

# Low-Temperature Confocal Microscopy on Individual Molecules near a Surface

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The spectral behavior of single terrylene molecules at a hexadecane–silica interface structure is studied. Different sample preparation techniques indicate that molecules close to a silica substrate in a hexadecane matrix are subject to additional dephasing or spectral diffusion. The temperature activation of the homogeneous line width of these molecules shows much reduced activation energies as compared to those of molecules in bulk hexadecane. These low-lying activation energies may be attributed to surface phonon states or local phonon modes particular to the interface structure. With the aid of a low-temperature scanning confocal microscope, the distance of single terrylene molecules to the silica surface can be measured with a precision of 60 nm. A drastic increase of the homogeneous line width for molecules with distances less than 150 nm to the silica surface is found.

## Introduction

The combination of optical microscopy and spectroscopy ranks among the most intriguing developments in optical spectroscopy during the past years. So far, different microscopic techniques, most notably near-field and confocal microscopy, have been combined with different spectroscopic techniques such as fluorescence,<sup>1</sup> time-resolved,<sup>2</sup> and Raman spectroscopy.<sup>3,4</sup> One of the major applications presently is single-molecule spectroscopy.<sup>5</sup> Individual molecules have been isolated and detected with room-temperature near-field and confocal microscopy as well as conventional microscopy at surfaces, at interfaces,<sup>6</sup> in gels,<sup>7</sup> and in fluid solution.<sup>8</sup> At low temperatures conventional far-field<sup>9</sup> and near-field<sup>10</sup> experiments have been reported. However, the intriguing possibility to correlate the spectral behavior and the spatial location of a single molecule has been hardly exploited so far. This is mainly due to the fact that most of the work has been carried out at room temperature, where single molecules exhibit broad unstructured lines such that spectral changes due to the environment are less clearly observed as is the case at low temperature. In addition, most samples studied so far do not show a lateral structure such that a pronounced change in the behavior of the individual molecules upon a variation in the lateral position on the sample is not expected. In contrast, all samples that consist of a thin layer of a matrix containing the molecules on a substrate do show an axial structure in the proximity of the surface of the substrate. At this interface between matrix and substrate either changes in the matrix structure with respect to bulk matrix properties or the mere vicinity of the substrate material will influence the spectral behavior of the probe molecules. For example, the proximity to a surface will change the phonon density of states (surface phonons) which influences the dephasing of the molecular electronic transition. Individual molecules such as terrylene are sensitive low-temperature probes for such changes, owing to their narrow optical line width. In addition, changes in the excited-state lifetime of single molecules at room temperature owing to differences in the dielectric function at the interface have been reported.<sup>6</sup> In the present paper individual terrylene molecules are selected by low-temperature confocal microscopy in a hexadecane matrix close to a silica surface, and their spectral behavior as a function of the distance to the surface is studied.

