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Communication

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Unhindered Brownian Motion of Individual Nanoparticles in Liquid Phase Scanning Transmission Electron Microscopy

Murat Nulati Yesibolati,[†] Kim I. Mortensen,[‡] Hongyu Sun,[†] Anders Brostrøm,[†]
Sofie Tidemand-Lichtenberg,[†] and Kristian Mølhave^{*,†}

[†]*DTU Nanolab, National Centre for Nano Fabrication and Characterization, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*

[‡]*DTU Health Tech, Department of Health Technology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*

E-mail: krmo@dtu.dk

Abstract

Liquid phase transmission electron microscopy (LPTEM) offers label-free imaging of nanoparticle (NP) processes in liquid with sub-nanometer spatial and millisecond temporal resolution. However, LPTEM studies have reported only on NPs moving orders-of-magnitude slower than expected from bulk aqueous liquid conditions, likely due to strong interactions with the LPTEM liquid-enclosing membranes. We demonstrate how scanning transmission electron microscope (STEM) imaging can be used to measure the motion of individual NPs and agglomerates, which are not hindered by such interactions. Only at low electron flux do we find that individual NPs exhibit Brownian motion consistent with optical control experiments and theoretical predictions for unhindered passive diffusive motion in bulk liquids. For increasing electron flux, we find increasingly faster-than-passive motion that still appears effectively Brownian. We discuss the

possible origins of this beam-sample interaction. This establishes conditions for the use of STEM as a reliable tool for imaging nanoscale hydrodynamics *in situ* TEM.

Keywords: Brownian motion, diffusion, liquid cell, scanning transmission electron microscopy, single particle, low flux

How nanoparticles (NPs) in a liquid suspension grow, move, and interact with each other and surrounding interfaces are of fundamental interest in, e.g., colloidal matter,^{1,2} biomedical applications,³ microfluidics,⁴ and artificial micro/nanoscale motors.^{5,6} Traditionally, imaging of such liquid processes has been limited to optical microscopy (OM). Bulk-level methods such as conventional OM and light scattering methods such as dynamic light scattering (DLS) cannot deliver nanometer-spatial-resolution at the single-particle level. Alternatively, optical localization microscopy can track the center positions of fluorescently labeled particles,^{7,8} if they are separated by more than the typically 200 nm optical resolution limit. In denser samples, various super-resolution microscopy modalities may be used to circumvent this limit on fluorescent samples.^{8–10} Recently, liquid phase transmission electron microscopy (LPTEM) has revolutionized the access to nanoscale, label-free imaging of a wide variety of liquid processes.^{11,12} Typically, the liquid cells used for LPTEM consist of electron-transparent silicon nitride (SiN_x) windows suspended on two Si chips, which enclose a liquid sample layer with a thickness ranging from a few hundred nanometers to a couple of microns (Figure 1a, and Supporting Information Note 1). With LPTEM, NP dynamics, e.g., nucleation and growth,^{13–16} self-assembly,^{17–23} and interactions^{16,17,23–27} have been studied with sub-nanometer spatial resolution and millisecond temporal resolution.

If the suspended particles are influenced only by thermal forces caused by the collisions with solvent molecules, with no additional external forces, each particle exhibits unhindered and passive Brownian motion with a diffusion coefficient, D , given by the Stokes-Einstein equation (Supporting Information; Methods). In this case, frame-to-frame step displacements of the particle follow a normal distribution and the mean-squared displacement (MSD) in d dimensions, $\text{MSD} = 2dD\Delta t^\alpha$, is proportional to the time lag Δt , i.e. with exponent

$\alpha = 1$.

