Nanoscale Interparticle Distance within Dimers in Solution Measured by Light Scattering

Roland W. L. van Vliembergen,†‡ Leo J. van IJzendoorn,†§ and Menno W. J. Prins*†‡§¶

†Department of Applied Physics, ‡Institute for Complex Molecular Systems, and §Department of Biomedical Engineering, Eindhoven University of Technology, 5612 AZ Eindhoven, Netherlands

Supporting Information

ABSTRACT: We demonstrate a novel approach to quantify the interparticle distance in colloidal dimers using Mie scattering. The interparticle distance is varied in a controlled way by changing the ionic strength of the solution and the magnetic attraction between the particles. The measured scaling behavior is interpreted using an energy–distance model that includes the repulsive electrostatic and attractive magnetic interactions. The center-to-center distances of particles with a 525 nm radius can be determined with a root-mean-square accuracy of 12 nm. The data show that the center-to-center distance is larger by 83 nm compared to perfect spheres. The underlying distance offset can be attributed to repulsion by charged protrusions caused by particle surface roughness. The measurement method accurately quantifies interparticle distances that can be used to study cluster formation and colloid aggregation in complex systems, e.g., in biosensing applications.

INTRODUCTION

The stability of nanoparticle dispersions lies at the basis of colloid science1 and is of prime importance for its applications.2,3 The stability behavior results from the sum of interparticle forces with different signs and length ranges, e.g., the Hamaker force,4 electrostatic forces,4 entropic forces such as the hydrophobic effect,5 and the depletion force.6 Calculations of these forces typically assume particles with chemically and physically homogeneous surfaces. In reality, the interparticle forces depend on the surface roughness and on the chemical heterogeneity of the particle surface. Particle clustering and particle–substrate interactions are sensitive to these heterogeneities, but experimental techniques to address this are scarce.

Methods have been developed to study interparticle forces as a function of interparticle distance, where distances are typically in the nanometer range, energies in the range of kBT, and forces in the pN range. A commonly applied tool has been colloidal probe microscopy,7 where a particle is attached to an atomic force microscope (AFM) tip and consequently brought near to the surface of another particle that is immobilized on a substrate. The method allows accurate quantification of the force and interparticle distance and has been used to test interaction models and fit the interaction force constants.8 However, a limitation of colloidal probe microscopy is that the precise position of the interaction planes of the outer surfaces of the particles cannot be measured with respect to the centers of the particles. Consequently, a potential distance offset of the interparticle interaction, for example, caused by particle surface roughness or by molecules adsorbed onto the particles, cannot be revealed. Another approach to determine the interparticle distance is to use a long chain of particles and use Bragg diffraction10 or video microscopy to measure the total length of the chain.11 These methods determine the average interparticle distance in a chain, which has contributions from particle polydispersity as well as roughness.

In a previous theoretical paper, we have predicted that it should be possible to accurately measure center-to-center distances in particle dimers based on angle-dependent measurements of optical scattering.12 Here we demonstrate experimentally that dimers of magnetic particles can be formed and aligned in solution using magnetic fields and that the center-to-center distance of the particles can be quantified with nanoscale precision from the optical scattering signals. By comparing Mie scattering calculations with measured signals, we are able to quantify the interparticle distance in the range between 10 and 150 nm. The data clearly indicate that the dimers have a repulsive distance offset of 83 nm, which can be understood from the surface roughness of the particles. We will present the experimental method, the optical scattering simulations, the model for interparticle forces, and the observed scaling relationships. Finally, we will discuss how the method can be applied to study effects of surface roughness and molecular adsorption on interparticle interactions, in solutions with complex molecular composition, and in bioanalytical assays.

Received: July 28, 2017
Revised: November 20, 2017
Published: November 28, 2017
**MATERIALS AND METHODS**

Light scattering of rotating particle dimers (Figure 1a) has been measured using the setup sketched in Figure 1b. Briefly, the setup consists of a square cuvette (internal size 1 mm) containing the sample solution, which is surrounded by an electromagnetic quadrupole. A laser (660 nm) is focused into the cuvette with a 150 mm lens. The depth of focus of the laser beam is 2.5 ± 0.5 mm, i.e., bigger than the size of the cuvette, so that the beam diameter is roughly constant inside the cuvette. Light is collected onto three photodiodes (PDs): one at a scattering angle of 30°, one at a scattering angle of 90°, and one in transmission. The scattering intensity is a lot smaller at these smaller scattering angles from contributing to the measurements, a slit is attached to the cuvette to block the refracted light from the rounded edges.