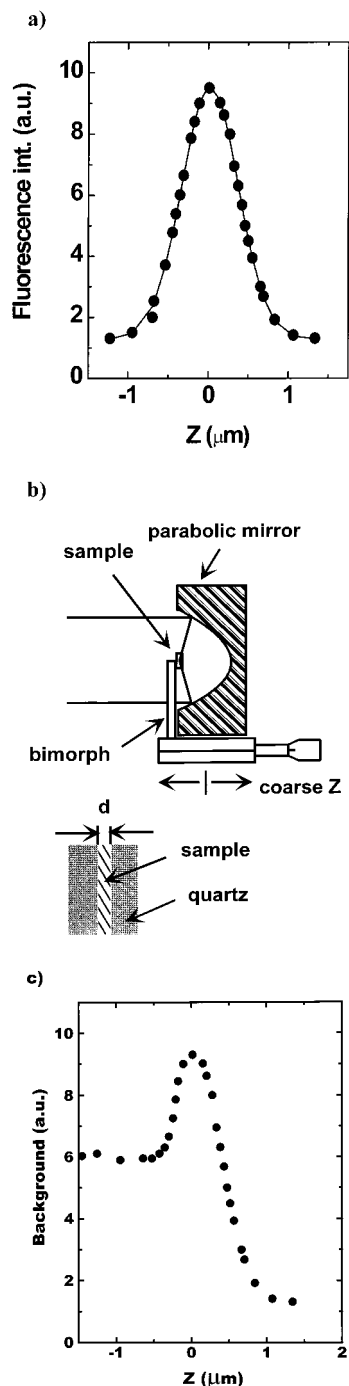
## Experiment

**Setup.** For the experiments described below two different kinds of experimental arrangements have been used. The excitation source in all experiments is a monomode dye laser (Coherent Radiation 699-29) continuously pumped by all lines of an argon ion laser (Innova 300). For the experiments presented in this paper, the built-in commercial frequency stabilization of the laser has been used. Amplitude stabilization of the excitation light was achieved by an electrooptic modulator (500 kHz bandwidth). Excitation of the sample has been carried out via either a single-mode optical fiber<sup>11</sup> or a high-quality parabolic mirror<sup>12</sup> (16 mm aperture, NA = 1) in a confocal setup. The fluorescence detection scheme was common to all experiments. Fluorescence photons were separated from stray excitation light by both a notch filter at 578 nm and a red pass filter (Schott RG610) and focused on the photocathode of a photomultiplier (RCA 31034 A02) or an avalanche photodiode (EG&G SPCA 200 PQ) operating in a single-photon counting mode. Excitation spectra were recorded on a multichannel analyzer. In addition, the fluorescence intensity autocorrelation function  $g^{(2)}(\tau)$  of single molecules was measured from  $\tau = 1$   $\mu$ s to  $\tau = 1$  s with a logarithmic autocorrelator (ALV 5000).

For the confocal experiments the parabolic mirror was illuminated along its optical axis by a parallel laser beam, enlarged to the aperture of the mirror. Under such conditions and due to negligible geometrical aberrations of the mirror, we measured a lateral spot diameter of the excitation laser about 1  $\mu$ m in the focus plane. The sample was mounted on the end of a bimorph with one of its flat sides facing the parabolic mirror. The bimorph itself was fixed on a scanning table (see Figure 1), which allowed us to adjust the coarse position of the sample in the focus of the mirror along its optical axis. We have measured the total bending range of the bimorph along the  $z$ -direction at liquid helium temperatures, obtaining a value of 22  $\mu$ m for voltages between  $-70$  and  $+70$  V. Thus, we were able to move the sample along the  $z$ -direction, relative to the (fix) focus of the parabolic mirror, and therefore to scan in the depth of the sample.

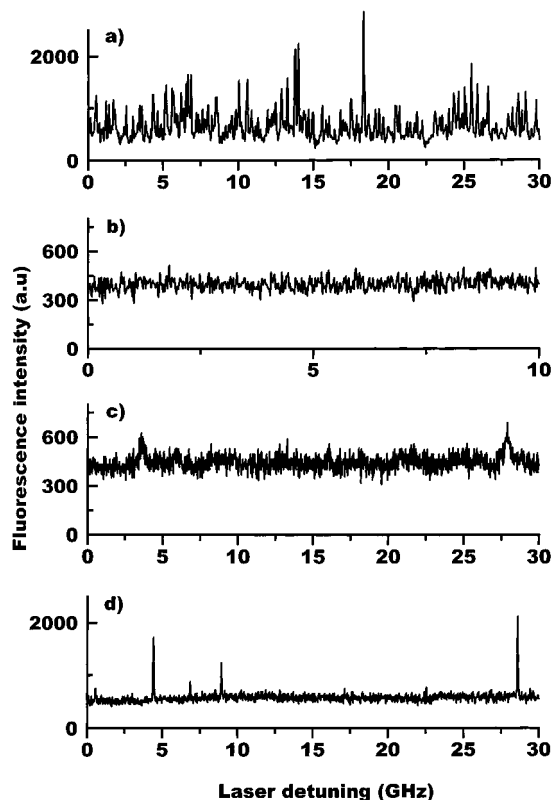
**Sample Preparation.** In preliminary experiments, we have used the excitation by a polarization preserving optical single-mode fiber on three different samples prepared from a common solution of terrylene in liquid hexadecane. Terryene is hardly solvable in hexadecane at room temperature, giving low

