_s - \Delta$. (a) Initially the tip and the sample are isolated from each other. (b) When they are electrically connected with each other, their Fermi levels E_{Ft} and E_{Fs} are equalized and consequently the potential in the vacuum space has a slope, hence an electrostatic force. (c) By applying an appropriate dc bias voltage, V_{dc}, the electrostatic force vanishes. If the polarization of the adlayer is altered from Δ_1 to Δ_2, V_{dc} required for compensation also changes.

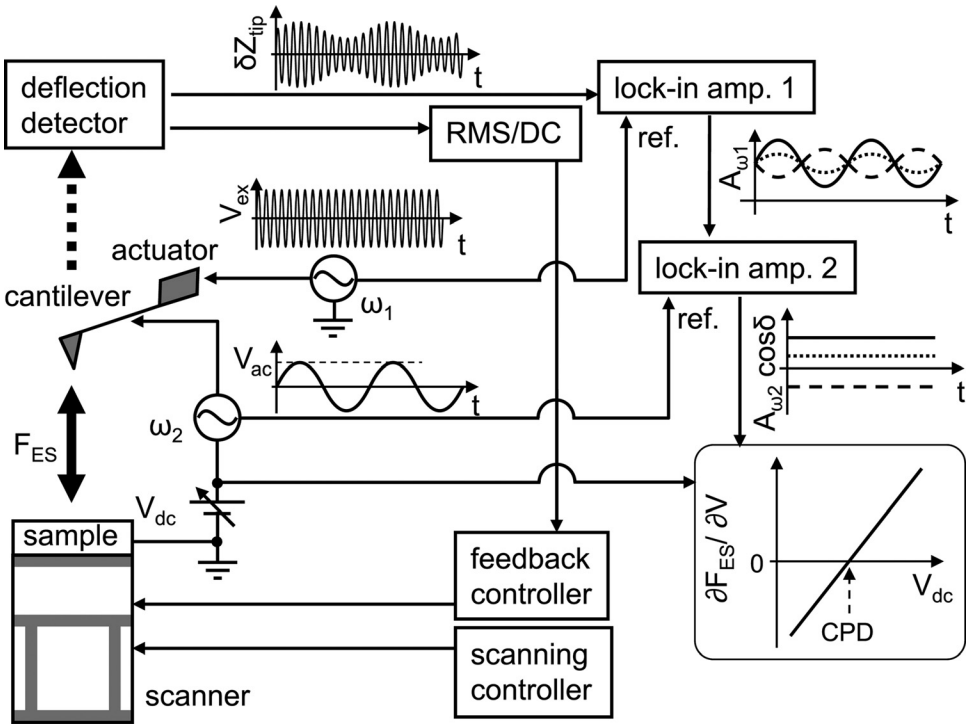

FIG. 5. Schematic diagram of the EFS measurement system. The cantilever is excited at a frequency ω_1 close to its resonance, while the bias voltage between the cantilever and the substrate is modulated at a frequency ω_2 much lower than ω_1. The envelope of the cantilever oscillation $A_{\omega 1}$ is detected via lock-in amp. 1 and its ω_2 component that reflects electrostatic force is extracted via lock-in amp. 2. The CPD is found as the bias voltage where the output of lock-in amp. 2 crosses zero.

both spiropyran and azobenzene. As a visible light source, a 532 nm, 30 mW solid laser was used for spiropyran and a 440 nm, 20 mW diode laser for azobenzene. Because the light power of these sources are not equal to each other and directivity of an LED light is lower than that of a laser, all the lights mentioned in the preceding text were defocused using convex lenses in order to roughly equalize the optical power density to about 5 mW/cm^2.

