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Accurate quantification of magnetic particle properties by intra-pair magnetophoresis for nanobiotechnology

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The application of magnetic particles in biomedical research and *in-vitro* diagnostics requires accurate characterization of their magnetic properties, with single-particle resolution and good statistics. Here, we report intra-pair magnetophoresis as a method to accurately quantify the field-dependent magnetic moments of magnetic particles and to rapidly generate histograms of the magnetic moments with good statistics. We demonstrate our method with particles of different sizes and from different sources, with a measurement precision of a few percent. We expect that intra-pair magnetophoresis will be a powerful tool for the characterization and improvement of particles for the upcoming field of particle-based nanobiotechnology. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4816429>]

Presently a generation of tools is being developed for the biosciences and *in-vitro* diagnostics based on acquiring data at the single molecule and single cell level with good statistics. An example is the flow cytometer, which collects data of single biological cells with high throughput and thereby establishes detailed histograms of cell population characteristics.¹ Another example is the field of high-throughput single-molecule DNA sequencing,^{2,3} where technologies are able to read the bases of individual nucleic-acid molecules with high throughput. An important advantage of single-cell and single-molecule data compared to ensemble-averaging measurements is that high sensitivities are achieved and that deeper insights are gained into the biological processes, e.g., into the development of population heterogeneities in space and/or in time.⁴

Superparamagnetic particles are a potent component in many single molecule analysis technologies^{5–8} because such particles can be manipulated and interrogated by magnetic fields without perturbing the biological matter under study. To be able to use magnetic particles in high-throughput methods with single-particle and single-molecule resolution, it is important that the size as well as the magnetic properties of the particles are accurately known. The size of single magnetic particles can be accurately determined, e.g., by transmission electron microscopy⁹ and scanning electron microscopy or pore sensing.¹⁰ However, the characterization of magnetic properties remains challenging. The magnetic moments of single particles have been measured by Brownian motion analysis,¹¹ field sensing,^{12,13} and magnetophoresis,^{14–16} but the reported methods all suffer from large measurement uncertainties (above 30%) because of large uncertainties in the fields and field gradients applied within the reference frames of the particles.

In this paper we describe a way to characterize the magnetic field dependent moments of magnetic particles with a high accuracy. The high accuracy is achieved by recording particle movement in the *high and local field gradients generated by the particles themselves* (see Figure 1). We measure the trajectories of pairs of particles, present in a liquid close to a physical surface in an applied magnetic field, and we deduce the magnetic moments from the time dependency of the particle pair separations. The applied magnetic field is generated by two electromagnets with soft-iron poles.¹⁷ The field is applied uniformly to the particles and is repeatedly switched between out-of-plane (Figure 1(b)) and in-plane (Figure 1(c)) orientations in order to repetitively induce separation and rejoining of the particle pairs at the surface (Figure 1(d)). In this intra-pair magnetophoresis method, (i) the field gradients and therefore the magnetophoretic velocities are high, (ii) the applied field strength is uniform and well-controlled, and (iii) many particles are simultaneously and repetitively measured. In the following we will demonstrate that pair-based distribution functions can be accurately measured and can be translated into single-particle based histograms of the magnetic moments, with a measurement uncertainty of only a few percent. Furthermore, we will show that repeated measurements can be performed on the same particles in order to determine field-dependent magnetization curves and grain size distributions within the magnetic particles.

The magnetic moments have been characterized for different types of superparamagnetic particles. Suspensions were made by dispersing the particles in phosphate buffered saline buffer with 1 mg/ml bovine serum albumin (to reduce particle-surface interactions) in a small fluidic chamber made of two glass cover slips, separated by a 100 μm thick spacer. The studied particles were $\varnothing$ 2.8 μm , Dynabeads M-270 COOH (Invitrogen), $\varnothing$ 1 μm , Dynabeads MyOne COOH (Invitrogen), and $\varnothing$ 2.6 μm Micromer PEG-COOH (MicroMod) particles. Imaging was performed using a