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**Figure 1.** Schematic representation of the confocal setup (panel b) and the fluorescence intensity of a single molecule versus the detuning of the bimorph (panel a). The thickness  $d$  of the sample is roughly  $5\ \mu\text{m}$ . The tuning range of the bimorph at  $4.2\ \text{K}$  is roughly  $22\ \mu\text{m}$ . Panel c shows the reflex from the silica surface as the focal spot is tuned across the interface.

concentrated solutions. Sample 1 was simply a drop of this mixture on the end of a freshly cleaved fiber and placed immediately inside the precooled cryostat to inhibit the evaporation of hexadecane. In a second experiment (sample 2), again, a drop of the same mixture was put on the end of the fiber. After several minutes at room temperature, the liquid hexadecane was totally evaporated. In a last step, the fiber end was cleaned with an impregnated tissue of acetone. With such a procedure we expect some terrylene molecules still to be present on the fiber end, adsorbed on the silica surface. Finally, a sample 3 was prepared in the same way as sample 2 except that, in an additional step, we placed a new drop of pure (without terrylene



**Figure 2.** Fluorescence excitation spectra of single terrylene molecules in hexadecane for samples 1–3 (see text). Part a shows the fluorescence excitation spectrum of a solution of terrylene in hexadecane, part b is the spectrum of sample 2, i.e., terrylene directly adsorbed on silica, part c shows a spectrum of sample 3 at  $T = 4.2\ \text{K}$  and part d shows the same spectrum at  $T = 1.5\ \text{K}$ .

impurities) hexadecane on the end of the fiber. Like sample 1, sample 3 was immediately cooled to liquid nitrogen temperature inside the cryostat.

For the experiment performed with the confocal setup, new samples were prepared from several drops of the same mixture terrylene in hexadecane used for the fiber experiments. In this case the sample was inserted between two silica plates (see Figure 1b). The thickness of the hexadecane layer was estimated from experiments to about  $5\ \mu\text{m}$ .

## Results

Terrylene in hexadecane belongs to the few known systems that fulfill the strict requirements<sup>11,13</sup> of SMS at low temperatures. The strong absorption of terrylene at  $\lambda = 571.9\ \text{nm}$  is inhomogeneously broadened ( $\Delta\lambda = 0.3\ \text{nm}$ ) at  $T = 1.5\ \text{K}$  in the Schpol'skii matrix hexadecane. In agreement with the high fluorescence quantum yield of this molecule ( $0.7^{14}$ ), intense zero-phonon lines with a nonsaturated line width of  $42\ \text{MHz}$  ( $T = 1.5\ \text{K}$ ) can be found in the excitation spectrum with very high signal-to-noise ratio. The very low intersystem-crossing rate to the triplet state of about  $1\ \text{kHz}$  explains the high saturation intensity of  $1.91\ \text{W}/\text{cm}^2$ .<sup>13</sup>

Figure 2a shows an excitation spectrum on a  $30\ \text{GHz}$  laser scan measured on sample 1 at  $T = 4.2\ \text{K}$ , close to the maximum of the 0–0 absorption band at  $\nu_{\text{laser}} = 17\,481\ \text{cm}^{-1}$ . A large number of intense and narrow zero-phonon lines with high signal-to-noise ratios are present in the spectrum. In contrast, no such structures were observed on a long scan from  $\lambda = 571.2\ \text{nm}$  to  $\lambda = 572.5\ \text{nm}$  in sample 2 (see Figure 2b) for terrylene molecules directly adsorbed on the silica surface of the fiber end without additional terrylene. We know from the next

