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Scanning Near-field Fluorescence Microscopy and Nanoscopic Fluorescence Spectroscopy in Combination with a Non-contact Scanning Force Microscope

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The resolution of scanning near-field optical microscopy (SNOM) is determined by the dimensions of the microscopic light source (or detector) and the probe-to-sample separation rather than the diffraction limit. In the present microscope, we took advantage of scanning force microscopy (SFM) with a vibrating cantilever holding an optical fiber tip not only to give a simultaneous SFM topographic image but also to control the separation for SNOM without mechanical damages of the sample. By the precise control of the separation of the tip within 100 nm from the sample surface, we demonstrate here that this combined non-contact SFM and SNOM method can be used as fluorescence microscopy and nanoscopic fluorescence spectroscopy with a resolution of ~ 100 nm.

The resolution of scanning near-field optical microscopy (SNOM)[1] is determined by the dimensions of the microscopic light source (or detector) and the probe-to-sample separation rather than the diffraction limit. As the light source, a microlithographic hole,[2] a small aperture in a metal film,[3,4] a micropipette,[5,6] a fluorescence probe[7] and an optical fiber tip[8-10] have been used. For the separation control, the steep decrease in a tunneling current,[11] a shear force[12] or the intensity of an evanescent wave[8-10] with an increase in the separation has been utilized. In the present microscope, we took advantage of non-contact scanning force microscopy (SFM named also atomic force microscopy (AFM)) with a vibrating cantilever[13,14] not only to control the separation for SNOM without mechanical damages of the sample but also to give a simultaneous SFM topographic image. By the precise control of the separation of the tip within 100 nm from the sample surface, we demonstrate here that this combined non-contact SFM and SNOM method can be used as fluorescence microscopy and nanoscopic fluorescence spectroscopy with a resolution of ~ 100 nm.

Since the invention of the scanning tunneling microscope (STM),[15] other scanning probe microscopes (SPM) such as SFM[16] have been devised to image surfaces from the micron scale down to the atomic scale. Many studies have already demonstrated that the STM and SFM can effectively image organic thin films and surfaces.[17] However, these two methods are rather insensitive to chemical composition. The friction force microscope (FFM)[18,19] and the scanning surface potential microscope (SSPM)[20] have proven useful for chemical differentiation.

Although friction and surface potentials are highly material dependent, another physical property that is more sensitive to surface chemical properties is desired for definitive chemical assignment. The spectroscopic

information is the most useful for this purpose. The lateral resolution of conventional microscopic spectroscopy[21] is, however, limited by the diffraction of the observed light, while the resolution of the SNOM is determined only by the probe size and the probe-to-sample separation.[1,12,22] Although the study of the SNOM has a long history,[1] the optical images have begun to be taken only within the last decade. One of the most progressed SNOMs uses the shear force to control the separation and an aluminum-coated tapered optical fiber.[12] The other is the combined SNOM and SFM in the contact mode based on a silicon nitride cantilever with a pyramidal tip.[23] In the latter microscope, the tip was used simultaneously as the SNOM optical probe and the SFM tip.

In the present work, an optical fiber tip sharpened and bent by heating was mounted on a stainless steel cantilever. The separation between the tip and the sample was controlled by an SFM operation with a laser beam deflection,[24,25] in which the cantilever was vibrated at the resonance frequency. The decrease in an amplitude of the vibrating cantilever by the van der Waals force[13,14] was used to control the separation. Figure 1 shows the schematic diagram of the present non-contact SFM-SNOM.

A test sample for scanning optical absorption measurements of the Ar ion laser beam with the non-contact SFM was a chromium checker-board pattern on a quartz substrate gifted by Dai Nippon Printing Co., Saitama. For the fluorescence microscopy and spectroscopy, the chromium pattern was coated by spin coating with a thin polyvinyl alcohol film containing fluorescein.

