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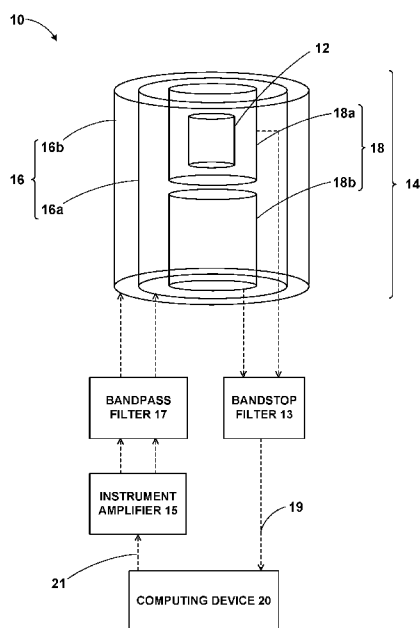


FIG. 1

(57) Abstract: An example system includes an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , to generate a magnetic response including a plurality of harmonics from the sample volume. A detection coil is configured to output a response signal indicative of one or both of the plurality of harmonics or a phase lag. The example system includes a computing device configured to receive the response signal, extract one or both of the phase lag or predetermined harmonic components of the plurality of harmonics, and determine an average saturation magnetization, an average hydrodynamic volume, or a core state of the plurality of magnetic nanoparticles based on one or both of the phase lag or the predetermined harmonic components.

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MAGNETIC NANOPARTICLE CHARACTERIZATION

TECHNICAL FIELD

[0001] This disclosure relates to characterization of magnetic nanoparticles, for example, magnetic nanoparticles such as superparamagnetic iron oxide nanoparticles.

BACKGROUND

[0002] Ferrofluids, composed of monodisperse magnetic nanoparticles (MNPs) in aqueous solutions, can be used in clinical and medical applications such as drug targeting and delivery, magnetic particle imaging (MPI), magnetic hyperthermia therapy, magnetic resonance imaging (MRI), etc. These applications are based on the nonlinear magnetic responses of MNPs to alternating current (AC) magnetic fields.

[0003] Superparamagnetic iron oxide nanoparticles (SPIONs) possessing strong magnetic moments that saturate at relatively low fields on the order of tens of milliteslas may be used as constituents of the ferrofluidic magnetoresponsive nanosystems listed above. SPIONs can be used for various applications, for example, bio-imaging contrast agents, heating sources for tumor therapy, and carriers for controlled drug delivery and release to target organs and tissues. SPIONs may be functionalized with polymer shells for providing colloidal stability and biocompatibility. Due to the large variability in medical treatments and uses, SPIONs may be specifically tailored for different types of applications, which may include tuning the physical and magnetic properties of the SPIONs. For example, MNPs with large magnetic moments may be desirable for drug delivery, MRI, and MPI, so the external gradient field is able to guide MNPs to target tissues. However, MRI applications may benefit from using relatively small diameter particles for *in vivo* cell tracking while drug delivery applications may benefit from using larger particles to ensure high magnetic moments.

SUMMARY

[0004] In some examples, the disclosure describes an example system including an excitation coil configured to simultaneously subject a sample volume including a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The first and the second excitation fields are configured to generate a magnetic response including a plurality of harmonics from the sample volume. The example system includes a detection coil configured to sense

the magnetic response and output a response signal in response to the magnetic response. The response signal is indicative of the plurality of harmonics. The example system includes a computing device. The computing device is configured to receive the response signal from the detection coil, extract predetermined harmonic components of the plurality of harmonics from the response signal, and determine an average saturation magnetization of the plurality of magnetic nanoparticles based on the predetermined harmonic components.

[0005] In some examples, the disclosure describes an example technique including sending, by a computing device, an excitation signal to an excitation coil configured to simultaneously subject a sample volume including a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The first and the second excitation fields are configured to generate a magnetic response including a plurality of harmonics from the sample volume. The example technique includes receiving, by the computing device, a response signal indicative of the plurality of harmonics, extracting, by the computing device, predetermined harmonic components of the plurality of harmonics from the response signal, and determining, by the computing device, an average saturation magnetization of the plurality of magnetic nanoparticles based on the predetermined harmonic components.

