Distance within colloidal dimers probed by rotation-induced oscillations of scattered light

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Abstract: Aggregation processes of colloidal particles are of broad scientific and technological relevance. The earliest stage of aggregation, when dimers appear in an ensemble of single particles, is very important to characterize because it opens routes for further aggregation processes. Furthermore, it represents the most sensitive phase of diagnostic aggregation assays. Here, we characterize dimers by rotating them in a magnetic field and by recording the angle dependence of light scattering. At small scattering angles, the scattering cross section can be approximated by the total cross-sectional area of the dimer. In contrast, at scattering angles around 90 degrees, we reveal that the dependence of the scattering cross section on the dimer angle shows a series of peaks per single $2\pi$ rotation of the dimers. These characteristics originate from optical interactions between the two particles, as we have verified with two-particle Mie scattering simulations. We have studied in detail the angular positions of the peaks. It appears from simulations that the influence of particle size polydispersity, Brownian rotation and refractive index on the angular positions of the peaks is relatively small. However, the angular positions of the peaks strongly depend on the distance between the particles. We find a good correspondence between measured data and calculations for a gap of 180 nm between particles having a diameter of 1 micrometer. The experiment and simulations pave the way for extracting distance-specific data from ensembles of dimerizing colloidal particles, with application for sensitive diagnostic aggregation assays.

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OCIS codes: (170.0170) Medical optics and biotechnology; (290.0290) Scattering; (290.5820) Scattering measurements; (290.5850) Scattering, particles.

References and links

1. Introduction

Aggregation processes of colloidal particles and macromolecules are scientifically of strong interest and are important for many technological applications. The aggregation of macromolecules such as amyloid proteins is related to several diseases, such as Alzheimer’s and Parkinson’s disease [1,2]. The aggregation and self-assembly of colloidal particles is being studied in order to develop new functional nanostructures [3,4]. Furthermore, macromolecules can play a key role in inducing the aggregation of colloidal particles. This is for example the case in aggregation assays where the formation of particle clusters reveals the presence of analyte in solution, for the sensitive and fast detection of diagnostic biomarkers [5–9].

Within aggregation processes, the earliest stages of aggregation (dimers, trimers) are most...
important to understand as they induce cascade pathways toward larger aggregates. For instance, the first phase of amyloid aggregation consists of the association of monomers, which then trigger the growth of fibrils [2]. And for the colloidal self-assembly of nanostructures, it is important to quantitatively understand the interaction forces leading to dimers and trimers, as these represent the nuclei around which larger structures are formed [4].

The earliest stages of aggregation are hard to resolve in aggregation processes, because low numbers of clusters need to be detected against a background of a high concentration of unclustered particles. An established analytical tool is Dynamic Light Scattering (DLS), which is able to quantify the average diameter of particles in a colloidal solution [10]. Unfortunately the method is not suited to study and quantify the earliest stages of particle aggregation, because the signals are dominated by the background of unclustered particles rather than the low numbers of clustered particles.

In earlier work we have developed a technique to study superparamagnetic dimers by rotating them in a magnetic field while measuring the laser light scattered by the dimers [7,8]. Because of the rotation, the dimers are alternatingly aligned with the laser beam and perpendicular to the laser beam, which gives an oscillating light scattering cross section. This is inherently a background-free detection method: individual particles do not rotate, nor have a scattering cross-section that depends on rotation, so only the dimers (and trimers, etc.) give a signal.

Ranzoni et al. [7,8] focused on 500 nm particles and scattering at small angles (30°), which produced scattering signals with high similarity to the geometric cross-section of the dimers. In this paper, we demonstrate that the use of larger scattering angles (mainly 90°) and somewhat larger particles (1 micrometer) leads to rapidly oscillating scattering signals which can be used to reveal detailed information about the dimers. Simulations and experimental results are presented, asserting the influence of the refractive index, size polydispersity, Brownian rotation, and inter-particle distance. These results show that the distance between the particles is a key parameter to explain the rapid oscillations in the rotation-induced scattering signals. Finally we conclude by discussing the impact and applications of these findings for the study of aggregation processes and for diagnostic aggregation assays.

