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Anisotropic diffusion of single molecules in thin liquid films

J. Schuster, F. Cichos, and Ch. von Borczyskowski

Institut für Physik 122501, Technische Universität Chemnitz, 09107 Chemnitz, Germany

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Abstract. Single molecule wide field imaging is applied to study the diffusion in ultrathin liquid films on solid surfaces. The results show a broad distribution of diffusion coefficients. This is tentatively ascribed to an anisotropy of the diffusion coefficient perpendicular to the surface and a slow exchange of molecules between regions of different diffusion coefficients. We have evidence, that these changes as well as the slow motion perpendicular to the surface are related to the molecular layering of the liquid close to the surface.

PACS. 68.08.-p Liquid-solid interfaces – 82.45.Mp Thin layers, films, monolayers, membranes

1 Introduction

It is known that liquids exhibit a layering structure at smooth solid surfaces. These density oscillations have been detected in a number of experiments as in ellipsometric measurements on wetting droplets [1], in surface force measurements on confined liquids [2,3], in desorption studies [4] and recently in X-ray diffraction experiments [5]. In addition it has been predicted and analyzed by various simulation studies (see *i.e.* [6,7,8]). Such a layering structure and the asymmetry of the molecular interaction at the interface immediately suggest that liquid dynamics have to be different from the bulk behavior too, since the potential of mean force – the potential in which the molecules move – will be different from the bulk. Shear force measurements in principle show this anisotropy of molecular mobility for confined liquid films [2,3,9]. However, to our knowledge there is currently no experimental data on anisotropic molecular diffusion in ultrathin liquid films on surfaces, even though this is a problem of tremendous interest in a wide range of scientific areas such as chromatography, rheology, tribology or wetting. Single molecule experiments [10] can provide such detailed information on molecular diffusion as shown *i.e.* for lipid diffusion [11] or for the motion of single viruses [12]. The main advantage of the single molecule technique is, that it allows access to distributions of observables as in the present case to the distribution of diffusion coefficients.

In this report we employ for the first time single dye molecules to probe the molecular diffusion in ultrathin liquid films of tetrakis(2-ethylhexoxy)silane – a liquid, which has been studied *i.e.* in X-ray diffraction experiments of Yu *et al.* [5]. Our experiments should thus allow to relate the diffusion to known inhomogeneities found in the diffraction experiments.

2 Experimental

We use a home-built fluorescence microscope consisting of a 100x/0.9NA (Zeiss Epiplan Neofluar) objective and a lens (f = 250 mm) to image the sample on an intensified frame transfer camera (Pentamax). Dye molecules in the sample are excited with the 514 nm line of an argon ion laser in a total internal reflection configuration as described elsewhere. Excitation light is focused to a spot of 20 μm diameter yielding an intensity of about 1 kW/cm^2 on the sample. A holographic notch filter in front of the detector is used to remove the excitation light from the fluorescence.

Samples are prepared on quartz cover slips, which are carefully cleaned before preparation. The deposition of thin films of tetrakis(2-ethylhexoxy)-silane (TEHOS) is carried out by dipping the substrates in a solution of TEHOS and R6G in hexane [5]. The film thickness is determined by measuring the surface plasmon resonance curve for a TEHOS layer deposited on a 50 nm gold film simultaneously. Additional ellipsometric measurements confirmed the determined film thickness. The films described in the following have a thickness of 4 (±1) nm and 17 (±2) nm. As shown in reference [5], films prepared in the same way show a clear layering signature in X-ray diffraction experiments.