[0006] In some examples, the disclosure describes an example system including an excitation coil configured to simultaneously subject a sample volume including a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The first and the second excitation fields are configured to generate a magnetic response having a phase lag relative to the first and the second excitation fields from the sample volume. The example system includes a detection coil configured to sense the magnetic response and output a response signal in response to the magnetic response, wherein the response signal is indicative of the phase lag. The example system includes a computing device. The computing device is configured to receive the response signal from the detection coil, extract the phase lag from the response signal, and determine an average hydrodynamic volume of the plurality of magnetic nanoparticles based on the phase lag.

[0007] In some examples, the disclosure describes an example technique including sending, by a computing device, an excitation signal to an excitation coil configured to simultaneously subject a sample volume including a plurality of magnetic nanoparticles

to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The first and the second excitation fields are configured to generate a magnetic response having a phase lag relative to the first and second excitation fields from the sample volume. The example technique includes, receiving, by the computing device, a response signal indicative of the phase lag, extracting, by the computing device, the phase lag from the response signal, and determining, by the computing device, an average hydrodynamic volume of the plurality of magnetic nanoparticles based on the phase lag.

[0008] In some examples, the disclosure describes an example system including an excitation coil configured to simultaneously subject a sample volume including a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The first and the second excitation fields are configured to generate a magnetic response including a plurality of harmonics from the sample volume. The magnetic response has a phase lag relative to the first and second excitation fields. The example system includes a detection coil configured to sense the magnetic response and output a response signal in response to the magnetic response. The response signal is indicative of one or both of the plurality of harmonics or the phase lag. The example system includes a computing device. The computing device is configured to receive the response signal from the detection coil, extract one or both of the phase lag or predetermined harmonic components of the plurality of harmonics from the response signal, and determine a core state of the plurality of magnetic nanoparticles based on one or both of the phase lag or the predetermined harmonic components, wherein the core state is one of a single-core state or a multi-core state.

[0009] In some examples, the disclosure describes an example technique. The example technique includes sending, by a computing device, an excitation signal to an excitation coil configured to simultaneously subject a sample volume including a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The first and the second excitation fields are configured to generate a magnetic response including a plurality of harmonics from the sample volume. The magnetic response has a phase lag relative to the first and second excitation fields. The example technique includes receiving, by the computing device, a response signal indicative of one or both of the plurality of harmonics or the phase lag, extracting, by the computing device, one or both of the phase lag or predetermined

harmonic components of the plurality of harmonics from the response signal, and determining, by the computing device, a core state of the plurality of magnetic nanoparticles based on one or both of the phase lag or the predetermined harmonic components. The core state is one of a single-core state or a multi-core state.

[0010] The details of one or more examples are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0011] FIG. 1 is a conceptual and schematic block diagram illustrating an example system including a computing device and a search coil for characterizing a sample volume containing a plurality of magnetic nanoparticles.

[0012] FIG. 2 is a conceptual and schematic block diagram illustrating an example of a computing device for controlling the system of FIG. 1.

[0013] FIG. 3 is a flow diagram illustrating an example technique for determining an average saturation magnetization of a plurality of magnetic nanoparticles based on a harmonic ratio extracted from a magnetic response of the plurality of magnetic nanoparticles to applied excitation fields.

[0014] FIG. 4 is a flow diagram illustrating an example technique for determining an average hydrodynamic volume of a plurality of magnetic nanoparticles based on a phase lag extracted from a magnetic response of the plurality of magnetic nanoparticles to applied excitation fields.

[0015] FIG. 5 is a flow diagram illustrating an example technique for determining a core state of a plurality of magnetic nanoparticles based on one or both of a phase lag or a harmonic ratio extracted from a magnetic response of the plurality of magnetic nanoparticles to applied excitation fields.