experiment described below that terrylene molecules must be present on the fiber end, but no absorption could be found from these molecules. Finally, for sample 3 (see Figure 2c), we observed broad and sparse structures in the excitation spectrum from  $\nu = 17\,486\text{ cm}^{-1}$  to  $\nu = 17\,490\text{ cm}^{-1}$  at  $T = 4.2\text{ K}$ . In a very few cases we observed intense isolated lines in this large spectral range, but the broad structures were by far predominant in the spectrum. Such structures are shown on the 30 GHz scan at  $17\,488\text{ cm}^{-1}$  in Figure 2c. By cooling to  $T = 1.5\text{ K}$ , these broad lines become narrow as is shown in the spectrum in Figure 2d. In a complementary experiment, we checked that no single-molecule fluorescence excitation lines were present in a frequency range from  $\nu = 17\,486\text{ cm}^{-1}$  to  $\nu = 17\,491\text{ cm}^{-1}$  in a drop of "pure" hexadecane on a freshly cut fiber. Consequently, in Figure 2c,d we observed excitation lines of individual terrylene molecules originating from the fiber end. As displayed in Figure 2d, only a few isolated lines were visible on a 30 GHz scan, an observation in agreement with the presumably low concentration of terrylene molecules in the sample due to the cleaning procedure of the fiber end.

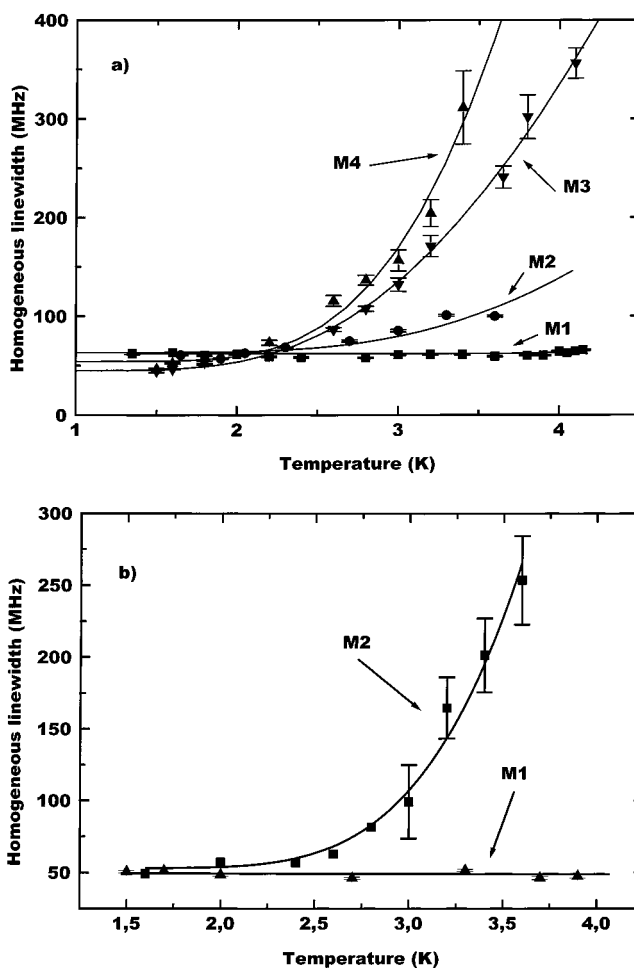
A comparison of parts c and d of Figure 2 readily indicates the steep temperature dependence of the fluorescence excitation line width of the individual terrylene molecules in sample 3. We have studied this width as a function of temperature for molecules in the bulk hexadecane matrix (samples 1) and those at or close to the silica surface (sample 3) between  $T = 1.3\text{ K}$  and  $T = 4.2\text{ K}$ . For sample 1, taking into account the experimental error, most of the lines do not show any variation of their width in this temperature range, the corresponding curves remaining flat. An example is displayed in Figure 3a for molecule M1. In contrast to this, the line widths of the molecules observed in sample 3 show a strong temperature dependence. Examples are also plotted in Figure 3a, where the homogeneous line width of molecule M3 and M4 increases by nearly a factor of 10 between  $T = 1.3\text{ K}$  and  $T = 4.2\text{ K}$ . The experimental values of the line width  $\Delta\nu$  at different temperatures are well-fitted by an exponential function

$$\Delta\nu = \Delta\nu_0 + C \exp(-\Delta E/k_B T)$$

where  $\Delta E$  is an activation energy,  $k_B$  the Boltzmann factor,  $T$  the temperature, and  $C$  a prefactor.  $\Delta\nu_0$  is the low-temperature lifetime limited value of the line width.