Interparticle distances have been measured for clusters (mainly dimers) of carboxylic MyOne particles (Thermo-Fisher). The particles are pipetted from the stock solution and magnetically washed three times in a 30 μM solution of sodium chloride, at a particle concentration of 1 mg mL⁻¹. As the stock solutions always contain a fraction of clustered particles, the colloid is then treated with an ultrasonic finger in order to break up these clusters. Finally, just before the experiments the particles are diluted an additional 10 times (to 0.1 mg mL⁻¹), using a solution of sodium chloride with a concentration in the range from 30 μM to 111 mM (to obtain 100 mM), and the solution is mixed and inserted into the cuvette using a syringe.

The zeta potential of the particles has been measured at various salt concentrations using a Malvern Instruments Zetasizer Nano ZS. At the lowest used salt concentration (30 μM) a value of ψ = −57 ± 3 mV was obtained, while at the highest salt concentration (100 mM) a value of ψ = −37 ± 2 mV was measured. Therefore, the particles are negatively charged in all experiments.

A magnetic field is applied to form and align dimers in the experiments: a rotating magnetic field (rotating at 5 Hz) is applied during 10 s, after which the field is turned off, also for 10 s. The rotation frequency of 5 Hz generates stably rotating dimers over the range of used magnetic fields (3–12 mT). The scattering signals are measured over 25 repeated field sequences (10 s field on, 10 s field off). The signals are averaged over these 25 consecutive sequences to get rid of most of the variations (mainly due to Brownian motion of the particles).

**RESULTS AND DISCUSSION**

The particles used in this paper have a radius of 525 nm, comparable to the wavelength of the light. As such they exhibit Mie scattering which results in a spatial scattering pattern with minimums and maxima at different scattering angles. For dimers, the scattered light from both individual particles is coupled and results in a spatial distribution of the scattered light that depends on their mutual distance and the dimer orientation with respect to the laser beam. The distance dependence is especially apparent for near-perpendicular scattering angles, which is why a photodiode at 90° is used.

In order to control the interparticle distance in the dimers, the electrostatic repulsion and magnetic attraction have been varied. The electrostatic repulsion was varied by altering the Debye screening length via the ionic strength of the solution, the balance of forces leads to an equilibrium distance h, which will be defined as their center–center distance L minus 2R, where R is the radius of a sphere of identical volume. The magnetic moments cause an attractive magnetic force. The surface charges cause electrostatic repulsion, partly screened by ions in the solution. The interparticle distances have been measured for clusters (mainly dimers) of carboxylic MyOne particles (Thermo-Fisher). The particles are pipetted from the stock solution and magnetically washed three times in a 30 μM solution of sodium chloride, at a particle concentration of 1 mg mL⁻¹. As the stock solutions always contain a fraction of clustered particles, the colloid is then treated with an ultrasonic finger in order to break up these clusters. Finally, just before the experiments the particles are diluted an additional 10 times (to 0.1 mg mL⁻¹), using a solution of sodium chloride with a concentration in the range from 30 μM to 111 mM (to obtain 100 mM), and the solution is mixed and inserted into the cuvette using a syringe.

The zeta potential of the particles has been measured at various salt concentrations using a Malvern Instruments Zetasizer Nano ZS. At the lowest used salt concentration (30 μM) a value of ψ = −57 ± 3 mV was obtained, while at the highest salt concentration (100 mM) a value of ψ = −37 ± 2 mV was measured. Therefore, the particles are negatively charged in all experiments.

A magnetic field is applied to form and align dimers in the experiments: a rotating magnetic field (rotating at 5 Hz) is applied during 10 s, after which the field is turned off, also for 10 s. The rotation frequency of 5 Hz generates stably rotating dimers over the range of used magnetic fields (3–12 mT). The scattering signals are measured over 25 repeated field sequences (10 s field on, 10 s field off). The signals are averaged over these 25 consecutive sequences to get rid of most of the variations (mainly due to Brownian motion of the particles).
and the magnetic attraction was varied by altering the magnetization of the MyOne particles via the magnitude of the applied magnetic field. Figure 1c shows the intensity of the scattered light as a function of time for two experiments: one at a low salt concentration (30 μM, in red) and one at a high salt concentration (10 mM, in black). The magnetic field strength is 10 mT in both experiments. The signals in Figure 1c show clear oscillations, and these depend on the salt concentration. The dependence is visible in the relative magnitude of the peaks, the number of peaks, and shifts of the peak positions along the time axis. In the 30 μM experiment, the central peak (around 9.63 s) has the largest magnitude, while in the 10 mM case the adjacent peaks are the highest, as marked by the arrows. Regarding the number of peaks, at 30 μM an additional peak is present at around 9.59 s (marked with an arrow), in contrast to the minimum at the same location at 10 mM. Finally, also the peak positions are shifted, as can be seen from the dashed lines connecting some of the peaks of the 30 μM experiment to the horizontal axis. The corresponding peaks occur further apart in the experiment at 10 mM.

