Supporting Information

Position-Dependent Three-Dimensional Diffusion in Nematic Liquid Crystal Monitored by Single-Particle Fluorescence Localization and Tracking

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1. Sample preparation

The LC used (RD-001, DIC Corporation, a mixture composed mainly of 4-Cyano-4'-pentylbiphenyl and 4-Cyano-4'-pentyltriphenyl) is nematic at room temperature, with nematic-isotropic transition at 77.7 °C. Dilute toluene solution of the CdSe quantum dots (LumidotTM CdSe/ZnS 560, Aldrich) was mixed with the LC and the excess solvent was evaporated. The resulting QD concentration was 1×10^{-10} M. For the cell, two cover glasses ($22 \times 22 \times 0.15$ mm) custom-coated with an indium tin oxide (ITO) layer (55 nm) were thoroughly washed and the ITO surfaces were rubbed using a rubbing setup (RM-50, EHC). 2 µL of the LC/QD mixture were dropped on the rubbed surface of the cover glass and covered with the other cover glass with the rubbing axes aligned. The resulting LC thickness was 5.0 - 5.5 µm.

2. Experimental setup

The experimental setup is based on an inverted fluorescence microscope (Olympus IX71). Fluorescence is excited with a circularly polarized 488 nm laser line of an Ar-Kr ion laser (with a typical power of 400 - 500 Wcm⁻²) and collected using an oil-immersion lens (Olympus UPlanFLN100×O2, 100x, NA = 1.3). The astigmatism is introduced in the detection path by a cylindrical lens (focal length 500 mm). Fluorescence images are detected using a band-pass filter 500-550 nm (Semrock) and an electron multiplication CCD camera (Andor, iXon+). The microscope is equipped with a three-dimensional piezo-stage (NS4312-C, Nano Control) which enables controllable positioning of the sample with nm accuracy. Calibration curves for the astigmatic imaging were measured using fluorescence beads emitting in the same spectral region (Envy Green, Bangs Laboratories Inc.) spin-coated on a cover glass. The calibration curve was obtained by moving the piezo-stage with the fluorescence beads sample along the *z* direction, determining the ratio W_y/W_x of the standard deviations from the Gaussian fits at each *z* location and by fitting the experimental data with a polynomial. More details can be found elsewhere [24].

3. Measurement and analysis of anisotropic single-particle fluorescence images

Examples of astigmatic image snapshots of a single diffusing QD are shown in Figure S1a. The change of the elongated pattern from horizontally to vertically oriented indicates diffusion along the z direction. All single QD fluorescence images were fitted with a two-dimensional (2D) Gaussian function as shown in the inset of Figure S1b. We verified that with the degree of astigmatism used the 2D Gaussian provides satisfactory approximation of the astigmatic point spread function. The centroids of the fits were used to determine time-dependent position of the QDs along the *x* and *y* directions. To determine the *z* position, standard deviations W_x and W_y along the *x* and *y* directions were evaluated from the fits, and the ratio W_y/W_z was compared with a calibration curve (Figure S1b). An example of the *x* and *z* positions of a QD evaluated as a function of time is shown in Figure S1c,d. The time interval (CCD camera recording time) was 30 ms. During this time the QD moves typically 100 nm which corresponds to about 1 pixel in the CCD image. Therefore, most of the time we do not observe streaks or other distortions of the images, as seen in Fig. S1 e,f. The occasional distorted shapes were not included in the analysis.



Figure S1.

a) Astigmatic fluorescence microscopic images of a single quantum dot at different times. b) Calibration curve (grey) and its fit (black) obtained using fluorescence beads. The inset shows fitting of the astigmatic image of a single quantum dot. \mathbf{c} , \mathbf{d}) Positions of a single quantum dot in the *x* - direction (\mathbf{c}) and *z* - direction (\mathbf{d}) obtained from the Gaussian fits. e, f) Two examples of raw images of a wide area of the sample showing both vertically and horizontally oriented astigmatic images, as well as molecules in the focal plane.

