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3D single-molecule tracking revealed that a translational diffusions of guest dyes in poly(2-hydroxyethyl acrylate) thin films on glass substrates was confined in a horizontal layer at a distance longer than 300-700 nm from the surface of the substrate. This peculiar long-range effect suggests that interaction between the host polymer and the interface could affect the property of polymers in much longer distance than conventionally estimated.

Polymer thin films are utilized in various nanofabrication[1-5] at the laboratory level as well as in industrial processes. The behaviours of guest chemical reagents depending on the internal structure and properties of host polymers are strongly related to the precision in the nanofabrication. In addition, the spatial distribution of guest reagents is not generally uniform in host polymers, which also affects the quality of the fine fabrication. It is therefore crucial to comprehensively elucidate behaviours of guest molecules in host polymer films. Detection of diffusion processes of guest dyes is a typical method for the investigation and, a variety of methods have been utilized such as mass measurement,[6,7] fluorescence recovery after photobleaching,[8] transient grating,[9] NMR,[10] etc. Although these measurements have provided diffusion coefficients of guest molecules as averaged values in space and time, it is rather difficult to directly correlate diffusion behaviours with spatial position for individual guest molecules in polymer thin films.

Single-molecule tracking (SMT) is one of excellent methods to obtain direct information on individual guest molecules.[11-19] The lateral motion of each guest molecule can be tracked at high spatial accuracy < 10 nm, revealing the diffusion dynamics of individual guests depending on their local environments in the host polymer.[20-25]

Although SMT has high localization accuracy in the lateral (XY) plane of fluorescence microscope, it has been difficult to attain super-resolved tracking along the optical (Z) axis of the objective. Because the properties of polymer solids is dependent on the distance from the interface[26,27] and, hence, the distribution of the guest molecule could be dependent on the Z-position, jthe application of SMT to the elucidation of depth-dependent properties seems of great significance. In the present study, we have applied three-dimensional (3D) SMT to the detection of the diffusion of guest molecules in polymer solids. On the basis of the diffusion dynamics in the 3D space, we discuss the specificity of the lateral diffusion of the guest dye in the polymer film.

As samples, thin films of poly(2-hydroxyethyl acrylate) (poly-HEA, M_n = 2670, M_w/M_n = 1.49, T_g = 290K) including small amount ($<10^{-9}$ M) of a perylenediimide derivative, N,N'-dipropyl-1,6,7,12-tetrakis(4-tert-butylphenoxy)-3,4,9,10-perylenetetra- carboxy-diimide (BP-PDI), were prepared on well-cleaned cover slips (NEO 24×32, Matsunami) by spin-casting of the ethyl lactate solution. As a standard for the precise determination of the Z-position, a perylenediimide derivative with methoxysilyl groups (msPDI) was attached onto

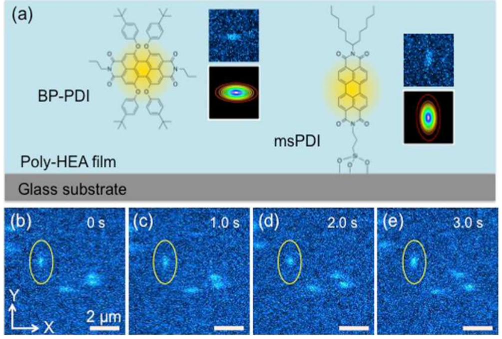

Fig. 1. (a) A schematic illustration of the sample. (b-e) A time course of fluorescence images of guest dyes in a polyHEA film obtained by the wide-field microscopic imaging system with an astigmatism setup.

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Electronic Supplementary Information (ESI) available: the details of sample preparation, the optical setup for the 3D imaging, and the localization accuracy of single molecules by using the imaging system used in the present study. See DOI: 10.1039/x0xx00000x

