

Novel Readout Method for Molecular Diagnostic Assays Based on Optical Measurements of Magnetic Nanobead Dynamics

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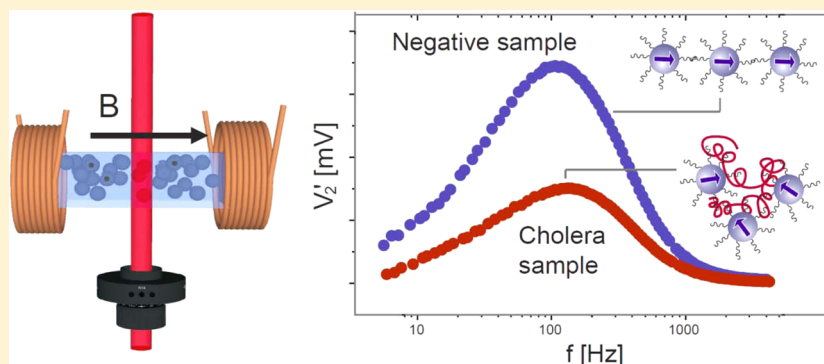
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Supporting Information



ABSTRACT: We demonstrate detection of DNA coils formed from a *Vibrio cholerae* DNA target at picomolar concentrations using a novel optomagnetic approach exploiting the dynamic behavior and optical anisotropy of magnetic nanobead (MNB) assemblies. We establish that the complex second harmonic optical transmission spectra of MNB suspensions measured upon application of a weak uniaxial AC magnetic field correlate well with the rotation dynamics of the individual MNBs. Adding a target analyte to the solution leads to the formation of permanent MNB clusters, namely, to the suppression of the dynamic MNB behavior. We prove that the optical transmission spectra are highly sensitive to the formation of permanent MNB clusters and, thereby to the target analyte concentration. As a specific clinically relevant diagnostic case, we detect DNA coils formed via padlock probe recognition and isothermal rolling circle amplification and benchmark against a commercial equipment. The results demonstrate the fast optomagnetic readout of rolling circle products from bacterial DNA utilizing the dynamic properties of MNBs in a miniaturized and low-cost platform requiring only a transparent window in the chip.

The detection of specific DNA sequences has facilitated the diagnosis and targeted treatment of several human diseases. Although great advances have been made in the past few years, the detection of certain pathogenic bacteria is still based on bacterial culture and colony counts or on the polymerase chain reaction (PCR).¹ Both methods are time-consuming, ranging from a few hours for PCR to a few days for certain bacterial cultures, and require expensive equipment and trained personnel. In resource-poor regions of the world, rapid diagnostic tests that provide high sensitivity and specificity at a low cost are of utmost importance.² For instance, for cholera diagnostics, early detection and confirmation of *Vibrio cholerae* bacteria is crucial for rapid implementation of control measures

in outbreaks. However, the reliability of the rapid commercial diagnostic tests presently available is still debated.^{3,4}

Several techniques have been proposed to address the need for simple and highly sensitive techniques to detect DNA in complex biological samples,⁵ for example, acoustic biosensors⁶ or electrochemical biosensors.^{7,8} Solutions based on magnetic particles to capture and detect multiple target analytes using a low-cost, sensitive readout method have shown to be attractive^{9–14} and sensitive molecular detection technologies using magnetic nanoparticles or magnetic nanobeads (MNBs)

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to tag and detect oligonucleotides have emerged.^{15–17} As readout elements, surface-functionalized magnetoresistive sensors^{17,18} or miniaturized nuclear magnetic resonance (NMR) detectors^{15,16} have been used. Optical (fluorescence based) detection of nonamplified target captured by magnetic particles has also shown sub-picomolar sensitivity.¹⁴

Combining a magnetic readout with isothermal amplification is also potentially very interesting, as it may allow for a high sensitivity, while simplifying the sample processing steps. A recently proposed simple homogeneous readout format exploits the change of the Brownian rotation dynamics of MNBs when these attach to DNA coils produced by rolling circle amplification (RCA) of circularized DNA formed by padlock probe recognition of the target DNA.^{12,19–21} The method has been shown to provide a sensitivity in the low picomolar range and has been successfully employed for the highly specific detection of bacterial DNA²² and spores²³ as well as for studies of drug resistance in *Mycobacterium tuberculosis*.²⁴ However, the real break-through of this approach has been hampered by the bulky and costly measurement systems used for the magnetic characterization.^{25,26} Miniaturized systems using magnetoresistive sensors, have also been demonstrated^{18,22,27} but these are only sensitive to magnetic particles in close vicinity of the active sensor area.

The use of an optical probe such as a laser beam for measurements of the magnetic dynamics of MNBs is highly attractive because the beam may interact with a large sample volume and because measurements can be performed without physical contact to the device containing the sample. Early studies proposed the use of magneto-optical (MO) activity displayed by ferrofluids, namely, polarization change of the transmitted light, exhibited by a suspension of magnetic nanoparticles.^{28,29} The observation of MO effects in transmission requires that the light passes two polarizers: one to polarize the incoming light and one to analyze the polarization of the transmitted light. A limiting factor of these methodologies is the small magnitude of the MO effects, which requires nanoparticle concentrations in the nanomolar range to be observable. Also, multicore commercial MNBs only show weak MO effects since the magnetic nanograins (usually ~10 nm diameter iron oxide nanoparticles) composing the MNBs have a wide distribution of orientations with respect to the effective MNB magnetic moment and are enclosed in a polymer shell. Instead, alternative optical approaches that do not directly rely on MO effects are more attractive, as they can be applied to a broader class of magnetic beads, yet guaranteeing high sensitivity with a comparatively simpler optical instrumentation.^{11,30} These approaches target optical scattering contrast mechanisms produced, for example, by the rotational dynamics of clusters and chains of MNBs formed under the influence of an external rotating magnetic field of constant amplitude.^{11,30,31} As a prominent result, Ranzoni et al. have demonstrated high-sensitivity protein biomarker detection directly in plasma.^{11,32}

Here, we present a novel methodology for optical detection of the dynamic magnetic response of MNBs and demonstrate its use for the sensitive quantification of RCA products formed from *Vibrio cholerae* DNA. The technique relies on measurements of the light transmission modulation caused by the AC magnetic field-stimulated optical anisotropy of the MNB suspension during a cycle of the uniaxial applied magnetic field. We show that the field-frequency dependence of the second harmonic component of the transmitted light is closely related to the Brownian relaxation dynamics of the individual

MNBs and that the dynamic response in suspensions with MNB concentrations down to 1 $\mu\text{g/mL}$ (~10 pM) can be measured reliably. This simple readout method is very sensitive to scattering contrast variations due to the suppression of the field-induced dynamics caused by MNB agglutination as we demonstrate for streptavidin coated MNBs vs concentration of biotinylated bovine serum albumin (bBSA). As a key result, we show that the method can be used to detect the hybridization of functionalized MNBs to DNA coils formed by padlock probe recognition and rolling circle amplification from *V. cholerae* DNA with high sensitivity down to target concentrations of 10 pM. The results are benchmarked against those obtained using a commercial equipment.

EXPERIMENTAL SECTION

Experimental Setup. Figure 1a and b shows schematics of the two experimental configurations used to measure the

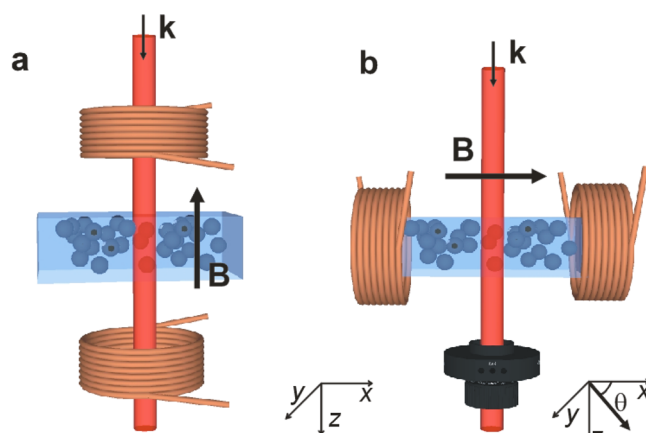


Figure 1. Measurement configurations: (a) The magnetic field is applied parallel to the laser beam ($B \parallel k$). (b) The magnetic field is applied perpendicular to the laser beam ($B \perp k$), and a polarizer is introduced in the xy -plane at an angle θ to the x -axis.

optical transmission when an oscillating uniaxial magnetic field is applied along the laser beam ($B \parallel k$) or perpendicular to the laser beam ($B \perp k$), respectively. In the latter case, a polarizer could be introduced to define a linear polarization of the light at an angle θ to the magnetic field direction.

