

## Electronic Supplementary Information

# Measuring a diffusion coefficient by single-particle tracking: Statistical analysis of experimental mean squared displacement curves

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### 1 Analysis of all trajectories

In the following, the results for all seven measured trajectories are summarized. Trajectory 1 corresponds to the one used to illustrate the analysis in the main paper.

#### 1.1 Relative error of the slope of the MSD curves

Fig. 1 shows the relative errors of the slopes of the MSD curves as a function of the number of fitting points for the segment lengths  $N_{seg} = 100$  and  $N_{seg} = 1000$ , respectively.

The data sets from the different trajectories feature qualitatively the same behaviour, and the best relative accuracy for the slope is obtained for 3 or 4 fitting points. The slight discrepancies between the data sets might reflect variations in the localisation errors. For the following evaluation of the data the number of fitting points that was taken into account was set to  $n = 4$ .

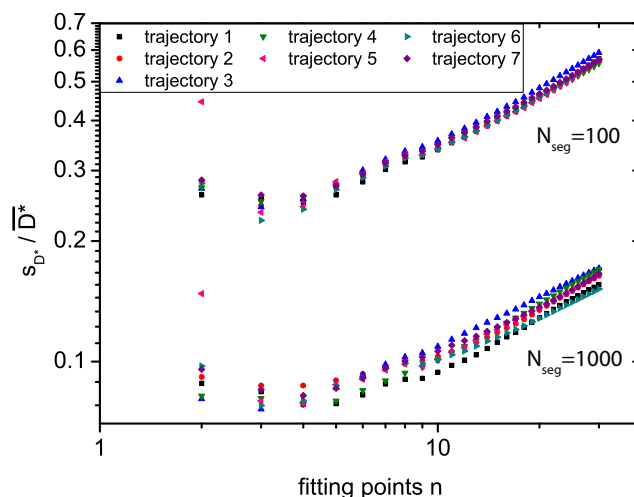
#### 1.2 Distribution of diffusion coefficients

The distributions of the determined diffusion coefficients from all seven data sets have been determined as a function of the lengths of the segments  $N_{seg}$ , and are shown in fig. 2. The results for trajectory 1 are discussed in length in the body of the paper and are not reproduced here. The setup of fig. 2 is similar to that of fig. 3 (left) and fig. 4 (right) in the main text. For all trajectories the distributions and accuracies are qualitatively similar. Discrepancies with respect to the absolute value of the diffusion coefficient are ascribed to size variations of the tracked particles.