[0016] FIG. 6A is a chart presenting examples of harmonic ratio as a function of frequency for simulated nanoparticles having different magnetizations.

[0017] FIG. 6B is a chart presenting an example of averaged harmonic ratio as a function of saturation magnetization for simulated nanoparticles.

[0018] FIG. 6C is a chart presenting examples of harmonic ratio as a function of frequency for simulated nanoparticles having different core diameters.

[0019] FIG. 6D is a chart presenting an example of averaged harmonic ratio as a function of core diameter for simulated nanoparticles.

[0020] FIG. 7A is a conceptual and schematic diagram illustrating an example of predominance of Néel relaxation exhibited by multi-core nanoparticles.

[0021] FIG. 7B is a conceptual and schematic diagram illustrating an example of predominance of Brownian relaxation exhibited by single-core nanoparticles.

[0022] FIG. 8A is a chart presenting an example of the relative contribution of Néel relaxation and Brownian relaxation to effective relaxation time as a function of core diameter for sample single-core nanoparticles.

[0023] FIG. 8B is a chart presenting an example of the relative contribution of Néel relaxation and Brownian relaxation to effective relaxation time as a function of core diameter for sample multi-core nanoparticles.

[0024] FIG. 9A is photograph illustrating an example search coil assembly including a sample vial, a detection coil, a low frequency coil, and a high frequency coil.

[0025] FIG. 9B is photograph illustrating the sample vial, the detection coil, the low frequency coil, and the high frequency coil of the example search coil assembly of FIG. 9A.

[0026] FIG. 10A is schematic and conceptual drawing illustrating the structure and dimensions of the sample vial, the detection coil, the low frequency coil, and the high frequency coil of FIG. 9B.

[0027] FIG. 10B is schematic and conceptual drawing illustrating the structure and dimensions of the sample vial of FIG. 9B.

[0028] FIG. 11A is a chart presenting examples of harmonic ratio as a function of frequency for sample nanoparticles having different core states.

[0029] FIG. 11B is a chart presenting examples of phase lag as a function of frequency for sample nanoparticles having different core states.

[0030] FIG. 12A is a chart presenting an example of the difference in amplitudes of the third harmonic for multi-core nanoparticles in liquid and frozen states.

[0031] FIG. 12B is a chart presenting an example of the difference in amplitudes of the third harmonic for single-core nanoparticles in liquid and frozen states.

[0032] FIG. 13A is a chart presenting examples harmonic ratio as a function of frequency for sample single-core nanoparticles in liquid and frozen states.

[0033] FIG. 13B is a chart presenting examples of phase lag as a function of frequency for sample single-core nanoparticles in liquid and frozen states.

[0034] FIG. 13C is a chart presenting examples of harmonic ratio as a function of frequency for sample multi-core nanoparticles in liquid and frozen states.

[0035] FIG. 13D is a chart presenting examples of phase lag as a function of frequency for sample multi-core nanoparticles in liquid and frozen states.

[0036] FIGS. 14A–14D are photographs illustrating example bright-field transmission electron microscopy micrographs of sample single-core and multi-core nanoparticles collected using dynamic light scattering.

[0037] FIGS. 15A–15D are charts presenting example statistical hydrodynamic size distributions for the sample single-core and multi-core nanoparticles shown in FIGS. 14A–14D.

[0038] FIGS. 16A–16D are charts presenting example core diameter distributions for sample single-core and multi-core nanoparticles shown in FIGS. 14A–14D.

[0039] FIG. 17A is a chart presenting x-ray diffraction spectra for sample nanoparticles.

[0040] FIG. 17B is a chart presenting magnetization curves for the sample nanoparticles of FIG. 16A.