2. Experimental

The experiments presented in this paper have been performed with a setup similar to that used by Ranzoni et al. [7], displayed schematically in Fig. 1(a). A square cuvette with internal size of 1 mm and glass walls of width (0.2 ± 0.1) mm is in the center of the setup. It is filled with a solution containing the magnetic particles. Around this cuvette is a magnetic quadrupole, which in the presented experiments is used to create a 5 mT rotating magnetic field. Finally, a laser beam is focused in the cuvette, and scatters from the particles.

The experiments have been performed using superparamagnetic carboxylic MyOne particles (Dynabeads MyOne Carboxylic Acid, Thermo Fisher). Without the presence of a magnetic field, the particles are non-magnetic and unbound, and move randomly due to Brownian motion. In the presence of a magnetic field, they attract each other, because of the magnetic dipole-dipole interaction, and form magnetic dimers. In contrast to the experiments described by Ranzoni et al [7,8], the particles have not been functionalized and no chemical bond is formed between the particles.

The particles are first magnetically washed five times in MilliQ water, and a sonic needle is used to break up any clusters. Then the particles are dispersed at a concentration of 0.1 mg/mL in a mixture of 0.09 % Phosphate Buffered Saline (PBS) solution and 99.91 % MilliQ water. Just before the experiment, the solution is vortex mixed, and inserted into the cuvette using a syringe.

The zeta potential of these particles has been measured in a 1 mM sodium chloride solution,
using a Malvern Instruments Zetasizer Nano ZS. The found zeta potential has an average of $(-61 \pm 2) \text{ mV}$ with a rms spread of $5 \text{ mV}$. A zeta potential of this magnitude means that the particles repel each other, and form a stable colloidal suspension. I.e. in absence of a magnetic field no aggregation of the particles occurs.

The light source used to illuminate the cuvette is a diode laser (Opnext HL6545MG) with a wavelength $\lambda = 660 \text{ nm}$, which is focused with a $150 \text{ mm}$ lens. The waist of the laser beam in the focus is $(15 \pm 5) \mu \text{m}$ and the depth of focus of the beam is $(2.5 \pm 0.5) \text{ mm}$, so that the beam diameter is roughly constant over the entire length of the cuvette. In the experiments, the laser beam is horizontally polarized, using the intrinsic polarization of the laser diode. For the scattering in the $(y,z)$ plane this implies that the incident light is $p$ polarized. Light scattered...
from the cuvette is measured using an amplified photodiode (PD, Thorlabs PDA36A). Before it reaches the photodiode it is collected by a 75 mm lens and passes a polarizer that blocks vertically polarized light, i.e. only the light having identical polarization to that of the laser is transmitted.

In Fig. 1(b) the scattering geometry is represented schematically. A rotating magnetic field (angle $\alpha_f$) is used to rotate the particle dimers in the $(x,z)$-plane and at a given time the dimers are oriented at an angle $\alpha_d$ with respect to the $z$-axis. The light is detected in the $(y,z)$-plane with the scattering angle $\beta_s$ with respect to the $z$-axis.

In the experiments a rotating magnetic field with a frequency of 5 Hz is used. While the dimers are rotating, they also experience a viscous drag from the solvent. As a result of this, the dimers lag behind the field by a constant phase lag $\alpha_f - \alpha_d$ [11], in this case $(16 \pm 3)\degree$. At higher field rotation frequencies than those used in the experiments, the drag forces can become so large that the dimers can no longer keep up with the field.

It is important to realize that the angle at which the light is scattered by the dimers is generally not identical to the angle of the scattered light outside of the cuvette, which is a whole set of angles $\gamma^{(i)}$, with $i$ an index used to enumerate them. This is illustrated in Fig. 1(c). The scattered light experiences refraction and reflections when it passes from water, through glass to air. As a consequence a fraction $f^{(i)}$ of the light scattered at $\beta_s$ leaves the cuvette in direction $\gamma^{(i)}$. Only for light scattered at $\beta_s = 90\degree$ the angle outside of the cuvette is the same as the scattering angle. As an example, for $\beta_s = 22\degree$: $\gamma^{(1)} = 30\degree$.