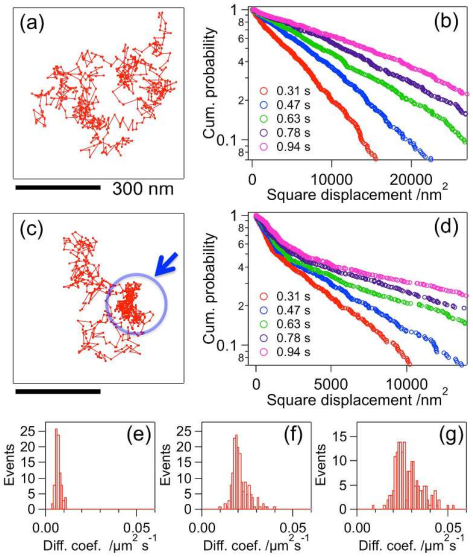

Fig. 2. (a) A typical 2D trajectory of single BP-PDIs exhibiting normal diffusion in a 100-nm thick polyHEA film and (b) corresponding histograms of SDs at several time bins. (c) A trajectory of a guest dye showing anomalous 2D diffusion in the same polyHEA film and (d) corresponding histograms of SDs at several time bins. (e-g) Distributions of the lateral diffusion coefficient of the guest dyes in (e) 100-nm, (f) 500-nm, and (g) 1000-nm thick polyHEA films. These histograms summarize the data of (e) 87, (f) 169 and (g) 177 molecules.

the surface of cover slips by silane coupling as schematically shown in Fig. 1. The details of the synthesis of the msPDI and the attachment onto the glass surface are described in the supplemental information. The polyHEA films were preserved at 323K for at least 12 h before the wide-field single-molecule imaging.

For the SMT of the guest dyes, we used a wide-field fluorescence imaging system with an inverted optical microscope.[28] (See details in S.I.) For the 3D SMT, we employed so-called astigmatism imaging method,[29,30] where the position of guest dyes along the optical (Z) axis is provided as the elongation of the fluorescent spot. In the present setup, the guest dyes in the vicinity of the glass substrate shows vertically elongated fluorescent spots as schematically shown in Fig. 1a. On the other hand, the dyes far from the glass surface exhibit horizontally elongated spots. In the present setup, the localization accuracy in the lateral (XY) plane was ca. 30 nm, while that along the Z-axis was ca.120 nm (see details in S.I.).

Figures 1b-1e show a time course of fluorescence images of guest dyes in a 1000-nm thick polyHEA film. One fluorescent spot with an elongated shape along Y-axis marked with an ellipsoid did not show any lateral motion. This spot is ascribable to the msPDIs immobilized on the surface of the glass substrate. On the other hand, three fluorescent spots with elongated shapes along the X-axis showed distinct lateral motions, indicating that these spots correspond to BP-PDIs in the bulk polymer film far from the interface.

Before the discussion on the 3D motion of the guest dyes, we first show translational motions of guest dyes analysed with the conventional 2D SMT on the XY plane. Figure 2a exhibits a typical 2D trajectory of BP-PDIs in 100-nm thick polyHEA films.

To precisely elucidate the translational motion in Fig. 2a, we plotted histograms of square displacement (SD) of the molecule at several fixed time lags, ΔT, in Fig. 2b, where normalized semilogarithmic plots of the histograms of the SD are shown. For the normal diffusion, the cumulative probability of the square displacement is given as P(SD) $\propto$ exp(-SD/<SD>). Hence, the logarithmic value of P(SD) shows linear decrease with an increase in the square displacement. The linear relation in Fig. 2b indicates that the lateral motion of the guest dye can be regarded as normal diffusion. Most (90%) BP-PDIs in the 100-nm thick films showed normal diffusion in the XY plane (Table 1). The result indicates the polymer film can be regarded as homogeneous medium in terms of lateral diffusion coefficient of the guest dye. This is in consistency with the molecular weight of the present polymer being much smaller than typical values (10^4-10^5)–inducing the entanglement of polymer chains.[31,32]

Table 1. Number of molecules showing normal and anomalous diffusions

Film thickness (nm)[a]	Num. of molecules analysed	Num. of molecules showing normal diffusion (Ratio)	Num. of molecules showing anomalous diffusion (Ratio)
100	87	9 (89.7%)	78 (10.3%)
500	169	161 (95.3%)	8 (4.7%)
1000	177	169 (95.5%)	8 (4.5%)

[a] Film thickness measured with an interference thickness meter (See detail in SI).

Although most BP-PDIs in the 100-nm thick polyHEA films showed normal diffusion, lateral motions of small number of the guest dyes were classified as anomalous diffusion whose P(SD) does not show a Gaussian distribution. A typical example is shown in Fig. 2c, where the translational motion circled with blue colour is apparently slow compared with those in other areas. Fig. 2d shows the normalized histograms for this guest dye at several time lags. The nonlinear shapes of the semilogarithmic plots indicate that the lateral motion includes at least two diffusion coefficients.