Numerous LPTEM studies have investigated the motion of NPs, but the NPs chosen for study were likely interacting with the enclosing liquid cell membranes: All previous LPTEM studies^{15,16,23,28-37} have found exceptionally slow diffusive motion of NPs compared to unhindered and passive diffusion. Even when Chee *et al.* considered only the fastest motion observed, the instantaneous diffusion coefficients were still 20 times smaller than predictions for bulk conditions.³² Thus, every diffusion coefficient observed in LPTEM has been 2-9 orders of magnitude smaller than that of unhindered and passive diffusive motion in bulk liquid. In a recent study reporting on highly viscous solutions, >100 times that of water, and thus under conditions far from the typically aqueous LPTEM studies, bulk level diffusion was observed using very low flux.³⁸

The reported several orders-of-magnitude reduction in diffusion coefficients cannot be explained by a simple increase in the hydrodynamic drag due to thin layer confinement, such as the Faxén correction, see Supporting Information Notes 2-5.^{28,39,40} In all cases, the dynamics had non-normally distributed step displacements, power-law distributed waiting times between flights, and MSDs that scaled with Δt as a power-law with $\alpha < 1$, which indicate sub-diffusive motion.⁴¹ Additionally, NPs were influenced by the beam causing them to move in⁴² or out⁴³ of the field of view (FOV). Among the suggestions for explanations are inhomogeneous charging of the SiNx³² or a thin liquid layer with high viscosity near the membrane interface,²⁸ but the mechanisms behind the varieties of observed slow dynamics are not yet fully understood.

While, slow-moving, surface-interacting NPs are well-suited for high-resolution imaging, the sole focus on such NPs raises a broader concern about the validity of LPTEM results as representative of bulk conditions,⁴⁴ due to the clear presence of a strong interaction that could influence many other experimental aspects than merely motion. Moreover, the lack of observations of unhindered diffusion in water could lead to the false perception that such NP motion does not occur in LPTEM.

In aqueous LPTEM, currently it is not known: (i) to what extent unhindered Brownian motion is observable, (ii) how beam-irradiation influences the motion of NPs moving unhindered between the two membranes, and (iii) if LPTEM could be appropriate as a high-resolution tracking method for NP dynamics in bulk liquid, free from uncertainties due to membrane interactions. If clarified, these aspects would form a foundation for LPTEM studies of processes with freely-moving NPs and provide a valuable model system for bulk liquid conditions, which could open for a range of new experimental designs.

To address this, we used LPTEM in STEM mode with a high-angle annular dark-field (HAADF) detector to image 150 nm Au citrate-stabilized NPs in a custom-built liquid cell (Supporting Information Note 1). To minimize influence from membrane surfaces, we imaged within the region with 3-4 μm thick liquid in the middle of the bulging cell. The theoretical values for the diffusion coefficients that we use for comparison below are the bulk values reduced by Faxén's mid-plane surface proximity correction at the thickness estimated for each experiment (Supporting Information Note 3-5). The liquid thickness is beyond the inelastic mean free path,⁴⁵ which leads to STEM contrast inversion from multiple scattering causing the Au particles to appear dark (Figure 1b, Supporting Information Note 6).^{46,47}

In order to minimize electron-beam effects on NP dynamics, we used electron fluxes of $0.14 - 3.5 e^-/(\text{\AA}^2 \times \text{s})$. These fluxes (Supporting Information Note 6) are low compared to typical values of $1 - 100 e^-/(\text{\AA}^2 \times \text{s})$ in the LPTEM literature focusing on NP dynamics.^{15,16,24,28,32,33}

In a slow scanning STEM image (Figure 1b), we found that many NPs stuck to the top (①) and bottom membranes (②), as in previous studies.²⁸ Slow "sticky" motion was present for NPs (Figure 1b, ③) interacting with a membrane, as earlier reported.^{15,16,23,28-36} Freely moving NPs are not readily observed in a slow-scan STEM image, as they diffuse too fast compared to the acquisition time. Only an occasional NP moving favorably with respect to the raster scan direction of the beam may be observed as a short streak (Figure 1b, ④)

We reduced the number of pixels and the pixel dwell time of each frame, to obtain a