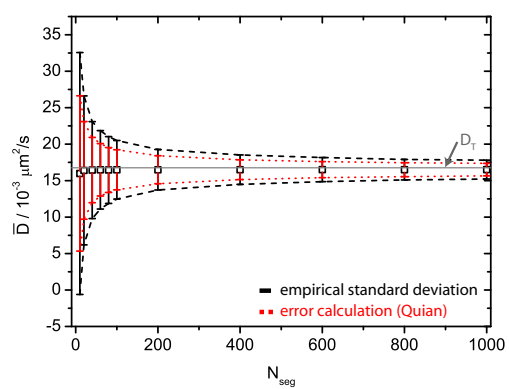
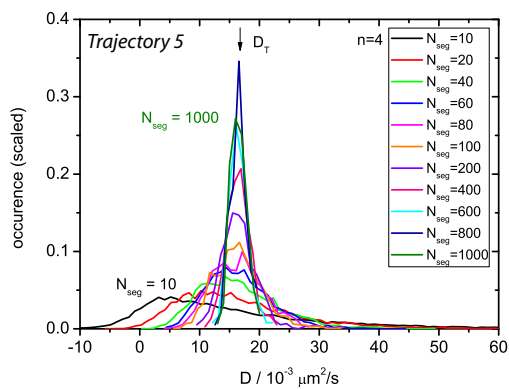
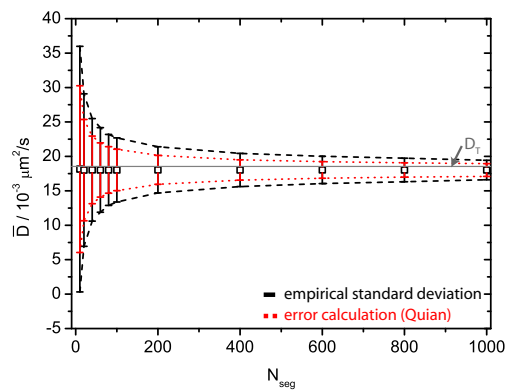
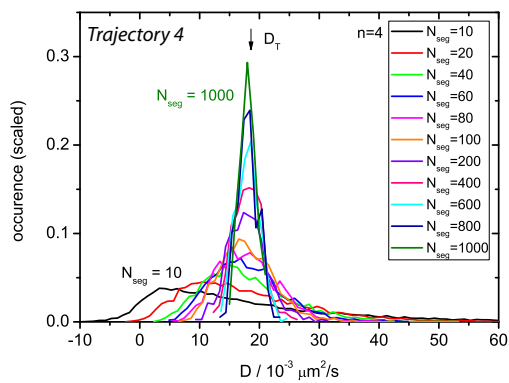
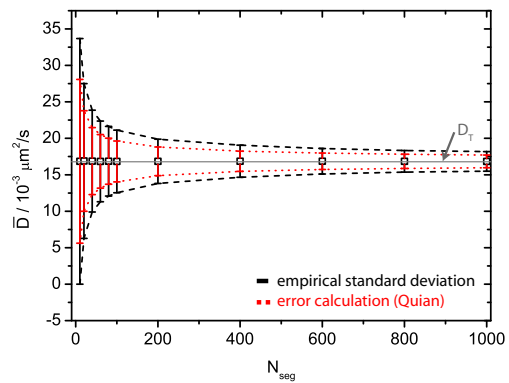
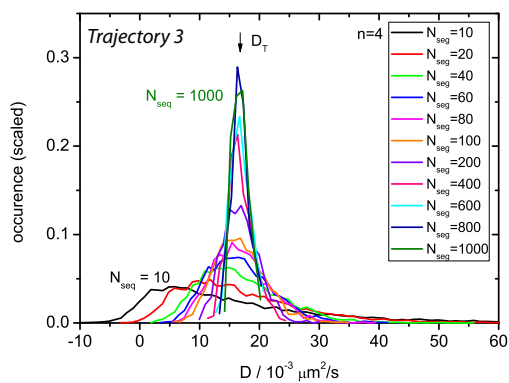
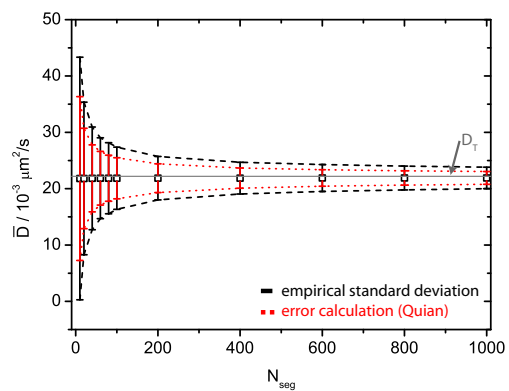
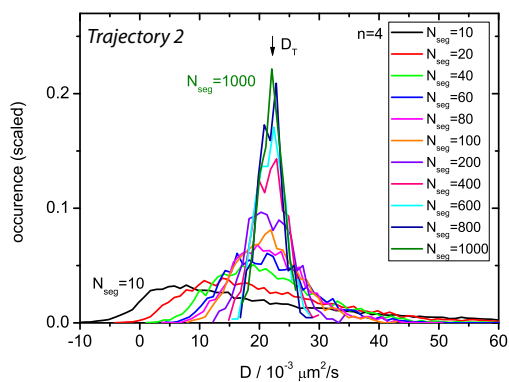
### References