The anomalous diffusion is, in general, ascribable to the inhomogeneity of the host polymer films. In addition, the motion along the Z-axis might induce the “anomalous” motion in the 2D trajectories, because the property of the polymer film near interfacial areas, such as the surface exposed to the air and the contact face to the glass substrate, differs from that in the bulk. To elucidate this effect, we prepared polymer films with different thicknesses and analysed single-molecule trajectories for more than 100 guest dyes in each of the films (Table 1). In the analysis for the thickness dependence, we estimated the diffusion coefficients D by using the following equation on the basis of the 2D analysis.

$$MSD = 2dDt_{frm} \qquad (1)$$

Here, d is the dimension of diffusion, t_{frm} is the time interval of images; t_{frm} is the sum of the exposure time of the CCD camera (30.5 ms) and the data transfer time of the camera (typically, 0.8 ms), and MSD is a mean square displacement at the time lag of t_{frm}. This analysis yields a single diffusion coefficient for a single trajectory even though the trajectory includes multiple diffusion coefficients as was shown in Fig. 2c.

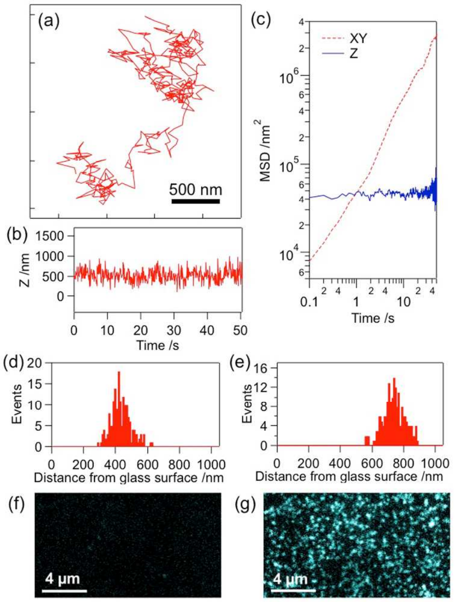

Fig. 3. (a, b) Trajectories of a guest dye in a 1000-nm thick film (a) on the XY plane and (b) along the Z-axis. (c) MSD vs time plots of the trajectories shown in (a) and (b). (d, e) Histograms of the average distance from the surface of glass substrates to guest dyes in (d) 500-nm and (e) 1000-nm thick polyHEA films. (f, g) Fluorescence images of a 1000-nm thick polyHEA film including BP-PDIs obtained by (f) the TIR microscopy and (g) the wide-field epi-illumination microscopy. For the TIR fluorescence imaging, an objective with an NA = 1.49 was used (See detail in SI).

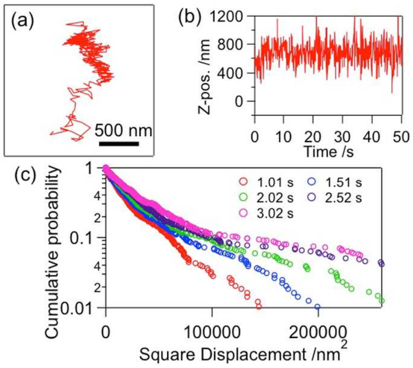

Fig. 4. (a, b) Trajectories of the lateral motion of a guest dye showing anomalous diffusion (a) on the XY plane and (b) along the Z-axis. (c) Corresponding histograms of SDs at several time bins.

Histograms of diffusion coefficients thus obtained for three polymer films with different thickness are shown in Figs 2e-2g, indicating that the diffusion coefficient of the guest dye increases with an increase in film thickness. This result implies that the viscosity of the polymer film and/or the mode of the diffusion of the guest dyes are dependent on the film thickness. It was reported that the glass transition temperature (T_g) of acryl polymer with polar groups in its side chains, such as poly(methyl methacrylate) (PMMA), on silicon oxide increased with decreasing thickness.[26] The effect is, however, restricted in the region near the interface; ca. 100 nm. Hence, the present dependence on the film thickness, as shown in Figs 2f-2g, could not be simply attributable to the effect of the interface.