In the initial experiment, modification with photochromic material was attempted onto a flat Si substrate while its polarization change was measured through electrostatic force via Pt-coated AFM probe. UV light (365 nm) and 532 nm visible lights were irradiated for 15 min alternately onto the substrate mounted on the AFM's scanner stage while the tip was retreated from it by several micrometers. After each irradiation, the tip was brought into interaction with the substrate and EFS measurement was carried out. Figure 6 shows the result of EFS measurement with a Si substrate modified with hydroxy-spiropyran and a Pt-coated AFM probe. Figs. 6(a)–6(c) show sequential changes in EFS profiles caused by each irradiation step of 365 nm UV, 532 nm visible, and 365 nm UV, respectively. The EFS profiles sometimes show deviation from linearity at V_{dc} values distant from the CPD as seen in Fig. 6(a). This is attributed to charge dynamics induced in the bulk Si of the probe or the substrate by a large electrostatic field and does not affect the EFS profile around the CPD value. Although each EFS profile is rather scattered mainly due to electric noise in the measurement system, the CPD value shows a significant change upon irradiation. The CPD value was determined by averaging out the noise in each profile. Although the slope of the profile varies with the probe-sample distance and hence does not always coincide between the repeated measurements, the CPD value itself was fairly reproducible irrespective of the slope. EFS measurement was repeated several times after each irradiation and the change in averaged CPD is summarized in Fig. 6(d). Upon the first irradiation to 365 nm UV light the CPD changed from 0.77 to 0.70 V. After the next irradiation to 532 nm light, the CPD increased to 0.82 V, that is, larger than the initial value. Upon exposure to the UV again, the CPD decreased to 0.70 V. Thus the polarization of the substrate modified with hydroxy-spiropyran proved to be reversibly switchable and to exhibit a mechanically detectable potential change. Because the CPD values measured after two 365 nm irradiation steps were almost same, it is concluded that the final state of the isomerization was well reproducible. On the other hand, the CPD value after 532 nm irradiation is larger than the one before the initial UV irradiation. This implies that some portion of hydroxy-spiropyran had already been transformed before the initial EFS measurement probably due to UV light contained in sunlight or laboratory illumination that could slightly be cast onto the sample. Then the reliable value for expected CPD change is the difference between "C" and "B" or "D" in Fig. 6(d), which is about 100 mV.

A similar EFS measurement was carried out for a Si substrate modified with azobenzene carbonic acid. Changes in typical EFS profiles induced by 15 min irradiation of 365 and 440 nm lights are shown in Figs. 7(a) and 7(b), respectively. As summarized in Fig. 7(c), the measured CPD change was around 50 mV, about half of the value obtained for the substrate modified with hydroxy-spiropyran. This difference should not be wholly attributed to the intrinsic difference between the two photochromic materials; a difference in optical conditions like irradiation intensity or quality of the beam focusing may also significantly affect the result. However, it is concluded that within the limitation of accessible light sources or focusing setup, hydroxy-spiropyran can

give a larger polarization change than azobenzene carbonic acid can.

Thus as a next step, modification with hydroxy-spiropyran was attempted onto the Si probe and an EFS measurement was carried out using a Au coated mica substrate. Both modification of the probe and the EFS analysis were carried out in the same manner as described in the preceding text. Figure 8 shows the result in the same manner as in Figs. 6 and 7. Compared with the data in Fig. 6, the EFS profiles in the present data seem to be noisier. We partly attribute this to the tip-substrate electrostatic force generated by the smaller number of hydroxy-spiropyran molecules whose thermal fluctuation more influenced the measured data. Apart from the resultant larger error in the derived CPD values of each state, however, the average differences between them induced by photoisomeization are around 100 mV and were almost same between the two directions. This result implies that the difference in the topographical condition between the two experiments does not essentially affect the photoisomerization.

IV. DISCUSSION

The irradiation duration of 15 min for each isomerization in Figs. 6–8 is not sufficiently short for probing the local polarization dynamics of biomolecules, etc. It should be noted that this time can be shortened more by improving optical conditions. If better-collimated light sources and an appropriate optical setup are used so that the incident light is focused into an area with a diameter of 0.1 mm around the tip apex, the light power per area will be enhanced by about 10^4, which will improve the quantum yield of isomerization

