Excitation-Polarization Effects on Single-Molecule Fluorescence on Quartz Surfaces

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The single-molecule fluorescence of dye molecules on quartz surfaces was studied by using a photon-counting video microscopy. We observed fluorescent spots including a countable number of molecules. The orientation of the transition dipole of the molecules on quartz surfaces was found to vary widely in contrast to that observed on Si surfaces where the transition dipole was highly ordered.

Single-molecule fluorescence microscopy and spectroscopy provide us information about nanoenvironment of individual molecules which is averaged over in bulk measurements. Recent advances in near-field [1-4] and far-field [5-8] fluorescence microscopy allowed us to visualize single-molecule fluorescence at air-substrate interfaces at room temperature.

Recently, we have visualized the single molecule fluorescence of rhodamine B (rhB) molecules adsorbed on a Si (100) surface covered with a native oxide and have found unique fluorescence characteristics.[7] In reference 7 fluorescence from rhB molecules appears in two different forms: (i) bright fluorescent spots grouping a countable number of independent single molecules and (ii) diffused fluorescence strongly quenched by the Si wafers. Furthermore, the fluorescence photocounts from bright fluorescent spots strongly depends on polarization of excitation light.

The fluorescence properties of single molecules on surface will reflect local and microscopic adsorption properties on the substrates. In this letter, we compared the fluorescence of single dye molecules adsorbed on quartz surfaces with that on Si surfaces especially with a view to examining excitation-polarization effect on isolated fluorescent spots. Quartz or glass substrate covered with a polymer film was selected as a standard surface in many studies of single-molecule fluorescence microscopy and spectroscopy at air-substrate interfaces.[1-4,6,8]

The experimental setup used was reported previously.[7] A frequency doubled cw Nd:YAG laser (10 mW, Coherent DPSS 532) was used to excite the sample without passing through the optical microscope used (Nikon Optiphot XP). The colatitude (θ) of the incident direction of the laser light was 65° with respect to the z-axis, if we set the z-axis to the microscope optical axis which is perpendicular to the sample surface. The polarization direction of p-polarized light is perpendicular to the incident direction and is in the plane of incidence, whereas that of s-polarized light is perpendicular to the p-polarized light. The laser power was same for circular and linear polarization. The fluorescence emission was determined with a 100 × microscope objective (NA 0.75) along the z-axis, and passed through a long-pass filter to be detected with a photon-counting video camera system (Hamamatsu C2400-40).

The sample was prepared by drop-and-drag treatment used in the previous study.[7] A drop of 2×10^{-7} M methanol solution of Texas Red (TR, sulforhodamine 110) was gently dragged on an optically polished quartz substrate with a piece of lens-cleaning paper. Because the partition ratio of dye molecules on the paper to those on the substrate is ~100:1,[7] the use of drop-and-drag treatment is equivalent in molecular density to direct spin-coating of a drop of $\sim 10^{-9}$ M solution. On the prepared submonolayer of

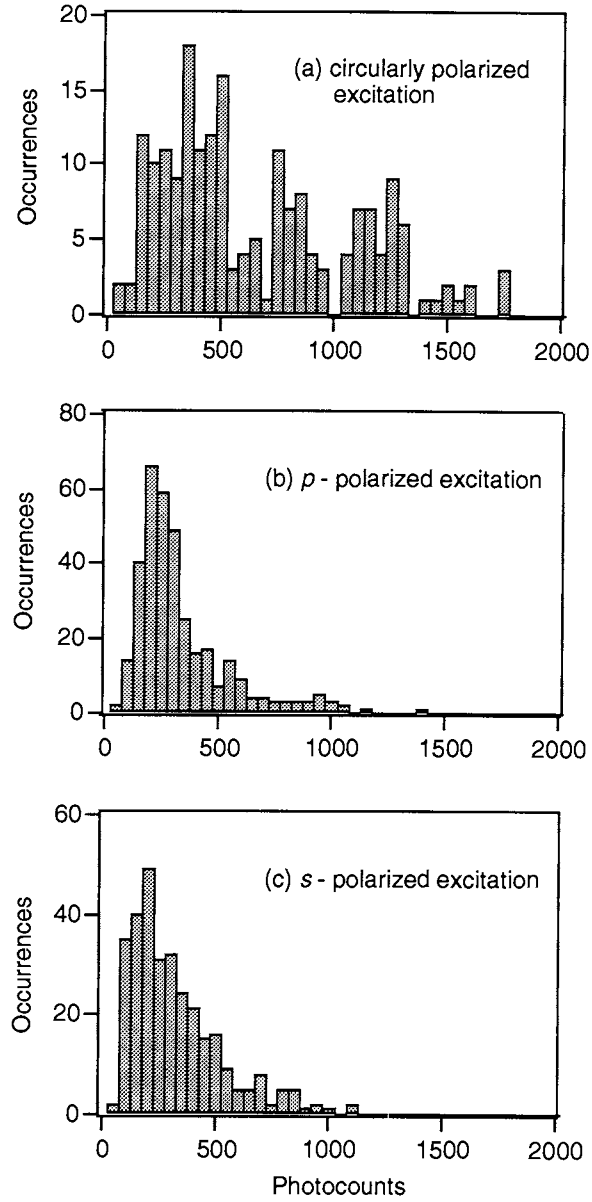

Figure 1. Histograms of fluorescence photocounts evaluated from observed individual fluorescent spots of Texas Red on quartz surfaces versus the frequency of occurences under the excitation of (a) circularly, (b) p-, and (c) s- poralized light.

