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Size-dependent ferrohydrodynamic relaxometry of magnetic particle imaging tracers in different environments

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Purpose: Magnetic particle imaging (MPI) is a recently developed imaging technique that seeks to provide ultrahigh resolution and tracer sensitivity with positive contrast directly originated from superparamagnetic iron oxide nanoparticles (NPs). MPI signals can be generated from a combination of Néel relaxation, Brownian rotational diffusion, and hysteretic reversal mechanisms of NPs in response to applied magnetic fields. When specific targeting of organs, such as carcinoma and endothelial cardiovascular cells, is needed, different behavior may be expected in immobilized NPs, due to complete or partial elimination of the Brownian motion. Here, the authors present an experimental investigation of the MPI spatial resolution and signal intensities as a function of a wide range of median core sizes of NPs under four representative conditions, including after immobilization in a tissue equivalent medium.

Methods: Monodisperse hydrophobic NPs with median core diameters ($d_0$) ranging from 7 to 22 nm were synthesized in organic media and subsequently dispersed in aqueous solution after a facile surface modification. Morphology, median size, size distribution, and magnetic properties of the NPs were investigated. Hydrophobic and hydrophilic NPs with various core sizes were immobilized in trioctyl phosphine oxide and agarose gel, respectively. Their size-dependent performance as MPI tracers for system matrix and $x$-space image reconstruction was evaluated using magnetic particle spectrometry (MPS) and compared with the free rotating counterparts.

Results: Immobilized NPs with core diameters smaller than $\sim$20 nm have similar spatial resolution, but lower signal intensities when compared with their free rotating counterparts. Compared to their performance in solution, spatial resolution was improved, but signal intensity was lower, when larger NPs with core size of 22 nm were immobilized in agarose. Same trends were observed in signal intensities, when considering either system matrix or $x$-space approaches. The harmonic and $dm/dH$ signal intensities changed linearly and the spatial resolution did not change with decreasing NP concentration up to 15 $\mu$g/ml.

Conclusions: The results show that the MPI signal is very sensitive to both NP size and environment. The authors’ calculations show that Brownian rotational diffusion is slower than the field switching cycle and, therefore, it has minimal influence on MPS signals. $dm/dH$ analyses show that Néel relaxation is the dominant mechanism determining MPI response in smaller NPs ($d_0 < \sim$20 nm). Larger NPs show hysteretic reversal when the applied field amplitude is large enough to overcome the coercivity. Linear variation of the MPS signal intensity with iron concentration but with uniform spatial resolution enables quantitative imaging for a range of applications, from high-concentration bolus chase imaging to low-concentration molecular imaging (while the authors’ instrument is noise-limited to $\sim$millimolar iron concentrations, nanomolar sensitivity is expected for MPI, theoretically). These results pave the way for future application of the authors’ synthesized tracers for immobilized or in vivo targeted MPI of tissues. © 2013 American Association of Physicists in Medicine.

Key words: magnetic particle imaging, magnetic relaxation, hysteretic reversal, targeting, iron oxide nanoparticles

I. INTRODUCTION

Magnetic particle imaging (MPI) is a quantitative tomospheric imaging technique for visualizing magnetic nanoparticle tracers in a wide range of potential diagnostic and therapeutic applications, such as cardiovascular imaging and early cancer detection. MPI features depth independent signal and outstanding contrast, since signal is generated solely by magnetic nanoparticles and there is no interfering signal from diamagnetic tissue. In theoretical predictions, MPI’s mass sensitivity is in the order of nanograms (Fe) and spatial resolution is 0.2–0.5 mm [assuming magnetite nanoparticle tracers of optimum size (20–30 nm), field gradient of about 3 $T\mu_0^{-1}m^{-1}$, and neglecting the effects of relaxation mechanisms], which makes it a very promising tool for full-body imaging, in comparison with other techniques such as MRI.
High sensitivity and contrast are promising for cancer imaging, for example, direct imaging of the intratumor distribution of therapeutic magnetic NPs to verify if they are distributed in the layers around the tumor cortex rather than the desired accumulation in the core for effective theranostic application.\textsuperscript{5} Linearity of the signals and higher tracer sensitivity without any interfering background signal makes MPI a potentially noninvasive technique for cellular imaging applications such as in vivo stem cell tracking.\textsuperscript{6,7}

In spite of all the advantages highlighted above, in practice, it remains a challenge to optimize MPI tracers for maximum resolution and signal intensity. Generally, in MPI, time-dependent magnetization of the nanoparticles (NPs) caused by an alternating magnetic field (typically in the range of 3–25 kHz) induces a voltage in a receiver coil.\textsuperscript{3,4} This signal is proportional to $\frac{dm}{dt}$, in which $m$ is the NPs magnetic moment. Furthermore, it is the nonlinear magnetization of superparamagnetic NPs that enables signal localization and makes MPI possible. A gradient field is applied which features a field-free point (FFP) or a field free line. To form an image, the FFP is scanned across the sample; NPs located at the FFP experience a rapid change in magnetization as the FFP passes and generate signal, while NPs spatially removed from the FFP have saturated magnetization, and therefore experience no change in magnetization and thus generate no signal.