For basic studies of the response of MNB suspensions, the sample container was a transparent circular fluid cell with a volume of 20 μL having a diameter of 5 mm and a height of 1 mm (corresponding to the optical path). The cell was fabricated in PMMA and sealed with two thin glass windows. For biodetection experiments, the microfluidic cell was substituted with commercial cuvettes (Malvern Z117, Malvern, US) to improve reproducibility between the measurements. In these, the optical path length was 2 mm.

Details on the optical setup components are given in Supporting Information section S1. Briefly, experiments were carried out using either a red ($\lambda = 635$ nm) or blue ($\lambda = 405$ nm) laser light source and the light transmitted through the sample contained was collected using a photodetector. The laser beam was expanded to match the size of the sample container using a beam expander.

The sinusoidal uniaxial magnetic field $B_{AC}(t) = B_0 \sin(2\pi ft)$ of amplitude B_0 up to 3 mT and frequency f up to 10 kHz was generated using two small sintered ferrite yokes. The AC

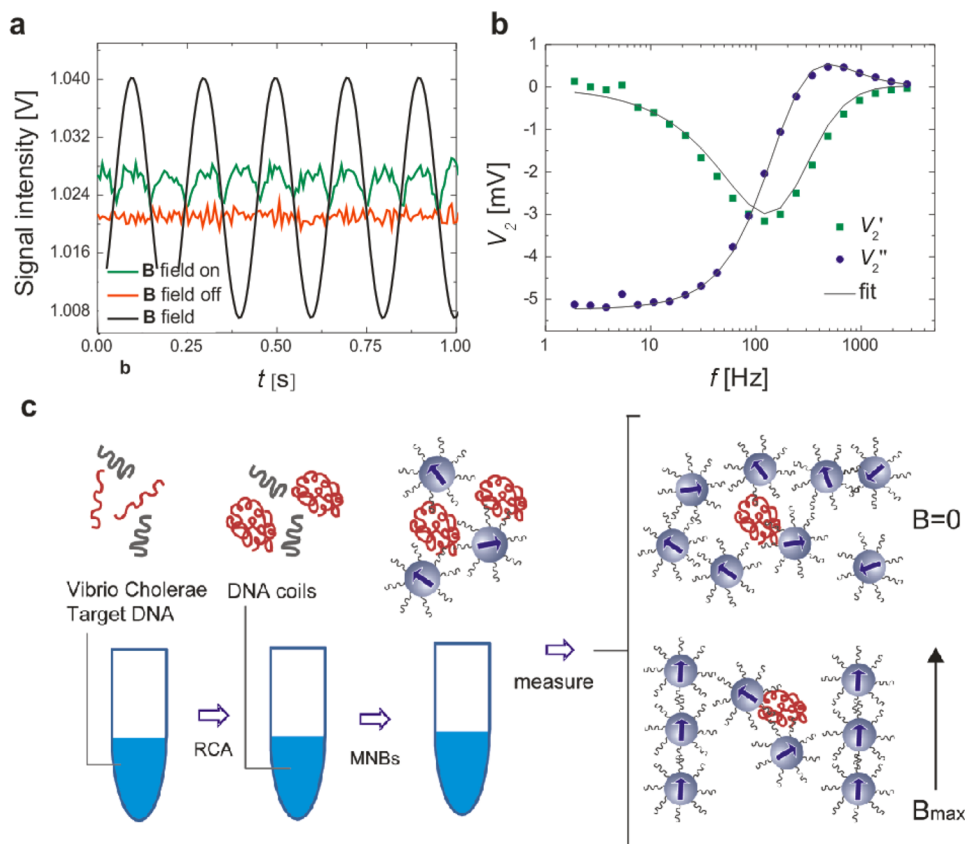


Figure 2. Light transmission measurements ($\lambda = 635$ nm) on suspensions of 50 nm MNBs in the $\mathbf{B} \parallel \mathbf{k}$ configuration with $B_0 = 1$ mT. (a) Transmitted light intensity vs time recorded on a 10 $\mu\text{g/mL}$ MNB suspension for a magnetic field excitation with $f = 5$ Hz. (b) In-phase and out-of-phase components of the complex second harmonic photodetector signal $V_2 = V_2' + iV_2''$ measured versus frequency for a 50 $\mu\text{g/mL}$ MNB suspension. The solid lines are curve fits obtained using the model presented in Supporting Information section S3. (c) Schematic illustration of the physical mechanism for detection of DNA coils. DNA coils of *V. cholerae* are formed by padlock-probe recognition and rolling circle amplification. The DNA coils are mixed and incubated with functionalized MNBs. MNBs bound to DNA coils cannot follow the external magnetic field variation. The resulting reduction of the modulated optical signal due to freely rotating magnetic beads (illustrated here as having formed chains) is used as a readout method.

magnetic field was measured in real time using a high-speed Hall probe that also provided the reference for a lock-in amplifier used to detect the second harmonic voltage output components from the photodetector, in-phase and out-of-phase with the AC magnetic field. Further details are given in Supporting Information section S1.

Magnetic Nanobeads. In the experiments, we used commercially available multicore MNBs from Micromod (Rostock, Germany) with nominal diameters of 50, 80, 100, 130, and 250 nm, respectively. These MNB types are of irregular shape³³ and consist of clusters of nanograins of iron oxide of which some are thermally blocked, i.e., the individual MNBs show a small remnant magnetic moment in the absence of an applied magnetic field. This remnant magnetic moment follows the physical orientation of the MNB, which fluctuates in time because of thermal agitation when the MNB is in suspension. At present, it is not known whether the individual MNBs display optical anisotropy (e.g., due to elongated MNB shape) and, if so, whether this optical anisotropy is linked to the orientation of the remnant magnetic moment.

Detection of Biotinylated Bovine Serum Albumin. In these experiments, 20 μL of a 500 $\mu\text{g/mL}$ suspension of streptavidin coated MNBs with a nominal diameter of 80 nm (BNF starch, Micromod) was mixed with 20 μL of sample containing different concentrations of biotinylated bovine

serum albumin (bBSA) and incubated for 10 min. Subsequently, the MNBs were collected within the reaction tube using a commercial magnetic separator and resuspended in PBS to a total volume of 200 μL (and a final MNB concentration of 50 $\mu\text{g/mL}$), which was the volume of the cuvette used for the characterization. The measurements were performed using a blue light laser emitting at a wavelength of 405 nm in order to amplify the scattering effect from MNBs and MNB clusters.¹¹

Detection of *Vibrio cholerae* Coils. Details on the coil formation from target DNA strands, the oligonucleotide sequences and the oligonucleotide conjugation to the MNBs are given in the Supporting Information section S2. The samples were prepared for analysis by gently mixing 15 μL of functionalized bead solution with 15 μL of DNA coil solution, followed by incubation at 55 $^\circ\text{C}$ for 30 min and thereafter diluted with 30 μL of a mixture containing equal volume of PBS (pH 7.5) and a solution containing 20 mM EDTA, 20 mM TRIS-HCl, 0.1% Tween 20 and 1 M NaCl. The measurements were performed using a blue laser emitting at a wavelength of 405 nm. Measurements of the frequency-dependent magnetic susceptibility were performed in a commercial DynoMag AC susceptometer (Acreo, Sweden) as described previously.³⁴ To normalize the magnetic response with respect to the amount of magnetic material in each sample, the data was normalized with the high-frequency value of the in-phase magnetic susceptibility