Figure 1: Illustration of the experimental LPTEM STEM setup and analysis. (a) A liquid sample containing Au NPs is sealed between two Si chips with 50 nm thick electron-transparent SiNx windows in a custom-built liquid TEM holder (Supporting Information Note 1). The windows bulge outward due to the vacuum in the TEM chamber (Supporting Information Notes 2 and 3). (b) Example of slow-scan STEM HAADF image of 150 nm Au NPs with an exposure time of 2 s. NPs are either stuck to the top (①) or bottom membrane (②), moving very slowly while interacting with a membrane (③), or moving rapidly between the two membranes, which is seen as a streak only when movement is favorable with respect to the scanning direction of the electron beam (④). (c,d,e) Sketch of analysis process of fast-scan STEM time-lapse movies (Supporting Information; Methods). NPs moving unhindered (arrows, ④) are preserved while NPs stuck to a membrane (① and ②) are removed by subtraction of a “background” image (temporal average of all frames) from each frame in the time-lapse movie. (f) NPs are detected automatically and the center position of each NP is localized in each frame in the time-lapse movie and thus tracked (Supporting Information; Methods). Typically, each trajectory (colored lines) is clearly separated from others in space and time.

33 Hz frame rate with a large FOV ($10\mu\text{m} \times 10\mu\text{m}$) (Supporting Information; Methods). This allowed us to image multiple, individual, freely-diffusing NPs (Figure 1c-e). Our image analysis selected for the freely-moving particles: Particles that were stuck to membranes (or exhibiting orders-of-magnitude slower motion) were excluded from the analysis by subtraction of an all-frames averaged “background” (Figure 1c-e, and Supporting Information; Methods).

The beam broadens as it passes through the liquid but only to the extent that NPs at the top membrane (Figure 1c,d, ①) and the bottom membrane (②) can be discerned in images by the edge sharpness and contrast. The contrast of freely-moving NPs vary with time within a range between the levels of particles stuck to the top and bottom, indicating varying beam broadening and corresponding z -position. This ensures consistent tracking in the xy -plane independent of the z -position (Figure 1e, ④). Due to the noise level, the contrast unfortunately does not allow quantitative tracking of particle motion along the z -axis (Supporting Information Note 7).

To characterize the lateral motion of the moving NPs, we tracked the NPs by determining their centroids in each frame of the time-lapse recordings (Figure 1f, Supporting Information; Methods, and Supporting Movies S1-S6). Note that lower electron flux leads to shorter trajectories as tracking is challenging due to the lower signal-to-noise ratio.

We found no significant indication of ($\alpha < 1$) sub- or ($\alpha > 1$) super-diffusion in the measured trajectories for individual NPs, as further discussed and verified below. Thus, it is appropriate to analyze individual NP trajectories as realizations of time-lapse recorded effective Brownian motion specified by a diffusion coefficient and $\alpha = 1$. Diffusion coefficients of individual particles (Figure 2a, and Supporting Information Note 8) were determined by the Covariance-based Estimator (CVE, Supporting Information; Methods),^{48,49} which is practically optimal, and superior to traditional analysis based on MSDs (Supporting Information Note 8), especially for shorter trajectories.^{48–50} For each particle at each experimental condition, we found similar diffusion coefficients in the x - and y -directions, indicating no influence

of beam-scan direction (Figure 2a) or significant NP variability in the population. This allowed us to obtain population-averaged diffusion coefficients ($\bar{D}_{x,y}$), by averaging the individual estimates of the 2D diffusion coefficients, using trajectory length as a weight factor (Figure 2b, and Supporting Information; Methods). Note that the CVE analysis implicitly measures and accounts for errors associated with the localization of particles (Supporting Information Note 9).

Since the diffusion coefficients of the individual particles were statistically identical for each experimental condition (Figure 2a), we combined all frame-to-frame displacements of all such particles (Figure 2c). For each experimental condition, the distribution of displacements is favorably described by normal distributions with parameters corresponding to the population-averaged CVE diffusion coefficients ($\bar{D}_x$ or $\bar{D}_y$), as expected if the motion effectively is unhindered Brownian motion. Intriguingly, the variance of the distribution of frame-to-frame displacements of NP trajectories increase with the electron flux (Figure 2c), indicating flux-driven increase of the effective diffusion coefficient. This can also be seen in the population-averaged MSDs (Figure 2d, dots) that show a linear relationship with the lag time within uncertainty—a hallmark of Brownian motion—however, with slopes that increase with increasing electron flux.

