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<td>Author(s)</td>
<td>Aoki, H; Hamamatsu, T; Ito, S</td>
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Kyoto University
Deep ultraviolet scanning near-field optical microscopy for the structural analysis of organic and biological materials

Hiroyuki Aoki, Toyohiro Hamamatsu, and Shizaburo Ito
Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Nishigyo, Kyoto 615-8510, Japan

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Scanning near-field optical microscopy (SNOM) using a deep ultraviolet (DUV) light source was developed for in situ imaging of a variety of chemical species without staining. Numerous kinds of chemical species have a carbon–carbon double bond or aromatic group in their chemical structure, which can be excited at the wavelength below 300 nm. In this study, the wavelength range available for SNOM imaging was extended to the DUV region. DUV–SNOM allowed the direct imaging of polymer thin films with high detection sensitivity and spatial resolution of several tens of nanometers. In addition to the polymer materials, we demonstrated the near-field imaging of a cell without using a fluorescence label. © 2004 American Institute of Physics.

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coupler (Newport) equipped with quartz objective and steering lenses. The gap between the probe end and the sample surface was regulated by a shear force feedback mechanism. The ultraviolet fluorescence signal from a specimen was collected with a microscope objective made of quartz, equipped with quartz objective and steering lenses. The gap between the probe end and the sample surface was regulated by a shear force feedback mechanism. The ultraviolet fluorescence signal from a specimen was collected with a microscope objective made of quartz (1.25 numerical aperture, 40×, glycerol immersion) and detected by a photomultiplier (R4220P, Hamamatsu Photonics). Optical filters (Omega Optical) were put in front of the detector to block the excitation light.

The polystyrene (PS) and poly(methyl methacrylate) (PMMA) were used as the test samples. The toluene solution of a polymer was spin cast on a quartz cover slip. A part of the sample film was scratched to remove the polymers, and the SNOM measurement was performed for the area including the edge between the polymer and the exposed substrate surfaces. Figure 1 shows the topographic and DUV fluorescence images for the PS and PMMA samples. The right- and left-hand sides of each image indicate the polymer and exposed quartz surfaces, respectively. The thickness of the polymer films was estimated to be ~200 nm from the height difference between the polymer and substrate surface for each sample. The PS domain was observed as the bright area in the SNOM image obtained by collecting the light at the wavelength from 280 to 350 nm [Fig. 1(b)]. The fluorescence from the PS thin film excited at 266 nm showed the maximum intensity at 310 nm, which is attributed to the phenyl ring excimer emission. Thus, the spatial distribution of PS was clearly imaged by the direct detection of the optical signal without any staining procedure. The measured fluorescence intensity from the PS film with a thickness of 200 nm was 45,000 photon counts/s. Since a few hundred photons per second is enough to obtain a clear image, the DUV–SNOM has such a high detection sensitivity as to measure the films of aromatic compounds as thin as 1 nm. Similarly to PS, other polymer materials having an aromatic ring such as polyester were clearly observed and characterized from the emission spectra by the DUV excitation. On the other hand, since PMMA does not absorb the light at the excitation wavelength of 266 nm, there is no contrast in the SNOM image [Fig. 1(d)]. Since PMMA has an absorption band attributed to electronic transition of the carbonyl group, it can be observed in the SNOM image by adjusting the excitation wavelength.

In order to demonstrate the spatial resolution beyond the diffraction limit, we performed the measurement of the PS latex beads with a diameter of 100 nm dispersed on a quartz substrate. The PS beads are commercially available as the “nonfluorescent” latex (Polybead carboxylate, Polyscience). Figure 2(a) depicts the fluorescence SNOM image of the PS nanoparticles. Bright fluorescence was detected from each bead with a volume on the order of attoliter; the SNOM image was taken within 15 min. The cross section profile for a PS particle is shown in Fig. 2(b). The particle was observed as the fluorescence spot with a diameter of 150 nm in the DUV–SNOM image, that is, the particle was broadened by 50 nm. This indicates that the point spread function is about 50 nm, which is beyond the diffraction limit.

The DUV–SNOM imaging is applicable not only to polymer materials but also to biological samples. Since nucleic acids and part of the natural amino acids have conjugated functional groups, they absorb the light in the ultraviolet region and emit the fluorescence at 300–400 nm. This indicates that DUV–SNOM can be used for imaging biomaterials such as protein and DNA. Figure 3 shows the DUV–SNOM image of the stromal cell, PA6. The fibrous structure under the cell membrane can be clearly seen in the SNOM image with a spatial resolution of 150 nm. At the excitation wavelength of 266 nm, it is thought that all of the protein and DNA in the cell were excited and emit the fluorescence in this image. Since the SNOM developed in this study enables us to use a broad wavelength range from DUV to near infrared, the selective imaging of a particular site is possible by the different excitation/collection wavelengths much wider than the conventional SNOM in the visible region.

In summary, the wavelength range of the excitation...
source of SNOM was extended to the deep ultraviolet region by the DUV transmittable probe and signal collection optics. By illuminating the specimens with the optical near field in the DUV region, most kinds of chemical compounds having aromatic substituents can be directly imaged through the fluorescence emission. The near-field imaging was demonstrated for thin films of polymer and biological materials without staining, and the spatial resolution of 50 nm was achieved. Moreover, the DUV–SNOM has high sensitivity to observe ultrathin films with a nanometric thickness because of the contrast mechanism of fluorescence detection. DUV–SNOM allows the direct observation of a variety of chemical species with a high resolution and sensitivity, and it will be a powerful microscopic technique in wide research fields.

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