4. Determination of the diffusion coefficient

Time dependent QD positions x(t), y(t), z(t) along the specific direction obtained from the microscopic fluorescence images were used to calculate the mean square displacement *MSD* as

$$MSD_z = \langle (z(t + \Delta t) - z(t))^2 \rangle \tag{1}$$

for the example of the direction z, and as

$$MSD_{3D} = \langle (x(t + \Delta t) - x(t))^2 + (y(t + \Delta t) - y(t))^2 + (z(t + \Delta t) - z(t))^2 \rangle$$
(2)

for three-dimensional (3D) diffusion. Experimentally, the time interval (CCD camera recording time) Δt was on the order of 30 ms, and the time average in Eq. (1) and (2) was constructed from 5 data points. The *MSD* values are related to a diffusion coefficient D_z as

$$D_z = \frac{MSD_z}{2\Delta t}$$
(3)

for the case of diffusion along one dimension (z in this example), and

$$D_{3D} = \frac{MSD_{3D}}{6\Delta t} \qquad (4)$$

for the 3D diffusion.

5. Cumulative distribution analysis

The complementary cumulative distribution function (cdf) of the diffusivity in the *z* direction at different positions (layers) were calculated according to ref. [S1] (ref. [17] of the main text). The advantage of the cdf treatment is that it can clearly reveal multimodal diffusion processes that are not obvious from the distributions of diffusion coefficients. Also, it works more reliably with fewer data points because it includes all square displacements between all consecutive images as opposed to *MSD* which requires (in our case) 5 images (4 displacements) for 1 resulting data point (value of diffusion coefficient). For each position (layer) the square displacements between all consecutive images were analyzed as

$$z^{2} = (z(t + \Delta t) - z(t))^{2}$$
(5)

for the time interval Δt = 30 ms. Diffusivity in the z direction d_z is related to z^2 as

$$d_z = z^2 / 2\Delta t \tag{6}$$

The obtained distribution of d_z for the time interval Δt , $p(d_z, \Delta t)$ is then integrated to obtain the complementary cumulative distribution as

$$C(d_z, \Delta t) = 1 - \int_0^{d_z} p(\delta, \Delta t) d\delta$$
(7)

The cdf's obtained by the above analysis for different distances from the cell wall are presented in Figure S2.



Figure S2.

Cumulative distribution function of the diffusivity along the z direction analyzed at different distances from the cell surface (thick lines) and the corresponding two-exponential fits (thin lines). The color codes correspond to the distance from the cell wall.



Figure S3.

Top: cumulative distribution function of the diffusivity along the z direction analyzed at the distance of 1 μ m from the cell surface (black) and the corresponding 2-exponential (left) and 3-exponential (right) fits to the data (red).

Bottom: Residuals of the 2-exponential (left) and 3-exponential (right) fits of the data above.

6. Error analysis

The error analysis is based on the theoretical treatment of single-particle diffusion and its analysis in terms of the mean square displacement (*MSD*) including the localization error of the particle position, ref. [S2]. In the case of 3D particle diffusion monitored by astigmatic imaging, the localization error is determined by the largest error, that is, the error in the z-direction. To quantify this error, we measured positions of an ensemble of QDs or single molecules immobilized on a glass surface. By analyzing the z-positions of an ensemble of QDs or single molecules with the same spatial orientation (to minimize the effect of the dipole orientation) we obtained a distribution of the center z-positions, as shown in Fig. S4. The standard deviation σ of the distribution in the Fig. S4 is 12.2 nm. The localization error in the z-direction is larger than that in the x and y directions. We therefore take as a conservative estimate a value of 20 nm as the localization error in all three directions.



Figure S4.

Distribution of center z-positions of an ensemble of single PDI molecules with the same spatial orientation analyzed as described in Section 3.