Figure 2e shows that only the diffusion coefficient obtained with the lowest electron flux was practically consistent with the value obtained in OM control experiments (Supporting Information; Methods, and Supporting Movie S6). Both these values are close to the theoretical value expected from unhindered and passive Brownian motion (black line, Figure 2e), which also agrees with recent results obtained at very low flux and very high viscosity.³⁸ The beam irradiation effect resulting in effective diffusion coefficients exceeding the theoretical value for bulk liquid has not been reported previously. Indeed, flux-related increases in diffusion coefficients have been reported only for NPs interacting with the membranes (such as ③ in Figure 1b), i.e. in a regime where diffusion coefficients remain orders-of-magnitude smaller than the bulk value^{23,32,35} and increases could be caused by reductions in membrane

Figure 2: Diffusion analysis of Au NPs in a water solution. (a) Example diffusion coefficients and (b) trajectory lengths of individual Au NPs, here at $0.89 \text{ e}^-/(\text{\AA}^2 \times \text{s})$ electron flux. (c) Frame-to-frame x -displacement distributions from trajectories of all individual Au NPs under various flux conditions and with OM (see legend, also indicating number of particles). The solid lines indicate normal distributions centered on the origin and with variances corresponding to the population-averaged diffusion coefficients obtained as in (a). Distributions for y -displacements are shown in Supporting Information Note 8. (d) Comparison of the population-averaged conventional MSDs (dots in various colors) to the population-averaged CVE $\bar{D}_{x,y}$ (solid lines in corresponding colors) and the theoretical MSD (dashed line). Error bars indicate SEM and increase with Δt . (e) Population-averaged diffusion coefficients at different electron fluxes and with OM. D_{theory} (dashed line) is the theoretical value for unhindered and passive diffusion with Faxén's mid-plane surface proximity correction (Supporting Information Note 5). Error bars indicate the standard error of mean (SEM). The data in (a) and (d) are offset slightly in the horizontal direction to show the error bars more clearly.

interaction, while in this case there is an additional force at play.

Note that direct determination of diffusion coefficients for individual particles from a fit of a straight line to MSD data is ill-advised (Supporting Information Note 8), due to the fact that the MSD values at different time lags are highly correlated, since they are estimated from the same trajectory.^{48,50} For the same reason, MSD plots of individual trajectories are notoriously difficult to use as rigorous basis for determining presence or absence of anomalous behavior (Figure 2d). While there are no indications of super- or sub-diffusion in the MSD plots, that all show a linear dependence on the lag time within uncertainty, this does not alone guarantee effective Brownian motion: one may occasionally observe linear MSDs when the underlying dynamics are governed by sub-diffusion with a heavy-tailed distribution of waiting times,⁵¹ but that would be accompanied by a non-normal distribution of displacements, which we did not observe (Figure 2c). This is in contrast to the distinctly non-normal distributions observed in other cases for NPs moving slowly near the membranes.³² As a statistically rigorous check for effective Brownian motion, we used a power spectrum analysis of the measured displacements⁴⁹ (Supporting Information Note 8). This showed that the motion indeed is effectively Brownian at all applied electron fluxes, but with effective diffusion coefficients that increase with the electron flux.

We corroborated these observations with a similar set of experiments performed with a viscous 70wt% glycerol in water solution. Also in this case, the observed diffusion coefficients increased with the electron flux reaching up to ten times higher diffusion coefficients (Supporting Information Note 10, and Supporting Movie S7).

Though the electron beam has a strong effect on the NP motion, this motion is consistent with effective Brownian motion at all fluxes. The motion does not depend on the beam scanning direction, NP position in FOV, or time of recording in each time-lapse movie (Supporting Information Note 11). A detailed analysis of the particle motion (Supporting Information Note 12) suggests that the diffusion coefficient appears similar on microsecond, millisecond, and second time scales. Hence the increase in diffusion coefficients of NPs is

not likely due to NPs interacting strongly and locally with the beam, but rather seems to be caused by an interaction present on longer distances and longer times, which results in a force that appears random at the time scale of our observations.

