Anisotropic and Hindered Diffusion of Colloidal Particles in a Closed Cylinder

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Video microscopy and particle tracking were used to measure the spatial dependence of the diffusion coefficient ($D_n$) of colloidal particles in a closed cylindrical cavity. Both the height and radius of the cylinder were equal to 9.0 particle diameters. The number of trapped particles was varied between 1 and 16, which produced similar results. In the center of the cavity, $D_n$ turned out to be 0.75$D_0$ measured in bulk liquid. On approaching the cylindrical wall, a transition region of about 3 particle diameters wide was found in which the radial and azimuthal components of $D_n$ decrease to respective values of 0.1$D_0$ and 0.4$D_0$, indicating asymmetrical diffusion. Hydrodynamic simulations of local drag coefficients for hard spheres produced very good agreement with experimental results. These findings indicate that the hydrodynamic particle—wall interactions are dominant and that the complete 3D geometry of the confinement needs to be taken into account to predict the spatial dependence of diffusion accurately.

1. Introduction

Understanding how confinement affects the diffusive behavior of colloidal particles is crucial to understanding various dynamic processes in biological and microfluidic systems.1–3 The first class of examples is given by particles that have to diffuse toward a flat wall before they become immobilized as needed for coating applications or for biomedical diagnostics in microfluidic chips.4,5 Here, diffusive processes under confinement have been found to have a direct influence on measurable quantities such as reaction rates and retardation times.6,7 Also, the confinement of particles in more than one dimension and/or involving wall curvature occurs; examples are the synthesis of colloids inside droplets,8 the trapping of particles inside pores,9,10 and the diffusion or directed transport of biomolecules or particles inside biological cells (or model systems for these11,12). For each of these cases, understanding the time-dependent particle dynamics is connected to the question of how confinement influences diffusion.13 Surprisingly, a majority of fundamental studies referring to this problem have been limited to simple geometries such as a particle approaching a flat wall or quasi-2D systems.14–17 Theoretically, the classical problem of a particle translating in the vicinity of a rigid flat wall was first treated in 1907 by Lorentz18 and then Frenk,19,20 which was later improved by Brenner21 and extended to the double-wall case by Goldman.22 The drag force along the axis of the cylinders was studied theoretically by Sano.23 For some geometries, the quantitative correspondence between differently obtained analytical expressions is still an issue, as evidenced by recent papers on methods to improve the analytical solution.24–26

(7) Frenk, H. The resistance against the movement of a rigour sphere in viscous fluids, which is embedded between two parallel layered barriers. Ann. Phys. 1922, 4 (10), 79–89.
Experimentally, single-walled and double-walled carbon nanotubes have been studied with optical tweezers, 27–29 total internal reflection microscopy, 15 and light scattering. 31,32 Although each of these techniques has its own challenges, the results of these studies coincided reasonably well with each other and with the theoretical expressions. Cylindrical geometries were also studied in a few (quasi-1D) cases: macroscopic measurements of the drag coefficient for a millimeter-sized sphere settling along the axis of a closed 3D cylinder 33,34 and microscopic measurements of diffusion along the axis of long cylindrical microchannels.

From this short overview, it is clear that both theoretical and experimental studies that extend beyond simple confining geometries are scarce. On the experimental side, this could perhaps be explained by the challenges in measuring local diffusion coefficients (or alternatively drag forces) in 3D confinements and in manufacturing suitable confining structures. However, recent developments in soft lithography 37,38 have provided new opportunities for the latter. Three dimensional microchannel-scale confinements can now be manufactured in a variety of geometries. 39 Moreover, these geometries can be also integrated into microfluidic systems in which liquid flow is controlled via pumps and valves. 37 These new capabilities offer unique opportunities for studying diffusion in 3D confinement because of the flexibility in the design of the confining geometry, active control over liquid flow, and good optical access to the confined volume.

In this article, we study the spatial dependence of the diffusion coefficient in 3D closed-cylinder geometry experimentally and compare our results with numerical simulations. Combination of soft lithography and real-time confocal scanning laser microscopy (CSLM) allowed us to observe the diffusion of micrometer-sized colloidal spheres in cylindrical cavities in which both the height and radius are 9 particle diameters. Microfluidic chips containing pressure-controlled structured membranes 41 were used to define the liquid environment and trap the particles. Diffusion coefficients were measured via particle tracking in two horizontal planes, one crossing the cavity center and one near the bottom. Good optical contrast and a large number of observations allowed us to resolve the radial ($D_r$) and azimuthal ($D_θ$) diffusion coefficients as a function of distance from the wall, with high resolution.

