Optically probing structure and organization : single-molecule spectroscopy on polyethylene films and a resonance Raman study of a carotenoid

Wirtz, Alexander Carel

2 Spincoated Polyethylene Films for Single-Molecule Optics

abstract – We present first results from single-molecule experiments on spincoated polyethylene films doped with terrylene (Tr) or 2.3,8.9–dibenzanthanthrene (DBATT). Perfectly clear films have been produced with a thickness of 100 to 200 nm. We have performed both polarization-dependent single-molecule spectroscopy and single-molecule position determination (microscopy) experiments on these samples. Of the two systems we tested, DBATT in polyethylene proved the most practical for single-molecule experiments. We aim to use this system to study the length-scale of locally increased order in the semi-crystalline polymer polyethylene.

The contents of this chapter have been published:
A. C. Wirtz, M. Dokter, C. Hofmann and E. J. J. Groenen, *Chem. Phys. Lett.*, 2006, **417**, 383–388.

2.1 Introduction

Polyethylene (PE) is the most well-known and frequently used of all polymers. Despite its wide range of applications many questions remain regarding the manner in which PE chains organize in the polymer matrix. This chapter presents our first results obtained in both single-molecule spectroscopy and microscopy experiments on spincoated thin (100 – 200 nm) PE films ultimately intended to investigate local order in the matrix.

Historically, PE found its first use in spectroscopy as a convenient inert host system for organic molecules for a variety of experiments in molecular spectroscopy. As such, PE has since been the subject of considerable scientific scrutiny. Such studies, both optical and non-optical, have shown that PE has a partly crystalline structure [58]. This implies local regions in the PE matrix with a high degree of order interspersed in largely amorphous regions. The degree of crystallinity is known to depend on the polymer chain length and linearity, but also on methods of crystallization [59, 60]. It is thought that dopant molecules may reside in the amorphous phase or be adsorbed on the surfaces of PE crystallites [42]. Little is known for certain about the approximate size of these regions of increased local order.

In optical spectroscopy, persistent spectral hole-burning (PSHB) studies were carried out to determine the temperature dependence of molecular excitation linewidths of chromophore guest molecules in PE. They showed a steeper dependence of linewidth on temperature than the characteristic $T^{1.3}$-dependence found for chromophores in purely amorphous glasses, indicating at least partly crystalline character of the chromophore environment [61, 62]. The first observations of single molecules in PE were of perylene [18, 63]. Single-molecule investigations of terrylene (Tr) molecules in pressed PE films have tentatively established the existence of two sub-populations of embedded Tr molecules. Studied films were generally of poor optical clarity and uniformity, having been produced by melting and pressing PE pellets. The molecules in these two populations display distinct spectral diffusion and dephasing on different time-scales [14, 20, 64]. Such a picture could be consistent with the description of dopant molecules effectively sensing the qualitatively different amorphous and crystalline environments in which they are embedded [42, 59, 65].

By extending the investigation of single molecules in PE films to single-molecule microscopy it would be possible to measure not only their spectral properties, but also to relate their orientations to positions in the matrix. This would open the way to study the local spatial order of PE chains, by using single molecules as sensitive probes of their immediate environment as

was done by Matsushita et al. [25] for n-alkane matrices. For conventional, thick PE films such experiments have not been reported. This is possibly due to a combination of inevitable spectral diffusion in PE with the poor optical quality of pressed samples.

We have devised a means of producing thin optically perfectly clear PE films by spincoating them. We have doped these with Tr or 2.3,8.9–dibenzanthanthrene (DBATT) molecules. On the latter system we have successfully done polarization-dependent single-molecule spectroscopy to determine the orientation of single chromophores. In addition we have performed microscopy measurements to determine the lateral positions of the single molecules with a sub-wavelength accuracy. For both of these techniques negligible scattering of the PE films was essential. The present results set the stage for an investigation of the length-scale of local order in PE films by using single molecules as nano-probes.

2.2 Experimental

Perfectly clear high density PE (HDPE) and low density PE (LDPE) films were prepared by spincoating at elevated temperatures (100 °C), from viscous hot (125 °C) solution of PE in 1:1 cis,trans-decalin (Aldrich, 99+ % pure). We used PE pellets from Wacker, with a crystallinity of about 80 % (HDPE) and 20 – 40 % (LDPE) after washing these in p.a. chloroform (Biosolve AR) to remove any impurities. Concentrations used for HDPE and LDPE in decalin were respectively 2.0×10^{-2} g/ml and 2.7×10^{-2} g/ml.