The activation energies deduced from such fits on five investigated molecules from sample 3 are distributed in an interval between 10 and 12  $\text{cm}^{-1}$  ( $\pm 0.5\text{ cm}^{-1}$ ). M2 in Figure 3a is one of the few examples of molecules with narrow and intense lines at  $T = 4.2\text{ K}$  in sample 3. These molecules also do show a less pronounced temperature dependence of the line width.

The differences in temperature dependence of the homogeneous line width for the molecules in samples 3 and 1 indicate an interaction with distinctly different local environments. One interpretation could be that the lines in sample 3 arise from molecules located directly at the silica surface or "close" to the interface silica/hexadecane. The few observed intense lines in the same spectrum possibly arise from molecules that have desorbed from the silica surface and spatially diffused into the liquid alkane hexadecane during the few minutes between the last step of the sample preparation and its freezing inside the cryostat. To verify the assumption that the spatial proximity of the molecules to the silica surface is correlated with the change in activation energy, the temperature dependence of the homogeneous line width of individual molecules was also studied with a confocal setup (see Figure 1). In these experiments the distance between molecule and interface was deter-



**Figure 3.** (a) Temperature dependence of the line width of single molecules in sample 3. Points are measured curves, and solid lines are fits according to an exponential activation behavior. (b) Temperature dependence of the line width of two molecules measured with the confocal setup. M2 had a distance of  $400 \pm 50\text{ nm}$  to the silica surface, and M1 had a distance of  $2.5 \pm 0.05\text{ }\mu\text{m}$ .

mined in two steps. First, after the selection of the fluorescence excitation line of a single molecule, we recorded the maximum fluorescence signal with the laser in resonance with a molecular transition as a function of the bimorph position (Figure 1a), i.e., by moving the position of the molecule relative to the focus of the parabolic mirror. The corresponding curve is a convolution of the Gaussian profile of the excitation spot at the focus and the spatial response function of the fluorescence excitation of the individual molecule. The lateral size of this spatial response function is given by the absorption cross section, which is between  $10^{-2}$  and  $10^{-3}\text{ }\mu\text{m}^2$  at low temperatures and can therefore be neglected compared to the size of the excitation spot, which is about  $1\text{ }\mu\text{m}$ . Thus, the curve in Figure 1a nearly reproduces the shape of the Gaussian beam in the excitation volume of the sample. A Gaussian fit of this curve gives the position of the maximum with a precision of  $\pm 30\text{ nm}$ , which mainly depends on the signal-to-noise ratio of the fluorescence signal since the reproducibility of the bimorph position is better than  $10\text{ nm}$ . From this measurement, we deduced the bimorph position for which the molecule coincides with the maximum of the Gaussian excitation spot. In a second step, we increased the excitation light, and we measured the intensity of the background (elastically scattered laser light) as a function of the bimorph position. The background, initially high when the focus lies in the Schpol'skii matrix (left part of the curve on Figure 1c), first increases when the focus moves toward the interface silica/hexadecane. We attribute the maximum back-

ground arising from the reflection of the excitation beam to the silica plate surface. The background drops off when the excitation spot is in the depth of the silica plate. The position of the hexadecane/silica interface is thus deduced from the maximum of the curve with again a precision of  $\pm 30$  nm. Finally, from these measurements, we were able to calculate the distance between the molecule and the interface with a total error of  $\pm 60$  nm. As described above, the sample consists of a layer of the terrylene/hexadecane mixture between two silica cover slips. The thickness of the layer has been estimated to be  $5\ \mu\text{m}$  using the same method to locate the opposite interface.