Because of the rounded corners of the cuvette, it would however also be possible that light scattered at different angles experiences refraction in the corners and is still emitted at 90 degrees. To counter this, a slit is cut out of black photographic tape and is attached to the front of the cuvette, as shown schematically in Fig. 1(c). This way, the light refracting in the corners is absorbed by the tape.

The conversion from $\beta_s$ to the photodiode angle, $\gamma_{PD}$, has been modelled for the idealized case of a perfectly non-rounded square cuvette, using the Fresnel coefficients to determine the fractions $f^{(i)}$.

In the experiments, the field is alternatingly on for 10 seconds, and off for 10 seconds. While the field is on, short chains of particles are formed, which fall apart again when the field is off due to Brownian motion. These short chains mainly consist of dimers, based on the measured $(16 \pm 3)\degree$ phase lag. This value is between the calculated 13$\degree$ for dimers and 19$\degree$ for trimers, and is consistent with about 75% dimers and about 25% trimers. Probably also some still longer chains are present, causing this estimate of the relative dimer concentration to be an underestimate.

During the magnetic actuation the scattered laser light is collected by a photodiode. The scattering signal of the ensemble of chains formed during each pulse is averaged over 32 pulses to improve the signal to noise ratio. Depending on the angle at which the photodiode is placed, signals appear with different shapes, see Fig. 1(d). Especially at PD angles around 90 degrees, many peaks appear: approximately 10 per full rotation of the field. At relatively small PD angles, up to around 60 degrees, the signals only contain two peaks per full rotation of the field. This behavior has previously [7] been approximated by the geometric cross section for two touching spheres (equation and derivation in appendix A). This approach cannot describe the oscillations in the dimer angle dependence that are observed at scattering angles larger than approximately 60 degrees. In order to obtain a better understanding of the angle dependent scattering of the dimers and the resulting frequency spectra, Mie scattering simulations have been performed that are described in the next paragraph.
3. Simulation code

Two-particle Mie scattering simulations have been performed, using the MSTM v. 3.0 code written by D. W. Mackowski [12].

The simulations have been performed for both s and p polarization of the light, with similar results. All figures in this article will be restricted to p-polarization, as was used in the measurements. The corresponding figures for s-polarization can be found in appendices C and D.

4. Input parameters

4.1 Diameter

The diameter of the particles \(D\) is 1.05 µm according to Fonnum et al. [13], with a coefficient of variation (CV) of 1.9 %. These values are in agreement with the manufacturer’s specifications, except for the CV for which the manufacturer-specified value of 3 % will be used.

4.2 Refractive indices

The refractive index of the MyOne particles is not specified by the manufacturer, nor are there any published measurements. An estimate can be obtained by calculating an effective refractive index based upon the contents of the particles. MyOne particles consist of polystyrene and iron oxides. These iron oxides predominantly consist of maghemite \((\gamma-\text{Fe}_2\text{O}_3)\) [13]. MyOne particles contain 255 mg/g [13] of iron. Assuming the iron is all contained in the form of maghemite (density 4.87 g/cm³ [14]) implies that including the oxygen, 365 mg/g of the particles consists of maghemite, i.e. 0.0749 cm³/g particles. The remainder then consists of polystyrene, which has a density of 1.05 g/cm³ [15], corresponding to 0.605 cm³/g particles. I.e. the volume fraction of maghemite is 11.0 %.

The refractive index of maghemite is approximately 2.63 [16]. Note that the wavelength for which this value was obtained has not been given in the reference, and also the refractive index of minerals varies depending on their origin. For these reasons a relatively large uncertainty range will be used in the refractive index of maghemite: \(n_{\text{Fe}_2\text{O}_3} = 2.6 \pm 0.5\).

Bulk polystyrene has a refractive index of 1.5855 at 660 nm [17], but it has been shown in microparticles that it can have a slightly lower refractive index of around 1.581 at 660 nm [18]. To incorporate both these values, the refractive index of polystyrene that is used is \(n_{\text{PS}} = 1.583 \pm 0.002\).