This article is further organized as follows. In section 2, we will explain how drag forces are calculated from the 3D Stokes equation and translated into diffusion coefficients. In section 3, we describe the colloidal fluid, the design and operation of the cylindrical cavities, the video particle-tracking experiments, and the data analysis. In section 4, we show how spatially dependent diffusion coefficients are obtained from image analysis and compare the diffusive behaviors for 1 and 16 enclosed particles to each other and to numerical calculations. Conclusions are drawn in section 5.

2. Numerical Calculations

In the low Reynolds number regime, we have a fundamental linear relation between the hydrodynamic force ($F_H$) and the particle velocity ($v_p$) via the diffusion tensor ($D$).

$$v = -\beta D \cdot F_H$$

(1)

Here $\beta = 1/k_B T$ and $D$ is a diagonal tensor with components $D_r$, $D_θ$, and $D_z$ corresponding to the principal directions in cylinder coordinates. The diffusion coefficient $D_θ$ in each direction can be expressed in terms of resistance and thermal energy.

$$D_θ = \frac{k_B T}{3\pi \eta d_p}$$

(2)

where $k_B$ and $T$ are the Boltzmann constant and the absolute temperature, respectively. For a spherical particle in an infinite medium, eq 2 becomes the well-known Stokes–Einstein equation

$$D_θ = \frac{2\pi \eta}{3\pi \eta d_p}$$

(3)

In our numerical simulations, the local diffusion coefficient is obtained by solving the Stokes equation to calculate the drag force $F$ for a given $v$. This can be done for a particle at any chosen position inside the cylinder. By imposing no-slip boundary condition on the container wall and giving the particle a velocity $v_p$, the drag forces and diffusion coefficients in the $r$ and $θ$ directions are obtained separately by applying the velocity in these separate directions. All simulations were implemented using the commercial COMSOL multiphysics package. 29 In these simulations, only the single-particle case is considered. Hydrodynamic interactions between multiple particles are not taken into account. The numerical calculations were validated by comparing the calculated diffusion coefficients with analytical solutions for well-known geometries: a spherical particle near a single wall and in between two walls.


3. Experimental Section

3.1. Colloidal Suspension. Polystyrene latex particles with a diameter \(d_p\) of 1.13 ± 0.05 μm, containing a red fluorescent dye and carboxylate surface groups, were obtained as an aqueous suspension from Polysciences. This suspension was sonicated and subsequently diluted with a 100 μM NaCl solution to a particle volume fraction of approximately 0.5%. The final salt concentration corresponds to a calculated electric double layer thickness of 30 nm, which is small compared to the particle size but large enough to maintain colloidal stability. Given the size and mass density \(\rho = 1.05\) g/mL of the particles, the sedimentation length is \(l = kT/6\pi\eta d_p^3 \approx 30\) μm, which is large enough to prevent significant particle settling. The refractive index of the PS-latex particles is 1.6 whereas that of the aqueous solvent is 1.33. All diluted suspensions were studied at 22 ± 2 °C.

3.2. Microfluidic Cylindrical Cavities. Capturing particles in 3D confinements was achieved by filling a microfluidic channel with a suspension and subsequently pressuring down a membrane with embedded cylinder-shaped cavities to trap fluid underneath (Figure 1). The number of particles that could be trapped varied between 1 and 16. The device used for this is a poly(dimethylsiloxane) (PDMS)-based multilayer microfluidic chip as described in ref.41. Briefly, the chip consists of two layers of channels running in perpendicular directions: one containing the fluid of interest and the other for control. The ceiling of the fluidic channel is an elastomeric membrane in which cavities are embedded. On pressurization of the control channel, the membrane deflects and touches the floor; releasing the pressure restores the original state. Cylinders are visualized through a 170-μm-thick glass slide covered with a 50 μm PDMS layer. This application using so-called integrated structured elastomeric membranes (iSEMs) allows the trapping of aqueous liquids in cavities of arbitrary shape and dimensions in the micrometer range. Also, the simultaneous study of multiple cavities is possible. Our microfluidic channel had a height and width of 16 and 200 μm, respectively. The cavities had a height \(H\) and diameter \(2R_{cylo}\) of 10 and 20 μm, respectively.