Based upon this simple principle, two theories for MPI image formulation have been developed: system matrix and $x$-space. In system matrix reconstruction, the signal’s higher-order harmonic content can be distinguished from the applied AC field using Fourier analysis.\textsuperscript{3,8,9} In the $x$-space reconstruction, an image is formed by relating signal intensity to FFP location at all points within the imaging volume.\textsuperscript{10–12} In $x$-space MPI, the point spread function (PSF) is a product of the derivative of the magnetization, $\frac{dm}{dH}$, which is purely a property of the tracer, and the instrument-dependent field gradient, $dH/dx$, in which $x$ is the distance.\textsuperscript{13,14} We emphasize that the principles of tracer optimization are the same for both reconstruction approaches, which are simply linked by the Fourier transform. The time-rate of change of tracer magnetization should be maximized for a given alternating applied field. Relevant to system matrix reconstruction, the number of measurable harmonics and their relative intensity will thus both be maximized. Relevant for $x$-space reconstruction, the $\frac{dm}{dH}$ height is maximized for sensitivity and its full width at half maximum (FWHM) is minimized for spatial resolution. Magnetic particle spectrometry (MPS) is now an established method for rapid screening of NPs to determine their suitability for MPI.\textsuperscript{12,15,16}

Superparamagnetic iron oxide is the most favorable tracer for MPI, due to its biocompatibility and adequate magnetic properties,\textsuperscript{17} with several formulations of superparamagnetic iron oxides having already been approved by the FDA for clinical applications such as MRI or iron delivery in anemia.\textsuperscript{18–20} However, iron oxide NPs prepared for MPI should be designed delicately to have the highest size monodispersity and lowest magnetic anisotropy, in order to provide highest spatial resolution and signal intensity during applications\textsuperscript{8,17} within the constraints of limits set by peripheral nerve stimulation and specific absorption.\textsuperscript{10}

Previously, we have investigated how the core-size, hydrodynamic-size, and the size distribution of the tracer determine the MPI resolution and intensity.\textsuperscript{3,12,21} These studies were performed when the NPs, dispersed in aqueous media, were able to move freely in response to the applied magnetic field. However, during in vivo imaging, the environment of the NPs is quite complex and free NP motion cannot be assumed. For example, in the case of specific targeted imaging, NPs may bind to the cell membranes and their magnetic relaxation behavior may be considerably different from their free state.\textsuperscript{22} We are interested in understanding very generally how NPs will respond to a MPI excitation field in a variety of potential circumstances, as determined by the NP’s magnetic properties, their surrounding environment, and the field amplitude and frequency. Therefore, it is important to understand the different magnetic reversal mechanisms, i.e., Brownian rotational diffusion, Neél relaxation, and hysteretic reversal, available to NPs when an alternating field is applied.\textsuperscript{17} Brownian rotational diffusion occurs by the free rotation of magnetically blocked NPs with respect to the fluid to achieve an equilibrium orientation under an applied field.\textsuperscript{23,24} On the other hand, Neél relaxation occurs by thermally activated reversal of the moment within the NPs, and involves no physical movement of the NPs. Finally, for magnetically blocked NPs, hysteretic reversal can also occur if the applied field exceeds the NP coercivity, $H_c$. The measurement time is also relevant, and when multiple mechanisms are available, the fastest is expected to dominate, where reversal time is determined by NP size, field amplitude, and field ramp rate.\textsuperscript{25,26} Therefore, in designing MPI tracers for any application, it is crucial to understand the contributions of these three reversal mechanisms, and how they relate to the NPs properties and environment, and to the frequency and amplitude of the applied AC field.\textsuperscript{15}

In this study, we investigated the size-dependent performance of MPI tracers under idealized local environments representing bound and free states, using MPS to evaluate tracer relevance in both the system matrix and $x$-space MPI formulations. To accomplish this, a thermal decomposition process was used to prepare hydrophobic iron oxide nanoparticles with various median core sizes, ranging from 7 to 22 nm, all with narrow ($\pm 3–5$ nm) size-distributions.\textsuperscript{21,27} The nanoparticles were then transferred from chloroform to aqueous media using an amphiphilic copolymer.\textsuperscript{27} To create idealized bound states, in which NP motion was prohibited and to monitor the contribution of the relaxation regimes prior to and after phase transfer, the NPs were homogeneously dispersed and immobilized in trioctyl-phosphine oxide (TOPO), which is solid at room temperature, and tissue equivalent agarose gel, respectively, and the resultant MPS signal intensities (obtained from both system matrix and $x$-space approaches) and resolution (obtained from $\frac{dm}{dH}$ measurements analyzed using $x$-space theory) were compared with freely moving NPs. Since the frequency is fixed (25 kHz) in our MPS system, we used different field amplitudes to study
the contribution of the hysteretic reversal in the received signals. These comparisons enabled us to investigate the size and environmental dependence of NP’s relaxation behavior. In particular, we hoped to better understand the NPs’ response during targeted-imaging, when Brownian rotation may be inhibited due to specific binding or perfusion into host tissue, as shown schematically in Fig. 1(a). This model shows that MPI has the potential to be an effective imaging modality even when the NPs are immobilized in tissues (e.g., cancer targeting) and cells (e.g., stem cells).
II. MATERIALS AND METHODS

II.A. Synthesis of different sizes of magnetite tracers

Iron oxide NPs were synthesized from pyrolysis of iron oleate (Fe–Ol) fatty acid salt, in the presence of oleic acid (OA, tech grade 90%, Sigma-Aldrich, St. Louis, MO) and 1-octadecene (ODE, technical grade 90%, Sigma-Aldrich, St. Louis, MO), according to established protocols. Fe–Ol was prepared by reacting sodium hydroxide (NaOH, 97%, Sigma-Aldrich, St. Louis, MO) with anhydrous iron chloride (FeCl₃, 98%, Alfa Aesar, MA) and OA, following the method reported before. The purified Fe–Ol was stored in ODE with a concentration of 18 wt. % Fe–Ol. NPs were prepared by refluxing a mixture of Fe–Ol and OAc at 320°C for 24 h under argon atmosphere. Median core sizes of the NPs were tuned accurately by adjusting the molar ratios of Fe–Ol:OA as 1:0, 10, 15, 18, 19, and 20.