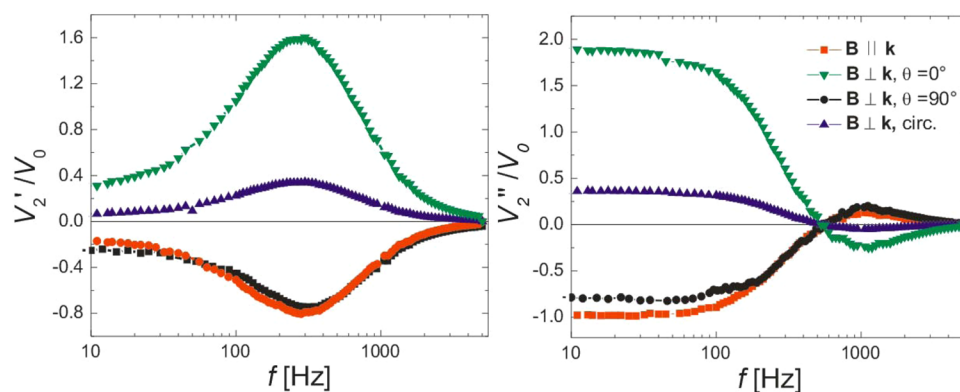


Figure 3. V_2' (left) and V_2'' (right) spectra recorded for a 10 $\mu\text{g/mL}$ suspension of 50 nm MNBs in the four configurations: $\mathbf{B} \parallel \mathbf{k}$, $\mathbf{B} \perp \mathbf{k}$ with $\theta = 0^\circ$, $\mathbf{B} \perp \mathbf{k}$ with $\theta = 90^\circ$, and $\mathbf{B} \perp \mathbf{k}$ using a circularly polarized beam ($\lambda = 635 \text{ nm}$) $B_0 = 2 \text{ mT}$. All data points are normalized with the measured average photodetector signal V_0 .

(χ_∞) measured at a frequency well above the Brownian relaxation frequency as this value is proportional to the total content of iron-oxide nanoparticles in a sample.³⁴

RESULTS AND DISCUSSION

Detection Method. In this section, we discuss the modulation of the transmitted light intensity in the time and frequency domains to shed light on the physical mechanism behind the optomagnetic effect and on how the optomagnetic signal can be used to detect a biological target.

Figure 2a shows the light transmitted intensity measured by the photodetector vs time for a 10 $\mu\text{g/mL}$ suspension of 50 nm MNBs for a magnetic field excitation ($f = 5 \text{ Hz}$, $B_0 = 1 \text{ mT}$) applied in the $\mathbf{B} \parallel \mathbf{k}$ configuration. Figure 2a also shows a corresponding signal trace obtained with no magnetic field excitation (solid red line in graph). The signal level with no magnetic field excitation is the same before and after an experiment. The signal with the field excitation (solid green line in graph) is dominated by a component at $2f$, and shows sharp minima when the applied field intensity is close to zero and maxima when the field magnitude is maximal, irrespective of the field direction (solid gray line in the plot). The signal level of the minima is close to that obtained when no magnetic field excitation is applied. The calculated fast Fourier transform (FFT) spectrum of the photodetector signal confirms that the signal is dominated by a component at $2f = 10 \text{ Hz}$.

Figure 2b shows the complex second harmonic photodetector signal $V_2 = V_2' + iV_2''$ measured by lock-in technique versus frequency f of the AC magnetic field. The measured in-phase signal (V_2') exhibits a clear peak at $\sim 100 \text{ Hz}$ and the out-of-phase signal (V_2'') shows a steplike transition with saturation at low frequencies and a value close to zero at high frequencies. This signal resembles the Brownian relaxation signal from MNBs recorded by conventional magnetic susceptibility measurements, except that the roles of the in-phase and out-of-phase components appear to be reversed. An analytical model to explain this effect is presented in Supporting Information section S3.

As at most one polarizer is used in our transmission geometries, ordinary magneto-optical effects that give rise to a rotation of the light polarization can be ruled out. Thus, the optical signal is due to the possible optical anisotropy of the individual MNBs, the formation of chainlike structures of MNBs, or both. Moreover, permanent irreversible agglomeration of MNBs because of their small remnant moment or

other interactions can be ruled out as no MNB agglomerates were observed in dynamic light scattering experiments. Hence, the modulation of the optical signal observed in Figure 2a must be due to the dynamics of the individual MNBs or due to the formation and disruption of optically anisotropic MNB suprastructures stimulated by the magnetic external field.

In the low-field regime, an applied magnetic field initially induces a physical rotation of the MNBs to partially align their blocked magnetic moments with the field. Above a certain field value (depending on temperature, the viscosity of the liquid, and the volume, shape and magnetic susceptibility of the MNBs), because of the increase in the effective MNB magnetic moment, the magnetic dipolar interactions dominate and chain-like MNB suprastructures oriented along the applied field may form. Upon reduction of the field magnitude and below this particular critical field value, thermal diffusion of the MNBs dominates and the MNB chains break up such that the individual MNBs lose their orientation and drift away from each other. In a slowly oscillating magnetic field, MNBs rotate and elongated clusters of dipole-interacting MNBs may thus form and breakup twice during a field cycle. In a harmonically varying magnetic field, MNB clusters formed at large fields break up at low magnetic fields. Upon a sign change of the magnetic field, the individual MNBs have to physically rotate to orient their blocked magnetic moments along the field before the formation of clusters because of magnetic dipole interactions again become favorable. The time scale of this dynamic formation/disruption process is directly connected to the Brownian relaxation time of the *individual* MNBs, as well as to the thermally diffusive motion that separates the chain constituents.

A target analyte bound on one or more MNBs may suppress the rotation of the individual MNBs, as well as the suprastructure formation/disruption process, and can then be detected by monitoring variations of the modulated optical signal. Considering the dynamic process depicted above, the light transmission for the $\mathbf{B} \parallel \mathbf{k}$ configuration is maximum when the externally applied magnetic field is large, because aligned MNB structures formed along the light path reduce the geometrical scattering cross-section of the suspension to the incoming light. When the magnetic field approaches zero, the intensity of the transmitted light is reduced due to the higher geometrical scattering cross-section of the now disordered MNB suspension. The qualitative description of the dynamic process and its influence on the signal measured on the

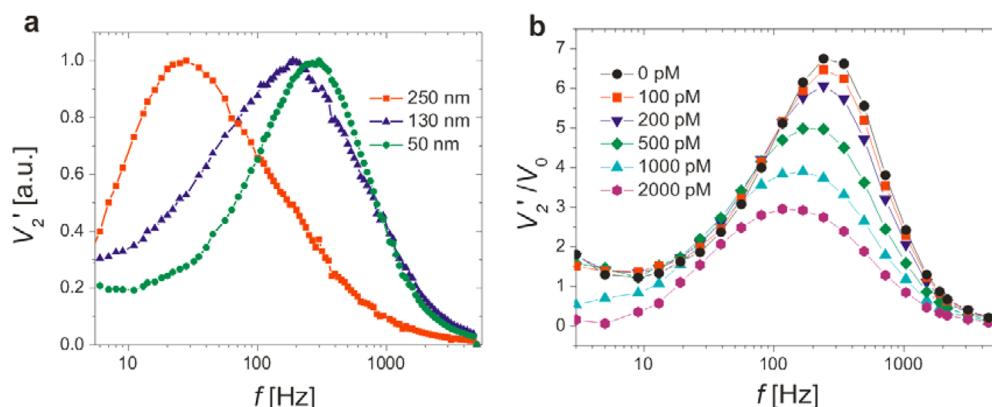


Figure 4. (a) V_2' spectra recorded for 10 $\mu\text{g/mL}$ suspensions of 50, 130, and 250 nm MNBs in the $\mathbf{B} \perp \mathbf{k}$ configuration ($\lambda = 635$ nm) with $\theta = 0^\circ$ and $B_0 = 2$ mT. (b) V_2' spectra of 50 $\mu\text{g/mL}$ suspensions of 80 nm streptavidin MNBs mixed with the indicated concentrations of biotinylated BSA. The spectra were recorded in the $\mathbf{B} \perp \mathbf{k}$ configuration using a cuvette with an optical path of 2 mm ($\lambda = 405$ nm) with $\theta = 0^\circ$ and $B_0 = 2$ mT. The solid lines are guides to the eye.

photodetector can be made semiquantitative. For a system consisting of identical MNBs, the Brownian relaxation dynamics is characterized by the relaxation frequency f_B which is inversely proportional to the hydrodynamic volume of a MNB. In the Supporting Information section S3, we present an analytical model accounting for the observed dynamics that can be used to extract the hydrodynamic particle size from the spectra measured by the optical technique. The continuous line in Figure 2b is the curve fit obtained with the proposed model using a single set of parameters to describe both the in-phase and out-of-phase components. The model is found to fit the data well and to provide robust estimates of the model parameters (details are given in Supporting Information section S6).

