



PiBMA by means of copolymerization of isobutyl methacrylate and the perylene-labeled methacrylate monomer as shown in Fig. 1. In addition to the Pe-labeled copolymer (PiBMA-Pe), we prepared a carbazole-labeled copolymer (PiBMA-Cz), which is an efficient donor of excitation energy to the Pe chromophore, yielding enhanced fluorescence from the Pe. The characterization of these copolymers is listed in Table I. The molecular weight and its distribution were measured with a gel permeation chromatography (GPC) system (L-7000, Hitachi) calibrated with standard polystyrene and PiBMA.

Table I Characteristics of polymer samples

|          | $M_n \times 10^{-3}$ | Mw / Mn | $f^* / \%$ |
|----------|----------------------|---------|------------|
| PiBMA    | 450                  | 2.94    | 0          |
| PiBMA-Pe | 2,690                | 1.25    | 1.3        |
| PiBMA-Cz | 52                   | 4.30    | 20         |

\* Composition of chromophoric unit in the copolymer.

**2.2 Apparatus.** The details of the apparatus were as described elsewhere.[6] The SNOM system used in this study is based on a commercially available instrument (SP-301, Unisoku). The distance between the tip to the sample surface was regulated by a shear force feed-back system. The UV-SNOM probe was made from an optical fiber with a pure silica core by a heating-and-pulling method followed by a chemical etching method. The end sharpened was metal-coated on the side to make an aperture of 50 - 100 nm in diameter.

A He-Cd laser (IK5351R-D) was used as the light source, which provided dual wavelength beams at 325 nm and 442 nm. The laser power was attenuated to a few mW before coupling into the fiber probe. The second harmonic light of a Ti:sapphire laser (Tsunami, Spectra Physics) was also used for excitation of the Pe chromophore. The fluorescence from the sample was collected by a high NA objective (1.3 NA, Nikon) and detected with a photomultiplier (R4220P, Hamamatsu).

**2.3 Sample Preparation.** Monolayers were prepared on a Teflon-coated trough equipped with a Wilhelmy-type film balance. A benzene solution (ca. 0.1 g L<sup>-1</sup>) of the labeled polymer was spread onto the surface of subphase water at 20 °C. After the monolayer was left for 20 min, it was compressed to a surface pressure of 5 mN m<sup>-1</sup>, and then transferred onto a glass substrate by the vertical dipping method.

### 3. RESULTS AND DISCUSSION

A polymer monolayer of PiBMA containing a small amount of the labeled polymer, PiBMA-Pe (the fraction  $F = 1/300$  in the monomer unit) was prepared on a glass plate, and observed by SNOM with excitation at 415 nm. This wavelength is fit to the second absorption band of Pe :  $\epsilon(415 \text{ nm}) = 27,100 \text{ L mol}^{-1} \text{ cm}^{-1}$ . Figure 2 shows the SNOM image taken with the Pe fluorescence through an appropriate combination of optical filters. Many small dots could be seen in the picture, although the topographic image simultaneously taken was very flat on

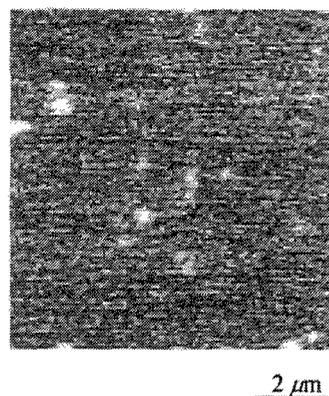


Fig. 2 SNOM image taken with Pe fluorescence:  $\lambda_{\text{ex}}=415 \text{ nm}$ .

the surface. Considering the number averaged degree of polymerization  $dp = 19,000$  and the composition  $f$  of Pe unit in the copolymer, a single chain bears a large number of Pe: ca. 247 on average. In single molecule experiments with low molecular weight dyes, a discrete jump of fluorescence intensity has been often used as an indication of single molecule detection,[9-11] because the intensity of a spot must be an integral multiple given by the number of dye molecules gathered in the spot. However, this is not the case for the present polymer system. Some were bright and others were dark in Fig. 2; obviously the intensity was changed continuously