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FIG. 1. Sketch of the intra-pair magnetophoresis methodology. (a) The separation S of magnetic particle pairs due to magnetic dipole-dipole interactions is measured in (b) out-of-plane and (c) in-plane magnetic fields. (d) Repeated separation and rejoining of magnetic particles S (black line) at different field strengths (blue line) with alternating in-plane and out-of-plane orientation (red line). (e) Microscopy images of M-270 superparamagnetic particles at different times, upon application of an out-of-plane magnetic field at $t = 0$. The image is a zoom-in of the total field of view.

microscope ($50\times$ magnification objective; Leica DM4500) and a high speed camera (100 frames per second; IDT MotionPro X3). Typically about 20 particle pairs were simultaneously imaged. Particle pairs were excluded from analysis when other particles were present within a distance of about $15\text{ }\mu\text{m}$. We applied a magnetic field that alternated between out-of-plane and in-plane orientations at a frequency of 0.5 Hz . Each measurement contained one four-second sequence of the resulting separation and rejoining of particles. In this way, several consecutive recordings were made, as shown in Figure 1(e). The applied field strength was uniform in the plane of the particles, within a few percent over a 1 mm^2 surface area.¹⁸ The magnet also creates a field gradient toward the surface, which keeps the particles at the bottom surface of the fluid chamber. Custom image processing software was used to determine the time-dependent intra-pair separations $S(t)$ (see Figure 2). Particle position

tracking was based on cross-correlation of the images with a reference image of a particle, which gives positional accuracies well below 100 nm .

In an out-of-plane oriented magnetic field, the intra-pair separation, S , increases due to the repulsive dipole-dipole forces between the particles with magnetic moments m_1 and m_2

$$F_{dd} = \frac{3\mu_0 m_1 m_2}{4\pi S^4}. \quad (1)$$

This force is opposed by the hydrodynamic drag that acts on the particles when they move parallel to the surface.¹⁹ The corresponding differential equation can be solved²⁰ resulting in the following time-dependence of the intra-pair separation S

$$S(t)^5 = \frac{45\mu_0 m_1 m_2}{64\pi^2 \eta R} t + S(0)^5. \quad (2)$$

Here η is the dynamic viscosity ($10^{-3}\text{ Pa}\cdot\text{s}$ for water) and R is the particle radius. Due to the very strong distance dependence of the magnetic dipole-dipole force, the perturbation of the intra-pair separation due to particles at a further distance is very small (a particle at a distance of $10\times$ the particle diameter from the particle pair exerts a force that is four orders of magnitude smaller than the forces exerted within the particle pair). The observed separation behavior is accurately described by Eq. (2), as shown in Figure 2. From the fit, the geometric mean of the magnetic moments of the particles is determined. In Eq. (2) the hydrodynamic particle-particle interactions have been neglected; this gives a slight underestimation of the magnetic moments, by about 0.8% for the $2.8\text{ }\mu\text{m}$ particles and about 0.2% for the $1.0\text{ }\mu\text{m}$ particles.²⁰ Furthermore, we verified that electrostatic forces do not play a role in our experiments.²⁰

In order to measure complete field-dependent magnetization curves, a magnetic actuation protocol was implemented in which the current through the electromagnets was increased in subsequent repulsion trajectories, as sketched in

FIG. 2. Intra-pair separation S as a function of time of a single pair of M-270 particles, determined for different field strengths (open circles). The solid curves correspond to fits based on the solution of the equation of motion [Eq. (2)]. The inset shows the same data, but now with the fifth power of the intra-pair separation (S^5) on the y-axis. The dynamic range of the intra-pair force in the experiment is on the order of 10^3 (ranging from $\sim 10\text{ pN}$ to $\sim 0.01\text{ pN}$ at 16 mT , obtained using Eq. (2) for the smallest and the largest distance).

FIG. 3. Magnetization curves determined for three individual particle pairs (M-270, denoted by open squares, open diamonds, and open circles; the error-bars are shown within the symbols). The data for all three pairs were collected within a timespan of 40 s. The solid circles correspond to VSM measurements (vibrating sample magnetometry) performed on an ensemble of the same batch of particles. The curves correspond to a log-normal distribution-weighted sum of Langevin curves. The best fitting log-normal distribution functions $f(m_{\text{grain}})$ of the magnetic moments of the particle grains are shown in the inset for the four experimental data-sets. From repeated measurements on the same particles, the error in particle magnetic moment per measurement point is found to be about 4%.