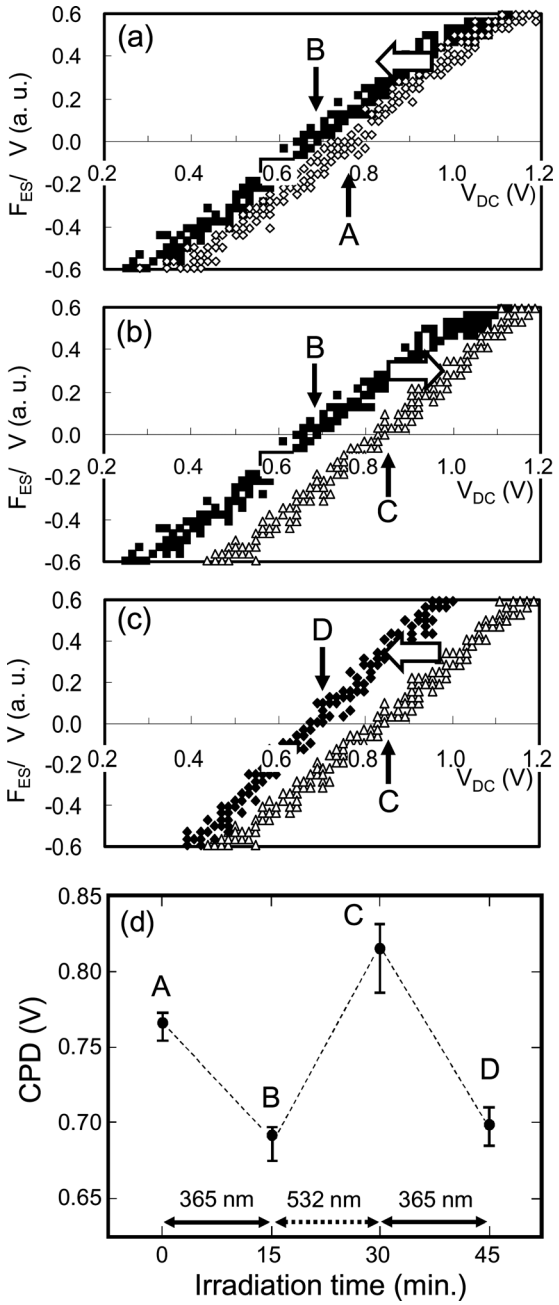

FIG. 6. Successive changes in EFS profiles of a Si substrate modified with spiropyran induced by 15 min irradiation of (a) 365 nm UV (from A to B), (b) 532 nm visible (from B to C), and (c) 365 nm UV (from C to D) lights. (d) Summary of the CPD values measured with a Pt-coated AFM probe by sweeping the V_{dc} several times after each irradiation step.

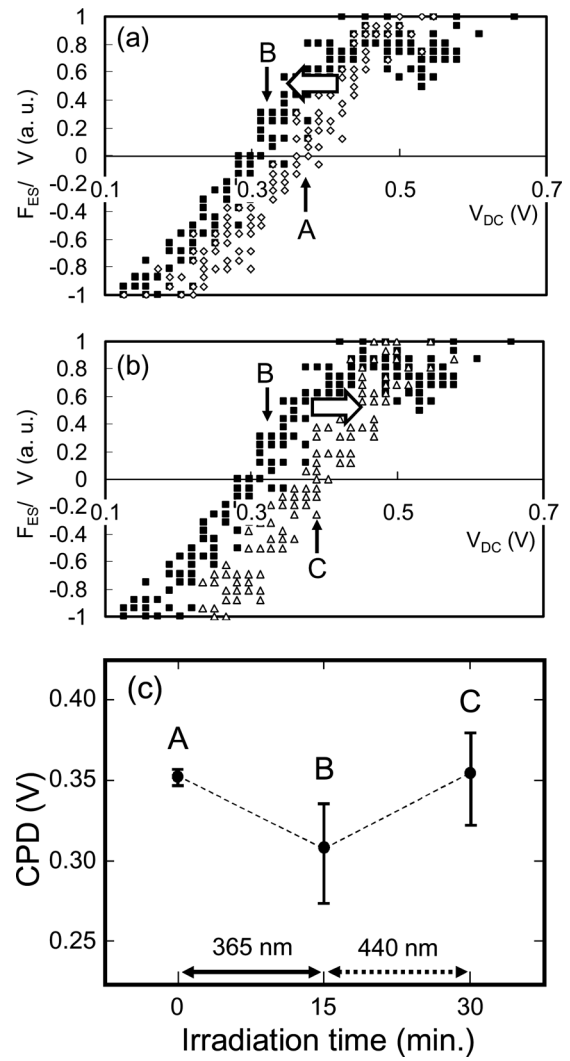

FIG. 7. Successive changes in EFS profiles of a Si substrate modified with azobenzene induced by 15 min irradiation of (a) 365 nm UV (from A to B) and (b) 440 nm visible (from B to C) lights. (c) Summary of the CPD values measured with a Pt-coated AFM probe by sweeping the V_{dc} several times after each irradiation step.