Each measurement records 500 successive images at a frame rate of 40 fps (25 ms exposure time). The images are analyzed automatically by a software developed in our laboratory. The software localizes single molecule spots and determines position, size and intensity of the spots by fitting a 2-d elliptical gaussian. Short blinking periods are removed by involving up to 3 successive frames in the analysis, in which the software locates spots nearby. Off periods longer than 75 ms, photobleaching or diffusion out of the observation area limits the trajectory length. The average signal to noise ratio is determined

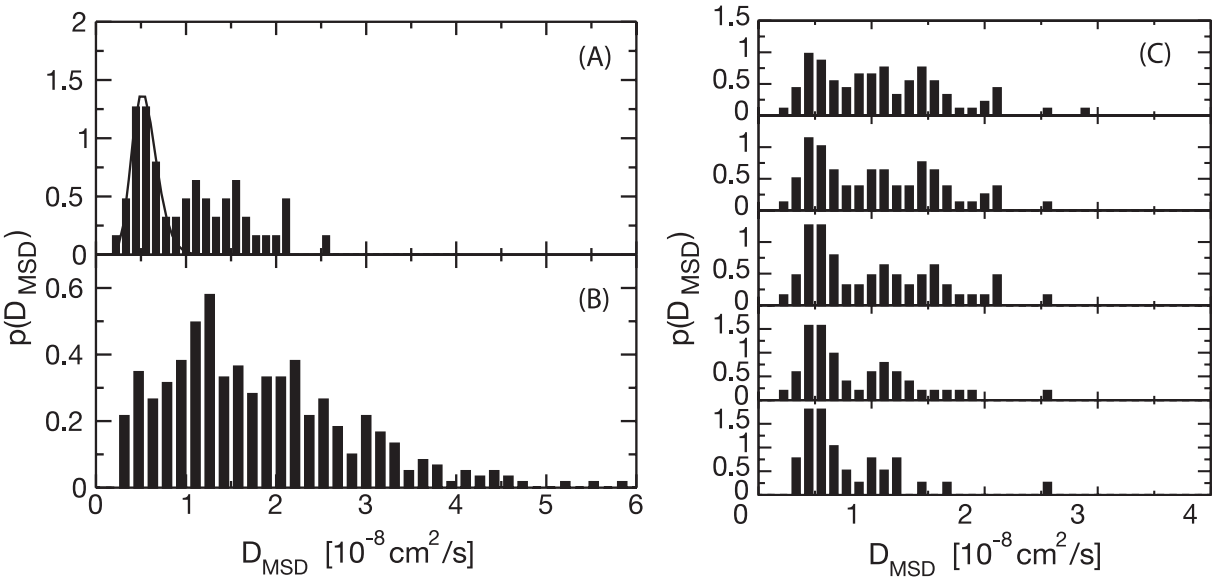

Fig. 1. (A) Distribution of diffusion coefficients D obtained for the 4 nm thick TEHOS film. The average diffusion coefficient is $\langle D\rangle = 1.0 \times 10^{-8}$ cm^2/s. The peak values in the distribution are D = 0.5×10^{-8} cm^2/s, D = 1.1×10^{-8} cm^2/s, D = 1.6×0^{-8} cm^2/s and D = 2.1×10^{-8} cm^2/s. The solid line shows the theoretical distribution for D = 0.5×10^{-8} cm^2/s according to reference [13]. **(B)** Distribution of diffusion coefficients obtained for the 17 nm thick TEHOS film. The average diffusion coefficient is $\langle D\rangle = 1.9 \times 10^{-8}$ cm^2/s. **(C)** Distributions of diffusion coefficients for the thin film sorted by trajectories longer than 20, 30, 40, 50 and 80 snapshots respectively (from top).

to be about 7 which leads to a localization accuracy of 20 nm. Typical trajectories consist of 20 to 400 snapshots with an total average of 40 snapshots. Lateral diffusion coefficients are obtained in two ways from the trajectories. For each trajectory the mean square displacement (MSD: $\langle r^2_{msd}(t)\rangle = \langle[\boldsymbol{r}(t) - \boldsymbol{r}(0)]^2\rangle = 4Dt$) is calculated as a function of time. A weighted linear fit yields then one diffusion coefficient per trajectory. The weights are chosen to reflect the variance in the diffusion coefficient coming from a measurement with limited trajectory length as described in reference [13,14]. Besides the MSD analysis we apply a new method developed in our group which evaluates the spot size in each single molecule image and relates it to a diffusion coefficient [15]. This method allows us for the first time to follow the time dependence of the diffusion coefficient in a single molecule trajectory. It is an additional tool to visualize changes in the diffusion coefficient, which are usually hidden by the extensive averaging of the MSD technique.