The measured signals can be further studied by analyzing their frequency spectrum with a Fourier transform, as shown in Figure 1d. The frequency spectrum contains peaks at multiples of the rotation frequency. The amplitude of the peaks in the frequency spectrum is proportional to the number of dimers but also depends on the interparticle distance. By evaluating the ratio of two Fourier components, the concentration dependence is divided out, making it a metric that should depend only on the distance. Figure 1d indicates that the amplitudes of the 8f and 10f Fourier components change significantly, with the 8f being larger than the 10f component for the 10 mM salt concentration and the reverse being the case for the 30 μM salt concentration. Hence, the ratio of the 8f and 10f Fourier components changes with salt concentration and might be a good metric for determining the interparticle distance.

In Figure 2a, the ratio of the 8f and 10f components is plotted against the salt concentration, at a constant magnetic field of 10 mT. This ratio increases by more than an order of magnitude from 0.2 to 4 as the salt concentration is increased from 30 μM to 10 mM. At an even higher salt concentration of 100 mM the ratio decreases to around 2. At two salt concentrations (100 μM and 1 mM) more than five measurements have been performed using different cuvettes. The observed cuvette-to-cuvette variability of the 8f/10f ratio is bigger than the error bars. Most likely, this is caused by small changes in the exact positioning of the cuvette and the slit blocking its edges.

In Figure 2b, the ratio of the 8f and 10f components is plotted against the magnetic field strength, at a constant salt concentration of 500 μM, showing a clear increase of the 8f/10f ratio with field strength. The error bars in the Fourier components have been determined by calculating the 8f/10f ratio for 25 individual rotating field pulses and determining the standard deviation.

Figure 2 shows that the ratio of the 8f and 10f Fourier components depends on both the salt concentration and magnetic field strength. An increased magnetic field generates higher attractive forces and thus a smaller interparticle distance. Likewise, an increased salt concentration corresponds to the repulsion decaying over shorter distances, causing a smaller distance between the particles. In both cases, the general trend in the measurements is an increasing 8f/10f ratio. In order to study this in more detail, scattering simulations have been performed to verify the dependence of the Fourier components on the interparticle distance. In addition, a model has been developed to estimate the distance between particles in a dimer for a given salt concentration and magnetic field strength.

**Scattering Simulations.** Two-particle Mie scattering simulations have been performed, using the MSTM v. 3.0 code written by D. W. Mackowski.\textsuperscript{13} The particles are represented in the simulations by perfect spheres with a distance \( L \) between their centers. The values for the parameters are identical to those in a previous theoretical paper.\textsuperscript{12} Specifically, the diameter of the particles is 1050 nm, the refractive index of the particles is \( n = 1.68 + 0.005i \), and the refractive index of the solution is 1.331. In the following, only scattering angles of 90° are considered, as these correspond to the measured PD signals at 90°.

These simulations have been performed for a range of rotation angles with a resolution of 0.3° and for interparticle distances from 0 to 250 nm with steps of 1 nm. Results for three values of the distance have been plotted in Figure S2a. By applying a Fourier transform of the simulated time traces (plotted in Figure S2b), the simulated 8f/10f ratio can be calculated as a function of distance, as plotted in Figure 3.

The ratio of the 8f and 10f Fourier components in the simulations increases for interparticle distances between 0 and 80 nm and between 180 and 250 nm. Between 80 and 180 nm interparticle distance, the ratio is decreasing. As Figure 2b shows, the ratio increased with a stronger magnetic field, at a constant salt concentration of 500 μM, i.e., with decreasing...
interparticle distance. Combining this result with Figure 3 implies that the corresponding value of $L - 2R$ in the experiments must have been between 80 and 180 nm. More specifically, a ratio of 1.2 implies $L - 2R = 141$ nm and a ratio of 4 implies $L - 2R = 110$ nm.

Comparing the simulation results to Figure 2a, it is clear that the decreasing values of the $8f/10f$ Fourier ratio at the highest salt concentrations correspond to values of $L - 2R$ less than 80 nm. The steep increase observed at the lower salt concentrations still gets mapped to the part between the 80 and the 180 nm intersphere distance. A value of 0.18 as observed for 30 μM salt concentration corresponds to $L - 2R = 180$ nm, and the value of 2.1 observed at 100 mM corresponds to $L - 2R = 30$ nm.