II.B. Phase transfer of the as-synthesized NPs

Phase transfer of the NPs was performed following our previous reports. Amphiphilic copolymer of poly(maleic anhydride-alt-1-octadecene) (PMAO, Mn = 30 000–50 000, Sigma-Aldrich, St. Louis, MO) and methoxy poly(ethylene glycol) (PEG, Mn = 5000, Sigma-Aldrich, St. Louis, MO) was used for coating and phase transfer of the iron oxide NPs. PMAO-PEG was prepared by acid catalysis of mPEG and its ester bonding with anhydride ring of PMAO as we described in Ref. 27. Purified NPs were collected by a strong magnet and dried in vacuum for 1 h. 10 mg of the hydrophobic NPs were dispersed in 1–2 ml of chloroform by sonication for about 1 h. 10 mg of PMAO-PEG copolymer was added to this mixture. After sonication for about 1 h the NPs were dried in argon and dispersed in 1 ml of tris-acetate-EDTA (TAE, 1x) buffer by sonication for 30 min, followed by size exclusion purification (SephacrylTM S-200 gel, GE healthcare Life Sciences, US) to remove unbound polymer molecules and transfer the NPs to water. The NPs were stored in the refrigerator (−4°C) for further analysis.

II.C. Characterization of the NPs

Inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Jarrell Ash 955, MA) was used to determine the concentration of iron in the samples. The hydrodynamic sizes of NPs dispersed in water were measured by dynamic light scattering (DLS, Zetasizer Nano, Malvern Instruments, UK), with mean diameters (intensity distribution) used for comparing samples and calculating Brownian relaxation times. Morphology and crystallographic phase structure of the NPs were investigated by bright field imaging and selected area diffraction, respectively, using a transmission electron microscope (TEM, FEI TecnaiTM TM G2 F20, 200 kV, Hillsboro, OR), equipped with a Gatan CCD camera (Pleasanton, CA). TEM samples were prepared by depositing and drying a drop of diluted NPs suspension on carbon coated copper grids. Liquid samples (~100 μl, containing 100–200 μg of NPs) were used for studying the magnetization behavior (M vs H) of the NPs by a room temperature vibrating sample magnetometer (VSM, Lakeshore, Westerville, OH). Agarose and TOPO dispersed samples were transferred to measurement vials before their gelation or freezing and then cooled down in the fridge in order to immobilize the NPs. The magnetization was fit to the Langevin function, using the Chantrell method, to determine the median magnetic core size (d₀) of the NPs. This method is volumetric and gives the median core size distribution of a statistically significant number of NPs, better representing the total sample than TEM measurements.

The Brownian rotational diffusion time constant, \( \tau_B \), is
\[
\tau_B = \frac{3V\eta}{k_BT},
\]
where \( V \) is the hydrodynamic volume of the NPs, \( \eta \) is the viscosity of the environment, \( K_B \) is the Boltzmann’s constant, and \( T \) is the ambient temperature (K) where small angles of rotation and small fields \( (H \ll H_K) \) are assumed. The Néel relaxation time (\( \tau_N \)) for thermal excitation of the magnetization over an anisotropy barrier (KV) under an applied field, \( H \), comparable in magnitude to \( H_K \), a property of the material, is given by
\[
\tau_N = \tau_0 \exp \left( \frac{KV}{k_B T} \left( 1 - \frac{H}{H_K} \right)^2 \right),
\]
where \( \tau_0 = 10^{-10} \), \( V \) is the volume of the core iron oxide, \( H \) is the magnetic field \( (|H|/\mu_0) \), and \( K \) (11 kJ/m³) is the magnetocrystalline anisotropy constant for bulk iron oxide. Note that for small field amplitudes, when \( H \ll H_K \), the term \((1-H/H_K)^2\) can be dropped in the exponent.

II.D. Immobilization of the NPs

Certified molecular biology agarose (BioRad, Hercules, CA) and trioctyl-phosphine oxide (TOPO, 99%, Sigma-Aldrich, St. Louis, MO) were used for immobilization of the hydrophilic and hydrophobic NPs, respectively. The water dispersed NPs were mixed with a 3 wt. % solution of the agarose (1:1 v/v) and were stored in the fridge for 2 h, allowing for mixture gelation. Final agarose concentration was 1.5 wt. %, which is a standard value defined for having tissue-equivalent characteristics. Pore sizes smaller than ~250 nm were reported for agarose gel prepared with the same concentration. Solid TOPO was placed in a water bath (~50°C) and after complete melting was mixed thoroughly with chloroform dispersed NPs (1:1 v/v). Solidification of the TOPO occurs after about 5 min at room temperature. Concentrations of the NPs were known in all liquid (1–2 mg/ml) and frozen (0.3–0.5 mg/ml) samples and vigorous mixing was used to assure their homogeneous dispersion in gel and TOPO. The saturation magnetization and MPI performance of these immobilized NPs were studied by VSM and MPS, respectively.
II.E. MPI signal testing of the NPs