The effective refractive index, \(n_{\text{eff}}\) for materials containing sub-wavelength particles is given by the Maxwell Garnett equation [19], for which the solution in the case of real refractive indices can be written as

\[
\frac{n_{\text{eff}}^2}{n_{\text{PS}}^2} = \frac{(1 + 2 f_{\text{Fe}_2\text{O}_3}) n_{\text{Fe}_2\text{O}_3}^2 + 2 (1 - f_{\text{Fe}_2\text{O}_3}) n_{\text{PS}}^2}{(1 - f_{\text{Fe}_2\text{O}_3}) n_{\text{Fe}_2\text{O}_3}^2 + (2 + f_{\text{Fe}_2\text{O}_3}) n_{\text{PS}}^2}, \tag{1}
\]

in which \(f_{\text{Fe}_2\text{O}_3} = (11 \pm 1)\%\) is the volume fraction of maghemite.

Using Eq. (1), the effective refractive index of the MyOne particles was found to be 1.68 ± 0.05. The uncertainty in this number originates mainly from the uncertainty in the refractive index of maghemite.

Another effective medium approximation, the Bruggeman equation, gives an identical value and uncertainty after rounding. Both the Bruggeman and Maxwell Garnett equations were used in a study by I. Bodurov et al. [20]. The work reports measurements on various metal oxide nanoparticles in water and shows that both equations are in good agreement with literature values of the refractive indices of these oxides.
There will also be a small imaginary part of the refractive index. For polystyrene this is around $5 \times 10^{-4}$ [18]. For maghemite it will probably be higher, like is the case for other iron oxides such as hematite, which has an imaginary part of the refractive index of 0.067 [21]. For now, a value of $n = 1.68 + 0.005i$ will be assumed for the particles.

Given the low salt concentration of the solution in which the particles are dispersed, the effect of the salt on the refractive index will be small. Therefore, the medium refractive index is assumed to be identical to that of distilled water, namely 1.331 [22] at a wavelength of 660 nm.

5. Mie scattering simulations for single dimers

First we investigate the influence of the scattering angle $\beta_s$ and the dimer angle $\alpha_d$ on the intensity of the scattered light. The particles in the dimers are assumed to be touching each other. In Fig. 2 the $p$ scattering intensity is plotted versus these angles. The scattering intensity has been normalized with the maximum for each scattering angle. The normalization divisor shows that the scattering intensity decreases by a few orders of magnitude as the scattering angle increases.

Fig. 2. Simulated scattering intensity as a function of the dimer and scattering angle, normalized for each scattering angle. On the right the normalization divisor is plotted.

For a relatively low scattering angle, like $\beta_s = 22^\circ$ (bottom dashed line in Fig. 2), the scattering intensity reaches a maximum when the dimer is perpendicular to the laser beam. On the other hand it is (almost) minimal when the dimer is aligned. As $\gamma^{(1)} = 30^\circ$ for $\beta_s = 22^\circ$ this simulation is a good first approximation of the PD signal at 30° as shown in Fig. 3(a).

The scattering intensity at higher scattering angles contains more oscillations. Up to $\beta_s = 140^\circ$ more and more peaks and valleys appear in Fig. 2. Figure 3(b) compares the simulation to
Fig. 3. Simulated scattering signals compared to the measured signals shown in Fig. 1(d).
(a) The PD signal at 30° compared to simulations with a scattering angle of 22°. (At $\beta_s = 22^\circ$, $\beta'_{PD} = 30^\circ$.) The deviation in the shape is probably due to the other scattering angles that after refraction also correspond to $\gamma_{PD} = 30^\circ$. (b) The PD signal at 90° compared to the scattering simulations at 90°. The peak positions do not overlap, as indicated by the dashed lines and arrows. The deviation is 4° for the peak around 300° and 10° for the peak around 330°.

the measured signal at 90° where the scattering angle $\beta_s$ equals the photodiode angle $\gamma_{PD}$. The simulation and the experiment have a similar number of peaks and valleys but they appear at different scattering angles, as indicated by the arrows. Also, the minimum in the simulations at $\alpha_d = 180^\circ$ is actually a small maximum in the experiments.