Apart from simulations on dimers of MyOne particles, also simulations on trimers and dimers of 100 nm diameter particles have been performed. These are plotted in Figure S2c–f. In general, bigger interparticle distances result in higher frequency components being present. This enables using a ratio of two Fourier components to determine the distance. While the $8f/10f$ ratio works quite well for dimers of MyOne particles, for differently sized particles, a different ratio will work better. For instance, for those 100 nm particles the $4f/2f$ ratio increases with distance between 75 and 225 nm. The trimer simulations, especially at larger interparticle distances, do not resemble our experimental results, while the dimer simulations do. As such, it can be concluded that the presented measurements are dominated by the dimer scattering.

**Interparticle Distance Model.** The distance between two particles in a dimer, $h$, can then be defined as the distance between the particle centers, $L$, minus the radii of both particles, where the radius is defined as the radius of a sphere of identical volume. For a dimer consisting of two identical particles with a net surface charge, the interparticle distance results from a balance between the electrostatic repulsion and attractive magnetic dipole–dipole interaction. Here we will model these interactions and calculate the equilibrium interparticle distance, $h_{eq}$. In addition, we will discuss the role of particle roughness and the influence of viscous drag on the interparticle distance in a rotating dimer. Comparing these distances to those obtained by matching the experiments to the simulations will also allow information on the roughness of the particles to be extracted, as explained in Figure 4.

**Particle Properties.** Before discussing the interactions, we will first review a few important properties of the carboxylic magnetic particles. We measured the zeta potential of the particles in the solutions that are used in the scattering experiments. For all salt concentrations, except the highest, the zeta potential was close to $-60$ mV; for the highest salt concentration (100 mM), the zeta potential was $\psi = -37 \pm 1$ mV (see Table S1). SEM images (see Figure 1a and Figure S1) show that the MyOne particles are rough spherical particles with protrusions that have a size up to 100 nm. The average diameter of the MyOne particles is 1.05 μm with a coefficient of variation (CV) of 1.9%, according to Fonnum et al. The manufacturer’s specifications report the same diameter, but a slightly higher CV (3%).

The magnetic properties of the MyOne particles have been described by Lipfert et al., who fitted the vendor supplied magnetization curve of an ensemble of particles with the Langevin function. In this study we convert the magnetization to a dipole moment $m(B)$ using the particle radius:

$$m(B) = \frac{4}{3} \pi R^3 M(B) = m_{sat} \left[ \coth \left( \frac{B}{B_0} \right) - \frac{B_0}{B} \right]$$

with the fit parameters $m_{sat} = 2.62 \times 10^{-15}$ A m$^2$ and $B_0 = 12$ mT based on Lipfert et al.

**Electrostatic Interaction.** The electrostatic interaction between particles is determined by the charge on the particles (related to the zeta potential) and the Debye screening length, $\kappa^{-1}$:

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_i \varepsilon_0 k_B T}{2 N_A e^2 I}}$$

with $\varepsilon_i$ the dielectric constant of water, $\varepsilon_0$ the permittivity of free space, $k_B$ the Boltzmann constant, $T$ the absolute temperature, $N_A$ the Avogadro number, $e$ the elementary charge, and $I$ the ionic strength of the solution. In the case of monovalent electrolytes, such as sodium chloride, the ionic strength is identical to the molar concentration of the electrolyte. Typical values for the Debye length range from 1.0 nm at 100 mM ionic strength to 56 nm at 30 μM.

An approximation for the interaction free energy valid for all $k\kappa$, large $k\kappa$, and up to moderately high surface potentials ($\psi \lesssim 4kT/(ze)$) is given by:

$$\Delta E_{es}(k) = 4\pi \varepsilon_i \varepsilon_0 \left( \frac{k_B T}{e} \right)^2 Y(h)^2 \frac{k^2}{2R + h} \ln[1 + \exp(-k\kappa)]$$

with $z$ the electrolyte valence, in this case $z = 1$, and
determined by distances at an energy of 1 spread in distance due to thermal motion by considering the dependence of the free energy on the distance also gives the solid line corresponds to eq 6 for the viscous drag, whereas the dashed line corresponds to eq 7. The inset shows the modeled distance at a a cannot physically overlap. Also, the electrostatic interaction is limit the minimal distance between the particles, as the particles particles. First, the protrusions caused by surface roughness of particle surface roughness on the distance between two dimer. This corresponds to the parameter \( \alpha_{\text{lag}} \) above 6 mT), both hydrodynamic limits lead to the same interaction between the particles would be dominant.