We used a custom-built MPS for MPI signal analysis of the NPs with various median core sizes, ranging from 7 to 22 nm as well as commercial NPs Resovist™ and Feridex™. The MPS excites magnetization in NPs using a transmit coil, and measures the voltage, V(t), induced in a receiver coil by the changing NP magnetization (details of the spectrometer can be found in our previous report). The MPS signals (dm/dH and harmonic spectrum) are calculated from the received voltage, V(t), and are used to evaluate the MPI performance of the NPs in the system matrix or x-space approaches, respectively. Procedures for obtaining these data are discussed in Secs. II.E.1 and II.E.2. For each measurement, 200 μl of the samples with known iron concentrations were transferred to a 0.6 ml microcentrifuge tube and each vial was inserted into the MPS coils, applying sinusoidal excitation fields of 5.1–18.6 mTμ0−1 (peak-peak, f0 = 25 kHz). The sample holder and instrument are designed to ensure that the sample’s position is always the same in the center of the coil. All MPS measurements were repeated three times and their average with standard deviation is shown in this paper. However, the error bars were sometimes very small and are not seen in the plots.

II.E.1. Determination of NP dm/dH

Similar to other imaging systems, in MPI the PSF describes variation of the signal intensity with distance in the space around a point source in the imaging volume. It is the smallest observable signal source in the system and, therefore, defines the spatial resolution of the image. The PSF can be recorded in one or more dimensions around this central point. An image can be formulated as a convolution of the PSF with the distribution of point sources (tracer NPs) within the image volume. Full width at half maximum of the PSF determines the image resolution.

The MPS, which has no field gradient but only a sinusoidal excitation field, measures dm/dH(t). The dm/dH is essentially the instrument-independent PSF, in that it measures purely the NP tracer response to the excitation field. In x-space MPI, the PSF is simply the product of dm/dH and the known field gradient of the instrument and, therefore, the dm/dH gives the spatial resolution and signal intensity, along the excitation field direction of an arbitrary imaging system. The applied magnetic field in our MPS can be described as

\[ H(t) = H_0 \sin(\omega t) \]  

Here, \( H_0 \) is the peak excitation amplitude listed above and \( \omega \) is the field angular frequency (2 π \( f_0 \)), in which \( f_0 \) was 25 kHz for all measurements described in this work.

The induced voltage can be calculated considering the rate of variation of the magnetic moment of the NPs with applied field, m(H), using the following equation:

\[ V(t) = -\mu_0 S \left( \frac{d}{dt} m(t) \right) \]

\[ = -\mu_0 S \left( \frac{d}{dH} m(H) \right) \left( \frac{d}{dt} H(t) \right) \]  

in which \( \mu_0 \) is the magnetic permeability of the vacuum equal to 4π × 10−7 Vs/Am and \( S \) is the coil sensitivity with unit of 1/m. Considering Eq. (3) and rearranging Eq. (4), we have

\[ \frac{d}{dH} m(H) = \frac{-1}{\mu_0 S \omega H_0} \frac{V(t)}{\cos(\omega t)}. \]  

Generally, dm/dH, which has units of (m³), should be normalized by the quantity of iron in the sample. The resulting data [m³/g Fe] are a useful metric for evaluating tracer behavior that is a function of only the applied field and the tracer properties. Here, we generated the dm/dH graphs according to Eq. (5) and normalized by iron mass as measured by ICP.

II.E.2. MPI harmonic spectra

The received voltage signal [V(t)] was divided by \( \mu_0 S \), defined above, to determine the magnetization as a function of time and then divided by field ramping rate (ωH0). Then, harmonic spectra were generated by Fourier transform of the resulting data. Again, all the harmonic values were normalized by the amount of the iron in each sample, to make the results comparable for all different core sizes.

III. RESULTS

We considered several environments to demonstrate the range of responses available from our tracers: hydrophobic magnetic particles, with only an oleic acid ligand coating, were dispersed either in chloroform or immobilized in TOPO; biocompatible particles coated with PMAO-PEG were suspended in water or immobilized in agarose gel. Gelatinous agarose is a tissue equivalent material that has been used extensively for modeling and evaluating NP relaxation behavior in MRI. Gelled samples represented the expected signal for NPs that have perfused into the tissue. TOPO is a dense solid at room temperature that inhibited the motion of the hydrophobic NPs.