DETAILED DESCRIPTION

[0041] In this disclosure, superparamagnetic iron oxide nanoparticles (SPIONs) are a particular type of magnetic nanoparticles (MNPs). Descriptions of systems and techniques for characterizing MNPs are applicable for characterizing SPIONs. The MNPs may include any nanoparticles exhibiting magnetism or a magnetic response to an excitation field. Characterizing MNPs, for example, SPIONs, in sample compositions, for example, determining properties such as average diameters, nanostructure, and magnetization, may utilize relatively expensive, complicated, and slow techniques such as transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), and dynamic light scattering (DLS). Such characterization may also utilize additional processing of the sample compositions, for example, drying, to remove fluid and produce dried particles, prior to characterization.

[0042] The use of multiple apparatuses and additional processing steps may be reduced or avoided by using alternative techniques for characterizing SPIONs, for example, detecting and using the magnetic response of SPIONs to predetermined magnetic fields to determine properties of SPIONs. For example, the nonlinear magnetic response of SPIONs to alternating current (AC) magnetic fields may induce harmonic signals that may be related to the properties of SPIONS.

[0043] Example techniques and systems according to the disclosure may implement frequency mixing to characterize MNPs, for example, by determining magnetic or

physical properties of MNPs. Techniques that implement frequency mixing may include magnetically exciting a sample using two excitation fields have two respective frequencies, a high frequency (f_H) and a low frequency (f_L). In response to the excitation fields, the sample may generate a magnetic response that includes various frequency components. The magnetic response may be detected by a detection coil, which may generate a response signal based on the magnetic response. Detecting linear combinations, for example, frequencies representing linear combinations of the high and low frequencies, $mf_H \pm nf_L$ (where m and n may respectively be the same or different positive or negative integers) may reduce or substantially eliminate noise generated at the fundamental frequencies f_H and f_L themselves. The low frequency excitation field may drive the MNPs into their nonlinear saturation region periodically, for example, by allowing the MNPs sufficient relaxation time to enter a state of magnetic saturation. The high frequency excitation field is swept in a predetermined frequency range to generate mixing frequency signals, for example, the linear combinations $mf_H \pm nf_L$. In some examples, the detection coil may have a higher output voltage amplitude at the higher mixed frequency. Thus, using frequency mixing may reduce noise, for example, white noise, or 1/f noise (also known as pink noise).

[0044] Without wishing to be bound by theory, the magnetic response of MNPs to the excitation fields with respective frequencies f_H and f_L includes frequency mixing components, for example, linear combinations $mf_H \pm nf_L$, that are induced at odd harmonics according to the dynamic magnetization models described elsewhere in the disclosure. The response signal generated by the SPIONs includes frequencies corresponding to odd harmonic components ($m + n = 1, 3, 5, \dots$). Frequency mixing may be implemented using example systems according to the disclosure that include an excitation coil for stimulating magnetic nanoparticles, and a detection coil for detecting a magnetic response of the nanoparticles to the excitation. A “search coil” may refer to a system, subsystem, or assembly that includes one or more of one or more excitation coils, detection coils, or sample vials or containers for containing a plurality of magnetic nanoparticles.

[0045] SPIONs may also exhibit different sizes, magnetic strengths, clustering (single-core particles or clusters of multi-core particles), and differ in the origin of their superparamagnetism—from intrinsic Néel motion (rotating spin inside a stationary particle) or from extrinsic Brownian motion (rotating the entire particle along with its spin).

[0046] Because the response of SPIONS to the excitation fields may be related to the magnetic and physical properties of SPIONS, one or both of the amplitude and the phase of one or more odd harmonic components may be used to determine magnetic and physical properties of SPIONS. For example, harmonic ratios of the third harmonic ($f_H + 2f_L$) over the fifth harmonic ($f_H + 4f_L$) are inversely proportional to saturation magnetization, M_s , and core diameter, D , of MNPs, according to the induced signal model and harmonic ratio model described elsewhere in the disclosure. The phase lag of magnetic moment to the driving fields may be monitored using harmonic phase angles, and is related to the hydrodynamic volumes of SPIONS, according to the relaxation time model and phase lag model discussed elsewhere in the disclosure. Thus, by analyzing the phase lag and one or more harmonic ratios in the magnetic response of SPIONS to search coil signals, properties such as saturation magnetization, the average hydrodynamic size, the dominating relaxation processes of SPIONS, and the distinction between single- and multi-core particles may be determined.