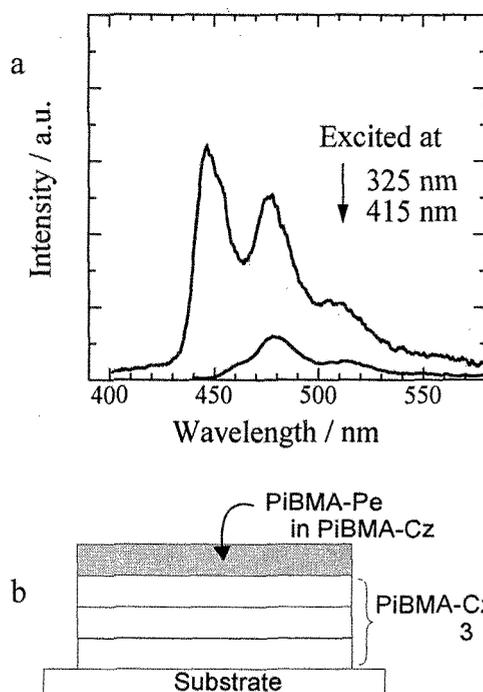


Fig. 3 (a) Fluorescence spectra of PiBMA-Pe monolayer measured with 325 nm and 415 nm excitation. The fluorescence intensities of the spectra were corrected for the intensities of excitation light. (b) Layer structure for the energy transfer experiments.

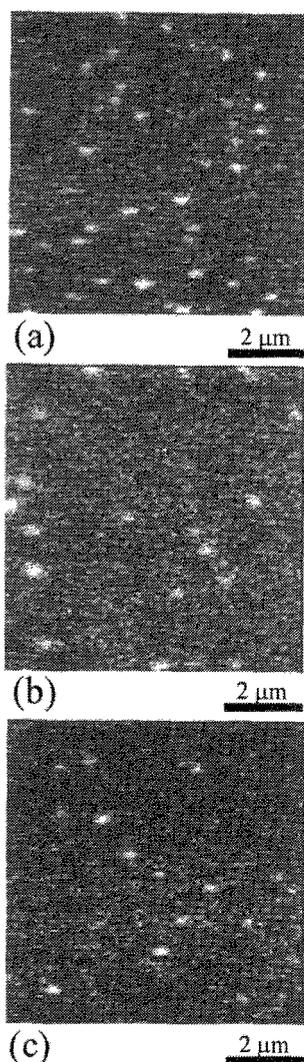


Fig. 4 SNOM images enhanced by the energy transfer mechanism:  $\lambda_{ex}=325$  nm. (a)  $F = 1/300$ , (b)  $F = 1/500$ , and (c)  $F = 1/1,000$ .

depending on the number of Pe chromophores. Important is that the number of dots observed was much smaller than the value expected from the plane density of PiBMA-Pe. These results suggest that the sensitivity of SNOM was insufficient to visualize all of the polymer chains, and a part of them having weaker intensities were missed under the noise level of the detector.

In order to enhance the intensity of Pe fluorescence and to improve the signal-to-noise ratio of the SNOM image, we employed the energy transfer phenomena between Cz and Pe chromophores. The emission band of Cz has a large overlap with the absorption band of Pe, resulting in efficient energy transfer with a large Foerster distance of 2.9 nm.[12] Furthermore, energy migration also takes place among Cz groups in such a highly concentrated system. Figure 3a depicts the fluorescence spectra of PiBMA-Pe recorded by a conventional far-field fluorescence spectrometer.

Observed was the four-layered PiBMA-Cz (energy donor) specimen, in the top layer, PiBMA-Pe (energy acceptor) being incorporated as shown in Fig. 3b. In this sample, PiBMA-Cz was used for all layers in order to increase the energy transfer efficiency. These spectra were measured under the same optical conditions as those of SNOM measurements, *i.e.*, excited at 325 nm and 415 nm and observed through the optical filters to remove the strong scattered light of the excitation wavelength and the Cz emission. Figure 3a indicates that the intensity of Pe emission under the 325 nm excitation is about 6 times that of the 415 nm excitation. Thus, the Pe fluorescence intensity was remarkably enhanced by the energy transfer mechanism, compared with the direct excitation of Pe units. Under the 325 nm illumination, the absorbed energy on Cz can migrate among many Cz units and finally transfers to the Pe unit. This is a kind of light harvesting system in which a Pe moiety is able to collect photon energies from a large number of surrounding Cz units. It should be noted that the lateral diffusion of energies may blur the SNOM image. However, the diffusion length of excitation energy in the Cz monolayer is known to be ca. 10 nm, in the previous experiments.[13,14] Considering the spatial resolution of the SNOM image, ca. 100 nm, the effect of energy diffusion is negligible. Therefore, the sensitization with the energy transfer mechanism seems to be an effective manner markedly improving the quality of image.