Figure 2c schematically illustrates the procedure for detection of the target DNA exploiting variation of the optical scattering contrast provided by the presented size-sensitive readout. First, the *V. cholerae* target DNA is amplified into micrometer sized coils (approximately 90 kbp, 60 min RCA time) via specific recognition of the cholera target molecule by padlock probes and followed by ligation and RCA.^{12,35} The DNA coils are mixed and incubated with MNBs functionalized with the target oligonucleotide. As opposed to free MNBs, MNBs bound to DNA coils cannot follow the external magnetic field variation at the same rate. This causes a reduction of the modulated optical signal near the characteristic frequency for the free MNBs.

Comparison of Different Measurement Configurations. For detection of low analyte concentrations, the use of a low concentration of MNBs is needed to enhance the relative target related signal variation. Figure 3 shows the in-phase and out-of-phase second harmonic photodetector signal measured versus frequency for four experimental configurations (cf., Figure 1): (1) $\mathbf{B} \parallel \mathbf{k}$ (polarization independent), (2) $\mathbf{B} \perp \mathbf{k}$ with a linearly polarized beam with $\theta = 0^\circ$, (3) $\mathbf{B} \perp \mathbf{k}$ with a linearly polarized beam with $\theta = 90^\circ$, and (4) $\mathbf{B} \perp \mathbf{k}$ with a circularly polarized beam. To facilitate comparison, the spectra are normalized to the average photodetector signal V_0 due to the transmitted light measured simultaneously with the spectra.

For the $\mathbf{B} \parallel \mathbf{k}$ configuration, the data were collected using a linearly polarized laser beam, but identical results were obtained for a circularly polarized beam (Supporting Information section S4). Hence, for this geometry there is no dependence on the polarization of the light. For the $\mathbf{B} \perp \mathbf{k}$ configuration, the data show a clear dependence on the polarization of the light. When

the light is polarized perpendicular to the magnetic field ($\theta = 90^\circ$), the spectra closely resemble those obtained for the $\mathbf{B} \parallel \mathbf{k}$ configuration. When the light is polarized parallel to the magnetic field ($\theta = 0^\circ$), the spectra display the same shape but have the opposite sign and a much larger magnitude. Thus, when the B field magnitude is high, the intensity of the transmitted light is reduced for $\theta = 0^\circ$ in the $\mathbf{B} \perp \mathbf{k}$ configuration. The optical contrast induced by the external magnetic field originates from the fact that the scattering is higher for light polarized along elongated MNB structures (individual MNBs or chains of MNBs) than for light polarized perpendicular to such structures. To further corroborate on the contrast mechanism, Figure 3 also shows the spectrum obtained in the $\mathbf{B} \perp \mathbf{k}$ configuration for circularly polarized light. It corresponds to the average value of the results obtained in the same $\mathbf{B} \perp \mathbf{k}$ configuration for linearly polarized light with $\theta = 0^\circ$ and $\theta = 90^\circ$, respectively. This confirms that a signal, although weaker, can be also obtained for an unpolarized light source, that is, the observed effect is of geometric rather than of magneto-optical origin.

From the results shown in Figure 3, we conclude that the $\mathbf{B} \perp \mathbf{k}$ configuration with $\theta = 0^\circ$ (Figure 1b) provides the largest signal. We therefore use this optimal configuration in the studies below. In Supporting Information section S5, we demonstrate that the shape of the spectra measured in this configuration does not depend on the MNB concentration and can be measured reliably for 50 nm MNBs in concentrations down to 1 $\mu\text{g/mL}$ corresponding to a molar MNB concentration of 2 pM.

Dependence on MNB Size. To demonstrate that the presented method can be used to distinguish between MNBs with different sizes, V_2' spectra have been recorded for MNBs with nominal diameters of 50, 130, and 250 nm and are shown in Figure 4a. To ease comparison of the measurements, the spectra are normalized to their maximum values. A clear dependence on the MNB size is observed such that the peak position shifts to lower frequency upon increasing MNB size. Supporting Information section S6 presents an analysis of the spectra and a comparison of the hydrodynamic MNB sizes obtained from fits of the spectra to independent determinations by dynamic light scattering and magnetorelaxometry measurements. A good correlation is found between the results obtained by the different techniques.

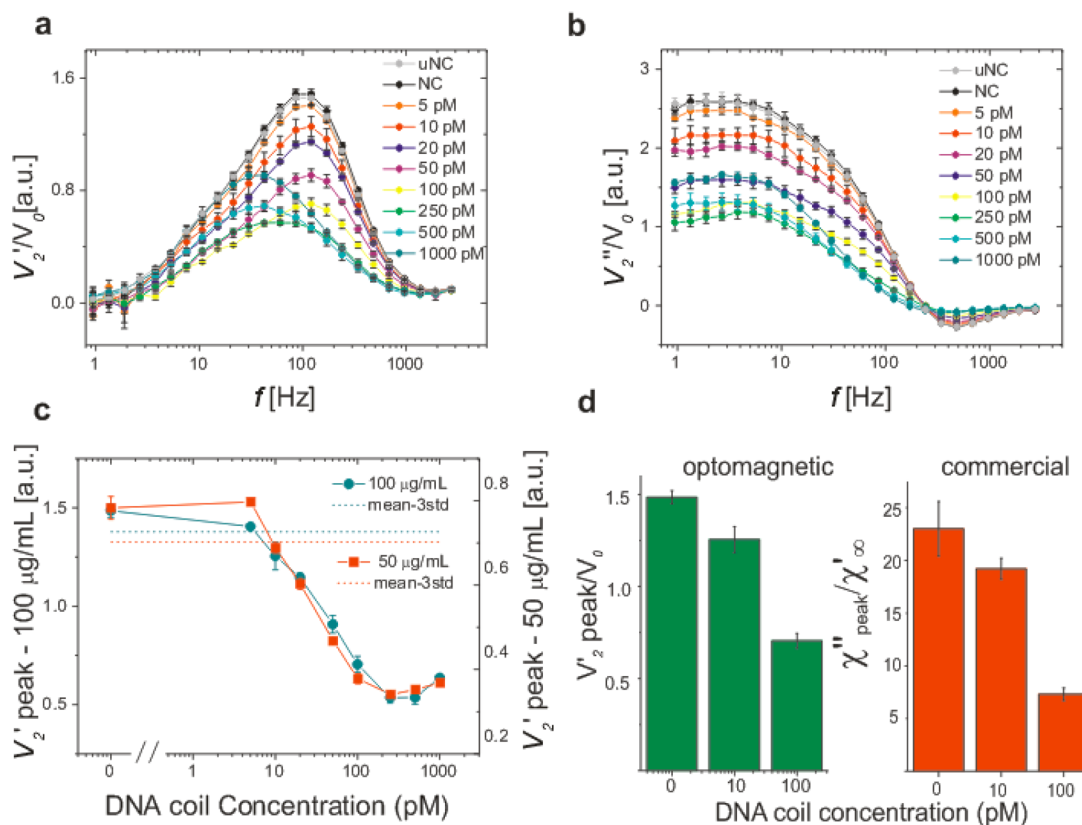


Figure 5. (a) V_2' and (b) V_2'' spectra of 100 nm MNBs (100 $\mu\text{g/mL}$) incubated with DNA coil suspensions of the indicated concentrations ($B \perp k$ configuration ($\lambda = 405$ nm) with $\theta = 0^\circ$ and $B_0 = 2$ mT). An unspecific negative control (uNC), corresponding to 5 nM coils from *E. coli* has been also included in the measurements together with the negative control (0 pM DNA coil concentration) (c) Dose-response curves calculated as the peak value of V_2' data for the MNBs concentrations of 100 and 50 $\mu\text{g/mL}$. (d) Comparison between the quantification of *V. cholerae* DNA coils obtained with the presented optomagnetic method (MNB concentration 100 $\mu\text{g/mL}$) and by magnetic susceptibility measurements in a commercial DynoMag AC susceptometer.