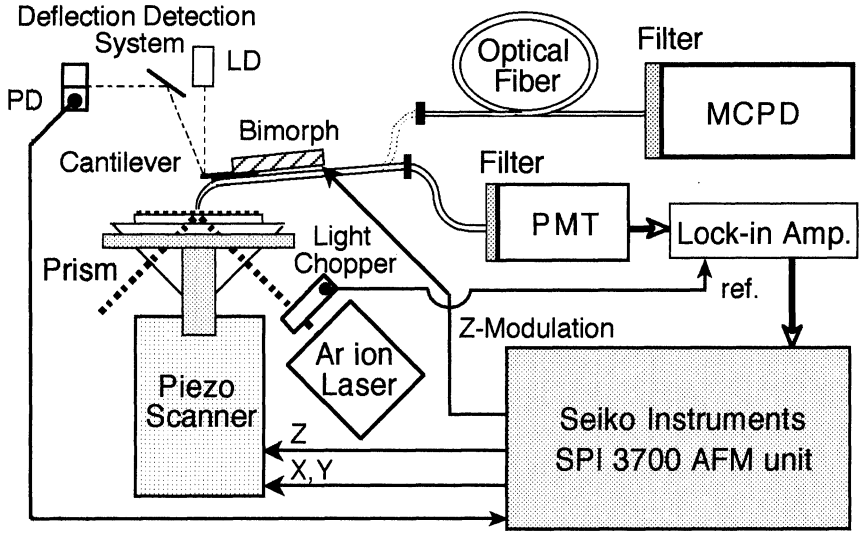

Fig. 1. The schematic diagram of the combined non-contact SFM-SNOM. The optical fiber tip picks up an evanescent wave of an Ar ion laser beam for excitation and fluorescence light from the irradiated sample surface on a prism. The laser beam was modulated by a light chopper and impinged on a sample-prism interface at the incidence angle of 45°. The picked-up light was introduced into a photomultiplier tube (PMT) through the optical fiber for SNOM. For recording a fluorescence spectrum, the optical fiber was introduced into a slit of an Otsuka Electronics IMUC-7000 intensified multichannel photodetector. Selection of the excitation or the fluorescence light was done by optical filters placed in front of the PMT window or the slit of IMUC-7000. Scanning of the tip and imaging of lateral distribution of the intensity of the evanescent wave or the fluorescence light were carried out with a Seiko Instruments SPI-3700 AFM unit which contains a non-contact SFM function and accepts a user's input signal. A lock-in amplifier was used to distinguish a modulated optical signal from optical noise.

The chromium checker-board pattern (ca. 25 nm in height) without the fluorescent film was imaged with a high resolution less than 100 nm by the non-contact SFM with the optical fiber tip. The map of the evanescent light intensity picked up by the tip gave the corresponding checker-board pattern with a resolution of ~ 100 nm. The light intensity was high on the chromium uncovered parts. The topographic height difference between the chromium covered and the uncovered parts was diminished by the fluorescent film coating as shown in Fig. 2a, but the chromium covered parts are likely to be still higher (ca. 7 nm) than the uncovered parts. The map of the total light intensity on the dye-film covered sample was shown in Fig. 2b., where the total light consists of the evanescent wave of Ar ion laser transmitted through the chromium pattern with the dye film and the fluorescence emitted by the dye. It is clear from the comparison between these two images that the lower light intensity areas in Fig. 2b correspond to the topographically higher areas in Fig. 2a where chromium seems to cover the quartz substrate. This agrees with the above observation on the sample without the dye film that the intensity of the evanescent wave behind the opaque chromium film is weak. The screening of the excitation beam by the chromium pattern also gave clear contrast in the fluorescence intensity mapping (Fig. 2c), where the excitation