Figure 4 shows the SNOM images for the samples illustrated in Fig. 3b. Since the fraction  $F$  of PiBMA-Pe was gradually diluted from 1/300 (Fig. 4a) to 1/1,000 (Fig. 4c), the number of dots observed was decreased. It is easy to calculate the plane density  $D$  of the PiBMA-Pe chain from the occupation area  $A$  of the monomer unit on the surface and the fraction  $F$  as follows.

$$D = F / (A \times dp)$$

where  $A = 0.27$  nm<sup>2</sup> / monomer unit,  $dp = 19,000$ , and  $A \times dp$  means the area occupied by a single PiBMA-Pe

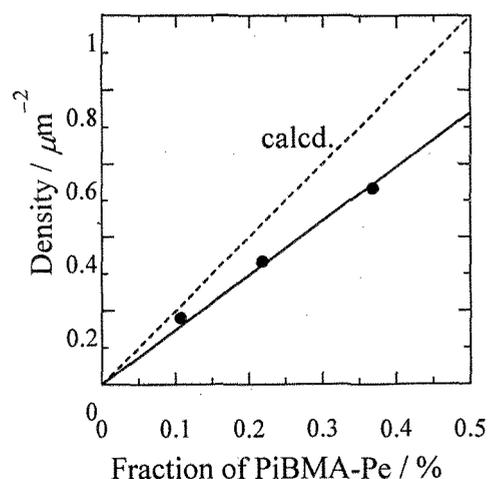


Fig. 5 Plot of the observed and calculated plane densities of PiBMA-Pe chains in the monolayer.

chain.

Figure 5 depicts the relationship between the observed and calculated plane densities of the labeled chains. The observed density was slightly smaller than the calculated value. This difference probably comes from the fact that the molecular weight dispersion of PiBMA-Pe was rather large: 1.25. The GPC chromatogram showed that the labeled sample consisted of polymers having a wide range of molecular weight from  $5 \times 10^5$  to  $1 \times 10^7$ . Therefore, a part of the low molecular weight components were not yet detected in the images of Fig. 4 because of the small number of Pe units. However, the small deviation from the calculated values indicates that the fluorescent spots observed correspond to individual polymer chains embedded in the monolayer.

In order to estimate the fraction of the hidden polymers, the intensity histogram of each spot was compared with the molecular weight distribution. Figure 6 shows the histogram of more than 150 spots together with the number averaged molecular weight distribution measured by GPC. The number of spots counted is insufficient to reach the statistical average. Consequently, the intensity distribution is still dependent of the frame treated. However, the threshold of the appearance and disappearance can be evaluated to ca.  $1.7 \times 10^6$  in the molecular weight as indicated with the broken line in Fig. 6. This means that the present system requires at least 150 Pe dyes for the single chain

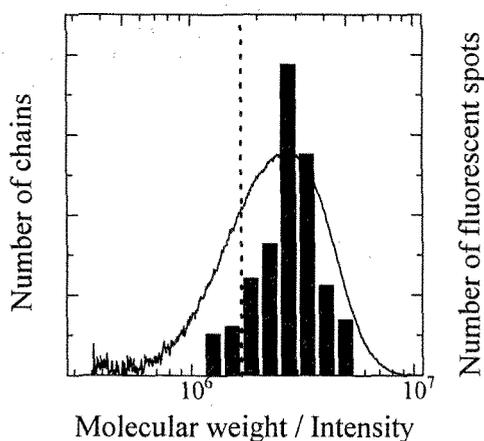


Fig. 6 Molecular weight distribution and histogram of the number of spots as a function of fluorescence intensity.

detection.

Finally, let us discuss the conformation of a PiBMA-Pe chain in the monolayer. The contour length of a polymer chain is about  $5 \mu\text{m}$  at the mean molecular weight. If the chain took a fairly expanded form, it should be observed as a long string in the SNOM image of  $100 \text{ nm}$  resolution. All of PiBMA-Pe chains were actually seen as circular spots with a small diameter less than  $200 \text{ nm}$ . Therefore, it is safely said that the polymer chains were not elongated, but took a rather contracted conformation in the two-dimensional monolayer.

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