MPS signals (both dm/dH and harmonic spectra) and hysteresis loops for different sizes of the NPs dispersed in four different media are shown in Fig. 2. Concentration normalized plots shown in Fig. 2 (left side columns) are generated according to Sec. II.E.1. Inset graphs show dm/dH after normalizing intensity to compare the full width at half maximum of the peaks and correlate it to nominal spatial resolution. The range of measured harmonics (from third to thirty ninth) is also presented in Fig. 2 (middle column). The harmonic signals are shown after normalization by the amount of iron in the samples. Corresponding median core sizes of the NPs and their standard deviations were determined using Chantrell32 fitting to the measured m(H) data shown in the right side column. As in previous experiments, we observed that NP size strongly influenced MPS performance. In each of the model environments we investigated, the equilibrium magnetization (m-H curve) and MPS signal (dm/dH and harmonics) both varied substantially with the magnetic core size as measured from 7 to 22 nm (Fig. 2).
FIG. 2. $\frac{dm}{dH}$ (left column) and harmonic spectra (middle column) divided by the amount of iron in each sample and normalized to one (inset, left column) and normalized VSM (right column) data for the NPs with different core sizes before (free in chloroform and immobilized in TOPO) and after (free in water and immobilized in agarose) the aqueous phase transfer. The data in (a)–(f) are for NPs ranging in core diameter from $\sim 7$ to 22 nm as indicated. The last two rows are data for commercial Feridex™ (g) and Resovist™ (h). Median core sizes and standard deviations ($\sigma$) were calculated from the VSM results, using Chantrell size fitting. (See Ref. 32.) Both $\frac{dm}{dH}$ and harmonics based MPS signal intensities increase with median size and decrease with standard deviation of the NPs, in agreement with the slope of the m-H curves. Néel relaxation is the dominant mechanism for NPs up to 20 nm, while a combination of Néel and hysteretic reversal generates the signal in the largest (22 nm) NPs.
Different values of peak excitation amplitude ($H_0$) were applied for analysis of the role of the hysteretic reversal on the behavior of the largest NPs ($d_0 = 22$ nm, $\sigma = 0.38$), in free-rotating (water) and immobilized (agarose) states. The corresponding $\Delta m/dH$ data and harmonic spectra are shown in Figs. 3(a) and 3(b). Plots of $\Delta m/dH$ are shown after scaling by iron concentration (mm$^3$/mg Fe), and scaled to one to enable comparison of their FWHM (inset). The harmonic spectra also show the intensity of the NPs responses at different amplitudes.

Generally, the magnetic properties of iron oxide NPs are highly sensitive to size, morphology, and crystallographic structure. Direct observation of the NPs with TEM revealed the typical morphology and structure of these NPs. As shown in Fig. 4, the median core sizes of the NPs increases from $\sim 7$ to 22 nm, with increasing the molar ratio of the oleic
FIG. 3. Effect of the excitation field amplitude on $\frac{dm}{dH}$ (left) and harmonics (right) spectra of the 22 nm NPs in water (a) and agarose (b). Hysteretic reversal appeared to dominate at larger field amplitudes. As a result, as the field amplitude was increased, it reversed ever larger size fractions of the NPs in the sample, resulting in greater signal intensity, a shift in the $\frac{dm}{dH}$ peak, and increased higher harmonics. This effect was observed both in water and in gel immobilized samples.

Acid to iron oleate from 0 to 20. The selected area electron diffraction patterns of the NPs (insets, Fig. 4) show bright and sharp spots, arranged as individual rings indexed as (200), (220), (311), and (400) of magnetite, from inner rings to outer rings, respectively. Finally, electron energy-loss fine structure measurements and analysis has confirmed that the nanoparticles are predominantly magnetite ($\text{Fe}_3\text{O}_4$).

Since the hydrated size of the NPs is very important in determining their biological performance, the hydrodynamic size distributions of the NPs in aqueous media are also shown in Fig. 4. Hydrodynamic size also determines the Brownian relaxation time of the NPs, which—according to Eq. (1)—varies between 84 and 283 $\mu$s for the measured range (55–95 nm) of diameters. The DLS results shown here are based on the intensity percentage distribution of the NPs after surface modification with PMAO-PEG copolymer. The results show that the hydrodynamic size (Z-average) of the NPs is distributed in a narrow range of 55–95 nm with polydispersity index (PDI) of 0.18–0.23 for all core sizes. A single peak in the DLS spectrum also shows that the phase transfer typically resulted in uniform hydrodynamic size distribution and individually polymer-coated stable NPs. Postsynthesis and long-term stability of similar NPs are discussed in more details in our other recently published paper.

Figure 5 shows the linear variation of the MPS signal with iron concentration. Water dispersed NPs were diluted up to $\sim 15 \mu\text{g/ml}$ and their MPS signals were measured three times. The full harmonics spectra, the average values of their third to thirty ninth harmonics versus iron concentration, and their corresponding $\frac{dm}{dH}$ peak widths (normalized to one) and intensities are shown in Figs 5(a) and 5(b), respectively.

IV. DISCUSSION

Previous researchers have shown that MPI offers the prospect of high resolution and high-sensitivity medical
MPI is sensitive to variations in tracer size, and it has also been shown that the MPI signal is sensitive to tracer fluid viscosity as well as freezing of the fluid. We are interested in optimizing tracers for specific MPI applications, such as cancer targeting, stem cell tracking, or angiography. Idealized models of bound and free tracer environments help to understand how MPI tracers behave, and how their performance may be impacted by, for example, perfusion of tracer particles into a tumor, or targeting to the surface of a tumor cell by specific affinity or functionalization. In our MPI experiments, where the excitation frequency was fixed at 25 kHz and field amplitude varied from 5.1 to 18.6 mTμ0⁻¹, the tracer’s environment did influence MPI performance, due to differences in tracer responses to applied magnetic field. Furthermore, we can conclude that MPI imaging of immobilized particles is certainly possible, and with optimized tracers, performance should equal that of free-moving tracers. Our results will be useful for tuning the size of the tracers based on the magnetic properties preferred for particular MPI imaging applications, such as cancer targeting, vascular imaging, and stem cells labeling and tracking, where the NPs are fully or partially immobilized.
We observed a transition in behavior with increasing NP core size, corresponding to a change from superparamagnetism to ferromagnetism, around 20 nm (Fig. 2). This matches well with the blocking size of the NPs (~20.6 nm) calculated using the following:

$$KV = k_B T \log \frac{\tau_{\text{mean}}}{\tau_0},$$  \hspace{1cm} (6)

where $K$ is the magnetocrystalline anisotropy constant [Eq. (2)], $V$ is the volume of the iron oxide core, $k_B$ is Boltzmann’s constant, $\tau_{\text{mean}}$ is the field switching time or the measurement time (20 $\mu$s), and $\tau_0$ is $10^{-10}$ s. Here, blocking means that for a given measurement time, the thermal energy is not sufficient to overcome the NP anisotropy energy barrier ($KV$). The aggregated or clustered NPs can effectively act like NPs of larger size and show ferromagnetic behavior exhibiting open loops in their m-H curves. This effect was much more pronounced in 20 and 22 nm NPs when they were dispersed in chloroform. In MPS measurements, these transitions caused a shift from zero field (Fig. 2) in small sized NPs to nonzero values of the field for larger NPs (20 and 22 nm), in the location of the dm/dH peak, which corresponds to maximum dm/dH [Fig. 1(b)]. The peak changed systematically with increasing NP magnetic size [Fig. 1(c)] and sometimes featured an asymmetric “tail” at larger sizes. Physically, we interpret these changes to correspond to an evolution in magnetic reversal, from Néel-dominated relaxation in the smaller sizes [Fig. 1(a)] to Brownian or hysteresis-dominated reversal for larger sizes.8,21 Assuming highly monodisperse NPs without any aggregates, Néel relaxation times of the NPs with core sizes smaller than 22 nm were calculated to be less than ~5 $\mu$s [Eq. (2), assuming zero applied field]. However, Brownian or hysteretic reversal is expected to lead to a shift in the dm/dH maximum toward later times (phase lag) or larger fields, respectively, relative to the time at which the applied field is zero (Fig. 1).

Generally, the relative contributions of Brownian and hysteretic reversal will be determined by the applied field amplitude and frequency. In these experiments, hysteretic reversal was expected to dominate compared to Brownian relaxation, due to the relatively large field amplitude ($\mu$H ~ 14.6 mT, of the order of $H_K$) and relatively high frequency (25 kHz) magnetic field. Assuming that the viscosity of water is $10^{-3}$ N s m$^{-2}$ at room temperature, the Brownian relaxation time, calculated for the range of measured hydrodynamic diameters (60–90 nm) is 84–283 $\mu$s—longer than the period of the applied field (~40 $\mu$s). (Note that in a single period of the applied field, the NP magnetization was observed to reverse twice, although only one reversal is shown in the figures.)8 While the Brownian relaxation time [Eq. (1)] is defined for small fields ($H \ll H_K$), it provides a 1st-order approximation of the relaxation time when it is assumed that the moment cannot rotate within the crystal itself: since rotation from saturation to saturation (180°) is desired in MPI, to begin the magnetic reversal some small initial rotation would need to occur by random collisions before the moment could experience a torque from the applied field. Given these factors, it is unlikely that Brownian relaxation is the dominant relaxation mechanism under the experimental conditions described here, however, as will be discussed below, it could contribute measurably by aligning the NP easy axes when they are dispersed in solution.
To further probe the reversal mechanism of the largest NPs, we varied the excitation field amplitude between 5.1 and 18.6 mT μ_0^{-1} (Fig. 3) (all other MPS results presented in this paper were performed using an excitation amplitude of 14.8 mT μ_0^{-1}). As shown in Fig. 3(a), the tails (ranging from ~7 to 15 mT) in the dm/dH data are more pronounced at higher field amplitudes (i.e., 14.8 and 18.6 mT μ_0^{-1}), showing more contribution from hysteretic reversal. Here, MPS intensity, which depends on the number of moments that are reversed by the driving field, increases with increasing field amplitude (the same trend is observed in dm/dH plots and harmonic spectra). We note also that, as the field amplitude increased, the dm/dH peak location shifted to higher fields, while the harmonic spectrum displayed an increasing slope with relatively greater contribution from higher harmonics (most notably after the 7th harmonic). These effects are likely due to reversal of the fraction of the sample with larger size by the higher field amplitudes\(^\text{25,26}\) and corresponding to their larger coercivity due to their larger volume and reversal energy barrier. Therefore, monodispersity of the NPs plays a key role to get the optimum signal intensity and spatial resolution. As discussed above, this effect is less pronounced for the immobilized NPs dispersed in gel. Note that, since the excitation frequency in all measurements was constant (25 kHz), increasing the field amplitude increased the field ramping rate. However, all the dm/dH and harmonic data were divided by field ramp rate [Eqs. (3) and (4)], and therefore the signal intensity is only dependent on tracer performance and it is not proportional to the ramp rate. It is important to note that similar evolution was observed with field amplitude for both gel and water samples (the causes of difference between the water and gel samples at each amplitude will be discussed later).