Table 1: Summary of effects that could cause apparent increases in diffusion coefficients. See details in Supporting Information Note 12

Possible factors	s_{water} [nm]	$s_{70\text{wt}\% \text{glycerol}}$ [nm]	Discussion
	~ 170	~ 333	Experimental observations
Local temperature increase	~ 2.6	~ 10	Temperature measurement and theoretical calculations show that temperature increase during electron beam scanning is less than 5K in FOV.
Hot NP	~ 0	~ 0	The fast-moving NPs are full-size resolved during STEM imaging. Therefore, the step displacement increase cannot be attributed to the NP being hot while irradiated.
Thermophoresis	~ 24	~ 5	Thermal gradients can be large and lead to thermophoretic diffusion, which is a possible contributing effect.
Electrostatic interactions	~ 0	~ 0	The electric field from the beam is largely screened by the solution. Therefore, the Electrophoresis(EP), Dielectrophoresis(DEP), Electroosmosis(EO), electron beam induced current(EBIC), and potentials from EBIC appear all to be negligible. Any small effect will also be averaged out as the electron beam scans faster than the NP diffusion.
Momentum transfer to the particle	~ 0	~ 0	Momentum transfer from beam electrons to the NPs is negligible.
Pixel force vortex	~ 10	~ 3	A vortex flow can be created in the surrounding liquid that could be influential
FOV force vortex	~ 0.3	~ 0.1	
Diffusiophoresis	~ 400 nm		A rough estimate of the radiolytic H^+ gradient being able to cause additional velocity of the order observed.

At the maximal value of the diffusion coefficient, the characteristic diffusion displacement length (Figure 2c) between consecutive time-lapse frames at $\Delta t = 30$ ms increases above the expected frame-to-frame displacement in bulk condition by about $s_{70\text{wt}\% \text{glycerol}} = 0.3$ μm in the glycerol solution, and $s_{\text{water}} = 0.2$ μm in water. The interaction thus effectively adds up to 6-11 $\mu\text{m/s}$ random velocity on top of Brownian motion. This could be caused by one or more of a number of effects that we consider in detail in Supporting Information Note 12, discuss below, and summarize in Table 1.

Thermal effects such as radiation induced local temperature increase or "hot" NPs seem

negligible, which is supported by a microsecond time-scale analysis (Supporting Information Note 12) and direct LPTEM temperature measurements (Supporting Information Note 12). Although absolute temperature increases in the system are too small, thermophoresis from temperature gradients around the scanning beam could be a minor contribution to the increased diffusive motion.

Electrostatic interactions, electrophoresis and electroosmosis are expected to be negligible given the short screening length in the electrolyte and the lack of a microsecond time-scale direct beam interaction (Supporting Information Note 12). Similarly an 'electron wind' effect from beam momentum transfer to NPs or liquid seems a limited contribution.

Among the possible influences, diffusiophoresis, caused by the potential gradients in the electrolyte due to gradients in ion concentrations,⁵²⁻⁵⁴ such as pH, appears to be a prime candidate capable of increasing the apparent diffusion coefficient. A charge concentration gradient can cause diffusiophoretic motion of the order 5 $\mu\text{m/s}$, for the fastest diffusing radiolytically produced ion H^+ at about 2% per micrometer. Stronger H^+ gradients have been estimated in simulations⁵⁵ for less intense TEM irradiation conditions than the focused STEM beam. A rough estimate gives a diffusiophoretic contribution of the order of the experimentally observed effect (Supporting Information Note 12). Diffusiophoresis is expected to be lower if viscosity is increased (Supporting Information Note 12), contrary to what is observed, but concentration gradients could also be very different. With no validated models for radiolytic pH gradients in STEM, reliable quantitative diffusiophoresis estimates are difficult to make. In addition, the scanning beam further complicates estimating the actual effects in all cases from static condition models.

In LPTEM, most processes of interest involve particles smaller than what we used here to allow direct comparison to OM data. As the particle size decreases, the diffusion coefficient increases, making imaging more challenging. For example, a 20 nm spherical particle can be imaged in 100 nm thick water with limited Faxén-correction of about 20% (Supporting Information Note 4), and a bulk diffusion coefficient of $D = 24 \mu\text{m}^2/\text{s}$ at room temperature.