[0047] The magnetization dynamics of MNPs may be characterized by effective relaxation time τ_{eff} , which is dependent on Brownian relaxation time τ_B and Néel relaxation time τ_N . Both relaxation processes are dependent on the frequency and amplitude of applied magnetic fields. The τ_{eff} of a nanoparticle governs its ability to follow the applied magnetic fields. For example, some types of magnetic nanoparticles may relatively rapidly respond to applied magnetic fields, for example, by generating a field in response, while other types of nanoparticles may exhibit a relatively large lag in response to applied magnetic fields. The effective relaxation time τ_{eff} is related to the Brownian and Néel relaxation times as follows: $\frac{1}{\tau_{eff}} = \frac{1}{\tau_B} + \frac{1}{\tau_N}$. The τ_{eff} of a magnetic nanoparticle governs its ability to follow, or generate a response field, to the applied alternating or excitation field.

[0048] SPIONS are characterized by core diameter D , saturation magnetization M_s and concentration c . For the purposes of the models, SPIONS are assumed to be spherical, and without mutual interactions. The magnetic moment of each particle is given by $m_s = M_s V_c = M_s \pi D^3 / 6$, where V_c is volume of the magnetic core, ξ is the ratio of magnetic energy over thermal energy, k_B is Boltzmann constant, and T is the absolute temperature in Kelvin. An analytical expression for Brownian relaxation time is given by $\tau_B = \tau_{B0} \frac{1 + \xi^2 - \xi^2 \coth^2 \xi}{\xi \coth \xi - 1}$, where τ_{B0} is zero-field Brownian relaxation time and $\tau_{B0} =$

$\frac{3\eta V_h}{k_B T}$, hydrodynamic volume $V_h = \pi(D + 2d)^3/6$, η is viscosity, and d is the thickness of nonmagnetic polymer coating layer. The analytical expression for Néel relaxation time is given by EQUATION 1.

$$\tau_N = \frac{\tau_{N0}}{\sigma_{eff}(1-h^2)} \left(\frac{\sqrt{\sigma_{eff}/\pi}}{1+1/\sigma_{eff}} + 2^{-\sigma-1} \right)^{-1} \times \left(\frac{1-h}{e^{\sigma_{eff}(1-h)^2}-1} + \frac{1+h}{e^{\sigma_{eff}(1+h)^2}-1} \right)^{-1} \quad (\text{Equation 1})$$

[0049] In equation 7, $\tau_{N0} = \frac{\beta(1+\alpha'^2)M_s}{2\gamma\alpha'}$, $h = \frac{\xi}{2\sigma_{eff}}$, and $\beta = \frac{V_c}{k_B T}$, where $\gamma =$

2.8×10^{10} Hz/T is electron gyromagnetic ratio, $\alpha' = 0.1$ is a damping constant for magnetite nanoparticles, and $\sigma_{eff} = K_{eff}V_c/k_B T$ is the energy barrier.

[0050] The phase lag is modulated by the low-frequency field and also can be monitored at the harmonic phase angles, $\phi(t) = \arctan(\omega\tau_{eff})$, where ω is the angular frequency of the field, and τ_{eff} is modulated by the oscillating magnetic field.

[0051] Each type of SPION may have its own signature of phase and amplitude and will respond differently to applied magnetic fields. The voltage and phase generated from SPIONs at specific frequencies are represented by a phasor: $A \cdot e^{j(\omega t + \phi)}$ (or expressed as $A\angle\phi$), where ω is the frequency of driving field, ϕ is the phase lag, and $j = \sqrt{-1}$, and t is time. In systems in which two alternating currents (ACs) are applied to the driving coils, first, the background noise is collected with external fields applied. The background noise can be expressed as $A_n\angle\phi_n$. Second, a sample vial containing a predetermined volume of SPION sample is inserted into the search coil and the overall signal is collected. The overall signal is expressed as $A_t\angle\phi_t$. This signal is the sum of two phasors: the background noise and the signal generated by SPIONs (namely,