**Viscous Drag.** The rotating particle pairs experience a viscous drag caused by the interaction with the fluid. As a consequence, the axis through the centers of the particles lags behind the field. For particles rigidly bound to each other, the torque from the hydrodynamic drag can be determined by adding the contributions from the particles’ translation and rotation. This results in a phase lag:

\[
\alpha_{\text{lag}} = \arcsin \left( \frac{4\pi L^3}{3\mu_0 m^2} [12\pi \eta R (L/2)^2 \omega + 16\pi R^2 \eta \omega] \right)
\]

with \( \eta \) the dynamic viscosity of the liquid, which for water at room temperature is 1.0 mPa s, and \( \omega \) the angular velocity, \( \omega = 10\pi \) rad s\(^{-1}\).

In the experiment of Figure 1, the particles are trapped in an electrostatic–magnetic interparticle potential and are not rigidly bound to each other. So another possible hydrodynamic configuration could be that the particles move around each other and individually do not rotate. In this case the viscous drag corresponds to only the Stokes drag, which results in a phase lag:

\[
\alpha_{\text{lag}} = \arcsin \left( \frac{4\pi L^3}{3\mu_0 m^2} [12\pi \eta R (L/2)^2 \omega] \right)
\]

This equation applies when two particles are far apart and do not experience any hydrodynamic coupling. However, in our experiment the particles are close together in a dimer, so there will be hydrodynamic coupling between the particles and hence the phase lag is expected to be between the limits of eqs 6 and 7.

Both limits for the viscous drag have been explored and are used to calculate the interparticle distance. This distance has been calculated by solving the distance at which the sum of the attractive and repulsive free energies is minimal. The interparticle distance is plotted in Figure 5a as a function of the magnetic field strength for different salt concentrations. As it turns out, if the magnetic field is strong enough (in this case above 6 mT), both hydrodynamic limits lead to the same interparticle distance.

For low magnetic fields, it is known that dimers can exhibit a wiggling motion with distance oscillations. Meanwhile, the

**Magnetic Dipole–Dipole Interaction.** The MyOne particles are magnetized by the rotating field of the electromagnet and each particle acquires a magnetic moment described by eq 1. The attractive dipole–dipole force between the particles induces the formation of clusters. The corresponding free energy for a rotating dimer is

\[
\Delta E_{\text{dip}} = -\frac{\mu_0}{4\pi} \frac{|m(B)|^2}{L^3} [2 \cos(\alpha_{\text{lag}})^2 - \sin(\alpha_{\text{lag}})^2]
\]

with \( \alpha_{\text{lag}} \), the phase lag between the applied field and the central axis of the dimer. This phase lag can be derived from balancing the magnetic torque and the torque from the hydrodynamic drag, which will be discussed later. For now, we can neglect the effects of the phase lag on the interaction, setting it to zero, an approximation that is accurate in the case of sufficiently high magnetic fields.

With the electrostatic free energy and the magnetic dipole–dipole free energy derived, the interparticle distance \( h_e \) can be determined by finding the minimum of the free energy. The dependence of the free energy on the distance also gives the spread in distance due to thermal motion by considering the distances at an energy of \( 1 k_B T \) over the minimum of the distribution. However, it will turn out that the thermal distance spread is much smaller than the distance variability due to particle properties.

**Particle Surface Roughness.** Here we analyze the influence of particle surface roughness on the distance between two particles. First, the protrusions caused by surface roughness limit the minimal distance between the particles, as the particles cannot physically overlap. Also, the electrostatic interaction is affected, as it is strong between protrusions due to the closer proximity. In contrast, the magnetic dipole–dipole force is not directly affected by surface roughness because it is volume-based rather than surface-based.

Here, we model the influence of particle surface roughness by an effective increase in the center-to-center distance of the dimer. This corresponds to the parameter \( \Delta L \) introduced in Figure 4. If roughness is dominant, it should be positive, with its value being a measure for the roughness of the particles. On the other hand if negative values are found, an attractive interaction between the particles would be dominant.
dimers with the highest magnetic content might still be able to rotate normally. As a combination of different behaviors is difficult to model, this regime is avoided and only magnetic fields of 3.6 mT and above are used in the experiments.