Fig. 1(d). Measurement results are shown in Figure 3, and for other particle types magnetization curves can be found in supplementary material.²⁰ The obtained magnetization curves fluctuate around the curve measured by VSM (Vibrating Sample Magnetometry) as is expected for single-particle based measurements compared to an ensemble-averaging method. By fitting the magnetization curves with a log-normal distribution-weighted Langevin equation, the grain size distribution can be obtained (for an explanation, see Mihajlovic *et al.*¹³ The inset in Figure 3 shows the obtained grain size distributions (colored lines) and the distribution obtained from the VSM measurements (black line).

We have recorded the magnetic moments for many particle pairs and different types of particles, yielding the histograms in Figure 4(a). For each pair-averaged distribution, there exists a unique original single-particle distribution, because pair-averaging causes an over-determination of the original distribution. For example: an original single-particle distribution with two peaks (m_1, m_2) gives a pair-averaged distribution with three peaks (m_1, m_2 , and $\sqrt{m_1 m_2}$). For the original distribution, we assumed a parameterized peak function with three underlying parameters, namely the peak position, the peak width and the skewness of the distribution. From the original distribution, we calculate the pair-averaged

FIG. 4. Histograms of the pair-averaged and single particle magnetic moments. (a) Measured distributions of pair-averaged magnetic moments for the different types of magnetic particles, i.e., M-270, MyOne, and Micromer particles at a field of 13 mT. Each histogram corresponds to about 50 individual measurements on different particle pairs. The curves are obtained by calculating the pair averaged moment distribution from the single particle moment distributions in panel (b). (b) Deduced single particle moment distributions. Asymmetric peak functions are shown of single particle moment distributions which lead to the optimal fit of the measured pair-based distributions in panel (a).

distribution, and we apply an iterative least-squares optimization routine²⁰ to find the best fit to the data. Figure 4(a) shows that the fitted histograms describe the measured data well. In Figure 4(b) the corresponding single particle distributions are shown. As expected, the single particle distributions are broader compared to the pair-averaged distributions, roughly by a factor $1/\sqrt{2}$.²⁰ The data and analysis in Figure 4 demonstrate that the intra-pair magnetophoresis method yields accurate distributions of single-particle magnetizations.

Table I summarizes the magnetic particle properties obtained by intra-pair magnetophoresis, including the properties determined by fitting the magnetization curves. Interestingly, the M-270 and the Micromer particles have a similar zero-field magnetic susceptibility, but the saturation magnetization differs by about a factor 5. We conclude that the Micromer particles have a lower magnetic content (explaining the lower saturation magnetization) but to achieve the same zero-field susceptibility, the grain size needs to be larger, in agreement with the distribution-weighted Langevin fit of the magnetization curves. We find that the saturation magnetization of MyOne particles is roughly a factor ten lower than that of M-270 particles. Assuming the same iron density within both particles, a

TABLE I. Magnetic properties of three different particle types quantified by intra-pair magnetophoresis.

Particle type	Particle diameter [μm]	Zero-field susceptibility χ	$m_{p,\text{sat}}^a$ [$\times 10^{-14} \text{Am}^2$]	$\text{FWHM}/m_{p,\text{max}}^b$ at 13 mT [%]	$m_{\text{grain,mean}}^c$ [$\times 10^4 \mu_B$]	$\sigma_{m,\text{grain}}^c$ [$\times 10^4 \mu_B$]
From supplier	From supplier	From magnetization curve		From histogram	From magnetization curve	
M-270	2.8 ± 0.1	1.2 ± 0.2	13 ± 1	19 ± 2	5.8 ± 0.8	1.1 ± 0.2
Micromer	2.6 ± 0.1	0.91 ± 0.15	2.7 ± 0.4	49 ± 7	21 ± 3	1.0 ± 0.3
MyOne	0.99 ± 0.04	1.7 ± 0.3	1.2 ± 0.1	34 ± 4	6.6 ± 2.0	0.57 ± 0.3

^a $m_{p,\text{sat}}$ is the average particle saturation magnetization.