Glass cover slides (Marienfeld, thickness 0.13 – 0.16 mm) were used as substrates for spincoating and were extensively ozone-cleaned and subsequently silanized using 1,1,1,3,3,3–hexamethyldisilazane (Aldrich, HMDS, 99+ % pure) to make them hydrophobic. This is required to obtain sufficiently uniform wetting of the substrate. Sample thicknesses obtained were 100 – 200 nm and reproducible. For thinner samples the solvent evaporated too fast to allow uniform deposition and films lost their optical clarity. A critical factor is the viscosity of the decalin/PE solution, which in turn depends on the concentration and temperature of the PE solution, as well as PE chain length. Hence, conditions vary slightly for making good quality HDPE or LDPE films. To remove excess decalin, samples were stored under vacuum in an exsiccator for longer than one week. The samples doped with terrylene (Tr) were prepared by dissolving Tr directly in the hot decalin (concentration: 1×10^{-6} M) used to dissolve the PE pellets, except for samples used to record emission and excitation spectra. These samples and those containing

2.3,8.9–dibenzanthanthrene (DBATT) were prepared by soaking PE films in a 1×10^{-6} M solution of Tr/DBATT in chloroform for over 24 hours to introduce chromophore molecules into the host. Samples are carefully rinsed with clean chloroform after soaking, to remove as many chromophore molecules from the sample surface as possible. Another interval of 24 hours under vacuum served to remove excess chloroform. The resulting concentration of Tr and DBATT is estimated at approximately 1×10^{-8} M.

In order to characterize the chromophore/host systems we measured ensemble emission and excitation spectra on spincoated samples. All experiments were performed both at 77 K and 1.8 K in a helium bath cryostat. Spectra from PE films (soaked in the same chloroform/chromophore solution) produced by pressing PE pellets at 130 °C were also recorded for comparison. Excitation experiments were conducted using light from a Xe-arc lamp passed through a monochromator (SPEX 1704). A photomultiplier tube was used for detection of fluorescence photons after a red-pass filter (Schott GG590/RG600 and RG630 for Tr and DBATT respectively). For emission experiments we used a Spectra-Physics Stabilite 2017 Ar-ion laser at 514 nm. The emitted light was collected at right angles, imaged onto the slit of a spectrograph (Acton SpectraPro-500i) and detected by a liquid nitrogen cooled back-illuminated CCD camera (Princeton Instruments Spec-10:400B).

Single-molecule experiments were performed at 1.8 K in a helium bath cryostat, using a standard home-built low-temperature confocal microscope. Excitation occurred by means of a narrow bandwidth continuous-wave single-mode dye laser (Coherent 899-21, Rhodamine 6G, linewidth 1 MHz), pumped with an Ar-ion laser (Coherent Innova 300, 6 W). Single-molecule excitation spectra were recorded by sweeping the excitation light continuously over a range of about 30 GHz ($1\,\mathrm{cm}^{-1}$). We used either an aspheric singlet lens (Lightpath 35390, NA 0.68) or an achromatic objective (Edmund NT38-340, NA 0.85). Fluorescent light was detected after a confocal pinhole ($50 - 100\,\mu$m) and red-pass filters (Chroma 7511 600 LP for Tr; Omega 610 and 620 EFLP for DBATT) by a single-photon counting Avalanche Photo-Diode (APD) (EG&G, SPCM-AQ-161). After each frequency sweep the linear polarization angle of the excitation light was incremented using a variable $\frac{\lambda}{2}$–retarder plate (Newport Liquid Crystal Retarder Device, LCR-VID-05, combined with a Bernhard Halle achromatic (460 – 680 nm) $\frac{\lambda}{4}$–retarder plate) in order to determine the projection of the transition dipole moment of a dye molecule in the equatorial plane $\vec{\mu}_{ip}$. In this way, we determined the orientation of the molecules that lie within a certain frequency range, and then acquired images of these same molecules by selectively exciting them at their individual resonance frequencies. For this we excited with circularly polarized light and used the back-

illuminated CCD camera (magnification factor 208) as a detector. Typical excitation intensities were in the range of 0.01 to 2 Wcm^{-2}.