6. Ensemble effects

The simulations presented thus far have focused on individual dimers, neglecting spread in properties that are present in real experiments. The particles used in the experiments are not perfectly monodisperse. Additionally, in the experiments the dimers exhibit an angular spread due to Brownian rotation. These two effects have been further analyzed.

6.1 Polydispersity

In order to model the polydispersity, the size distribution has been assumed to be Gaussian with standard deviation $\sigma$, and has been discretized by taking $\sigma/5$ intervals (≈ 6nm ≈ $\lambda/100$ for the MyOne particles). Simulations have been performed for all combinations of particle sizes, and an ensemble average has been calculated. It turns out that the polydispersity can have a strong effect on the amplitude of the PD signal at 90°, as can be seen in Fig 4(a). The more polydisperse the particles are, the lower the effective amplitude of the signal gets. However, the peak positions are hardly shifted by the polydispersity.

Apart from the lower amplitude, at a coefficient of variation of 10 % the peak positions are very slightly shifted, but not near to the extent that it can explain the measurements.

At a polydispersity of the MyOne particles used in this research (< 3%) the signal is roughly identical to the monodisperse signal, although the amplitude is slightly lower: around 85 % of the value for monodisperse particles. As such, the polydispersity will have a relatively small effect in the current experiments and it cannot explain the peak shifts of the measured signal compared to the simulations. Polydispersity is however relevant when selecting particles for future experiments.
Fig. 4. (a) Influence of size polydispersity on the scattering signals (3 % CV is typical for MyOne particles), (b) Angular spread due to Brownian rotation (5 mT is used in the experiments), (c) Scattering intensity as a function of refractive index and the dimer angle, normalized for each refractive index. The blue dotted lines indicate the shift of the peak positions, which can be up to 6° (3° within the error margin) for the peak around 30° (330° in Fig. 3(b)), and almost nothing for the peak around 60° (300° in Fig. 3(b)). (d) Scattering intensity as a function of inter-particle distance and the dimer angle, normalized for each inter-particle distance. The blue dotted lines indicate the change in dimer angle of the signal peaks with the distance: up to 12° for the peak around 30°, and up to 5.5° for the peak around 60°.
6.2 Brownian rotation

Another difference between the simulations and the experiments is the presence of Brownian rotation of the dimers. The angular spread from this Brownian rotation can be estimated from the magnetic energy involved with this rotation. The ensemble-averaged magnetic moment of MyOne particles as function of the external magnetic field can be well described with a Langevin function [23]. Using the values from Lipfert et al. and a particle diameter of 1050 nm, results in \( m(2.5 \text{ mT}) = 1.82 \times 10^{-15} \text{ A m}^2 \) and \( m(5 \text{ mT}) = 3.60 \times 10^{-15} \text{ A m}^2 \).

In order to simplify the calculations, the system is treated for low rotation frequencies, where the dimers’ magnetic torque can become much larger than their viscous torque, so that the clusters are nearly aligned with the field. For comparison, in the experiments the phase lag is \((16 \pm 3)^\circ\).

In this approximation, the magnetic potential energy \( E \) for a dimer at an angle \( \delta \) with respect to the external magnetic field is given by

\[
E(\delta) = \frac{\mu_0}{4\pi} \frac{[m(B)]^2}{r^3} \left( \sin^2 \delta - 2 \cos^2 \delta \right),
\]

with \( r \) the center-to-center distance between the particles and \( m \) their magnetic moment (derivation in appendix B). The particles are assumed to be touching each other, so \( r = D = 1050 \text{ nm} \), the particle diameter.

A Boltzmann distribution is used to determine the angular spread and is plotted in Fig. 4(b). For a magnetic field of 2.5 mT the full width at half maximum (FWHM) is 6.65°. At the magnetic field used in these experiments (5 mT), the angular spread has a FWHM of only 3.35°. This angular spread causes the peaks to be less sharp in the experiments than in the simulations. The measured scattering signals are a convolution of the simulations and this angular spread probability distribution.