**Experimental Fourier Ratio versus Modeled Distance.**

Using the distance model, the magnetic field strengths and ionic concentrations from the experiments have been converted into distances. Figure 5b shows all measured 8f/10f Fourier ratio values as a function of the modeled distance between the particles. The 8f/10f ratio changes by more than an order of magnitude, and interestingly the data points for all salt concentrations lie on one curve (the dashed line serves as a guide to the eye). The measured 8f/10f ratio shows a similar behavior to the simulated ratio in Figure 3. In both cases the ratio increases to a maximum, at $h_E \approx 25$ nm and $L - 2R \approx 80$ nm, and then decreases to a minimum, at $h_E \approx 100$ nm and $L - 2R \approx 185$ nm, after which the ratio increases again. Comparing the distances at which the minimum ratio occurs, $\Delta L$ should be about 85 nm. The experimental position of the maximum ratio occurs for $h_E < \Delta L$, so it will be affected by the protrusions on the particles, so for a determination of $\Delta L$ the maximum is not meaningful.

The minimum in the measured 8f/10f ratio is less sharp than in the simulations (around 185 nm); this is caused by the 8f component not going to zero in the experiments. This can be attributed to the spread in particle properties such as the surface charge and magnetic content (also related to the size distribution). Also, due to the low interaction energy at these larger distances, angular spread due to Brownian motion and a small amount of dimer breaking and re-formation could play a role in generating a nonzero 8f component at large interparticle distances. The size distribution of the particles itself has barely any effect; as for the 3% polydispersity of the shape of the signal, and thus the ratio of Fourier components, is almost identical to that for perfectly monodisperse particles.12

**Determining the Distance Using More Fourier Components.**

Figures 3 and 5b show that a 8f to 10f Fourier component ratio does not uniquely relate to one distance. One way to avoid this ambiguity is to include more Fourier components. In order to determine the distance we therefore investigated the following minimized sum of the squared residuals:

$$S = \sum \left( \frac{\langle nf \rangle_{\text{meas}} - A \langle nf \rangle_{\text{sim}}(L_{\text{fit}})}{\text{fit}} \right)^2$$

where $A$ is an amplitude factor, and $L_{\text{fit}}$ is the fitted center–center distance, and both for the measured, $\langle nf \rangle_{\text{meas}}$ and simulated Fourier amplitudes, $\langle nf \rangle_{\text{sim}}$ the 6f, 8f, 10f, 12f, 14f, and 16f Fourier components have been summed; i.e., the sum is over $n$ with $n$ even and from 6 until 16.

These fitted distances are compared to the modeled distances in Figure 6. As can be seen there is a strong correlation between the fitted distances and the modeled distances. The dashed line corresponds to the points where the fitted distance equals the modeled distance. On that line, no measured points are observed. This indicates that an additional distance is present, as indicated by the dash-dotted line with $\Delta L = 83$ nm.

Now we analyze possible origins of this value of $\Delta L$. First, the average size of the particles might be different from that specified by the manufacturer, for example, because of batch-to-batch variations. In order to verify this, simulations have been done of the scattering of particles of different radius (see Figure S3). These simulations indicate the scattering is not strongly dependent on $L$. Even more so, near the distance where the 8f/10f ratio is minimal, the 8f/10f ratio does not change for particles 50 nm larger than those specified. So the only way to get a $\Delta L$ of 80 nm in this case would be if the particles are actually 80 nm larger for identical $h_E$. A discrepancy of this order of magnitude is highly unlikely. A second explanation could be the inaccuracy in the measured zeta potential. An increase in zeta potential of 10% causes an increase in the electrostatic energy of 8%. For the lowest salt concentration ($\lambda_D = 56$ nm) this would cause a 4 nm increase in the distance between the particles, which is very small. Third, the magnetic content might be different, for example, due to batch-to-batch variation. If the magnetic content would be lower by 10%, the magnetic energy would be lower by almost twice that amount because the energy scales with the square of the magnetization. This would, at the lowest salt concentration with $\lambda_D = 56$ nm, cause an increase in distance of 12 nm, which is again a very small distance.

Therefore, we attribute the 83 nm distance offset mainly to the presence of particle surface roughness. The value of $\Delta L = 83$ nm provides good agreement for most of the data points, so the used approximations are quite accurate. In principle, $\Delta L$ might depend on the interparticle distance. For the regime where the data points agree, $L_{\text{fit}} - 2R > \Delta L$, the standard deviation of the difference between $h_E$ and $L_{\text{fit}} - 2R$ is 12 nm, making this a measure for the distance accuracy of this method. This small value of the standard deviation is remarkable, in view of the significant amount of roughness on the particles. Probably the ensemble averaging inherent to the averaging of the pulses in a pulse train, and the presence of multiple dimers in the laser volume leads to very reproducible values for the distance.