To more directly and comprehensively elucidate the origin of the anomalous diffusion, we analysed the 3D trajectory whose example was shown in Fig. 1b. Figs 3a and 3b show typical time courses of the 3D position of a guest dye. Most of the BP-PDIs show a normal 2D diffusion on the XY plane as observed in 2D analyses, while, interestingly, the Z-position of a guest dye is almost constant and it randomly fluctuates around the average value. These motions are more quantitatively exhibited in Fig. 3c, where mean square displacements (MSDs) on the XY plane and that along the Z-axis are plotted. The MSD for the 2D motion on the XY plane linearly increases with a slope of unity, while the MSD plot of the motion along the Z-axis shows almost constant values in the time range. This result indicates that the motion along the Z-axis is confined in a narrow range, typically 600-900 nm from the surface of the glass substrates in the 1000-nm thick films. The guest dyes in the 500-nm thick films exhibited similar behaviours; most of their 2D motions on the XY plane exhibited normal diffusion, while their Z-positions were confined in a narrow layer, typically 300-500 nm from the glass surface.

Figures 3d and 3e show histograms of averaged Z-positions of the individual guest dyes. These results clearly show that the present guest dyes do not approach the surface of the glass substrates. This specific diffusion is more directly confirmed in Fig. 1, where all of diffusing fluorescent spots showed similar shapes, which were elongated along the X-axis. This result also indicates that almost constant distances from the surface were kept while diffusing. To confirm these specific diffusion processes along the Z-axis by other experiment, fluorescence image of a 1000-nm thick polyHEA film including larger number of BP-PDIs than the sample for 3D SMT was taken using the microscope with a total internal reflection (TIR) illumination system (see S.I. for the detail of the setup). Figures 3f and 3g show fluorescence images of the polyHEA film photoexcited by the TIR configuration (Fig. 3f) and by the epi-illumination (Fig. 3g). The TIR fluorescence image shows very small number of fluorescent spots with low intensities. The penetration depth of this TIR illumination was ca. 200 to 300 nm, indicating that guest dyes did not exist in the region within 300 nm from the surface of the cover slips. On the other hand, the fluorescence image obtained with the epi-illumination wide-field microscope includes large number of bright fluorescence spots corresponding to single BP-PDIs. This result is consistent with the result obtained by the 3D astigmatism imaging method and ensures the successful 3D tracking of individual molecules.

Similar confined motion along the Z-axis was also confirmed for guest dyes showing anomalous diffusion in the XY plane. A typical 3D trajectory clearly demonstrates this mechanism. Figure 4a shows the 2D (on the XY plane) trajectory of a guest dye in a 1000-nm thick polyHEA film. Figure 4b shows the time course of the Z-position of the guest dye, indicating that the dyes did not approach the surface of the cover slip during the measurement. Figure 4c shows the corresponding normalized histograms of SDs at several time bins. The curved histograms clearly show that the lateral diffusion is anomalous one in spite of the separate Z-position from the surface. This result directly indicates that the anomalous diffusion is not due to the translation along the Z-axis but ascribable to a locally viscous area in the polymer film.

Finally, it is worth noting the origin of the extraordinary behaviours of the guest molecule in position and diffusion along the Z-axis. The experimental result on the guest dye being restricted in the specific position implies position-dependent property of the polymer, such as the polarity of the surrounding area of guest dyes. As a result, the solubility of the guest dye could be dependent on the distance from the interface. As already mentioned, the property of the polymer near the interface (≤ ca. 100 nm) was reported to be different from that in the bulk region[26,27]. The present result, however, indicates that the distance of the location of the dye from the interface is much longer than the scale arising from the short-range interaction between guest dyes and glass surface. In other words, the present result suggests that the effect of the interface hierarchically affects properties of the polymer in much longer distance (>> 100 nm) from the interface. Actually, the location and the lateral diffusion coefficient of the guest dyes depending on the film thickness (Figs 2e-2f) supports the gradual change of the polymer properties in rather long distance along the Z-axis.

The interaction between the host polymer and the guest dye is generally sensitive to chemical properties. Our preliminary studies with the different guest dye with polar groups showed the different distribution of the location along the Z-axis; the location was rather close to the glass surface. This result implies that the polarity is also dependent on the distance from the interface, as predicted by the TIR measurement of the fluorescence of the guest molecules.[33]

In anyhow, the above discussion suggests that the location of the guest dye and the diffusion process are regulated through the interaction between the guest molecule and the host polymer, which is dependent on the distance from the interface. On this point, detailed investigation is underway by changing aging times and by using various guest dyes, host polymers with larger molecular weight, and different kinds of substrates, result of which will be published soon.

Notes and references

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