The smallest NPs (∼7 nm) showed the weakest MPS signal (Fig. 2), measured by dm/dH height, and the fewest measurable harmonics (up to 15th harmonics). In general, as size increased, the dm/dH height increased, dm/dH FWHM decreased, and the number of harmonics and their intensity increased, such that larger NPs featured spectra with good SNR even up to the 39th harmonic. The evolution in MPS results was consistent with the observed increase in the slope of the equilibrium m-H curves of the samples, as measured by VSM over the same range of fields probed in the MPS.\(^\text{46}\) The maximum signal intensity was observed for NPs in the range of 20 nm. The decrease of the signal in 22 nm NPs can be described based on the larger size distribution (standard deviation, \(\sigma = 0.373\)) of the 22 nm NPs, as shown in m-H graphs.\(^\text{12}\)

The equilibrium magnetization slopes are largest in 20 and 22 nm NPs, accompanied by appearance of an open hysteresis loop. For the given measurement time (∼100 s), this behavior is due to a superparamagnetic to ferromagnetic transition with increasing core size.\(^\text{17}\) The transition could be further enhanced by the magnetic interactions between the nanoparticles when they are not coated with PMAO-PEG, which can lead to interparticle interactions that tend to align the particles into chains (Fig. 1(b)).\(^\text{47}\) The open loop behavior observed for oleic acid coated particles is not seen after coating of the NPs with the copolymer. Similar evolution was observed in the MPS data of these samples, due to hysteretic reversal of these aggregates. Prior to coating with PMAO-PEG, the oleic acid coated NPs featured a narrow dm/dH centered at ∼8 mT and flat harmonic spectra with high intensity, whereas PMAO-PEG coated particles showed a broader peak centered at 0 mT and weaker harmonics. TOPO immobilized NPs typically showed the weakest MPS response. However, NPs smaller than 18 nm did not show such dramatic signal variations before and after coating, either in their MPS or VSM measurements (Fig. 2). This is likely due to their smaller size; these NPs experience weaker interaction forces in comparison with 20 and 22 nm NPs. However, compared with samples dispersed in chloroform, MPS signal intensity decreases after coating for all different sizes, due to the presence of the hydrodynamic layer around the NPs that stabilizes the particles and limits their interaction.

The observed dependence in M(H) and MPS behavior on the suspending matrix could have several origins, including changes in the size distribution during phase transfer, clustering caused by interparticle interactions, and the ability of the NPs to align their easy axes by rotation in liquid suspensions. For example, the dm/dH FWHM (insets of Fig. 2) decreases after transferring the as-synthesized NPs from chloroform to water, which can be clearly seen for 12, 16, and 18 nm NPs. We interpret this peak narrowing phenomenon to a narrowing of the size distribution of the NPs during phase transfer to water, due to elimination of larger and smaller NPs by filtration and size exclusion purification after their coating with PMAO-PEG copolymer. Such a narrower size distribution was also confirmed by smaller standard deviation values (\(\sigma\)) calculated for the NPs in water, in comparison with their chloroform dispersed counterparts (data not shown). Also, again it is important to consider the potential for strong interaction between uncoated NPs in chloroform, where steric stabilization is poor and local aggregation can occur, especially when an external field is applied, such as during MPS measurements. For the NPs larger than 20 nm, which are strongly interacting in chloroform, the dm/dH was fully changed after freezing the NPs motion in TOPO with maximum values shifted to zero field. NPs frozen in TOPO show the lowest signal intensities for all core sizes. Their equilibrium magnetization also shows a distinctive change that matches with the dm/dH data: when the NPs are allowed to align by rotation, such as in chloroform, a sharp transition is noted in the dm/dH, harmonic spectrum, and m-H loop. Furthermore, chaining of the nanoparticles in chloroform may serve to enhance this effect. However, freezing the NPs in random orientations, such as by cooling a TOPO dispersion in the absence of an applied field, results in a change in saturation field and susceptibility in the equilibrium measurement that corresponds to a low intensity, broad dm/dH, and low-intensity harmonics. This change could be due to alignment of the free NPs along their easy axis, compared to randomly immobilized NPs in TOPO and is in accordance with the Stoner-Wohlfarth model for magnetization reversal of single domain nanoparticles with different orientations with respect to the direction of the applied field.\(^\text{48}\)
Unlike differences between chloroform and TOPO, immobilization of the aqueous NPs in agarose gel did not change the \( \text{dm/dH} \) peak width considerably. The observation of the same peak width in water and gel is indeed an interesting finding, regarding tracer design for tissue targeted MPI. However, this similarity was mainly observed in NPs smaller than 20 nm. For the largest NPs with median size of 22 nm, the full width at half maximum of the \( \text{dm/dH} \) in gel is smaller than in water. This might be due to clustering of these larger NPs during the phase transfer and their stronger magnetic interactions and alignments, when the field is applied, a phenomenon which is prohibited when their field induced movements are limited in nanoporous gel structure.\(^{49}\) These clusters are larger than 100 nm and form about 5–10 volume percentage of this sample, as shown by DLS data in Fig. 4(f). Also, free NPs in water can align with each other, when the magnetic field is applied and form clusters with large coercivities, acting like larger NPs. The hysteretic reversal occurs for these large size NPs at higher fields, resulting in a wider \( \text{dm/dH} \) peak and a small change in the slope of their harmonics spectra (Figs. 2 and 3). Also, the NPs dispersed in water show higher signal intensities in comparison with gelled samples. This could also be due to alignment of the free NPs and/or clusters in water, a phenomenon which does not occur in gelled samples and as mentioned above, it can be described based on the Stoner-Wohlfarth model.\(^{48}\)