Nevertheless, due to the small amount of angular spread, no systematic shifts of the peaks in the scattering distributions are found, and angular spread due to Brownian rotation cannot be the cause of the mismatch between the experiments and the simulations.

7. Different refractive index

As has been discussed in the section on the input parameters, there is some uncertainty in the value of the effective refractive index of the particles. To check whether a different refractive index can explain the measurement results, simulations have been performed where the refractive index was systematically varied. As shown in Fig. 4(c), a change in the refractive index indeed shifts the peak locations. The location of the peak around 330° in Fig. 3(b) can deviate by 3° within the error margin of the refractive index. The location of the peak around 300° in that same figure does not depend on the refractive index within the simulated range.

Obviously, the calculated shift is not sufficient to explain the observed shifts in the experiment. Even by changing the refractive index by twice the calculated error range does not create a (pair of) valley(s) near \( \alpha_d = 360^\circ \) as shown in Fig. 3. And also the other peak positions do not shift as much as needed. In conclusion, the error margins of the refractive index are not so large that the experimentally measured scattering behavior can be explained.
Fig. 5. The simulation result with an inter-particle distance of 180 nm (dashed line in Fig. 4(d)) compared to the measurement with the photodiode at 90 degrees. The peak positions are in good correspondence.

8. Inter-particle distance

In the calculations presented so far, the particles in the dimers were assumed to be touching each other with a center-to-center distance of 1.05 µm (= D), so an inter-particle distance \( h = 0 \). In the experiments, the inter-particle distance is a consequence of the force equilibrium that is reached between on the one hand the magnetic dipole-dipole attraction and on the other hand the electrostatic repulsion between the particles in the dimer. Also the surface roughness of the particles can play a role.

To investigate the influence of inter-particle distance, simulations have been performed at a constant scattering angle of \( \beta_s = 90^\circ \) as a function of distance between the particles. Figure 4(d) shows that the peaks shift apart at a larger distance, and for inter-particle separations above \( h = 150 \) nm a new peak appears. At a distance of around \( h = 180 \) nm (indicated by the dashed line) the peak positions in the experiment coincide with the peaks found in the simulations at \( \gamma_{PD} = 90^\circ \).

In Fig. 5 the simulation at \( h = 180 \) nm is compared directly with the measurement. As can be seen, including the inter-particle distance in the simulations gives a very good agreement of peak positions in simulations and in the experiment.

The repulsive interaction between particles depends on their surface charge and separation. As measured (section 2), the zeta potential of the MyOne particles is \((-61 \pm 7)\) mV in a similar buffer as used in the experiments. The attractive interaction is determined by their magnetization (given in section 6.2) and separation.

A simple balance of electrostatic repulsion and magnetic dipole-dipole attraction results in a distance of around 95 nm. Additionally it seems quite likely that surface roughness increases this distance since the electrostatic repulsion is the strongest for the charges that are closest together, i.e. on protrusions. From SEM images it has been observed that there are many protrusions on these particles, of up to 100 nm in size [24]. Consequently a particle separation of 180 nm in the experiments is well within the realm of possibilities.

There are still some differences between the experimental signal (Fig. 5) and the simulations. The most significant difference is the slow oscillation with periodicity of 180°, which has a
maximum at 270°. Simulations have been performed to verify whether this is caused by distance spread (see appendix E), but it turns out that this is most likely not the case. Distance spread does slightly reduce the amplitude of the signal, and actually increases the scattering around 180°.

A possible explanation for the differences is related to the cuvette shape. To suppress the collection of light refracted by cuvette side walls, the corners on the front side of the cuvette were covered with black photographic tape. However, light can also reflect from the rounded corners at the back of the cuvette. This means that a part of the light leaving the cuvette at γ = 90° actually was scattered at angles other than 90°. Because the cross section for low angle scattering is an order of magnitude larger than the cross section for scattering at high angles, the background signal originating from the rounded corners is expected to have a dependence on rotation angle that is dominated by the low angle scattering contribution, i.e. the background signal is expected to have slow oscillations. This indeed is the case for the measured signal in Fig. 5: it shows rapid oscillations superposed on a slow background oscillation.