2.3 Results and Discussion

Films obtained by spincoating are of such perfect clarity they are invisible to the eye. Examination under a microscope showed an apparently flawless film containing the occasional speck of dust, as would be expected for any standard spincoated polymer layer. This holds for both LDPE and HDPE films. Atomic Force Microscopy (AFM) was used to determine the thickness and uniformity of samples obtained by spincoating. Figure 2.1 shows an AFM image of the edge of a cut made by a razor blade in an LDPE film. The cross-section shows that this particular sample is a little over 180 nm thick. On closer inspection there appear to be cracks in the film surface. Islands of about 10 μm across are formed. There also appear to be slightly thicker regions at the center of some of those. We assume that the cracks were initially not present, but are caused by the rubber glue used to fix the sample substrate for the AFM. In this procedure, the spincoated glass substrate is glued onto a steel disk. The glue is left to dry overnight, exposing the film to its caustic fumes. This results in a slight deterioration of the optical quality of the film, visible to the naked eye. This effect does not occur when films are left exposed to air for approximately one week, after cooling down to 1.8 K or after many months of storage under vacuum.

Figure 2.2 shows the excitation and emission spectra for Tr in a spincoated HDPE film. These spectra are similarly resolved as those obtained for terrylene dissolved in organic solvents and glasses. The mirror image of excitation and emission is characteristic of spectra of condensed aromatic molecules. The Tr excitation spectrum shows a blue shift of the $0-0$ transition in PE (568.5 nm) [64], compared to e.g. Tr in n-hexadecane (572 nm) [66]. The $0-0$ transition of DBATT in PE, however, was found to shift to the red (591 nm) compared to the position of the main site in n-hexadecane (589 nm) [67]. As a result of the Tr blue shift, the $0-0$ transition is located precisely between the dye laser gain curves of two laser dyes, R6G and Rhodamine 110, that are convenient for single-molecule experiments (see below). No significant differences were observed between the spectra of these chromophores in either LDPE or HDPE. Both spincoated and pressed PE films yielded virtually identical spectra.

Most of our single-molecule experiments in PE were performed on samples doped with DBATT. We have found that for our experiments DBATT is a

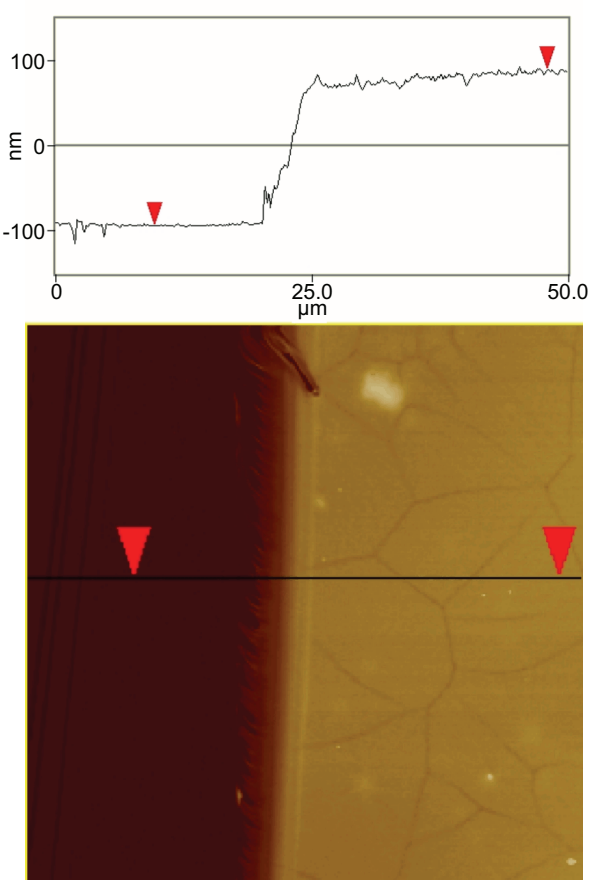

Figure 2.1: AFM image and cross-section of the edge of a razor blade cut in an LDPE film. The vertical distance between the two arrows in the cross-section corresponds to about 180 nm.

more convenient probe molecule than Tr. The position of the $0-0$ transition of DBATT in PE at 591 nm allows excitation in a large spectral range, whereas for Tr the available range is limited by our dye laser. DBATT is a high quantum-yield fluorophore with a saturation intensity only slightly below that of Tr [67, 68] and is likely to display a similar (lack of) solubility in PE.