3.3. Confocal Scanning Laser Microscope. Images were recorded with an UltraView LCI 10 CSLM system (PerkinElmer) consisting of a Nikon Eclipse inverted microscope, a Yokogawa (Nipkow disk) confocal unit, and a Hamamatsu 12-bit CCD camera. All recordings were made in fluorescence mode using a 25 mW laser with \(\lambda = 641\) nm and a 100× oil objective with NA = 1.3. The pixel size corresponding to the images was 0.135 μm, and the frame rate was 10 s⁻¹. Different focal planes and different numbers of particles per cavity were studied; for each condition, 6 subsequent movies of ~8000 frames each were recorded.

3.4. Particle Tracking. The majority of our image analysis (section 3.5) is based on the accurate localization and tracking of particles; this was done using the IDL implementation of the algorithm from Crocker and Grier.43 Additional procedures that are commonly used for data inspection and error analysis have been described in detail44 and are not repeated here. We will now discuss a few specific aspects of the present study.

Figure 1. (a) Three-dimensional representation of our device with integrated structured elastomeric membranes (iSEMs). The device consists of three major structures: a control channel (a1) that can be pressurized, a membrane (a2) in which cylindrical structures are defined, and a fluidic channel (a3) via which particles and solvent are delivered. The control and fluidic channels intersect each other perpendicularly. (b) Applying pressure to the control channel leads to the deflection of the membrane and the trapping of the particle suspension. (c) Schematic illustration of the experiment in which the particles are imaged in a chosen focal plane of a confocal microscope.
Because of the refractive index mismatch between particles and solvent, image distortion occurred and the optical contrast also deteriorated with increasing depth (Z). However, because of the low particle concentration, sufficient visibility could be obtained at all Z values of interest. At Z = 5 μm above the PDMS floor, the localization accuracy in the (X, Y) focal plane was ~30 nm as in an earlier study.45 The effective focal depth relevant to particle tracking was estimated to be 2.0 μm from an intensity versus Z scan of a particle that was stuck to the bottom. A more detailed discussion of the effective focal depth is given in the Supporting Information. The same immobilized particle also allowed an estimation of the mechanical drift of the cavities; we found it to be less than 1 pixel per 100 s, which is negligible for our purposes.

3.5. Image Analysis. 3.5.1. Cavity Geometry. To inspect the 3D geometry of the cavity, we employed two different methods. In the first method, a fluorescently dyed aqueous liquid was pumped into the chip and directly visualized by recording horizontal (X, Y) images at various vertical (Z) locations. This allowed a fairly accurate measurement of the contour in the horizontal plane and an estimate of the cavity height (Supporting Information). A second, independent check of the (X, Y) contour line was constructed by connecting the outermost points. A careful smoothing was applied, which resulted in an estimate of the cavity height (Supporting Information). A second, independent check of the (X, Y) contour line was constructed by connecting the outermost points. A careful smoothing was applied, which resulted in a contour of the wall $R_{\text{max}}$ as a function of the angle $\theta$. The transformation from Cartesian (X, Y) to polar (R, $\theta$) coordinates was made by localizing the cavity center and subsequently decomposing the relative vectors ($\Delta X, \Delta Y$) into R and $\theta$ components. Performing this operation also on the calculated contour line resulted in an $R_{\text{max}}(\theta)$ function that turned out to have a specific dependency for individual cavities. To facilitate the description of the contour, we described it with a Fourier series

$$R_{\text{max}}(\theta) = R_0 + \sum_{m} A_m \sin(m \theta) + B_m \cos(m \theta)$$

with a truncation at $m = 4$. The quality of this fit turned out to be very good.