Figure 3b shows two experimental examples of the temperature dependence of the fluorescence excitation line width of individual molecules with different distances to the silica surface. For molecule M2 the distance to the interface was  $400 \pm 60$  nm and  $2.5\ \mu\text{m}$  for molecule M1, respectively. The temperature dependence of the homogeneous line width of these molecules is in agreement with the experiments performed with the fiber and confirms our assumption: molecules close to the interface show a large temperature dependence of the homogeneous line width between  $T = 1.3$  K and  $T = 4.2$  K while molecules in the bulk do not exhibit any temperature dependence in this range.

The optical properties of dye molecules adsorbed on surfaces with high-resolution optical techniques, i.e., hole burning and fluorescence line narrowing, have been investigated for several systems. Two phenomena have been observed:

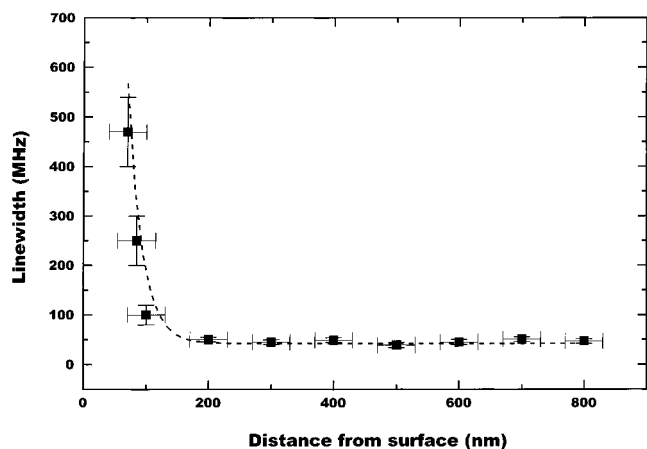
(i) The homogeneous line width as measured by hole-burning spectroscopy on an ensemble of molecules increases significantly when the molecules are adsorbed on a surface as compared to the same molecular species diluted in a glass or polymer.<sup>15–17</sup> The degree of broadening depends on the type of adsorption, i.e., binding to the surface<sup>15,18</sup> and thus on the surface material and on the dye molecule. In some cases an additional layer of matrix material has been used to cover the dye molecules on the surface.<sup>15</sup> In this case it has been shown that the dephasing properties of the dye molecules, i.e., the hole width and its temperature dependence, are determined by the layer material. Dye molecules incorporated in Langmuir–Blodgett films have been studied at various axial positions in the film by hole-burning spectroscopy.<sup>17,19</sup> A significant dependence of the hole width on the axial position and the correspondent temperature dependence has been found. Most notably, molecules directly in contact with the glass substrate the Langmuir–Blodgett film has been adsorbed on show a narrow low-temperature hole width with a steep increase at higher temperatures whereas molecules on the surface of the film show a large low-temperature line width with a smaller increase at higher temperatures.<sup>19</sup>

(ii) The vibronic structure on the fluorescence emission spectrum of adsorbed molecules remains unchanged,<sup>15,20</sup> indicating that the structure of the molecules is preserved upon adsorption. The Debye–Waller factor of perylene adsorbed on a  $\gamma$ -alumina surface has been measured to decrease by 40% as compared to the same molecular species incorporated in poly(vinylbutyral).<sup>20</sup>

Different models have been discussed to explain the increase in hole width of molecules adsorbed on surfaces. Fast spectral diffusion is one of them.<sup>16</sup> However, no clear picture has emerged from the literature yet. Broadening of the zero-phonon line of a single molecule is caused by dephasing or spectral diffusion. Besides the spontaneous emission which limits the coherence lifetime  $T_2$ , the additional contribution to the damping of the electronic coherence created by the resonant laser field, i.e., (pure) dephasing, originates from fluctuations of the