For individual DBATT and Tr molecules we carried out measurements to determine both saturation count rates and lower limits of the linewidths (Figure 2.3). To eliminate errors in determining the excitation intensities, we have plotted the fluorescence count rate versus the optical linewidth in Figure 2.3 (b) for a typical Tr molecule in PE. At higher excitation powers light-

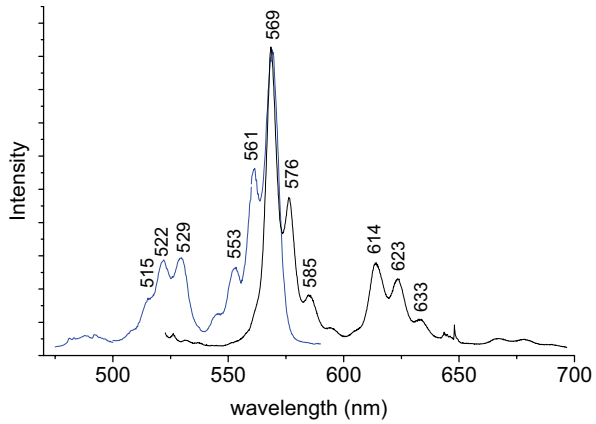

Figure 2.2: Emission and excitation spectra of Tr in a spincoated HDPE film at 1.8 K. The spectra have been normalized with respect to the $0-0$ peaks.

induced spectral diffusion occurs and may dominate, making it difficult to reliably measure count rates and linewidths. DBATT in PE showed similar saturation behavior, both for the linewidths and the count rates. Maximum count rates between about 40000 and 150000 cps were found for both chromophores. The spectral stability of the molecules was found to improve with time if samples were stored under vacuum. The linewidths measured are indicative of the nature of these chromophore/PE systems. Linewidths close to the lifetime-limited values of 17 MHz for DBATT (S_1-lifetime 9.4 ns) [67] and 40 MHz for Tr in n-hexadecane (S_1-lifetime 4.4 ns) [69] were not observed during any measurement in PE. For both chromophores, spectral diffusion causes broadening of the optical lines to values between 80 MHz and 200 MHz in our experiments. This is typical for molecules embedded in polymers [36, 70–72]. In our experiments, we have not observed significantly smaller linewidths for DBATT compared to Tr, as one might expect on the basis of their fluorescence lifetimes. No extensive characterization of linewidths was carried out, however, and we cannot rule out the occurrence of more narrow lines of DBATT in PE. The polarization and microscopy experiments were carried out at excitation intensities at least an order of magnitude below the saturation intensities of $\sim 3\,\mathrm{Wcm^{-2}}$. At these intensities spectral diffusion is not absent, but less frequent.

Figure 2.4 (bottom) illustrates how the orientation of DBATT molecules may be determined. In fact, it is the projection of the molecular transition-dipole moment in the equatorial plane ($\vec{\mu}_{ip}$) that is found in the polarization-dependent single-molecule experiment. We have for convenience sake referred

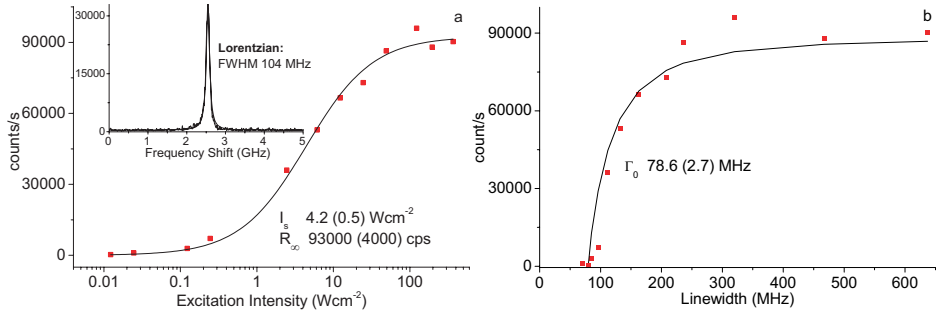

Figure 2.3: (a) Plot of fluorescence count rate versus excitation intensity of a single Tr molecule in HDPE. The solid curve is a fit according to the formula: $\frac{\mathrm{R}}{\mathrm{R}_\infty} = \frac{1}{1+\frac{\mathrm{I}_\mathrm{S}}{\mathrm{I}}}$. Inset: Excitation spectrum of a single Tr molecule ($\mathrm{I}_\mathrm{exc} = 0.7\,\mathrm{Wcm}^{-2}$) from $\lambda_\mathrm{exc} = 572.598\,\mathrm{nm}$ in spincoated HDPE (1.8 K) with a fitted Lorentzian. The single-molecule excitation peak is not perfectly symmetric as a result of spectral diffusion. Fits to several subsequent excitation spectra were averaged to determine linewidths and emission count rates as a function of excitation intensity for individual Tr molecules. (b) Plot of count rate versus linewidth for a Tr molecule. The solid line is a fit according to the formula: $\frac{R}{R_\infty} = 1 - \left(\frac{\Gamma_0}{\Gamma}\right)^2$, with $R_\infty = 89000\,\mathrm{cps}$ and $\Gamma_0 = 78.6\,\mathrm{MHz}$.