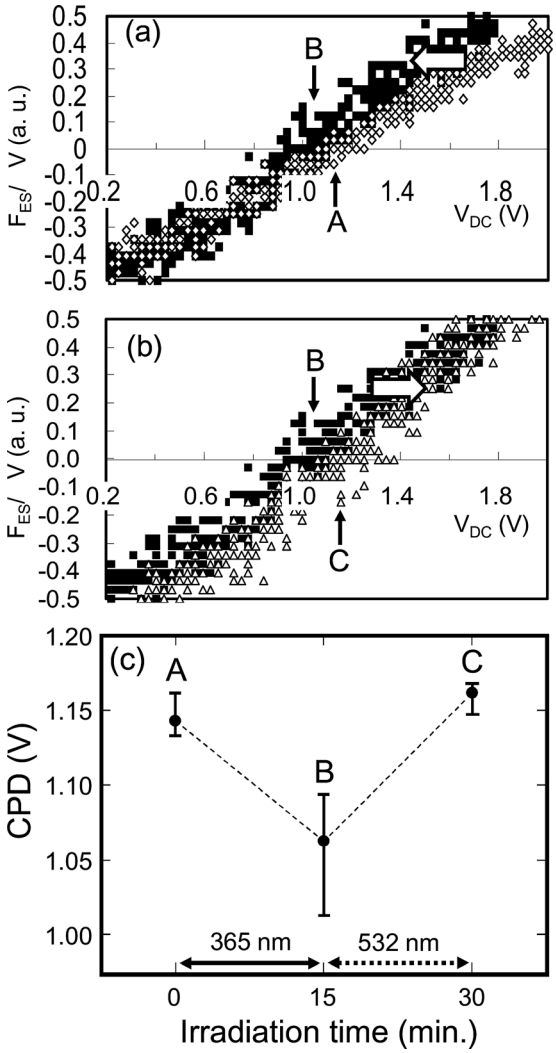

FIG. 8. Successive changes in EFS profiles of a Si probe modified with spiropyran induced by 15 min irradiation of (a) 365 nm UV (from A to B) and (b) 532 nm visible (from B to C) lights. (c) Summary of the CPD values measured with a Au-coated mica substrate by sweeping the V_{dc} several times after each irradiation step.

accordingly. Combining this method with the high-speed AFM imaging of, for example, a motor protein molecule, it might be possible to obtain its electrostatic force mapping with a probe that switched optically with a time scale of 1 s. Then by subtraction between the images of the two states, it will be possible to correlate the potential distribution with the molecular motion.

For practical use of the present method for polarization detection in an aqueous environment, a screening effect by water molecules is also anticipated. The effect of a polarized water layer on the CPD value measured with KPFM has been discussed in several works.[23–25] These commonly describe that several layers of adsorbed water on a hydrophilic surface screens out electrostatic potential contrast on the surface. They also seem to suggest, however, that when the adsorbed water is as thin as 1-2 layers, detection of the underlying potential is still possible. This is attributed to the fact that the interaction between the hydrophilic substrate and the water molecule prevents the latter from rotating freely, leading to substantial drop of the effective specific dielectric constant of the undermost water layer from the bulk value of ca. 80. If the AFM cantilever is oscillating with an amplitude much larger than a water molecule, which is the case for most of AM–AFM measurement, the dominant force interaction is the one at the moment when the probe comes closest to the sample during the course of oscillation. In such a moment, the number of water molecules between the tip and the substrate is considered to be extremely small and the constraining effect against the rotational motion of water molecule may still leave the possibility for the electrostatic potential contrast to be detected. Of course the possibility depends on the accuracy in regulation of the probe-sample distance to bring the probe as close to the sample as possible. Although this is another important technical requirement for biological imaging with AFM, it is out of scope of the present article.

Finally, no matter how these effects actually hinder the measurement in aqueous environment, enhancement of polarization change is essential for further progress of the present method. The experimental values of photo-induced CPD change are about 100 and 50 mV for spiropyran and azobenzene, respectively, both being much smaller than the values estimated from the dipole model described in the preceding text. One possible explanation for this difference is that the polarization change does not take place along the surface normal direction. Another possibility is that the transformation of the molecule is restricted by steric hindrance caused by neighboring molecules. It will be possible to obtain higher values by optimizing bonding configuration of the derivatives. Thus a more systematic study to optimize the surface modification method is required for further progress.

V. SUMMARY

As a prototype for an AFM probe with an optically switchable polarization, modification with a single molecular layer of photochromic molecules was attempted so that the polarization change accompanying their photoisomerization was utilized. Polarization switching caused by alternate irradiation of UV and visible lights were detected as change in the CPD with respect to a Pt-coated AFM tip using the EFS technique combined with AM–AFM measurement. Both substrates modified with derivatives of spiropyran and azobenzene mediated by silane coupling agents exhibited reversible polarization switching that caused changes in CPD of about 100 and 50 mV for spiropyran and azobenzene, respectively. Modification with spiropyran was also attempted onto a Si probe for AFM. The resultant change in CPD with respect to a Au substrate was also about 100 mV. Thus it was confirmed that modification of AFM probe or substrate with a single layer of photochromic molecules can reversibly switch the surface polarization to modulate the electrostatic force with a mechanically detectable level.

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