9. Conclusions and outlook

Colloidal dimers are formed during the initial phase of aggregation processes. In this article we have investigated the scattering properties of an ensemble of rotating dimers consisting of magnetic particles with a diameter of one micrometer. At a scattering angle of 90 degrees multiple peaks of the scattering intensity appear during a single revolution of the dimer. Mie scattering calculations have been performed to reproduce the experimental data and to show that the intensity fluctuations strongly depend on the properties of the dimers.

For an ensemble of particles, it turns out that size polydispersity, and misalignment of the dimers due to Brownian rotation, have almost no effect on the angular positions of the measured peaks. A deviation of the refractive index does induce a small shift of the angular positions, but the magnitude of this effect is too small to reproduce the measurements. Finally, variation of the inter-particle distance was found to have a large influence on the peak positions, large enough to explain the measurements. Therefore we conclude that the rotation-induced scattering oscillations provide a means to obtain the interparticle distance of the rotating dimers.

Being able to measure the inter-particle distance opens many potential applications. In self-assembly studies of colloidal particles, this could enable more detailed information on the forces acting on the particles in the early stages of assembly. Also in the field of particle-based biosensing [7,8,25], knowing the distance between particles can serve to distinguish e.g. specifically bound from non-specifically bound particles, thereby improving the performance of these bioanalytical assays.

Appendix A: Geometric cross-section

The geometric cross-section is the area of the particles illuminated by the laser beam. Therefore it depends on the x- and y-positions of the particles (see Fig. 1(b)).

During the rotation, \( x_1 = (R + \frac{1}{2}h) \sin \alpha_j \), \( x_2 = -x_1 \), \( y_1 = y_2 = 0 \). Therefore the difference between the particle centers in the x-direction is the only parameter that determines the geometric cross section. This distance is \( \Delta = |x_1 - x_2| = (2R + h) \sin \alpha_j \).

Some of the limiting cases of the geometric cross section have been plotted in Fig. 6.
In Fig. 6(a) the geometric cross section is simply the sum of the particles’ visible areas, i.e. \( A_{\text{geometric}} = 2 \pi R^2 \). In Fig. 6(c), one of the particles is completely hidden by the other, i.e. \( A_{\text{geometric}} = \pi R^2 \). The intermediate case (Fig. 6(b)) is the hardest to solve. The area of the lens-shaped region of overlap has to be determined. But using some geometry,

\[
A_{\text{geometric}} = R^2 \left( 2 \pi - 2 \arcsin \left[ \left( 1 - \frac{\Delta}{2R} \right)^2 \right]^{1/2} \right) + \frac{\Delta}{R} \left[ 1 - \left( \frac{\Delta}{2R} \right)^2 \right]^{1/2} \right) .
\]

(3)

For the special case of no distance between the particles, this expression simplifies to

\[
A_{\text{geometric}} = R^2 \left[ 2 \pi - 2 \arcsin \left| \cos \alpha_d \right| + \left| \sin(2\alpha_d) \right| \right] .
\]

(4)

In Fig. 7 the geometric cross section is plotted for touching particles, and for particles separated by \( h = 200\text{nm} \). As the distance between the particles increases, the cross section is maximal for a longer time. Basically, a larger range of values of \( \alpha_d \) results in values of \( \Delta > 2R \).
Appendix B: Magnetic dipole-dipole energy

In this section the magnetic dipole-dipole energy is derived that is responsible for aligning the dimer of superparamagnetic MyOne particles with the applied magnetic field. If an external magnetic field is present, these particle get magnetized. Because the maghemite grains are small and homogeneously spread over the particles the magnetization can be approximated to be homogeneous.

For homogeneously magnetized spheres, the field outside of the particles is identical to that of pure dipoles [26]. As a consequence, for the interaction of two magnetized spheres only their center-to-center separation $r$ and magnetic dipole moments $m$ play a role. These dipole moments are assumed to be aligned with the external field as is the case for ideal non-interacting superparamagnetic particles. Additionally, the moments are assumed to be equal in magnitude for both particles. If the particles have a different dipole moment the geometric mean of their dipole moments should be used as the effective dipole moment.