3.5.2. Diffusive Behavior. As explained in section 2, local diffusion coefficients $D_{\alpha}$ were calculated from the resistance and thermal energy. Experimentally, they can be measured from the mean square displacement ($\Delta r^2$) using the Einstein–Stokes–Sutherland equation

$$\langle \Delta r^2 \rangle = 2D_\alpha \tau$$

with $\Delta r = \Delta Re + RA|\Delta \Phi| + \Delta Ze$, and $D = D_xe_x + D_ye_y + D_ze_z$. Here, $\tau$ is the lag time and $\Delta r_{\alpha}$ is the $\alpha$ component of the vector that describes the 2D displacement of a particle during that time. In our case, the brackets indicate an averaging over different times and particles at a given location. In eq 2, $D_{\alpha}$ can be expressed as a diagonal tensor that has components of $D_x$, $D_y$, and $D_z$ corresponding to the principal directions in cylindrical coordinates. In our experiments, we focused on the in-plane MSD, hence only $D_x$ and $D_y$ are measured. The diffusion coefficient was measured from the initial slope corresponding to short-time behavior.

A fundamental question is how to define the location where $D_{\alpha}$ is measured, given that $D_{\alpha}$ itself is measured via a displacement. If the length scale over which the MSD can show spatial variation is much larger than the typical magnitude of $\Delta r_{\alpha}$ (as in ref 46), then this is only a minor issue. In the present study, where strong variations in $D_{\alpha}$ can be expected close to the wall, we optimized the spatial resolution by considering $\Delta r_{\alpha}$ only for the smallest (i.e.,

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unit) time step $t_1 (= 100$ ms$)$. This generated a minor secondary issue: because the exposure time of the camera per image ($\sigma$) was only slightly smaller than $t_1$, the magnitude of $\langle \Delta r_\alpha^2 \rangle$ that was found had to be corrected by subtracting $\sigma/3$ from $t_1$ as prescribed by eq 14 in ref 47. Our $\sigma$ was measured from a plot of $\langle \Delta r_\alpha^2 \rangle$ versus $t$ for a bulk viscous liquid (Supporting Information).

Unless mentioned otherwise, the location of $D_a$ was assigned to the midpoint of $\Delta r_\alpha$. Rounding the measured $X$, $Y$ locations to discrete (pixel) numbers was done to make a pixel map that could be presented as an image; for a cavity with 16 particles observed for $\sim 5 \times 10^9$ frames, a pixel was typically “hit” 20 times. To distinguish between the fundamentally different diffusion directions in a cylinder and to allow averaging over equivalent pixels, $\Delta r_\alpha$ was decomposed into radial ($R$) and azimuthal ($\theta$) directions. This produced values of $D_t$ and $D_b$ as functions of $R$ and $\theta$. In our analysis, the distance $\Delta R$ from the cylindrical wall is a more appropriate variable than $R$. In view of the slight deviations from perfect cylindrical shape (section 3.5.1), we used the Fourier description (eq 5) of $R_{\max}$ ($\theta$) to calculate $\Delta W(\theta)$ (or its normalized form $\bar{\xi}$):

$$\Delta W(\theta) = R_{\max}(\theta) - R \text{ and } \bar{\xi} = \Delta W/d_\theta$$

Hereafter, eq 7 was used to group data for $D_t$ and $D_b$ measured at the same $\Delta R$.

4. Results and Discussion

4.1. Spatial Dependence of Diffusion Coefficients. To start with an overview, we first show representative maps for the normalized diffusion coefficient (Figure 2a) and the occurrence frequency (Figure 2b). Both maps were measured in the midplane ($Z_{FP} = H/2$) of the cylinder. It turns out that even farthest away from the walls, $D_a$ is significantly lower than that of a free particle in bulk liquid. Moreover, it is clearly visible that $D_a$ is progressively reduced as the cylindrical wall is approached. The occurrence map looks fairly homogeneous except for a depletion zone in the vicinity of the cylindrical wall (which will be discussed in section 4.2).

A closer inspection of Figure 2b reveals two issues that had to be addressed. First, at two $(X, Y)$ locations the occurrence probability was significantly larger: near the cylindrical wall at $(X = 22, Y = 23)$ and near the center at $(18, 15)$. Both particles appeared to stick to a wall for at least part of the time. The particle near the center appeared to be in the bottom plane and was detected because of the enhanced brightness of such particles. The other particle was clearly stuck to a cylindrical wall. Overall, such sticking events were rare; in 60 h of recording only three such cases were found. Second, slight temporal fluctuations of the cavity contour were also observed. This was manifested as small differences between the contours found in subsequent movies and shows up in the time projection of Figure 2b as the increased width of the particle depletion zones in the lower and right-hand parts. Both issues (whenever they occurred) were resolved by excluding the problem areas from the analysis. Omitting this filtering operation resulted in significantly different results (in Figures 3–5) in the vicinity of the walls.