3 Results and discussion

Over 500 trajectories in total were analyzed for both films. Within our optical resolution the film appears to cover the whole substrate, since R6G molecules access all parts of the image. Figures 1A and 1B show the obtained distributions of diffusion coefficients. The distributions are asymmetric with a long tail towards larger diffusion coefficients. Specially for the thick film (B) the diffusion coefficients cover at least one order of magnitude (from 3×10^{-9} cm^2/s to 1×10^{-7} cm^2/s). Average diffusion coefficients of $\langle \mathrm{D}^{MSD}_{thin}\rangle = 1.0 \times 10^{-8}$ cm^2/s and $\langle \mathrm{D}^{MSD}_{thick}\rangle = 1.9 \times 10^{-8}$ cm^2/s are calculated. This is approximately one order of magnitude smaller than the diffusion coefficient measured for Rhodamin 6G in a TEHOS droplet by fluorescence correlation spectroscopy in our lab. Thus the diffusion is considerably slower than in the bulk liquid and slows down with decreasing distance from the surface, since the average diffusion coefficient is smaller in the thin film. Further we observe that molecules with low diffusion coefficients mainly have longer trajectories (1C), which points to a kind of deeper potential of mean force which keeps the molecules in these slow diffusion regions. These findings are in principle consistent with the observation of increasing shear viscosity with decreasing film thickness measured for confined films [2,3]. However, in our experiment the film is not confined by a second surface and one directly observes molecular mobility.

Distributions of diffusion coefficients are expected in particle tracking experiments even when only a single diffusion coefficient would be appropriate such as in a homogeneous medium. This is due to the limited length of the measured trajectories. However for both films studied, the measured distributions are much broader than expected from theory [13,14]. Figure 1A displays the calculated diffusion coefficient distribution for D = 0.5×10^{-8} cm^2/s and proofs, that the observed distribution of D must be inherent to the film. Thus regions which obey different diffusion coefficients should exist. Since a fast exchange (faster than the experimental time resolution) between such regions would result in an average diffusion coefficient with its corresponding distribution to be measured, it is obvious, that the observed broad distribution can only be measured if the exchange is slow compared to the time