The only regime where the data points deviate from $\Delta L = 83$ nm is the regime where $L_{\text{fit}} - 2R < \Delta L$, i.e., the regime where the protrusions might be so close that actually permanently

![Figure 6. Fitted center-to-center distance based on the 6f, 8f, 10f, 12f, 14f, and 16f Fourier components plotted versus the modeled separation distance $h_E$. The bottom dashed line corresponds to perfectly spherical particles with $L_{\text{fit}} - 2R = h_E$: this line does not match the data. The top dash-dotted line corresponds to particles with a center-to-center distance that is offset by $\Delta L = 83$ nm. We attribute this offset to protrusions on the particles, related to surface roughness (see Figure 4b). The data plotted with open circles correspond to experiments at high salt concentrations where a high magnetic field is or has been applied, presumably generating particle pairs with physical contact between the protrusions.](image)
bound dimers can be formed. The corresponding data points are shown as open circles. What is especially interesting are the points around \( h_0 = 50 \) nm. These correspond to experiments where the same solution was exposed to three sets of 25 pulses, with the field strength first low, then high, and then low again. Initially a high value (>1150 nm) for \( L_{\text{mod}} \) is observed. When applying the higher field, the particles get bound and have a \( L_{\text{mod}} \approx 1100 \) nm. While applying the original field again, \( L_{\text{mod}} \) remains small instead of returning to the initial value, indicating that an irreversible binding has occurred. As such, the modeled distance in these cases is no longer applicable, as other forces such as the Hamaker force are responsible for keeping the particles together. Nevertheless, it is very interesting that this technique is able to distinguish between different kinds of dimers: the solvent and used magnetic fields are identical; still clearly different signals are observed before and after the irreversible binding.

**CONCLUSIONS**

We have described a method to quantify the interparticle distance in particle dimers rotating in solution. The center-to-center distance between the particles was obtained from measured optical scattering signals, and the interparticle distance was modulated by systematically varying the ionic strength of the solution and the magnetic attraction between the particles. The comparison between model and experiment leads to the conclusion that the center-to-center distance is larger by 83 nm compared to perfect spheres, which is attributed to repulsion by charged protrusions caused by particle surface roughness. To our knowledge this represents the first measurement of nanoscale distance offsets between colloidal particles in solution.

The method can now be used to study how interparticle distances in solution are influenced by changes of particle properties, e.g., surface roughness of the particles, adsorption of macromolecules (e.g., protein corona), soft nanoparticles (e.g., viruses or polymers) or hard nanoparticles (e.g., inorganic nanoparticles), or how interparticle distances are influenced by properties of the solution in which the particles are suspended. The distance resolution of the technique may be improved by using spherical particles with smooth surfaces, so that even a submonolayer coverage of polymers or proteins might become detectable. This will be interesting for fundamental research as well as for applications in solution-based cluster assays for protein detection.\(^{21}\) Also, nonspherical particles will be interesting to study, e.g., particles with well-defined charge inhomogeneities or even Janus particles. While this paper demonstrated the method for particles in solution, it will also be applicable for measuring interparticle distances within dimers at fluid–fluid interfaces. Controlled rotation of dimers at a fluid–fluid interface should lead to angle-dependent scattering signals that can be interpreted in terms of the nanoscale interparticle distance. Finally, while in this paper dimers of magnetic particles have been studied, we expect that this method of determining interparticle distances may also be applicable beyond the magnetic domain, e.g., using alignment and rotation based on optical traps, electrical fields, or acoustical fields.\(^{24,25}\)

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b02634.

SEM images of the particles, measured zeta potentials, simulated scattering signals, and the influence of the particle diameter on the 8f/10f ratio (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

E-mail: m.w.j.prins@tue.nl (M.W.J.P.).

**ORCID**

Menno W. J. Prins: 0000-0002-9788-7298

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work is supported by NanoNextNL, a micro and nanotechnology consortium of the Government of The Netherlands and 130 partners.

**REFERENCES**

(1) Hardy, W. A preliminary investigation of the conditions which determine the stability of irreversible hydrosols. Proc. R. Soc. London 1899, 66, 110–125.

(2) Green, H. A photomicrographic method for the determination of particle size of paint and rubber pigments. J. Franklin Inst. 1921, 192, 637–666.

(3) Zhang, L.; Gu, F.; Chan, J.; Wang, A.; Langer, R.; Farokhzad, O. Nanoparticles in Medicine: Therapeutic Applications and Developments. Clin. Pharmacol. Ther. 2008, 83, 761–769.

(4) Hamaker, H. C. The London—van der Waals attraction between spherical particles. Physica 1937, 4, 1058–1072.

(5) Derjaguin, B. On the repulsive forces between charged colloid particles and on the theory of slow coagulation and stability of lyophobe sols. Trans. Faraday Soc. 1940, 35, 203–215.