TR, poly (methyl methacrylate) (PMMA) solution was then spin coated to immobilize the adsorbed molecules. In addition to immobilization, we found that the background counts were reduced by a factor of two. Without PMMA coating, we could not distinguish fluorescent photocounts from the background counts. The polymer film may be effective in covering up the roughness on the substrate, thereby making the surface smooth and reducing light scattering.

Figure 1 shows the histograms of fluorescence photocounts of TR on a quartz-PMMA surface excited by (a) circularly, (b) *p*- , and (c) *s*-polarized light. For excitation by circularly polarized light, where the molecules are uniformly excited, the histogram has three maximums around ~400, ~800, ~1200 photocounts. The quantized histogram observed shows the emission from one, two, and three molecules. Distributions of fluorescence photocounts attributed to groups of more than three molecules were not observed in this study in contrast to the previous study.[7] The photocounts of ~400 / 30s per molecule are reasonable comparing those photocounts to the photocounts from rhB on Si surfaces (~100 counts / 30s per molecule).[7] In some experiments these maximums were not clearly obtained especially when signal-to-noise (S/N) ratio was not good enough for identifying fluorescent spots. The poor S/N ratio was due to surface irregularities of PMMA coating. Nevertheless, this digitized histogram similar to those reported for fluorescent spots on Si surface, together with the reasonable photocounts obtained, shows that fluorescence photocounts were detected at single molecular level. We found again digitized histograms in submonolayers of TR on quartz surfaces. The grouping of countable numbers of single molecules is not intrinsic to rhB and Si wafers. Probably the drop-and-drag treatment is due to the grouping, because the grouping is rarely found in our study using the standard spin coating preparation.[6]

The histograms using *p*- and *s*-polarized excitation light (Figure 1 (b) and (c)) are diffused in appearance but are resemble each other. This is explained that either parallel or perpendicular orientation is not particularly preferred for the transition dipole of TR on quartz surfaces. The possibility of random orientation on glass surfaces covered with a PMMA film was also suggested by the previous studies.[1,8] This observation is in good contrast with the case of Si surface, where photocounts obtained by *p*-polarized excitation reproduced a digitized histogram, whereas the number of photocounts using *s*-polarized light for a Si surface were too small to make a histogram.[7] The transition dipole of a single molecule on Si surfaces is not parallel to a surface but is forced into a tilted position on the assumption that fluorescence intensity observed is entirely determined by the absorption efficiency.[7]

To obtain polarization dependence for the individual fluorescent spots, we examined the photocounts under the irradiation of *p*- and *s*-polarized excitation. Table 1 shows the photocounts P and S, and the ratio P/S of those photocounts. The ratio P/S for quartz surfaces varied from smaller to larger values than unity (P/S = 0.373 - 3.66). This observation is consistent with the similar histograms for *p*- and *s*-polarized excitations (Figure 1 (b) and (c)). In contrast to that, all of the P/S values of 20 fluorescent spots on Si surfaces reported (P/S = 5 - 13) were much larger than unity, indicating small colatitude angles (θ = 20 - 30 °) of the transition dipoles.[7]

Table 1. Photocounts (counts/30s) of representative 18 fluorescent spots under the irradiation of *p*- and *s*- polarized excitation

P	S	P / S	P	S	P / S
428	117	3.66	248	665	0.373
345	297	1.16	184	430	0.428
496	383	1.30	162	321	0.505
364	273	1.33	200	229	0.873
231	475	0.486	218	248	0.879
136	336	0.405	415	579	0.717
339	734	0.462	230	206	1.12
109	206	0.529	176	253	0.696
115	187	0.615	307	254	1.21

The obtained P/S ratio for quartz surfaces show a sharp fluctuation of a factor of 10 from spot to spot. The degree of fluctuation is larger than those for Si surfaces which are within a factor of 3.[7] When the transition dipole is immobilized on a surface within the acquisition time, the P/S value may vary according to the azimuth even though its z-component is the same. We found larger fluctuation of P/S ratio for the quartz surface covered with a PMMA film than that for Si surface, and confirmed an immobilization effect of PMMA. If we assume that the transition dipole is completely immobilized and its azimuth angle φ is 45°, its colatitude θ is estimated to be ~71° to reproduce the P/S ratio of 0.37. In the same conditions, θ is ~34° for the P/S ratio of 3.6.

In the previous report, we tentatively concluded that the bright fluorescent spots on Si surfaces are on small intrinsic bumps on the surfaces. The origin of the sites is still unknown, but the unusual polarization dependence of the bright spots become clear in this comparative study. Although the grouping of countable numbers of adsorbed molecules reported for Si surfaces was also observed for quartz surfaces, the highly ordered orientation of contained molecules was confirmed to be intrinsic for Si surfaces.

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References and Notes

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