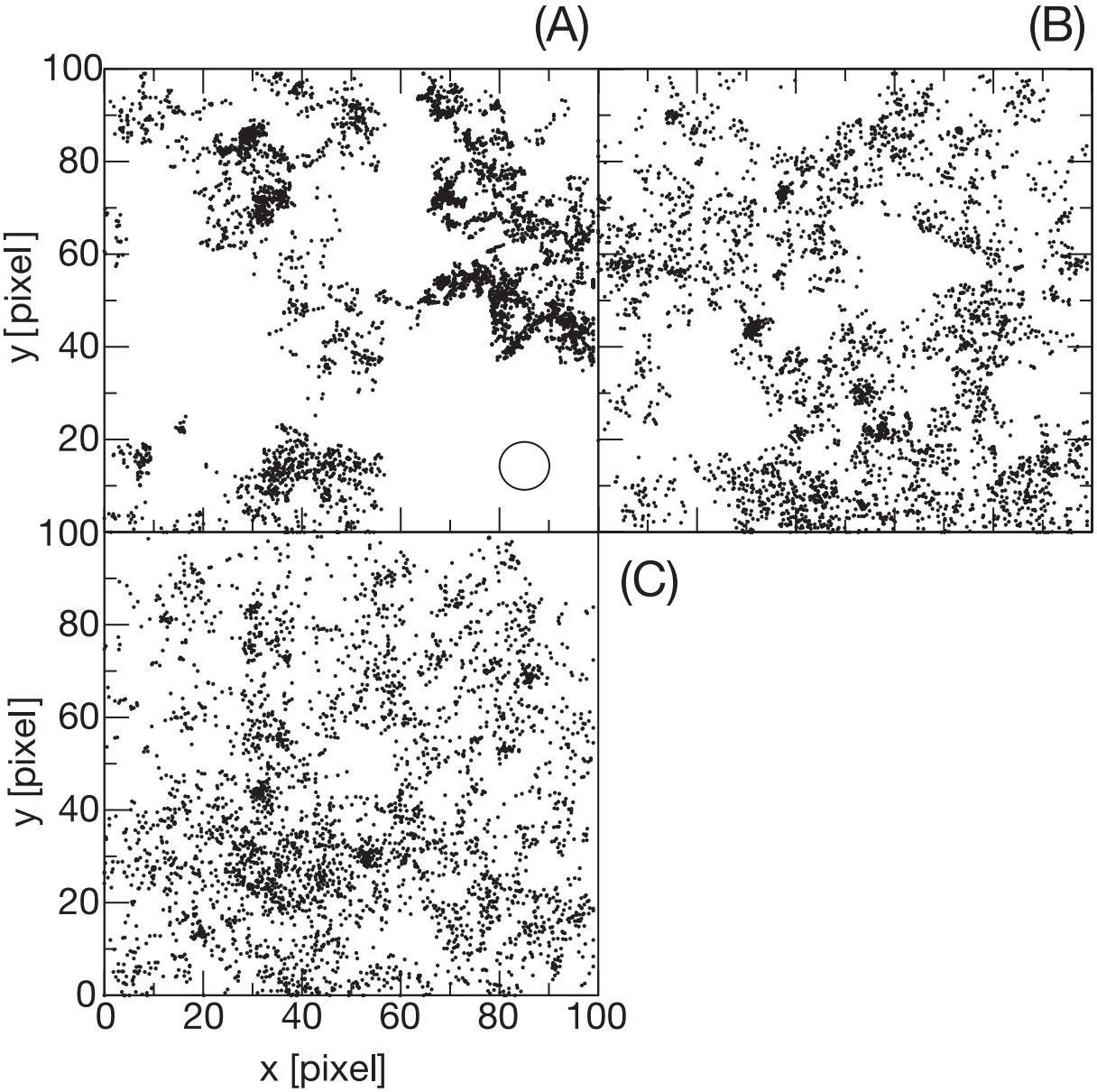

Fig. 2. Trajectory points of single molecule trajectories in the 4 nm thin film categorized by their diffusion constant D. **(A)** $D < 0.8 \times 10^{-8}$ cm^2/s, **(B)** 0.8×10^{-8} cm^2/s$< D < 1.4 \times 10^{-8}$ cm^2/s, **(C)** $D > 1.4 \times 10^{-8}$ cm^2/s. The circle in Figure (A) corresponds to the estimated domain size if static lateral surface inhomogeneities would exist (see text).

between two images. Further the MSD calculation blurs frequent changes of the diffusion constant within a trajectory, since the MSD calculation is a highly averaging procedure. The weighted linear fit mainly accounts for the first 4 to 5 points of the MSD. Therefore we conclude, that the exchange has to be even slower than 100 ms to 125 ms.

Such regions could be spatial domains either parallel or perpendicular to the surface caused by inhomogeneities of the surface or the layering structure of the liquid. If these regions correspond to lateral domains on the surface, the minimum size of these domains can be estimated from the diffusion coefficient and the time between subsequent images. For a diffusion coefficient of $D = 1 \times 10^{-8}$ cm^2/s and 25 ms between two images we calculate a diameter of 600 nm for such domains (1.2 μm for 100 ms!), which is at least one order of magnitude above the localization accuracy of our measurement. To proof if such regions exist, we have splitted the distribution of diffusion coefficients into three distributions ranging from 0 to 0.8×10^{-8} cm^2/s, 0.8×10^{-8} cm^2/s to 1.4×10^{-8} cm^2/s and 1.4×10^{-8} cm^2/s and above. In Figure 2 we plotted the positions of molecules which are belonging to one of the distributions (for the 4 nm film). Thus domains, which show only *i.e.* slow diffusion should appear as holes in the other distributions. However, since we observe different diffusion coefficients in the same spatial regions of the film (see Fig. 2), such lateral domains cannot be responsible for the observed distribution.