^b $\text{FWHM}/m_{p,\text{max}}$ is the full width at half maximum of the single moment distribution, relative to the peak position of the distribution.

^c $m_{\text{grain,mean}}$ and $\sigma_{m,\text{grain}}$ are the mean and standard deviation of the log-normal distribution of the magnetic moment of the grains within the particles.

decrease of $(2.8/1)^3 \cong 22$ would be expected. Since the average grain size is similar in the two particle types, the iron density within the MyOne particles must be higher compared to the M-270 particles, in agreement with the higher susceptibility found for the MyOne particles. We conclude that intra-pair magnetophoresis yields detailed quantitative information about the magnetic properties of the different types of particles.

We can compare intra-pair magnetophoresis to other magnetophoresis techniques. In existing magnetophoresis techniques the field gradients are generated by magnets and/or magnetic structures that are external to the particles. The applied fields are non-uniform in magnitude and in orientation, which complicates the magnetophoretic analysis, limits the analyzable field of view, and gives large uncertainties in the fields and field gradients experienced within the reference frames of the particles. In intra-pair magnetophoresis, the field gradients are locally very large while the applied field is constant over the surface. The uniformly applied field is well-controlled, and the analyzable field of view is large. Furthermore, the field gradients are easily reversed by changing the orientation of the applied field, allowing repeated measurements on the same particles, including measurements at different field strengths so that complete magnetization curves can be established.

Finally, we discuss the outlook for further development of the intra-pair magnetophoresis method. First, it will be interesting to extend the method to the characterization of smaller particles, i.e., sub-micrometer and nanometer sized particles. Magnetic particles can be optically tracked down to very small particle sizes.²¹ However, the accuracy of the magnetic moment quantification will decrease if the magnetic forces become smaller, because Brownian motion becomes more important in the particle trajectories. For example, the in-plane pair alignment prior to application of the out-of-plane magnetic field is important to reproducibly achieve repulsion between the particles. Comparing the pair alignment energy by magnetic dipole-dipole interaction to the thermal energy, we expect that magnetic particles with a size down to 100 nm should certainly be analyzable by the intra-pair magnetophoresis method.²⁰ Another interesting experiment will be to apply intra-pair magnetophoresis to mixed particle types. When a first particle type has been accurately characterized, it can be used to analyse a second particle type in a mixed-particle experiment. It will be particularly useful to analyze broadly distributed particles using particles with a sharp distribution of magnetic properties. Commercially available magnetic particles are known to have broad distributions of magnetic properties, since in industrial settings the magnetic properties are monitored only by ensemble-averaging techniques such as Vibrating Sample Magnetometry. The intra-pair magnetophoresis method is accurate, rapidly yields statistically relevant data, and it is easy to use. We expect that the analysis method will support the development of fabrication processes for next generations of particles with sharply defined magnetic properties. Used in nanobiotechnological research settings, we expect that the intra-pair magnetophoresis method will improve the accuracy of highly parallel single-molecule measurements in magnetic tweezers,⁸ magnetic force-

induced dissociation experiments,²² measurements of the mechanical response of cells to actuated particles,^{23,24} and of particle-based biosensing techniques to measure protein concentrations.^{25,26}

In summary, the intra-pair magnetophoresis method enables the characterization of magnetic particle properties with an accuracy of a few percent, with good statistics, yielding histograms and full magnetization curves over a large range of field strengths. The technique makes it possible to characterize particles with different sizes and does not require complicated fabrication techniques. In addition to applications in industry, the intra-pair magnetophoresis method will be easily adopted by academic research groups, as groups working with magnetic particles already have the required infrastructure like microscopes and cameras. We envisage that the intra-pair magnetophoresis methodology will be a very convenient tool to characterize and improve particles in the emerging field of particle-based nanobiotechnology.

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