electronic energies. In a crystal, these fluctuations are related to the coupling of the electronic states with phonons. The electronic density change occurring after an electronic transition has the effect of shifting the equilibrium positions of the nuclei, which in turn modify the electronic energies. Adiabatic processes will cause dephasing only if the total energy of the phonon bath remains unchanged after the interaction between electron and phonon. Consequently, following the Born–Oppenheimer approximation and assuming small vibration amplitudes of nuclei, it follows that, in the Taylor expansion of the electronic energies as a function of the nuclear coordinates, only the terms that give rise to multiphonon processes will contribute to dephasing. For instance, the quadratic coupling responsible for two-phonon processes will give a contribution to dephasing according to a  $T^2$  power law in a crystal in the presence of acoustic phonons and an Arrhenius law in the presence of optical phonon modes. Assuming that the cause of homogeneous line width broadening for molecules close to the surface is dephasing, both processes could explain the experimentally observed temperature behavior of the line width. In this picture the strong differences observed between the temperature dependence of the homogeneous line width of single molecules close to the interface silica/hexadecane and the ones far from the interface could be explained by a structure change of delocalized vibrations of the crystal in the vicinity of the interface, i.e., by surface phonons. Another explanation could involve the formation of localized vibrations around the molecules, i.e., a local phonon mode particular to the interface structure.<sup>17</sup> It has been shown that coupling to localized vibrations leads also to an exponential temperature dependence of the zero-phonon line width.<sup>21</sup> The activation energies of around  $12\ \text{cm}^{-1}$  found in our experiments are on the order of what is expected for such a dephasing process. Spectral diffusion can also contribute to the broadening of the homogeneous line for molecules close to the surface. Fast cooling of our samples and silica surface corrugations can create crystal dislocations at the interface where some molecules or groups of atoms could move between two or several equilibrium positions with nearly the same energies. Such motions have been directly observed in recent SMS experiments performed on polymeric matrixes.<sup>22</sup> In polyethylene, some defects have been shown to make phonon-assisted tunneling between two preferred states<sup>23</sup> at very low temperatures. Our experiments could also involve such slow configurational rearrangements of the matrix occurring near the interface. The zero-phonon lines of molecules close to the interface exhibit spectral jumps or drift randomly during the time scale of our laser scan to measure the excitation spectrum. This interpretation seems to be supported by our measurements of fluorescence intensity autocorrelation  $g^2(t)$  on single molecules close to the surface. Indeed a large number of investigated molecules that show a steep temperature dependence of the line width also exhibited a slow component between 100 ms and 1 s in the autocorrelation function. For the other molecules assumed far from the interface, no such contribution to  $g^2(t)$  was observed under similar conditions.

It is worth mentioning that we did not succeed in detecting individual molecules directly adsorbed on a silica surface without an additional hexadecane layer. Presumably, this is due to a considerable increase in homogeneous line width for those molecules even at  $T = 1.5$  K which prevents the detection of individual fluorescence excitation lines by frequency selection. To quantify this proximity effect, we have measured the homogeneous line width of single molecules for different distances to the silica surface (see Figure 4). Each point of the



**Figure 4.** Homogeneous line width of molecules with different distances to the silica surface at  $T = 1.5$  K. For the different distances from the surface a number of single molecules lines have been measured, and the vertical error bars represent the width in the distribution of the line width of the individual molecules found. The dotted curve is a fit with an exponential increase of the homogeneous line width versus  $d$ , the distance to the surface (see text).

curve represents the homogeneous line width of a couple of different molecules selected in the excitation spectrum at the corresponding bimorph position. All the measurements have been performed at  $T = 1.5$  K. The homogeneous line width, first nearly constant for distances greater than  $200 \pm 100$  nm, increases by almost 1 order of magnitude within  $150 \pm 50$  nm when approaching the silica surface. The dotted line in Figure 4 shows an exponential decrease of line width for increasing distance which can be fitted with the empirical relation

$$\Delta\nu = \Delta\nu_{\infty} + \Delta\nu_0 e^{-(d/d_0)}$$

Here  $\Delta\nu_{\infty}$  is the line width of terrylene in the bulk hexadecane matrix,  $\Delta\nu_0 + \Delta\nu_{\infty}$  is the extrapolated value for molecules directly on the surface, and  $d_0$  is the characteristic thickness of the surface layer where additional dephasing occurs. An exponential increase in line width would follow from a linear increase in electron phonon coupling if the surface is approached. The value found for  $d_0$  is 150 nm, and the line width of a single terrylene directly adsorbed on the silica surface  $\Delta\nu_0$  is determined to be 15 GHz. However, one should keep in mind that the small thickness of the interface layer together with the limited accuracy in the determination of the axial position does not allow for a precise determination of the behavior of the homogeneous line width of individual molecules as a function of their distance to the surface. Thus, the value for  $\nu_0$  given above has to be considered with great care.