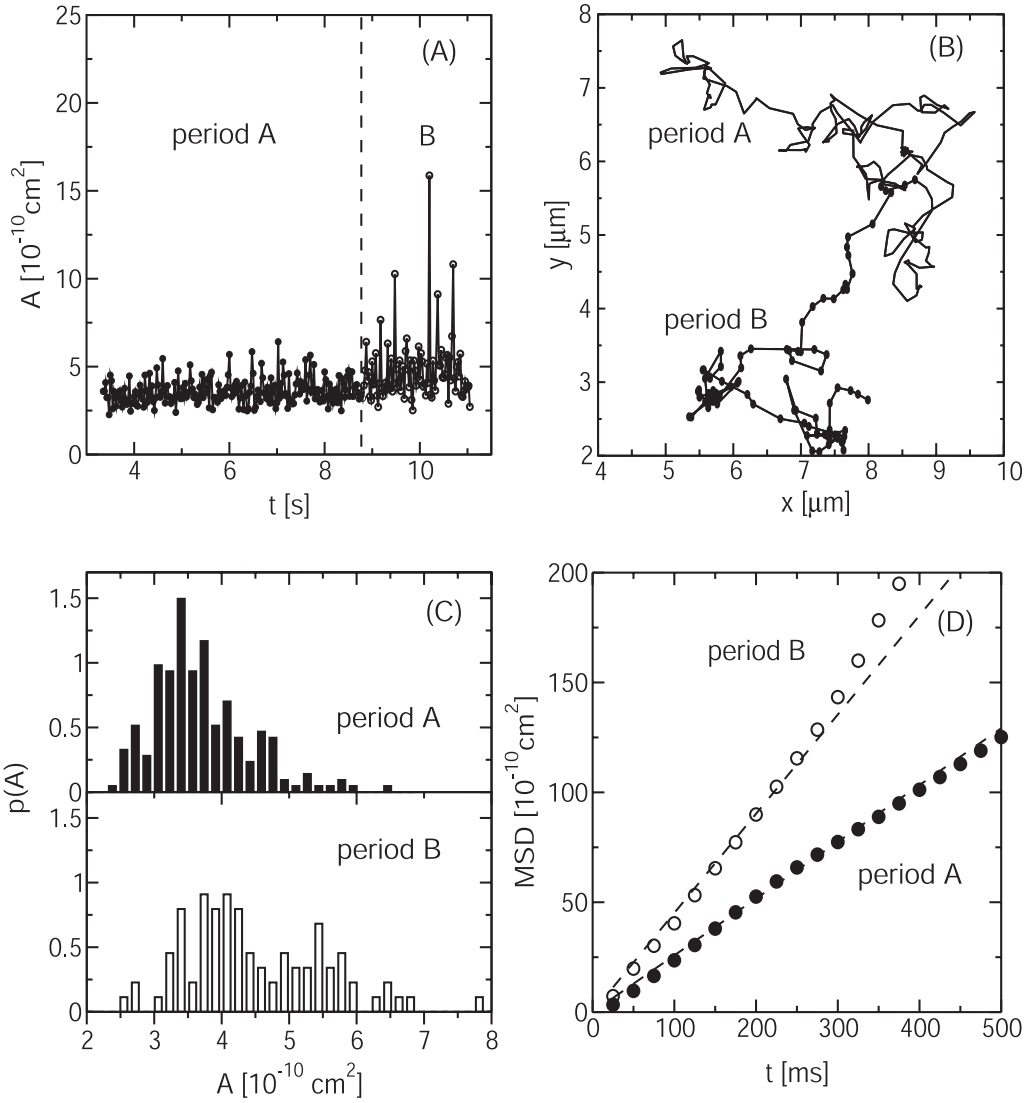

Fig. 3. (A) Spot size trajectory of a single molecule with a jump in the diffusion coefficient at 8.8 s. The time between datapoints is 25 ms. **(B)** Trajectory of the molecule in (A). The trajectory is split into periods A and B **(C)** Spot size histograms of the periods A and B. **(D)** Mean square displacement as a function of time in periods A and B. A linear weighted fit (dashed lines) leads to diffusion coefficients of $D_A = (0.56 \pm 0.1) \times 10^{-8}$ cm^2/s and $D_B = (1.0 \pm 0.1) \times 10^{-8}$ cm^2/s.