to this orientation as the orientation of the molecule. In this polarization trace we clearly see molecular resonances with a distinct dependence on the excitation polarization. Such a trace is recorded in approximately 6 minutes. Note the spread in linewidths, intensities and stabilities for the various molecular resonances. Figure 2.4 (top) shows excellent contrast between fluorescence intensity crests and troughs. The slight instability of the molecule in question, which is not untypical for these samples, barely hampers the fit and therefore the determination of the direction of $\vec{\mu}_{ip}$. We estimate this is accurate up to about 5°. These data are quite representative and show that the stability and count rates obtained at this excitation intensity are sufficient.

The small linewidth of the excitation light compared to the molecular zero-phonon linewidths allows for spectral selection of individual molecules in the confocal volume (cf. Figure 2.4). If the molecular density in the probed volume is not too high, therefore, we can be sure to be addressing single molecules when recording an image. In practice we tested this by observing single-step return to the background level in time traces. Figure 2.5 (a) shows how despite potential instabilities, images with good signal to noise ratio may be acquired. A point source should not give a Gaussian photon distribution, but one described by an Airy-diffraction pattern in the ideal case. This is

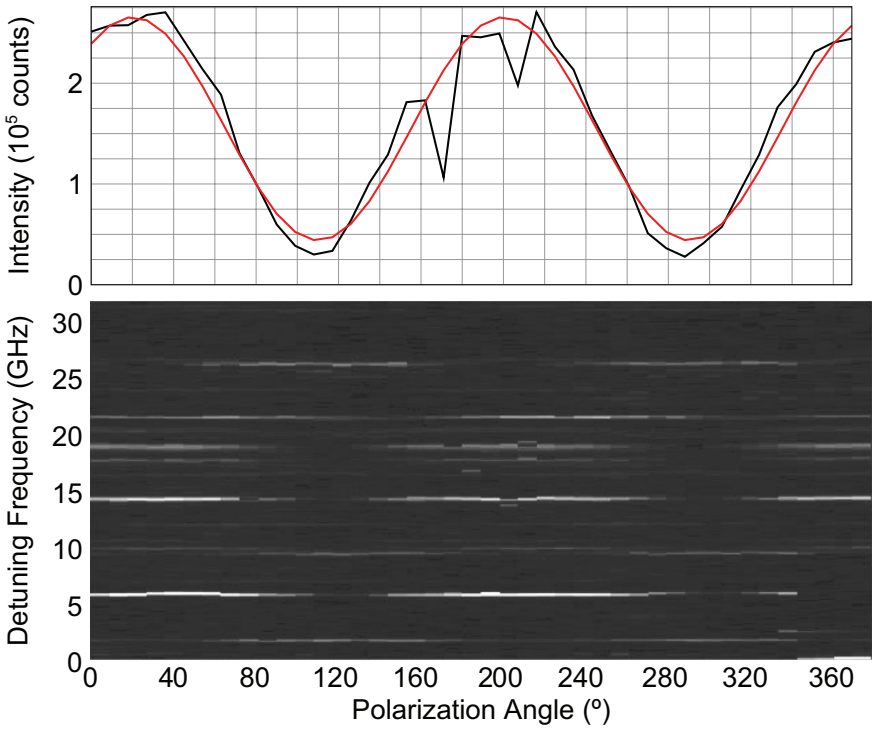

Figure 2.4: (bottom) Fluorescence intensity of DBATT in a spincoated HDPE film (1.8 K) as a function of excitation frequency and polarization angle: the linear polarization angle of the excitation light is incremented by 9° after every 31.7 GHz frequency scan. $I_{exc} = 0.3\,\mathrm{Wcm^{-2}}$. (top) Summed counts in 200 MHz frequency interval around the center of the resonance signal at 18.8 GHz as a function of polarization angle, with a $\cos^2$-function fitted to it.