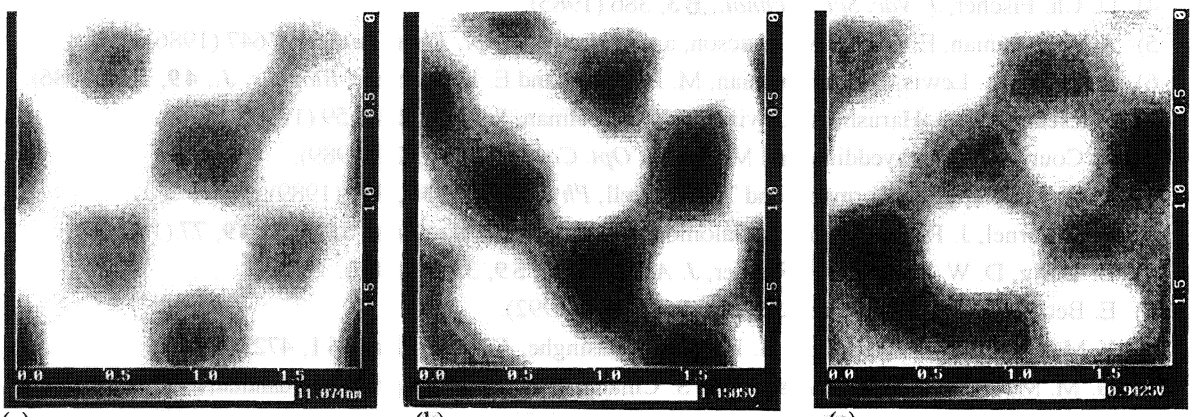

Fig. 2. (a) A 2 x 2 μm^2 non-contact SFM topography image of a chromium checker-board pattern coated with a polyvinyl alcohol thin film containing fluorescein. (b) A 2 x 2 μm^2 map of the total light intensity picked up by the optical fiber tip on the same surface as shown in (a). (c) A 2 x 2 μm^2 map of the fluorescence intensity detected with a Fuji Photofilm cut filter SC-52 of the same sample as shown in (a) and (b), but a different location.

beam picked up by the optical fiber was cut by the filter in front of the PMT in Fig. 1. For the use of the present SNOM as scanning near-field fluorescence microscopy, a light irradiation mode through the optical fiber tip[12] is preferable to avoid appreciable photodamage and bleaching of the dye and is now under investigation.

Figure 3 shows a fluorescence spectrum recorded with an Otsuka Electronics IMUC-7000 intensified multichannel photodetector by positioning the tip above the chromium uncovered area. A fluorescence band of fluorescein appearing around 525 nm was in good agreement with that observed with a

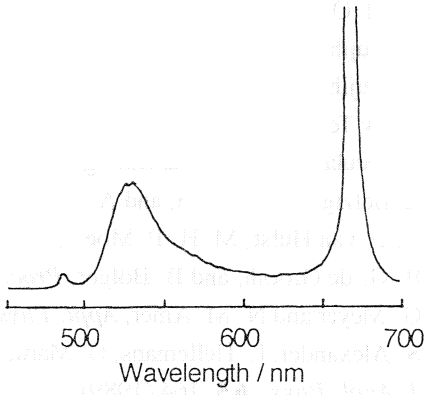

Fig. 3. A fluorescence spectrum recorded with the SC-52 filter by positioning the optical fiber tip above the area showing the high fluorescence intensity in (Fig. 2c).

large area sample using an ordinary Hitachi 850 fluorescence spectrophotometer with the same optical filter. In addition to the fluorescence band, a peak at 488 nm due to Ar ion laser passing through the cut filter is seen together with a strong peak at 670 nm due to the laser diode for the SFM beam deflection.

In conclusion, the scanning fluorescence microscopy and fluorescence spectroscopy of the localized area were successfully performed with a high resolution of ~ 100 nm[26] by the present combined non-contact SFM and SNOM. By the separation regulation with SFM in the non-contact mode, the present SNOM will be applicable to soft samples like biological materials without serious mechanical damages.

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26) The resolution will be increased by the use of an aluminum-coated tapered optical fiber down to 12 nm.[12] The sharpened tip used in the present work was not coated with metal.

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