Using the above approximations, the interaction energy is given by Ref. [26]:

$$ U = \frac{\mu_0}{4\pi} \frac{1}{|r|^3} \left[ m \cdot m - \frac{3 (m \cdot r)^2}{|r|^2} \right]. \quad (5) $$

If the dimers are at an angle $\delta$ with respect to the external magnetic field and thus to their magnetization, the inner product $m \cdot r = |m| |r| \cos \delta$, so that the above expression simplifies to:

$$ U = \frac{\mu_0}{4\pi} \frac{|m|^2}{|r|^3} \left( 1 - 3 \cos^2 \delta \right). \quad (6) $$

For small values of $\delta$ this can be approximated by the first terms of a Taylor series, which gives the following harmonic potential:

$$ U \approx \frac{\mu_0}{4\pi} \frac{|m|^2}{|r|^3} \left( -2 + 3 \delta^2 \right). \quad (7) $$

Appendix C: Angle dependence for $s$ polarization

As stated in the main text, for $s$ polarization, similar trends are observed as for $p$ polarization.

Firstly, the simulation result analogous to Fig. 2 is plotted in Fig. 8. Here everything but the polarization direction is identical. Note that just like before, higher scattering angles correspond to more rapid oscillations of the signal.

One of the at first more striking differences is that the rapid oscillations continue until scattering angles of almost $165^\circ$, while for the $p$ polarization they end already around $140^\circ$.

Another difference is that around $\beta_s = 90^\circ$ the maximum scattering actually occurs at $\alpha_d = 0^\circ$, which will become more noticeable in the next section, appendix D.

Appendix D: Distance dependence for $s$ polarization

Another interesting thing to consider is the inter-particle distance dependence for $s$ polarization. This is plotted in Fig. 9, while Fig. 4(d) contained the analogous analysis for $p$ polarization. As can be seen, also for $s$ polarization, there is a strong distance dependence. However, because it starts with a maximum at $\alpha_d = 0^\circ$, it does not have the separation of the peaks close to $0^\circ$.

On the other hand, the position of the peak around $\alpha_d = 60^\circ$ behaves exactly identical: the dotted line shown is exactly the same as that in Fig. 4(d). Therefore, even though the signal itself looks slightly different, it seems highly likely that a similar method of distance extraction works for $s$ polarization.
Fig. 8. Scattering intensity for s polarization as a function of the dimer and scattering angle, normalized for each scattering angle. On the right the normalization divisor is plotted.

Fig. 9. Scattering intensity for s polarization as a function of the dimer angle (horizontal) and inter-particle distance (vertical), normalized for each distance.
Appendix E: Distance spread

In the experiments of this paper, there is no molecular bond present between the particles, so the inter-particle distance is determined by the particle properties. This includes local roughness, surface charge, and magnetic content. Therefore, it is highly unlikely that all of the dimers have the same inter-particle distance; probably there is a distribution of distances.

In order to study this, simulations have been performed varying the inter-particle distance with steps of 1 nm. Gaussian distributions have been assumed for the inter-particle distance, around an average value of 180 nm. By using these distributions as weighting functions, it is possible to get a distribution-averaged signal. These are plotted in Fig. 10 for 3 different values of the standard deviation of the distance, $\sigma_h$.

![Fig. 10. Scattering signals for Gaussian distributed distances, for 3 standard deviations. In black, no distance spread, in red $\sigma_h = 20$nm and in blue $\sigma_h = 50$nm.](image)

For all three plotted values of the standard deviation of the distance, the peak positions remain unchanged. This was quite surprising seeing that the peak positions do clearly change when changing the average distance. What is different between the different values of $\sigma_h$ is the relative height of the peaks. The scattering at $\alpha_d$ around $180^\circ$ is higher for more distance spread, while the peaks around $90^\circ$ are lower. This is the opposite of the difference between experiment and simulations, so distance spread is very unlikely to be the cause of the observed difference.

Acknowledgments

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