The homogeneous line widths at  $T = 1.5$  K measured for terrylene molecules in the bulk hexadecane matrix were distributed between 50 and 65 MHz around an average value of 60 MHz, which is slightly higher than the radiative line width of 42 MHz.<sup>13</sup> We carefully tried to avoid power broadening, and drifts of our laser frequency stabilization can be ruled out as a possible cause of this extra broadening since we have measured homogeneous line widths at  $T = 1.5$  K between 40 and 50 MHz for molecules at the interface region. Although our statistic presently is poor (10 investigated molecules, five molecules coming from each sample), we would like to discuss an electrodynamic effect as a possible reason for the smaller low-temperature line width of molecules close to the interface. We can indeed expect a change of the radiative lifetime for molecules in the vicinity of the silica surface. The electric field emitted by the molecular emission dipole is reflected due to

the refractive index difference between the two media ( $n = 1.8$  and 1.34 for solid hexadecane and silica, respectively) and react in turn on the oscillating dipole. The modification of the radiative lifetime by such an interference effect has been experimentally observed for atoms close to mirrors<sup>24</sup> and others nonmetallic surfaces<sup>25</sup> and recently in single-molecule experiments at a polymer–air interface.<sup>6</sup> Moreover, our observation is supported by recent low-temperature SMS experiments on the system hexadecane/dibenzanthanthrene.<sup>26</sup> Following the treatment in ref 27, a change in lifetime between 70% and 10% depending on whether the molecular emission dipole is perpendicular or parallel to interface can result. Our value of a 40% increase in lifetime is consistent with this interpretation.

## Conclusion

Confocal microscopy is of considerable potential for spectroscopy because of its ability to raster scan samples in three dimensions with high spatial resolution. Whereas lateral scans can be performed with other microscopic techniques either with subwavelength resolution (near-field) or with diffraction limited resolution (wide field), the axial resolution of a confocal microscope is unsurpassed. If the point spread function of the microscope is known and a sufficient signal-to-noise ratio can be achieved during the experiment, the position of individual molecules can be determined with a precision higher than the standard resolution ( $\approx 200$  nm) of the microscope.<sup>28</sup> In the present work the fluorescence intensity of individual terrylene molecules was sufficient to achieve a precision of  $\pm 60$  nm in the determination of the axial position of the molecule. This resolution allowed us to select individual molecules with different distances to a dielectric interface such as silica–hexadecane. The main observation is a nearly exponential increase in line width upon decreasing distance to the silica surface. Within the accuracy of our statistics (10 molecules), the tendency for spectral diffusion also increases with decreasing distance to the interface. This observation points toward an increase in disorder at the silica–hexadecane interface where the crystalline order of the bulk hexadecane is not present. This interface region is roughly 200 nm thick. At closer distance to the silica surface it becomes increasingly difficult to observe individual terrylene molecules owing to an increased line width. Experiments with terrylene directly adsorbed on silica and without an additional hexadecane layer did not show any reproducible signals from single molecules presumably because of a drastic increase in line width. This is in accordance with earlier experiments by hole burning where a significant increase in hole width upon the adsorption of dye molecules on surfaces is observed.

The present work demonstrates a way to deconvolute distributions of observables usually measured in single-molecule spectroscopy by microscopic techniques. If in addition lateral scans are used, it becomes possible to isolate individual molecules such as Rhodamine 6G at cryogenic temperatures, which is not possible by pure spectral selection. This opens a new class of systems to low-temperature single-molecule spectroscopy. Such molecules can also be observed at room temperature by confocal microscopy. One thus will have the opportunity to investigate the behavior of one molecule from cryogenic temperatures to room temperature in a single experimental run and thus freeze out different degrees of freedom of the molecule and the surrounding matrix step by step.

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