Figure 3: Translational dynamics of a three-particle agglomerate in LPTEM. (a) Time-lapse STEM images of a single three-particle agglomerate diffusing and rotating between two SiNx membranes (Supporting Movie S8). The red arrow indicates an Au NP stuck to the top membrane, and the dark-blue arrow one stuck to the bottom membrane. The three-particle agglomerate (orange arrow) diffuses between the membranes, which can be seen from the contrast and focus variation. (b) The lateral, translational trajectory of the three-particle agglomerate. The inserted image is a STEM image acquired as the three-particle agglomerate is close to the top membrane showing sharp contours. The solution was 60 wt% glycerol in water solution, and the electron flux was $4.6 e^-/(\text{\AA}^2 \times \text{s})$. The scale bar is 300 nm.

A suitable frame rate for reliable tracking corresponds to about a few diameters diffused distance per frame, which would be at around 100-1000 frames per second (FPS). This is possible in modern bright field TEM cameras with low electron flux capability, though motion blur may be an issue when the exposure time is not as short as STEM scanning over a single particle. Diffusion can also be deliberately slowed by increasing viscosity,³⁸ lowering temperature, or by thin liquid layers as described by Faxén^{39,40} (see Supporting Information Note 5).

Studying motion of complex structures is possible in the present setup, as exemplified by the three-particle agglomerate of NPs in Figure 3 (Supporting Movie 8). With this, e.g., rotational hydrodynamics may be probed, which until now has been reserved to methods with much lower spatial resolution.^{56–58} Such clusters provide better imaging resolution than individual particles, since they diffuse slower due to their size. The three-particle agglomerate was not bound to any membrane, but diffuses and rotates freely between two SiNx membranes. This is indicated by its contrast, which varies between that of particles stuck to the top and bottom membranes. The lateral, translational diffusion coefficient was found to be $0.25 \pm 0.1 \mu\text{m}^2/\text{s}$ in a 60 wt% glycerol solution that has a 10-times higher viscosity than water. This compares favorably to an estimate of $0.2 \mu\text{m}^2/\text{s}$, derived from a spheroidal three-particle model that in pure water has $1.95 \mu\text{m}^2/\text{s}$.⁵⁶ While the estimates agree within the uncertainty, a higher experimental value is expected due to the electron flux influence discussed above.

In conclusion, unhindered diffusion has for the first time been observed in LPTEM and quantified with particle tracking. Diffusion coefficients measured at low electron fluxes are consistent with values expected from the Stokes-Einstein equation and OM control experiments at bulk conditions. Optimized LPTEM in STEM mode can hence be used for measuring dynamics of NPs in their native bulk environment at the nanoscale with high spatiotemporal resolution. This opens up for bulk dynamics studies in LPTEM, also e.g. rotation and interactions between particles and substrates.

Beam-induced faster-than-bulk diffusion was observed with effective diffusion coefficients increasing with the electron flux, still with Gaussian distributions of displacements. The beam influence can be substantial, up to more than a 10-fold increase for the 70% glycerol solution. Possible explanations were considered, where contributions were likely caused by thermal gradients leading to thermophoretic diffusion and beam momentum transfer to the liquid by an "electron wind" effect. The main contribution was most likely from diffusiophoresis caused by beam induced concentration gradients of e.g. radiolytic H^+ .

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Supporting Information Available

Supporting Information (.pdf) includes Methods and materials, Supporting Information Notes 1 to 12, Figures S1 to S21, Tables S1 to S3, Legends for Supporting Movies S1 to S8, and References detailing the custom-made liquid cell, liquid thickness estimation under OM, STEM data at different electron flux and extended analysis, and discussion.

Supporting Movie(.avi) includes 8 trajectory-overlay example movies from various electron fluxes and optical measurements in different liquid solutions.

Conflicts of interest

There are no conflicts to declare.

Author Contributions

M.N.Yesibolati, H.Y.Sun, and K.Mølhave proposed and directed the research; M.N.Yesibolati and H.Y.Sun conducted experiments. M.N.Yesibolati, K.I.Mortensen, and K.Mølhave carried out data analysis and wrote the manuscript; A.Brostrøm and S.Tidemand-Lichtenberg assisted with the data analysis. All authors discussed the results and approved the final version of the manuscript.

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