4.1.1. Midplane. More detailed information was obtained by binning data as plotted in Figure 2a according to the distance from the cylindrical wall and decomposing the diffusion coefficient into radial and azimuthal components as explained in section 3.5.2. We first consider one particle in the midplane ($Z_{FP} = H/2$) of the cylinder. The experimental data in Figure 3 reveal several aspects. In the center of the cavity, $D_t$ and $D_b$ are equal and the total $D_a$ is $\sim 25\%$ lower than the bulk value $D_0$. As the particle approaches the side wall along the radial direction, its diffusion coefficient follows a plateau, down to $\sim 3$ particle diameters from the wall where a gradual decrease sets in. This decrease is stronger for $D_t$, which is qualitatively in line with expectations from the known diffusive behavior of a particle near a single flat wall: here the perpendicular drag force is always greater than or equal to the parallel drag force.$^{21}$

Also shown in Figure 3 are numerical calculations (section 2) that take into account the specific 3D geometry of our experiments. The theoretically predicted trends in $D_t$ and $D_b$ as a function of

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displacement of this choice are shown in the inset of Figure 4. The differences for the 16 particles show a slightly less steep decay near the wall, bottom (ZFP = 0.5d₀, blue triangles) for the case of 16 particles in the cavity. Open symbols correspond to D₀, and closed symbols correspond to Dᵣ. Diamonds connected by solid lines show the numerical solution of the 3D Stokes equation. Open symbols correspond to D₀, and closed symbols correspond to Dᵣ. The inset shows histograms for the intensity measured at ZᵢFP = 0.6d₀ (solid line) and ZᵢFP = H/2 (red dashes). This intensity corresponds to the brightness of the fluorescent image of a particle, which depends on its distance to the Z–ZᵢFP focal plane as well as on ZᵢFP itself.

ξ correspond remarkably well with those of the experiments. Concerning the magnitudes, a 10% discrepancy is found between the model and the experiments. We think that this should mainly be ascribed to the error introduced by the normalization step with D₀: the latter quantity may have been measured at a slightly lower temperature. Another experimental error could be due to the slight uncertainty in the size of the individual particle in the cavity. Finally, we remark that the signal/noise (S/N) ratio of the experimental data is fair, even for ~5 × 10⁴ observations.

The case of 16 particles in the cavity suffers less from poor statistics, but the question is to what extent the diffusive behavior is the same. The still rather low particle volume fraction (∼0.004) suggests that close-range hydrodynamic interactions (collisions) between particles will rarely occur. A comparison between the cases of 1 and 16 particles per cavity is shown in Figure 4. The corresponding curves for Dᵣ and D₀ look fairly similar. The data for the 16 particles show a slightly less steep decay near the wall, and in the center of the cavity, D₀ appears to be 5% smaller. The latter discrepancy falls within the experimental error. Whether the former small difference could perhaps reflect interparticle hydrodynamics remains to be elucidated and would require a different simulation method. In any case, the better statistical accuracy of the experiment with 16 particles allows us to address several additional questions. We first address the accuracy that can be reached in measuring local diffusion coefficients with our particle-tracking method. Uncertainties exist in both the magnitude of D₀ and in the location where it is measured, and the errors can be either systematic or stochastic.

The first issue is related to the assignment of a location to the measured D₀. Different choices can be made: the beginning, end, or midpoint of the diffusive displacement Δᵣ. The consequences of this choice are shown in the inset of Figure 4. The differences are small, which is rationalized by the fact that the typical displacement of τᵣ = 0.1 s is only (0.1–0.2)ξ. At the smallest ξ, the value of Dᵣ based on the midpoint is smaller than at the beginning and end. This can be understood by observing that all radial displacements that begin or end in the ring closest to the wall only those that are small will also have the midpoint in that ring. Larger steps will result in midpoints at larger ξ. This biasing effect applies only to radial displacements in the outer ring.