The localization error σ is further used to determine a reduced localization error ξ as

$$\xi = \frac{\sigma^2}{\overline{D}\Delta t} \tag{8}$$

where \overline{D} is the average diffusion coefficient and Δt is a time lag between two consecutive frames. Using the parameters of the current system we obtain the value of the reduced localization error of $\xi = 0.2$. The theoretical treatment of the ref. [S2] shows that for a specific value of ξ there is an optimal number of points *n* on the

diffusion trajectory of a single particle that should be used to minimize the relative error in the fitting parameters of the *MSD* in the eqn. (3) and (4). For the value of ξ between 0.1 and 1 it has been verified experimentally, ref. [S3] that the optimum number of points for the *MSD* analysis is 4. In our work, 4 independent *MSD* values were used to determine the diffusion coefficient for all particles studied. According to the ref. [S2], for small values of the reduced localization error ξ the relative error (relative standard deviation) of a distribution of diffusion coefficients is given by the number of points *n* used to fit the *MSD* and by the total number of particles analyzed in the distribution. Using this approach, we calculated the errors of the average diffusion coefficients $\overline{D}_x, \overline{D}_y, \overline{D}_z$ and plotted the error bars in the Fig. 2e. We note that none of the relative errors exceeds 15% even within a conservative estimate. The errors of $\overline{D}_x, \overline{D}_y, \overline{D}_z$ were further used in the error propagation analysis to determine the errors of the tilt angle and these have been plotted in the Fig. 2g.

To estimate the errors of the average diffusion coefficient $\langle D_z \rangle$ in Fig. 3b obtained from fitting of the cumulative distribution function, we used the errors of the individual fitting parameters to calculate the overall error. The results are added as error bars in the Fig. 3b. Here, the largest error does not exceed 10%.

A possible source of a systematic error in the *z*-position determination is the use of the fluorescence beads adsorbed on a glass for measuring the calibration curve. The error arises from the fact that the presence of the glass changes the emission pattern of the beads compared to inside the LC. Another source of the systematic error could be spherical aberration, ref. [S4] of the objective lens when used deep inside the sample. However, we believe that with the current LC sample, the large thickness (5 μ m) and rather course sectioning (500 nm) allows neglecting these systematic errors.

7. Comparison of diffusion coefficient of quantum dots and an organic dye

To support the explanation of the results obtained on the QDs as due to specific interactions of the QD with the LC molecules (reorientation of the LC molecules in the vicinity of the QD) we performed control experiments on diffusion of single molecules of the dye Rhodamine B (RhB). The results for the diffusion of RhB along the x and y axes are shown in the histograms in the Fig. S5. The analysis provides the average diffusion coefficients for the RhB dyes of $D_x = 15.9 \ \mu\text{m}^2\text{s}^{-1}$ and $D_y = 33.5 \ \mu\text{m}^2\text{s}^{-1}$. These values are more than a **factor of 300** larger than the diffusion coefficients of the QD in the *x* and *y* directions averaged over all layers ($D_x = 0.051 \ \mu\text{m}^2\text{s}^{-1}$, $D_y = 0.111 \ \mu\text{m}^2\text{s}^{-1}$). At the same time, the diffusion anisotropy values D_y/D_x are very similar, with the values of

2.11 for the RhB and 2.18 for the QD. On the other hand, assuming a radius of the RhB dye of approximately 0.5 nm and taking into account the QD radius of 3 nm, the Stokes-Einstein relation predicts an increase of the diffusion by a **factor of 6** from the QD to the PDI. Thus, the QDs are diffusing by about a **factor 50** slower than predicted by the Stokes-Einstein relation and the difference can be attributed to the QD - LC interactions. A similar **factor of 40** is also predicted from the Stokes-Einstein relation for the difference in diffusion between the true (3 nm) and effective (19.4 nm) radius of the QD.



Figure S5.

Distributions of displacements of single RhB molecules along the x (left) and y (right) axes recorded during the time interval of 30 ms.

References

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