(6) Asakura, S.; Oosawa, F. On Interaction between Two Bodies Immersed in a Solution of Macromolecules. J. Chem. Phys. 1954, 22, 1255–1256.

(7) Kappl, M.; Butt, H.-J. The Colloidal Probe Technique and its Application to Adhesion Force Measurements. Part. Part. Syst. Charact. 2002, 19, 129–143.

(8) Ducker, W. A.; Senden, T. J.; Pashley, R. M. Measurement of forces in liquids using a force microsce. Langmuir 1992, 8, 1831–1836.

(9) Andersson, K. M.; Bergström, L. DLVO Interactions of Tungsten Oxide and Cobalt Oxide Surfaces Measured with the Colloidal Probe Technique. J. Colloid Interface Sci. 2002, 246, 309–315.

(10) Calderon, F. L.; Stora, T.; Mondain Monval, O.; Poulin, P.; Bibette, J. Direct measurement of colloidal forces. Phys. Rev. Lett. 1994, 72, 2959–2962.

(11) Li, D.; Lam, C. N.; Biswal, S. L. Measuring short-range repulsive forces by imaging directed magnetic-particle assembly title. Soft Matter 2010, 6, 239–242.

(12) van Vliembergen, R. W. L.; van IJzendoom, L. J.; Prins, M. W. J. Distance within colloidal dimers probed by rotation-induced oscillations of scattered light. Opt. Express 2016, 24, A123–A138.

(13) Mackowski, D. A general superposition solution for electro-magnetic scattering by multiple spherical domains of optically active media. J. Quant. Spectrosc. Radiat. Transfer 2014, 133, 264–270.

(14) van Ommering, K.; Somers, P. A.; Koets, M.; Schleipen, J. J. H. B.; van IJzendoom, L. J.; Prins, M. W. J. Mobility and height detection of particle labels in an optical evanescent wave biosensor with single-label resolution. J. Phys. D: Appl. Phys. 2010, 43, 155501.
(15) Fonnum, G.; Johansson, C.; Molteberg, A.; Mørup, S.; Aksnes, E. Characterisation of Dynabeads by magnetization measurements and Mössbauer spectroscopy. *J. Magn. Magn. Mater.* 2005, 293, 41−47.
(16) Lipfert, J.; Hao, X.; Dekker, N. H. Quantitative Modeling and Optimization of Magnetic Tweezers. *Biophys. J.* 2009, 96, 5040−5049.
(17) Sader, J. E.; Carnie, S. L.; Chan, D. Y. C. Accurate Analytic Formulas for the Double-Layer Interaction between Spheres. *J. Colloid Interface Sci.* 1995, 171, 46−54.
(18) Griffiths, D. J. *Introduction to Electrodynamics*, 3rd ed.; Pearson Education, Inc.: 2008.
(19) Ranzoni, A.; Janssen, X. J. A.; Ovsyanko, M.; van IJzendoorn, L. J.; Prins, M. W. J. Magnetically controlled rotation and torque of uniaxial microactuators for lab-on-a-chip applications. *Lab Chip* 2010, 10, 179−188.
(20) Domínguez-García, P.; Melle, S.; Calderón, O. G.; Rubio, M. Doublet dynamics of magnetizable particles under frequency modulated rotating fields. *Colloids Surf.*, A 2005, 270−271, 270−276.
(21) Ranzoni, A.; Schleipen, J. H. B.; van IJzendoorn, L. J.; Prins, M. W. J. Frequency-Selective Rotation of Two-Particle Nanoactuators for Rapid and Sensitive Detection of Biomolecules. *Nano Lett.* 2011, 11, 2017−2022.
(22) Zhang, P.; Hernandez, D.; Cannan, D.; Hu, Y.; Fardad, S.; Huang, S.; Chen, J. C.; Christodoulides, D. N.; Chen, Z. Trapping and rotating microparticles and bacteria with moiré-based optical propelling beams. *Biomed. Opt. Express* 2012, 3, 1891−1897.
(23) Fan, D. L.; Zhu, F. Q.; Cammarata, R. C.; Chien, C. L. Controllable High-Speed Rotation of Nanowires. *Phys. Rev. Lett.* 2005, 94, 247208.
(24) Zhang, X.; Zheng, Y.; Hu, J. Sound controlled rotation of a cluster of small particles on an ultrasonically vibrating metal strip. *Appl. Phys. Lett.* 2008, 92, 024109.
(25) Baasch, T.; Leibacher, I.; Dual, J. Multibody dynamics in acoustophoresis. *J. Acoust. Soc. Am.* 2017, 141, 1664−1674.