A second issue is the drift (i.e., nonzero average displacement) due to the gradient of the diffusion coefficient (dDᵣ/ξ) in the radial direction, which can make a contribution to (Δᵣ²) proportional to ξ². We simulated this effect for the case of a particle near a flat wall and found (Supporting Information) that on using the Einstein–Stokes equation the local Dᵣ (perpendicular to the wall) had to be replaced by

\[
D_{\text{eff}}(\xi) = \left( \frac{2\xi - 1}{2\xi} \right) \left[ 1 + \frac{4\xi^2 - 3(2\xi - 1)}{8\xi^2 - 1} \xi' \right]
\]

with ξ' being the normalized lag time (ξ' = τ/τ₀, where τ₀ = d₀²/(2D₀), which amounts to 0.028 in our case. The term in parentheses incorporates the effect of the wall on the drag coefficient, and the term in square brackets represents the drift effect. The latter term is always >1 but becomes <1.1 for ξ > 0.58. This means that the correction term plays a role only very close to the wall (~1 camera pixel in our case). Although the correction is thus only minor in our case, it is also clear from eq 8 that this is due to the small value of τ.

A third issue is how the magnitude of D₀ is affected by localization errors. The measured coordinates of the contour and center of the cavity suffer from inaccuracies that are larger than that of the particle localizations, and it is obvious that the strongest influence of these errors is expected near the wall. We examined this issue by repeating the image analysis but now increasing or decreasing the local contour radii R₉₀(θ) by 0.15d₀.

Also, the X and Y coordinates of the cavity center were varied by the same amount. Comparing D₀ values at the same ΔR for the different data sets thus created allowed us to estimate a ΔR-dependent standard deviation. To obtain the total standard deviation, these errors were combined with the statistical inaccuracy that arises from the finite number of observations N within each ΔR ring. The relative error due to this effect amounts to (2/N)¹/², and it is also ΔR-dependent. The resulting error bars for individual cases are shown in Figures 3 and 4. It turns out that the typical relative error in D₀ is 8% for 1 particle per cavity and 4% for 16 particles per cavity.

4.1.2. Bottom Plane. For the case of 16 particles, the diffusive behavior at the bottom of the cavity could also be examined, albeit an additional data-filtering step was needed to ensure that only particles very close to the bottom are taken into account. The need for this step arises from the fact that the Z range in which particles are detected is ~2 μm (Supporting Information). With the focus on ZᵢFP = 4.5 ± 1 μm, this effect is unimportant because the distance ΔZ of a particle to the nearest wall varies only between 4 and 5 μm. However, at ZᵢFP = 0.5d₀, ΔZ varies between 0.5 (contact) and 2 μm. In this range, Dᵣ and D₀ can be expected to show a strong dependence on the Z position.

To exclude particles that are not very close to the bottom, we collected the intensities of all ~10⁵ particles tracked at ZᵢFP = 0.5d₀ into a histogram (inset of Figure 5). Two distinctive peaks that differ in intensity by a factor of ~3 were found. We then assumed that only this secondary peak corresponds to particles at the bottom and restricted our analysis to this subset. The fact that

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this data collection gave substantially lower values for $D_D$ and $D_D$, whereas a similar filtering at $Z_{FP}$ = $H/2$ had negligible effects, seems to justify our assumption.

The thus-measured dependencies of $D_D$ and $D_D$ on $ξ$ at the bottom plane are presented in Figure 5, together with the data observed in the midplane (and with numerical simulations). For $ξ > 3$, the proximity of the bottom plane is manifested as an ~25% reduction in the $D_D$ values as compared to the midplane. As the cylindrical wall is approached ($ξ < 3$), a transition takes place to a regime in which the drag force becomes strongly dominated by the cylindrical wall. This is evident from the quantitative correspondence at small $ξ$ of the $D_D$ and $D_D$ curves measured at the bottom and midplanes.

The comparison to numerical calculations revealed some interesting aspects. Because the inaccuracy in $Z_{FP}$ was estimated to be $0.2 μm$, calculations were performed for $Z_{FP}$ = $(0.6–1.5)d_p$. The results (shown in the Supporting Information) revealed that the plateau levels for $D_D$ and $D_D$ depended strongly on $Z_{FP}$ and that the best agreement with our experiments was found for $Z_{FP}$ ≈ 0.6 μm. It also turned out that all calculated $D_D$ curves showed a very similar limiting behavior for $ξ < 1$, which was, however, significantly steeper than that of our experiment. This is also shown by the numerical $D_D$ curve in Figure 5. Part of this discrepancy could perhaps be attributed to a slightly less steep $ξ$ dependence for the case of 16 particles (as remarked before). Besides that, we have no explanation for the discrepancy. For the $D_D$ curves, a comparison between simulation and experiment produced a better correspondence than for $D_D$.