Considerably higher \( \text{dm/dH} \) signal intensities (\( \sim 2.5x \) and \( 5x \), respectively) were observed in our 16.5 nm (\( \sigma = 0.185 \)) synthesized NPs, in comparison with two typical commercially available NPs, Resovist (median size of \( d_0 = 13.82 \) nm and \( \sigma = 0.49 \)) and Feridex (\( d_0 = 7.6 \) nm and \( \sigma = 0.38 \), Fig. 2). Resovist has been extensively used as a gold standard for developing the MPI technique. However, increasing the magnetic core size to 20.16 nm with narrow size distribution (\( \sigma = 0.29 \)) increased the signal intensity by a factor of four. This increase in signal can be related to the larger magnetic core size (Fig. 4) and lower \( \sigma \) values of our synthesized NPs. As it can be seen in their VSM data, low magnetization rates of these commercial NPs agree with their weaker MPS signal. It is noteworthy that the MPS signal intensities of Resovist and Feridex are also very low after immobilization in agarose.

For many clinical applications of NPs, such as cell tracking and a wide range of tissue targeted imaging and related therapeutic approaches, it is necessary to quantify the internalized iron in tissues at very low concentrations without interference of iron-containing proteins (e.g., hemoglobin, transferrin, ferritin) supplied by the blood.\(^{50}\) This is more challenging for the organs with low level of NPs accumulation, such as kidney, brain, and tumors. MPI signal is only sensitive to iron oxide NPs and there is no conflicting signal from the endogenous iron. Also, it is critical to see linearity in the variation of the MPS signal with iron concentration in the order of tens of micrograms per milliliter.\(^{6,50}\) Figure 5(a) shows the harmonic spectra of these NPs at different concentrations. Note that our spectrometer is able to measure up to 39 harmonics. Decreasing the concentration equally shifts all the harmonics in each spectrum to lower intensities. The inset shows the linear variation of the 3rd to 39th harmonics of the NPs (\( d_0 = 22 \) nm) down to very low iron concentrations (i.e., \( 15 \mu \text{g Fe ml}^{-1} \)). Also, Fig. 5(b) shows that the \( \text{dm/dH} \) peak width does not change with decreasing concentrations of the NPs. Again, the inset shows linear decrease of the \( \text{dm/dH} \) peak height with concentration. Larger error bars in the low-concentration \( \text{dm/dH} \) graphs and nonlinearity of the 29th and 39th harmonics appear because the MPS signal for these samples is close to the background noise level of the spectrometer.

Generally, when NPs are expected to bind to cells during MPI imaging, it is necessary to consider how immobilization will affect their magnetic response. For applied fields at 25 kHz and field amplitudes greater than 5 mT\( d_0^{-1} \), which have been introduced as a safe range for clinical applications,\(^{10}\) the Brownian relaxation is much slower than the hysteretic reversal, which was observed to dominate. Synthesizing the data we have presented above, we can make some general remarks about optimizing tracers for MPI. We observed that signal intensity could be increased by increasing the median core size (up to our 20 nm sample); increasing the field amplitude also enhanced the signal intensity when there was a distribution of sizes. However, resolution and intensity should both be improved by using monodisperse NPs with a uniform coercivity and a sharp switching field. Such a sample would also have the advantage that its performance would be invariant in water or in tissue. While making large, monodisperse samples presents a practical challenge, given the tendency for the nanoparticles to cluster due to strong interactions, it represents the best course for further improving the MPI performance. In other words, highest MPI image quality is achievable through efficient surface functionalization of the larger and monodisperse NPs to avoid formation of aggregates during the NPs processing or applications.

V. CONCLUSIONS

NPs with approximate median core sizes of 7, 12, 16, 18, 20, and 22 nm and narrow polydispersity values were synthesized and successfully functionalized. We immobilized the NPs to study their MPI performance and found that signals obtained from smaller sizes of the NPs (\( \sim 12–18 \) nm) are less sensitive to their environment, while NPs larger than about 20 nm that are best for MPI are highly sensitive to the surrounding media. To verify this crucial finding, the chloroform dispersed NPs were frozen in TOPO prior to phase transfer and the water dispersed NPs were immobilized in agarose gel after functionalization. Peak narrowing was observed when the NPs were transferred from chloroform to water due to their smaller size distribution after phase transfer. Open loop magnetization hysteresis was observed in hydrophobic NPs larger than 20 nm, suggesting magnetic interaction of the NPs, which was fully suppressed after coating. The relaxation mechanism of the hydrophilic NPs was changed from Néel for smaller NPs to a combination of Néel and hysteretic reversal for NPs larger than 20 nm. Immobilization in agarose did not change the spatial resolution based on the FWHM of \( \text{dm/dH} \) and the slope of the harmonic spectra for NPs smaller than 20 nm. For larger sizes (i.e., 22 nm), hysteretic reversal of the large size fraction of the NPs shifted the peak to larger fields,
a phenomenon that was eliminated after preventing the NPs alignment in gel. This shows the key role of monodispersity of the synthesized NPs in order to get the highest MPI signal, especially for larger sizes. Harmonics and dm/dH intensities varied linearly with concentration, while the dm/dH peak width remained constant, confirming consistent MPI performance of the synthesized tracers down to very low concentrations comparable to the usual ranges observed in targeted tissues. The results of the model designed here help to improve the image quality in a broad range of tissue- or cell-targeted MPI applications in future.

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