Perpendicular to the surface, domains are given by the molecular layering of the liquid and as noted above the lateral diffusion coefficient changes with the distance from the surface. In addition the lateral diffusion coefficients measured in our experiment will not be valid for the vertical motion. The vertical motion of molecules could be much slower, since the observation of X-ray diffraction from molecular layers in [5] relies on a rather deep potential of mean force forming the layering structure, where the X-ray data appearently underestimates the layering strength [5]. Thus we conclude that the vertical motion of R6G molecules in the film samples the film thickness on a timescale of several ten to hundred milliseconds and thus cause the observed broad distribution of diffusion coefficients. In the picture of molecular layering the vertical motion is equivalent to a hopping between different molecular layers. Since this motion occurs on a millisecond timescale, it should be possible to observe changes in the diffusion coefficient within a single molecule trajectory. To do so we have applied the spot size analysis (SSA) [15], which allows the determination of the diffusion coefficient from a single image of the trajectory. Figure 3A shows an example of a spot size trajectory with a clearly visible jump at $t = 8.8$ s. The change in the spot size and in the mobility is also visible by direct inspection of the image sequence. We note again that slower and faster diffusion occur in the same spatial areas of the sample, so that no static lateral inhomogeneities of the surface could cause the different diffusion coefficients. Periods A and B in Figure 3 possess different histograms of the spot size (Fig. 2C) with average diffusion coefficients in period A and B of $D_A^{SSA} = 0.56 \times 10^{-8}$ cm^2/s and $D_B^{SSA} = 1.8 \times 10^{-8}$ cm^2/s as calculated from the spot size analysis. The histograms also show, that period B