Detection of Biotinylated BSA. To establish the applicability of the method for biodetection on a biological model system for controlled agglutination, we study the clustering of streptavidin functionalized MNBs in the presence of different concentrations of biotinylated bovine serum albumin (bBSA), which has an average of three to four binding sites for streptavidin.¹¹

Figure 4b shows V_2' spectra of suspensions of 80 nm streptavidin magnetic beads after incubation with bBSA of the indicated concentrations. For high bBSA concentrations (up to a few nM), a dramatic reduction of the signal amplitude as well as a lowering of the peak position are observed as compared to a negative reference without bBSA. This indicates that most MNBs are part of larger agglomerates. These have larger hydrodynamic sizes corresponding to lower values of the peak position and are also likely to have a reduced optical anisotropy. For lower bBSA concentrations, a smaller reduction of the signal amplitude and a smaller shift of the peak position are observed. The changes are observed consistently for bBSA concentrations down to 100 pM, implying that the presence of even a low amount of clustered MNBs can be sensed by this technique.

Detection of DNA Coils Formed from *Vibrio cholerae* DNA. As a specific demonstration and clinically relevant case, we target the detection of *V. cholerae* DNA strands volume-amplified to micrometer sized coils and demonstrate that our approach is sensitive down to a target concentration of 10 pM.

Figure 5 shows the results of the experiments with coils formed from *V. cholerae* DNA, as shown in Figure 2c. Figure 5a shows curves measured for DNA coil concentrations from 0 to 1 nM using 100 nm MNBs diluted at two different concentrations (100 and 50 $\mu\text{g/mL}$). The experiments were done in triplicate. Moreover, an unspecific negative control (uNC) sequence (5 nM DNA coils from *E. coli* genome) was studied to verify that the oligonucleotide probes on the MNBs do not bind unspecifically to other amplified DNA coil types. It is observed from Figure 5 that the uNC is indistinguishable from the sample with 0 pM (negative control, NC) *V. cholerae* coils. The peak value is reduced upon increasing the DNA coil concentration (the concentrations given below are the final concentrations after mixing). This reduction is due to MNBs bound to the micrometer sized DNA coils as these cannot contribute to the dynamics on the same time scale as free MNBs.¹² For the investigated DNA coil concentrations, the reduction of the peak value is proportional to the coil concentration (Figure 5b). Interestingly, f_B slightly increases with increasing DNA coil concentration, as observed previously by Strömberg et al.,³⁶ suggesting that larger MNBs may hybridize more efficiently to DNA coils during incubation and hence are selectively depleted. For high DNA coil concentrations, a detectable low-frequency signal arising from the rotation of DNA coil-MNB agglomerates is expected from the magnetic susceptibility data.¹² This is observed in our data as a strong shift of the peak position at lower frequency for target concentrations above 250 pM. The intensity of the low-

frequency peak clearly increases with increasing target concentration up to 1 nM.

Dose–response curves have been calculated from the data by plotting the V_2' values obtained at the frequency of the peak ($f = 170$ Hz). Error bars correspond to standard deviations of triplicate experiments. Figure 5c shows the results for samples with the indicated DNA coil concentrations for 100 nm MNB concentrations of 100 and 50 $\mu\text{g/mL}$, respectively. The limit of detection, estimated as the blank (NC) signal plus 3 standard deviations, indicates that a coil concentration of 10 pM is detectable. For both MNB concentrations, the dose–response curve saturates at high DNA coil concentrations (Figure 5d). We mainly attribute this to the simple analysis method, since the spectra measured at high target concentrations clearly depend on the concentration. A more sophisticated data treatment¹⁸ based on the full V_2' and V_2'' spectra may further reduce the LOD and increase the dynamic range of the sensor.

Figure 5d shows $V_2'(170 \text{ Hz})$ for 0, 10, and 100 pM DNA coil concentrations incubated with 100 nm MNBs (100 $\mu\text{g/mL}$) obtained using the presented method and corresponding data obtained using a DynoMag system (Acreo, Göteborg, Sweden) that measures the magnetic susceptibility of a 200 μL sample volume.

The frequency sweep measured using the optical method was obtained within 3 min whereas the measuring time in the DynoMag system was about 30 min. The sensitivity, the relative variation vs concentration and the standard deviation of triplicate measurements are rather similar for the two techniques. However, the presented optical approach is considerably cheaper, faster, and more sensitive in the low-frequency range. In addition, since much lower concentrations of MNBs can be used, the limit of detection and sensitivity of the presented optical technique may likely be subject to significant further improvement.

Comparison to Literature. For an MNB concentration of 100 $\mu\text{g/mL}$, we obtain a limit of detection of around 10 pM and a dynamic range of about 2 orders of magnitude, which is comparable to previous work utilizing MNBs and DNA coils in a homogeneous assay format.^{12,18} Similar sensitivities have been demonstrated amplifying the target oligonucleotide via PCR, coupling it to MNBs and using a miniaturized nuclear magnetic resonance readout. The present work presents a significant improvement as it accesses the same sensitivity range while relying only on standard optical components and an electromagnet combined with a low-cost disposable plastic chip. A DNA amplification method is required to target clinically relevant bacteria concentration, but the one shown, based on the use of padlock probes and RCA, has the potential to be technically simpler to realize on-chip than PCR since only two temperature steps are needed. The method provides a highly robust genotyping, since the padlock probe recognition results in a highly specific detection that robustly resolves mutant sequence variants.^{24,37} In addition, RCA is less sensitive to contaminants/inhibitors than PCR.^{19,20}

The limit of detection using instruments based on fluorescence microscopy of fluorophore labeled DNA coils is extremely low (around 10 fM)²⁰ but these instruments require high-power lasers as well as fast and sensitive line-cameras, and therefore do not represent a potential readout technology for a point of care instrument.

Compared to the previously reported magneto-optical methods, the presented measurement configuration is simpler and more versatile. Moreover, commercial MNBs having a

polymer shell, can be utilized and detected even at very low concentrations. Agglutination assays for immunodetection have been previously presented^{38,39} and a similar readout method was recently presented by Ranzoni et al.^{11,32} They measured the critical frequencies for relaxation of short chains (dimers and trimers) of superparamagnetic beads formed due to the presence of the target using a rotating magnetic field and detected the signal arising from the scattered light. For superparamagnetic beads, the magnetic moment of a single bead will follow the field rotation and is not linked to the physical orientation of the bead. Chains of beads, however, will rotate because it is energetically favorable for the chains to be oriented along the magnetic field (shape anisotropy). Superparamagnetic beads bound to DNA coils will generally be well separated and will thus not exhibit this shape anisotropy. Hence, no modulation of the optical signal is expected from superparamagnetic beads bound to DNA coils.

In the present work, we use transmitted light and MNBs with a remnant magnetic moment. These MNBs produce a modulation of the optical signal that reflects the rotation dynamics of the MNBs even in the absence of a biological target. We detect the presence of DNA coils primarily via the decrease of the dynamic signal because of free MNBs because the dynamics of MNBs attached to DNA coils is shifted to much lower frequencies. Thus, this approach is fundamentally different from that of Ranzoni et al.

Our approach can also be used for the direct detection of proteins via proximity ligation to form DNA coils of the same type as those investigated in the current work or for the detection of larger entities such as cells and bacteria where the quantification of the amount of free MNBs in a sample is relevant.