nicely illustrated by Figure 2.5 (b) in which the fitted 2-D Gaussian has been subtracted from the single-molecular photon distribution. We clearly see remaining diffraction rings. The accuracy of the lateral position determination is limited by the number of fluorescent photons collected [29, 73, 74]. This makes explicit the need for samples with negligible optical scattering. The strongly reduced confocal volume in these thin spincoated films, compared to conventional pressed PE films, ensures a higher axial resolution. Combined with the optical clarity of the film it also virtually eliminates scattering. This image shows that the optical quality of our samples allows us to determine the position of dopant molecules in PE with a high degree of accuracy (≤ 0.1 pixel $\simeq 10$ nm), surpassing the Rayleigh limit by more than an order of magnitude.

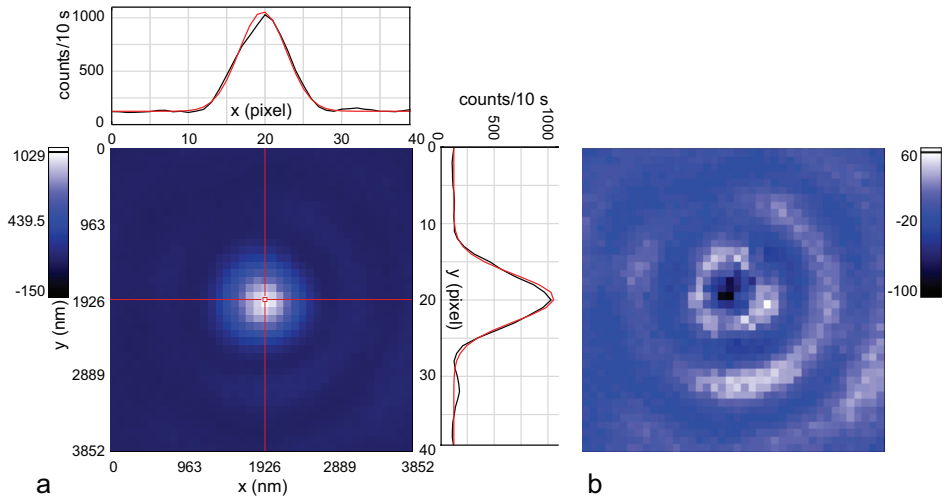

Figure 2.5: (a) Diffraction-limited fluorescence image of a single DBATT molecule in spincoated HDPE (1.8 K, magnification factor 208, acquisition time 10 s, circularly polarized $I_{exc} = 0.7\,\mathrm{Wcm}^{-2}$) and x– and y–cross-sections of the image (black line) and of the fitted 2-D Gaussian (red line). Corresponding nanometer scale axes are given. The intensity scale was adjusted to maximize image contrast. The diameter (FWHM) of this spot equals 698 nm. Prior to image acquisition, we measured the orientation of the $\vec{\mu}_{ip}$ of this molecule with an accuracy of about 5°. (b) Residual image after subtracting the 2-D Gaussian from the original image.

2.4 Conclusion

We have demonstrated the possibility of producing optically clear thin PE films by means of spincoating. Such spincoated PE films doped with DBATT molecules offer a promising system for single-molecule spectroscopy and microscopy. Our results demonstrate the feasibility of using these thin films to study the length-scale of local order in the organization of a PE matrix. Single-molecular nano-probes offer an opportunity for taking a closer look at what PE is like at the molecular length-scale. At 1.8 K the dye molecules will be oriented along the PE chains [42] and by reporting their position and orientation, they will report on their immediate environment. The unprecedented thinness of our samples offers two clear advantages compared to pressed PE films. First of all, scattering by the PE films is negligible and more accurate lateral position determination is possible, because of better photon statistics. Furthermore, the axial position of a local probe molecule is more precisely known in a film of reduced thickness. In order to be able to come to statistically relevant

conclusions, the correlated single-molecule position and orientation measurements described above need to be repeated for as many molecules as possible ($\gtrsim 250$) within one confocal volume, and for various confocal volumes in each sample. Such measurements are currently being performed in our laboratory for DBATT in PE.

Acknowledgments

We are grateful to Matthew Osbourn for recording the AFM images. We thank Michel Orrit for his continued interest and useful discussions. We acknowledge the group of E. J. R. Sudhölter of Wageningen University for its helpful suggestions regarding hydrophobic glass coverslides.