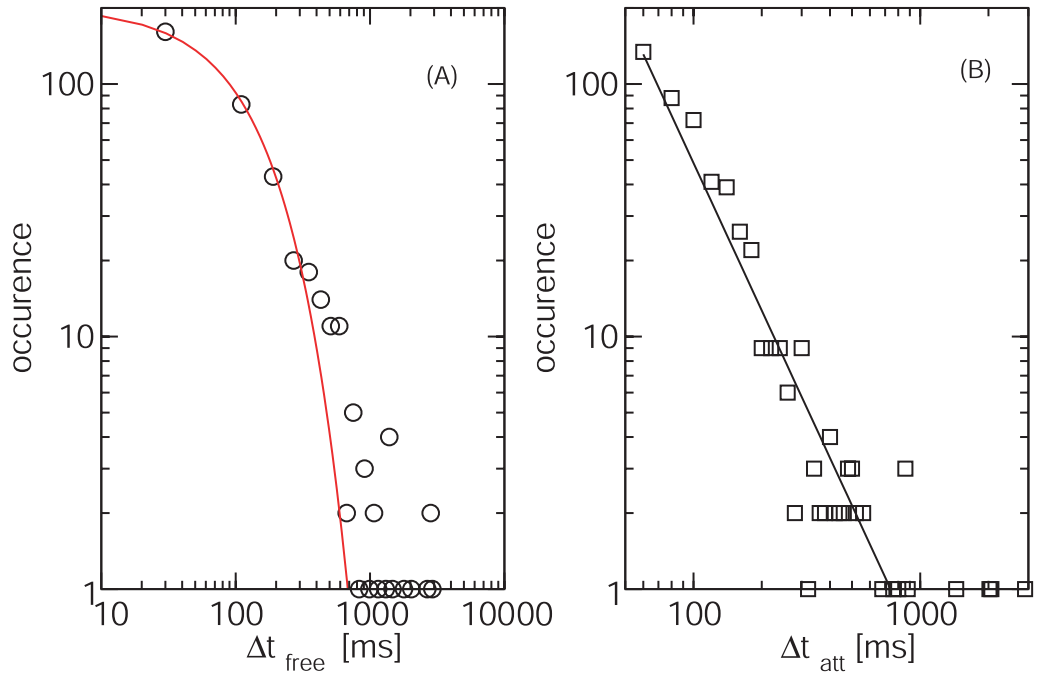

Fig. 4. (A) Diffusion duration (Δ t$_{free}$) statistics compiled from the trajectories of the 4 nm and 17 nm film. The line is the result of an exponential fit (exp($-\Delta$t$_{free}/\tau$)) to the data with an characteristic timeconstant of $\tau = 128$ ms. **(B)** Statistics of the attachment duration (Δt$_{att}$) for the thick and the thin film. The line is the result of a power law (Δt$_{att}^{-\alpha}$) fit to the data. The obtained exponent is $\alpha = 1.9$.

is actually broken into several parts interrupted by excursions to larger diffusion coefficients, which cause additional structure in the spot size histogram. However a direct determination of the residence times at certain spot size levels is not possible due to the limited statistical accuracy of the spot size analysis. Compared to the SSA, the MSD analysis of the two periods yields diffusion coefficients of $D_A^{MSD} = (0.56 \pm 0.1) \times 10^{-8}$ cm^2/s and $D_B^{MSD} = (1.0 \pm 0.1) \times 10^{-8}$ cm^2/s. The latter one is quite different from the value obtained by the SSA, since the MSD averages out the short excursions to higher mobility values. The observation of sudden changes in the diffusion coefficient along a single molecule trajectory thus supports the hypothesis, that the structure in the diffusion coefficient distribution could be related to an inhomogeneity such as the molecular layering.

A further detailed analysis of single molecule positions within a trajectory reveals periods without any change in position with respect to our localization accuracy. In addition quite a number of molecules are not moving within our observation time. The attachment periods can be characterized in two ways – with their duration (Δt_{att}) and the free diffusion period between two attachments (Δt_{free}). The duration gives an idea of the depth of the trapping potential. The time between two attachments displays the probability of accessing the trapping region in the film. Figure 4 shows an histogram of the duration of free diffusion (A) as well as the attachment duration (B). While the statistics of diffusion durations is close to a exponential behaviour, the statistics of attachment durations can only be properly described by a power law. The results thus suggest that to reach a trapping site on the surface molecules have to overcome a well defined barrier, while to escape from the trapping site the molecules need to overcome a barrier which is different for each trapping site and further widely distributed. However, at the current stage we can only speculate about the nature of the trapping site. The existence of attachments could further be a simple explanation for the found heterogeneity in diffusion. To slow down diffusion considerably frequent attachment periods have to exist. This would commonly lead to anomalous diffusion [16], since the waiting times in the trap are distributed by a power law. However, no signs of anomalous diffusion are observed, which rules out the heterogeneity due to attachments.

4 Conclusions

Combining all results, the measured diffusion of dye molecules in ultrathin liquid films shows strong signatures of spatial inhomogeneities which we tentatively ascribe to molecular layering. Dye molecules are slowly moving perpendicular to the surface by traveling a distance of some nanometers on a millisecond timescale. Since molecular layering should determine the structure of the film perpendicular to the surface, the vertical motion of molecules should be a hopping between minima of the layering potential which has a depth of several hundred meV for residence times in the millisecond range (700 meV correspond to 100 ms residence time in a layer). Further evidence for this hopping is obtained from sudden changes in the diffusion coefficient along a single molecule trajectory.

The motion parallel to the surface is considerably slower than in the bulk. Due to the low diffusion coefficient close to the surface, the liquid also appears to be more viscous with an effective viscosity of 0.44 Pa·s. This viscosity is *effective* since it is induced by the presence of the surface and not a property of the liquid. The effective viscosity decreases with increasing distance from the surface. It should in principle be possible to relate the viscosities to data from measurements with a surface force apparatus. A still puzzling result of our measurements is the occurrence of surface attachments, which has to be evaluated in more detail in further experiments.

Concluding, the presented single molecule wide field imaging experiments clearly show, that they could provide a wealth of detailed information about the nanoscopic dynamics in soft systems as demonstrated here for the anisotropic diffusion in ultrathin liquid films.

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