■ CONCLUSION

We have presented a novel approach to the readout of a homogeneous assay based on optical transmission measurements of the complex second harmonic spectra of the MNB dynamics in a uniaxial applied AC magnetic field. We have demonstrated sensitive detection of variations of this dynamic behavior because of changes of MNB concentration and MNB size and because of the slowing down of the dynamics of functionalized MNBs when they cluster in the presence of target biomolecules. As a key result we have shown, that *V. cholerae* DNA strands amplified into coils can be precisely and reliably detected with picomolar sensitivity.

Our current work aims at integrating all sample preparation steps from DNA extraction to coil formation onto a single chip, as recently demonstrated for PCR amplification.¹⁶ The simple optical readout presented here requires only a transmission window in the chip, it utilizes only low-cost optical and magnetic components, and measurements can be carried out within minutes. Thus, the presented readout will be easy to integrate in a low-cost portable system for point of care detection of, for example, bacteria. Future work aims to establish a deeper understanding of the physical mechanism of the optomagnetic signal, to establish the method for the readout of protein and small molecule targets and to integrate the readout with automated sample extraction and processing.

■ ASSOCIATED CONTENT

📄 Supporting Information

Schematic of the setup used for the experiments, the data measured for circular polarized light, a description of the

analytical model, and the magnetic nanobead size determination study using the presented method, dynamic light scattering, and magnetorelaxometry. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written with contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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SUPPORTING INFORMATION

Novel readout method for molecular diagnostic assays based on optical measurements of magnetic nanobead dynamics

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S1. Schematic of the setup

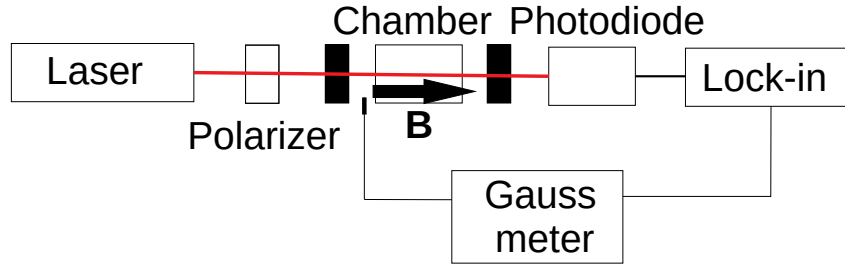


Figure S1 Schematic of the setup for the $\mathbf{B} \parallel \mathbf{k}$ configuration. The signal of the photo diode is filtered by the lock-in amplifier that is locked to the frequency of the applied field by the signal recorded by a high-speed Hall probe inserted in the system.

In the setup, sketched in Figure 1 and Figure S1, the light source was provided by a 635 nm wavelength red laser diode (LabLaser 635nm 5mW ULN, Coherent Inc., Santa Clara, CA, USA) or a 405 nm wavelength blue laser diode (LDH-D-C-405, PicoQuant GmbH, Berlin-Adlershof, Germany). The signal was collected by a Thorlabs PDA36A photo detector while the beam was expanded to a final diameter of 5 mm via an adjustable beam expander (Laser Beam Expander bm.x VIS-YAG 4x, Linos Inc., Milford, CA, USA). Two electromagnets were used to produce the field either parallel or perpendicular to the laser beam. The magnetic field was measured in real-time using a high-speed Hall probe transducer (F1A Magnetic Field Transducer, SENIS AG, Baar (Zug), Switzerland). The self-inductance of the coils was corrected for via software to ensure a constant amplitude and phase of the magnetic field excitation. The optical signal modulation was measured using a Signal Recovery model 7225 computer-controlled lock-in amplifier. Dynamic light scattering measurements were performed with a Malvern Zetasizer Nano (Malvern Instruments, Ltd. Worcestershire, UK). Measurements were repeated three times and averaged. All magnetic bead samples were purchased from Micromod

(Rostock, Germany). The beads of 130 nm and 250 nm were of type Nanomag-D, while the beads of 50 nm and 80 nm and 100 nm were of type BNF-Starch. All bead types had a sizeable remnant magnetic moment and a linear low-field magnetic response. In the bBSA and DNA coil detection experiments, the setup sketched in Figure 1b was used where the microfluidic system was substituted with commercial cuvettes (Malvern Z117, Malvern, US) in order to ensure reproducibility between the measurements.

S2. Sample preparation

Padlock probe target recognition, ligation and RCA

The sequences used are given in Table S1. For synthesis of a 100 μ L batch of 5 nM DNA-coils (*Vibrio cholera* or *Escheria coli*), a 50 μ L ligation mix (20 nM) was prepared by mixing 5 μ L of 10 $\times$ phi29 DNA polymerase buffer, 2.5 μ L of ATP (20 mM, Thermo Fisher Scientific, USA), 1 μ L of phosphorylated padlock probe (1 μ M, Biomers, Germany), 3 μ L of target (1 μ M, DNA Technology, Denmark), 1 μ L of T4 DNA ligase (1 U/ μ L, Thermo Fisher Scientific) and 37.5 μ L of MilliQ water followed by incubation at 37°C for 15 min. A 60 μ L RCA mix was prepared by mixing 25 μ L of ligation mix, 6 μ L of 10 $\times$ phi29 DNA polymerase buffer, 4 μ L of dNTP (2.5 mM, Thermo Fisher Scientific), 6 μ L of BSA (2 μ g/ μ L, sterile filtered, NEB, USA), 0.4 μ L of phi29 DNA polymerase (Thermo Fisher Scientific) and 18.6 μ L of MilliQ water followed by incubation at 37°C for 60 min (RCA reaction occurring) followed by 65°C for 5 min (inactivation of enzymes). A hybridization buffer consisting of 4 μ L of Tris-HCl (1 M, pH 8.0, Sigma-Aldrich), 8 μ L of EDTA (0.5 M, Sigma-Aldrich), 2 μ L Tween-20 (10%, Sigma-Aldrich), 20 μ L NaCl (5 M, Sigma-Aldrich) and 6 μ L of MilliQ water was added

to the RCA mix to obtain the final batch of DNA coils. Dilution series of DNA coils were prepared by stepwise dilution of the 5 nM DNA coil batch with hybridization buffer.

Conjugation of detection oligonucleotides to magnetic nanobeads For the detection of DNA coils, 150 μ L of MNBs (100 nm BNF-Starch streptavidin, Micromod, Germany, 10 mg/mL of solid content, 6×10^{12} beads/mL) was washed in 50 μ L of 1 $\times$ Wtw buffer (10 mM Tris-HCl, 5 mM EDTA, 0.1% Tween20, 0.1 M NaCl) (all materials from Sigma-Aldrich) two times. 9.45 μ L of detection oligonucleotide (Table S1) solution (Biomers, Germany, 10 μ M) was added to the bead suspension (63 times oligo excess with respect to total number of beads) followed by incubation at room temperature for 30 min with tube rotation. The MNBs were finally washed 1 $\times$ Wtw buffer three times and finally resuspended in 150 μ L 1 $\times$ PBS pH 7.5 (Sigma-Aldrich) to a magnetic bead solid content of $\sim$ 10 mg/mL.

Table S1. Sequences of Targets, Padlock Probes and Detection Probes used in this study.