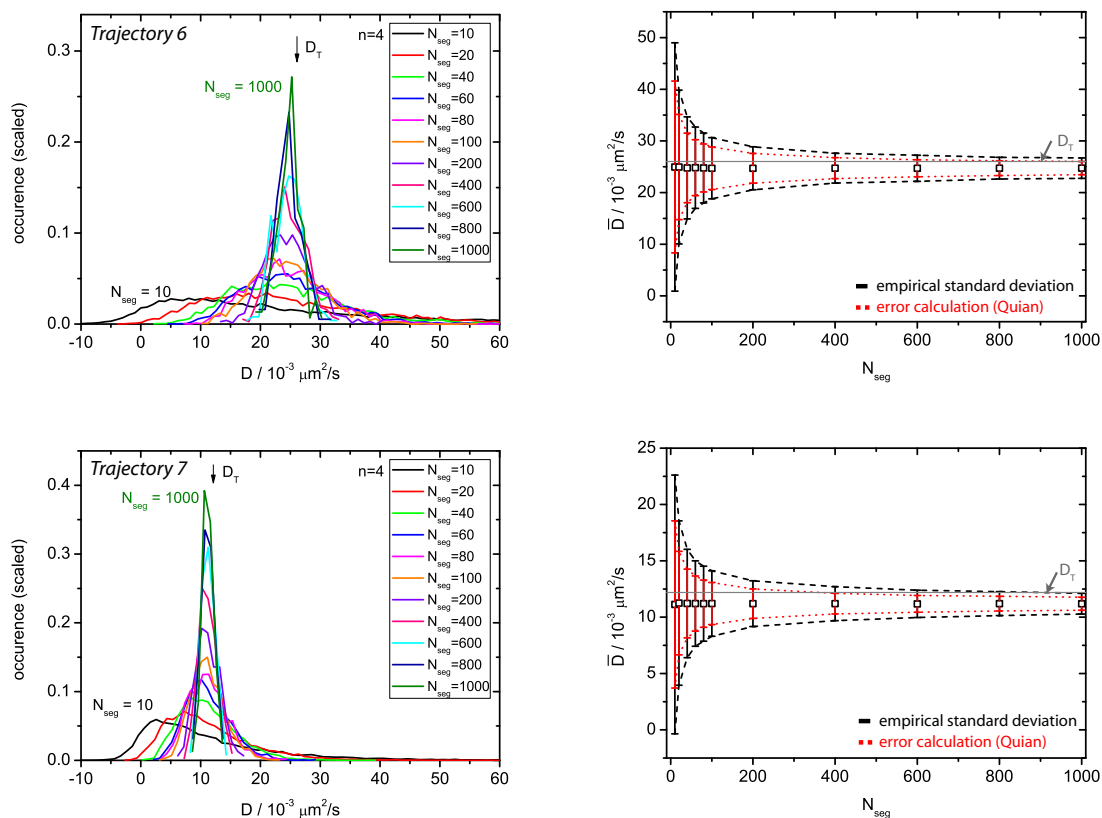
- 1 H. Qian, M. P. Sheetz and E. L. Elson, *Biophys. J.*, 1991, **60**, 910.

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**Fig. 1** (Color online) Relative error of the slopes  $D^*$ , obtained from unweighted linear fits to the MSD curve as a function of the number of fitting points  $n$  for the segment length  $N_{seg} = 100$  and  $N_{seg} = 1000$  for all measured trajectories. More details are given in the text and the main paper.





**Fig. 2** (Color online) Summary of the distributions of the diffusion coefficients for all measured trajectories. Left: Distributions of the diffusion coefficients as a function of the segment lengths  $N_{\text{seg}}$ . All the diffusion coefficients were obtained from linear fits to the MSD curve using the optimal number of fitting points of  $n = 4$ . The arrow on top of the distributions indicates the diffusion coefficient of the corresponding full trajectory. Right: Mean values of the distributions of the diffusion coefficients  $\bar{D}$  (open squares) and empirical standard deviations (black error bars) as a function of the segment length  $N_{\text{seg}}$ . Theoretical values for the standard deviations (red error bars) have been calculated according to<sup>1</sup> using  $\sigma = \pm \bar{D} [2n/3(N_{\text{seg}} - n)]^{1/2}$  and  $n = 4$ . As a guide for the eye the error bars are connected by the dashed (experimental data) and dotted (calculated data) lines. The grey line corresponds to the diffusion coefficient that is determined from a linear fit to the MSD curve of the corresponding full trajectory.