4.1.3. Comparison with Other Geometries. To emphasize further the effect of confining geometry on the spatial dependence of the local diffusion behavior, we performed additional numerical calculations. In panel a of Figure 6, we compare the radial and azimuthal diffusion coefficients for three distinct geometries: (1) our closed cylinder ($R_{cyl} = 9d_p$, $H = 9d_p$) at the midplane ($Z_{FP}$ = $H/2$), (2) an infinitely long cylinder with $R_{cyl} = 9d_p$, and (3) a single flat wall. In comparing cases 1 and 2, we find that for small $ξ$ (< 2) the diffusive behavior is largely dominated by the cylindrical wall; this is similar to the experimental trend in Figure 5. However, as the central axis of the cylinder is approached, the limiting behaviors are clearly different: even at 4.5 particle diameters away from the top and bottom surfaces, the latter boundaries still lower the value of $D_D$ by 8% compared to the infinite cylinder case. To reduce this difference to 1%, the distance of the end planes from the center has to reach 20 particle radii (data not shown).

To corroborate further the importance of end planes, we also compared the previous cases (1 and 2) with calculations for a cylinder with $R_{cyl} = 9d_p$ and $H = 9d_p$ but now with one end open. Figure 6b shows once again that in the vicinity of the cylindrical wall all curves collapse. Closer to the center of the cylinder, the diffusion behavior is clearly influenced by the absence of end planes. Each added boundary further decreases the limiting value for both $D_D$ and $D_D$.

Finally, we also examine the influence of wall curvature. The comparison between the cases of an infinitely long cylinder and a single flat plane reveals that very close to the wall (ξ < 1.5) the diffusivities of the particle are essentially the same in Figure 6a. This indicates that very close to the wall the curvature of the latter can be neglected for $R_{cyl}/d_p ≥ 9$. However, close to the radial axis the diffusion behavior is notably different. In summary, the calculations given in Figure 6 illustrate once more that, as a rule, the complete 3D geometry needs to be considered to obtain a quantitative description of the spatial distribution of local diffusion.
of the wall by comparing for each movie two sets of $R_{\text{max}}(\theta)$: the “smooth” contour obtained from fitting with eq 5 and the “noisy” primary data. The difference $\Delta R_{\text{max}}$ did not show any systematic dependence on $\theta$, and its distribution $P(\Delta R_{\text{max}})$ had a standard deviation $\sigma$ of 140 nm. This $\sigma$, which includes the contribution of dynamic wall fluctuations, matches the found depletion range very well. To illustrate this further, we took a step function $P(\xi)$ as expected for hard spheres and walls and convoluted it with the distribution $P(\Delta R_{\text{max}})$. As can be seen in Figure 7, the resulting curve agrees very well with the experimental $P(\xi)$ data. Finally, we also did a computer simulation in which a screened electrostatic repulsion between particles and a wall is included. Here it turned out that the additional change in our $P(\xi)$ due to a potential with a contact value of $8k_B T$ and a Debye length of 30 nm has a negligible effect.

The implications of these findings for the present study are the following: (i) it is corroborated that the thermodynamic interactions between particles and wall are essentially like that of hard surfaces and (ii) fluctuations of the PDMS wall cannot be excluded, but if so, their amplitude does not exceed 150 nm. This latter aspect was already taken into account in the error analysis above. The complete 3D geometry of the confinement needs to be taken into account.

A current limitation of the iSEM-based cavities is that particles could not be trapped in large numbers. The ability to reach higher volume fractions inside 3D cavities would be very interesting because it would allow us to study at which number of particles or volume fraction the effect of particle–particle hydrodynamics becomes manifest and how these interactions will change the local diffusive behavior. This will be a topic of further study.

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Supporting Information Available: The following issues are discussed: Size and shape of the cavity. Effects of finite shutter time. Effective depth of focus. Computer simulations on the effect of confining boundaries. Dynamics of particles with space-dependent mobility. This material is available free of charge via the Internet at http://pubs.acs.org.