Name	Sequence
<i>V. cholera</i> target	5'-CCCTGGGCTCAACCTAGGAATCGCATTTG-3'
<i>V. cholera</i> padlock probe	5'-TAGGTTGAGCCCAGGGACTTCTAGAGTGTACCGACCTCAGTAGCCGTGACTATCGACTTGTTGATGTCATGTGTGCGACCAAATGCGATTCC-3'
<i>V. cholera</i> detection probe	Biotin-5'-TTTAGTAGCCGTGACTATCGACT-3'-FAM
<i>E. coli</i> target	5'-ACGTCGCAAGACCAAAGAGGGGGACCT-3'
<i>E. coli</i> padlock probe	5'-CTTTGGTCTTGCGACGTCAGTGGATAGTGTCTTACACGATTTAGAGTGTACCGACCTCAGTAGCCGTGACTATCGACTAGGTCCCCCT-3'
<i>E. coli</i> detection probe	Biotin-5'-TTTTTTTTTTTTTTTTTTTTTTGTGGATAGTGTCTTACACGA-3'-FAM

S3. Analytical model

The rotation dynamics of an entity with hydrodynamic volume V_h is characterized by the Brownian relaxation frequency f_B

$$f_B = \frac{k_B T}{6 \pi \eta V_h}, \quad (1)$$

where k_B is Boltzmann's constant, T is the absolute temperature and η is the dynamical viscosity of the fluid. In this work, we consider the Brownian relaxation of MNBs with a remnant magnetization, i.e. a blocked magnetic moment. Upon excitation by a weak alternating magnetic field $B_{AC}(t) = B_0 \sin(2\pi f t)$, the magnetization $m(t)$ of the MNBs changes due to their physical rotation. In the time domain, the magnetization can be written

$$m(t) = m_{AC} \sin(2\pi f t - \phi), \quad (2)$$

where ϕ is the phase lag between the magnetization response and the excitation and m_{AC} is the frequency-dependent amplitude of the magnetization. In the frequency domain, the MNB response is characterized by the complex magnetic susceptibility $\chi = \chi' - i\chi'' = |\chi|(\cos \phi - i \sin \phi)$ with in-phase component χ' and out-of-phase component χ'' . At low enough frequency, the response of the magnetization of the MNB is in-phase with the applied magnetic field. Upon increasing frequency, the MNB magnetization lags behind the applied field and χ' decreases monotonically. Correspondingly, χ'' initially increases, attains its maximum value for $f = f_B$, and decreases above f_B . Cole and Cole [1] have shown that the magnetic susceptibility of a bead ensemble is well described by

$$\chi = \chi' - i\chi'' = \frac{\chi_0 - \chi_\infty}{1 + (if/f_B)^{1-\alpha}} + \chi_\infty, \quad (3)$$

where χ_0 and χ_∞ are the magnetic susceptibilities for $f = 0$ and $f \rightarrow \infty$, respectively, and α ($0 \leq \alpha < 1$) is a parameter accounting for the polydispersity the MNBs ($\alpha = 0$ for a monodisperse ensemble of MNBs) [1]. For convenience, we define the normalized magnetic susceptibility as $\tilde{\chi} = \chi/\chi_0 = \tilde{\chi}' - i\tilde{\chi}'' = |\tilde{\chi}|(\cos \phi - i \sin \phi)$, which fulfills that $\tilde{\chi}(f = 0) = 1$ and $\tilde{\chi}(f \rightarrow \infty) = \chi_\infty/\chi_0$ (< 1).

The photo detector signal only depends on the amplitude of the AC magnetic field and not its sign. In the main text, we have argued that the rotation of the individual MNBs determines the time scale for the optical signal modulation. We therefore make the following assumptions:

- (1) The photo detector signal can be described by

$$V(t) = V_{\text{offset}} + V_{\text{AC}} \sin^2(2\pi ft - \phi) \quad (4)$$

where V_{AC} is the frequency-dependent amplitude of the signal modulation, V_{offset} is the signal offset and where we have neglected possible higher order terms of even powers of $\sin(2\pi ft - \phi)$. With this convention, a positive V_{AC} corresponds to a higher intensity of transmitted light when the magnetic field is large.

- (2) V_{AC} has generally the same frequency dependence as the magnetic susceptibility squared, such that we can write $V_{\text{AC}} = V_0 |\tilde{\chi}|^2$, where V_0 is the amplitude for $f \rightarrow 0$.

With these assumptions (following the procedure from Ref. [2]) we calculate the complex 2nd harmonic photo detector signal $V_2 = V_2' + iV_2''$ measured by lock-in technique to

$$V_2' = -\frac{1}{2\sqrt{2}}V_0 |\tilde{\chi}|^2 \sin(2\phi) = -\frac{1}{\sqrt{2}}V_0 (\tilde{\chi}')(\tilde{\chi}'') \quad (5a)$$

$$V_2'' = -\frac{1}{2\sqrt{2}}V_0 |\tilde{\chi}|^2 \cos(2\phi) = -\frac{1}{2\sqrt{2}}V_0 [(\tilde{\chi}')^2 - (\tilde{\chi}'')^2] \quad (5b)$$

where we used that $\tilde{\chi}' - i\tilde{\chi}'' = |\tilde{\chi}|(\cos \phi - i \sin \phi)$. These expressions were combined with Eq. (3) to produce the fits in the main text. As a key result the model can precisely fit both signal components and explain the sign change observed at high frequencies in the V_2'' plot. Our future work aims at making the above semi-quantitative model quantitative and at establishing a clear link between the model assumptions and parameters and the physical properties of the magnetic nanobeads and their interactions.

S4. Data for linearly and circularly polarized light

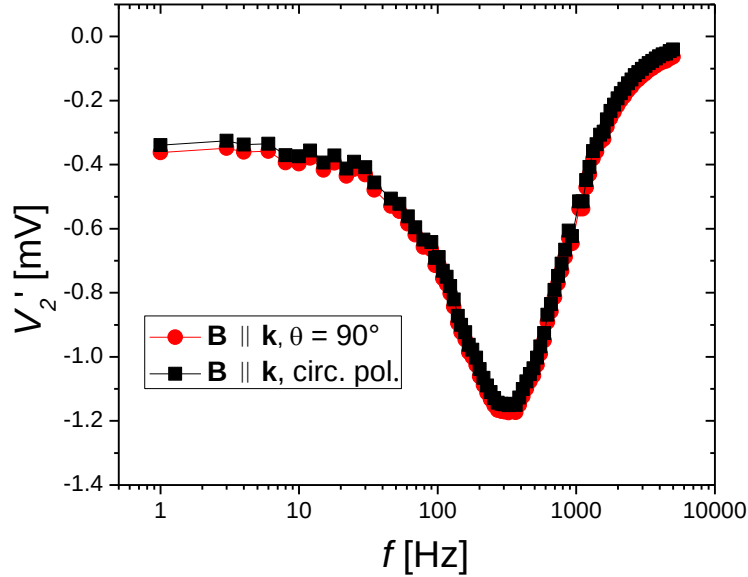


Figure S2 V_2' spectra recorded in the $\mathbf{B} \parallel \mathbf{k}$ configuration using a linearly polarized beam and a circularly polarized beam. No significant difference can be observed between the two spectra.

S5. Dependence on MNB concentration

Figure S3 shows the V_2' spectra measured for suspensions of 50 nm MNBs with MNB concentrations ranging between 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$. The signal is found to scale approximately linearly with the MNB concentration and the shape of the spectra is remarkably independent of the MNB concentration. The lowest investigated concentration of 1 $\mu\text{g/mL}$, which still gives rise to a clearly measurable spectrum, corresponds to a MNB molar concentration of 2 pM.

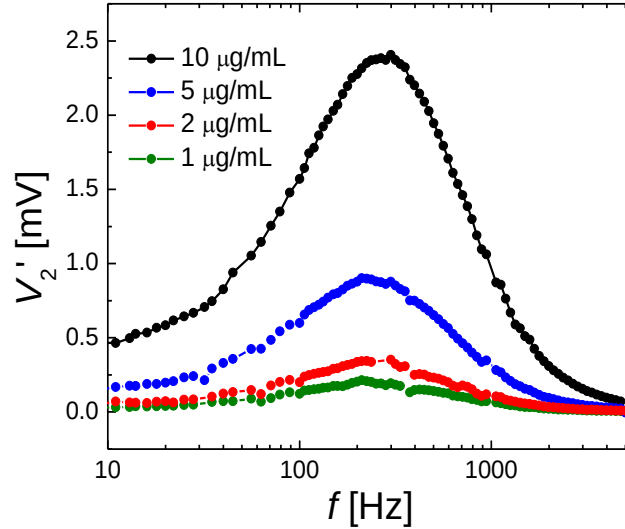


Figure S3. V_2' spectra recorded for suspensions of 50 nm MNBs with MNB concentrations ranging from 1 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$ ($\mathbf{B} \perp \mathbf{k}$ configuration with $\theta = 0$ and $B_0 = 2$ mT and $\lambda = 635$ nm).

S6. Size determinations

In this section, we compare the particle size determined by the presented optical transmission measurement method with determinations by dynamic light scattering and magnetorelaxometry. The comparison is carried out for samples with nominal MNB sizes of 50, 130 and 250 nm.

Magneto-optical transmission spectra (presented method)

Figure S4 shows the V_2' spectra measured using the presented optical transmission method in the $\mathbf{B} \perp \mathbf{k}$ configuration with $\theta = 0^\circ$ and $B_0 = 2$ mT. The solid lines are fits of the analytical model presented in section S3 to the data.

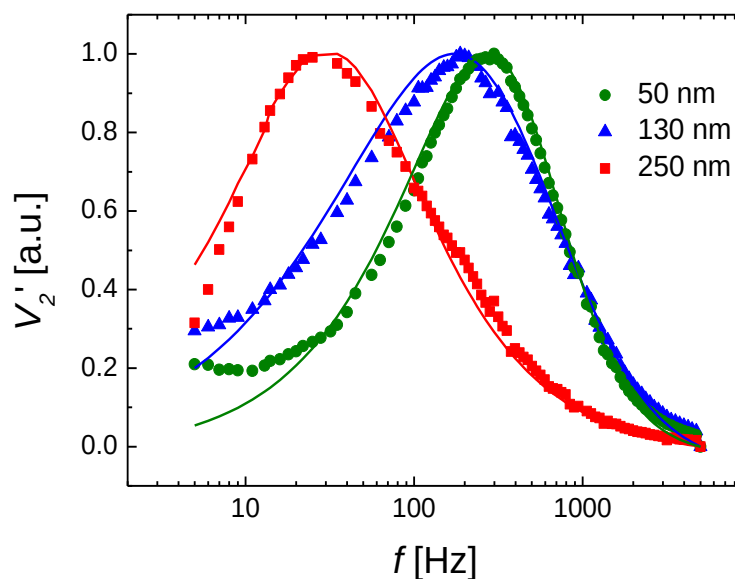


Figure S4. V_2' spectra recorded for 10 $\mu\text{g/mL}$ suspensions of MNBs with nominal dimensions of 50, 130 and 250 nm ($\mathbf{B} \perp \mathbf{k}$ configuration with $\theta = 0^\circ$ and $B_0 = 2$ mT). The lines represent fits obtained using the model presented in section S3.

The resulting values of f_B , the corresponding hydrodynamic diameter and the Cole-Cole parameter α are given in Table S2 below.

Dynamic light scattering data

Figure S5 shows dynamic light scattering data obtained on the same particle suspensions in a Malvern Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK). Measurements were repeated three times and averaged. The resulting average particle diameters are given in Table S2 below.

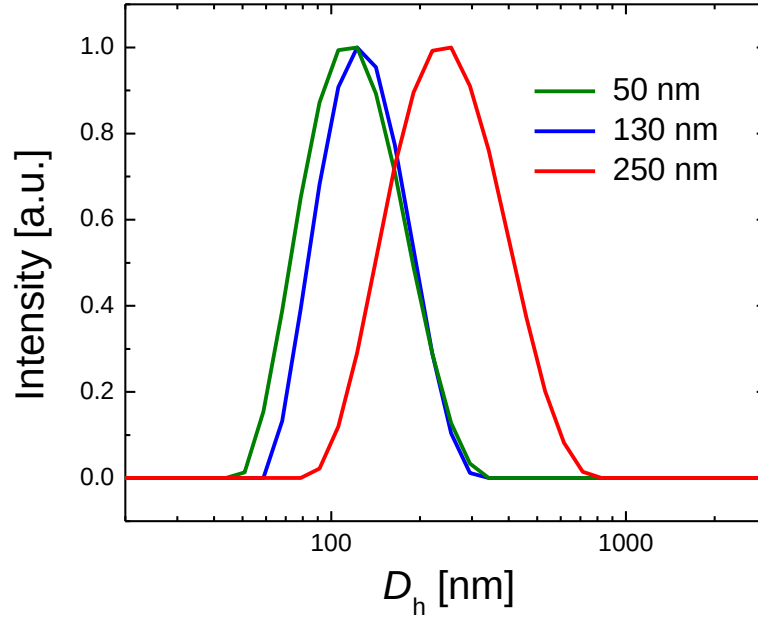


Figure S5. Dynamic light scattering measurements on the three types of MNBs with the indicated nominal sizes.

Magnetorelaxometry

The same samples were also characterized in a chip-based system for magnetorelaxometry as described by Østerberg *et al.* [3] (Fig. S6). The results for the MNBs with nominal sizes of 130 and 250 nm magnetic in Ref. [3] were obtained on the same batch as used in the present study. The results on the 50 nm beads were obtained for the same concentration and for the same instrument settings as the two other bead sizes. The resulting values of f_B , the corresponding hydrodynamic diameter D_h and the Cole-Cole parameter α are given in Table S2 below.

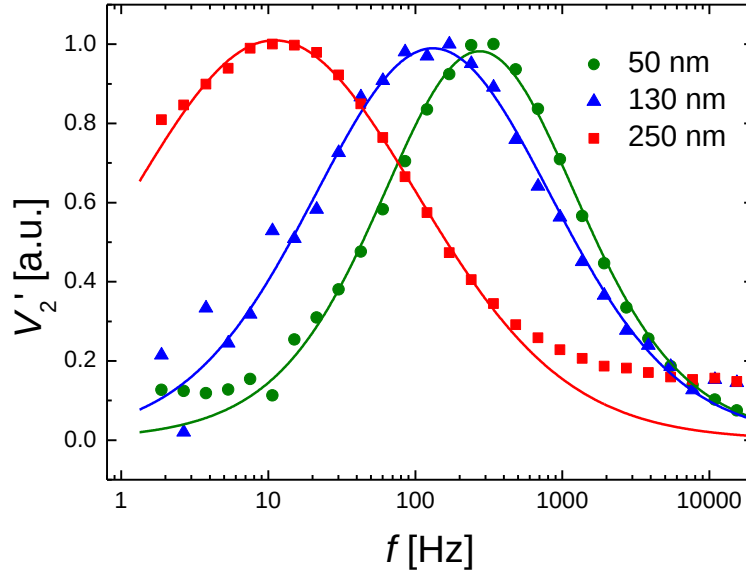


Figure S6. Magnetorelaxometry measurement results on the three types of MNBs with the indicated nominal sizes.

Table S2 Nominal particle sizes and sizes determined by dynamic light scattering (average size $\langle D \rangle$), the presented method (hydrodynamic size D_h), and by chip-based magnetorelaxometry (hydrodynamic size D_h). For the two latter methods, the table also gives the Brownian relaxation frequency f_B and the Cole-Cole parameter α extracted from the fits.

Nominal value	Presented method			DLS	Magnetorelaxometry		
	f_B [Hz]	D_h [nm]	α	$\langle D \rangle$ [nm]	f_B [Hz]	D_h [nm]	α
50 nm	553(2)	92(2)	0.14(2)	114	274(4)	120(1)	0.18(1)
130 nm	471(4)	100(2)	0.32(2)	126	131(4)	153(2)	0.29(1)
250 nm	32(3)	240(1)	0.11(2)	250	11.1(3)	349(3)	0.40(1)

Discussion

The values of f_B obtained by the fitting using the analytical model from section S3 correspond to sizes that are generally ~ 5 -20% lower than those obtained by dynamic light scattering and significantly lower than the sizes obtained from magnetorelaxometry

measurements. These differences may be attributed to the different weighting of a particle size distribution by the different methods. The hydrodynamic size obtained by magnetorelaxometry for the 250 nm beads is significantly overestimated due to bead sedimentation [2,3]. The results demonstrate a good correlation between the particle dimensions obtained by the presented optical method and in particular dynamic light scattering measurements.

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