$A_p\angle\phi_p$): $A_n\angle\phi_n + A_p\angle\phi_p = A_t\angle\phi_t$, which reduces to the equation set

$$\begin{cases} A_n \times \cos\phi_n + A_p \times \cos\phi_p = A_t \times \cos\phi_t \\ A_n \times \sin\phi_n + A_p \times \sin\phi_p = A_t \times \sin\phi_t \end{cases}$$

[0052] By solving the equation set above, the harmonic amplitude A_p and phase lag ϕ_p of each type of SPIONs at different frequencies can be determined.

[0053] In the presence of oscillating magnetic fields, SPIONs are magnetized and their magnetic moments tend to align with the fields. For a ferrofluid system of monodispersed, noninteracting SPIONs, the magnetic response obeys the Langevin function: $M_D(t) = m_s c L(\xi)$, where, $L(\xi) = \coth \xi - \frac{1}{\xi}$, $\xi = \frac{m_s H(t)}{k_B T}$. The external magnetic fields may be expressed as $H(t) = A_H \cos(2\pi f_H t) + A_L \cos(2\pi f_L t)$, where

A_H, A_L, f_H, f_L are the amplitude and frequency of high and low frequency fields, respectively.

[0054] In cases where a variation in particle diameters may be expected, another model that may be used is a log-normal size distribution model. The probability density function $\rho(D; D_0, S)$ is given by $\rho(D; D_0, S) = \frac{1}{S\sqrt{2\pi}D} \exp\left[-\frac{(\ln D - D_0)^2}{2S^2}\right]$, where D_0 is the median core diameter and S is the standard deviation of $\ln D$. Therefore, the mean and standard deviation of MNP core diameters are given by $D_0' = \exp\left[\ln(D_0) + \frac{S^2}{2}\right]$ and $S' = \exp\left[\ln(D_0) + \frac{S^2}{2}\right] \times \sqrt{\exp(S^2) - 1}$. The cumulative distribution function (CDF) for this log normal distribution is $CDF(D) = \frac{1}{2}\left[1 + \operatorname{erf}\left(\frac{\ln D - D_0}{S\sqrt{2}}\right)\right]$. Herein, the revised magnetization model of bulk ferrofluid is given by $\tilde{M}(t) = \int_0^\infty \rho(D; D_0, \sigma) M_D(t) dD$.

[0055] According to Lenz's law, the induced voltage in a pair of detection coils expressed as: $u(t) = -S_0 V \frac{d}{dt} M_D(t)$, where V is volume of SPION suspension and where $M_D(t)$ is the magnetic response governed by the Langevin function. Detection coil sensitivity S_0 equals to the external magnetic field strength divided by current.

[0056] EQUATION 2 sets forth the Taylor expansion of $M_D(t)$, including the major frequency mixing components,

$$\begin{aligned} \frac{M_D(t)}{m_s c} &= L\left(\frac{m_s H(t)}{k_B T}\right) = \frac{1}{3}\left(\frac{m_s}{k_B T}\right) H(t) - \frac{1}{45}\left(\frac{m_s}{k_B T}\right)^3 H(t)^3 + \frac{2}{945}\left(\frac{m_s}{k_B T}\right)^5 H(t)^5 + \dots \\ &= \dots + \left[-\frac{1}{60} A_H A_L^2 \left(\frac{m_s}{k_B T}\right)^3 + \dots\right] \times \cos[2\pi(f_H \pm 2f_L)t] \\ &+ \left[\frac{1}{1512} A_H A_L^4 \left(\frac{m_s}{k_B T}\right)^5 + \dots\right] \times \cos[2\pi(f_H \pm 4f_L)t] + \dots \end{aligned} \quad \text{(Equation 2)}$$

[0057] The mixing frequency components corresponding to the third and fifth odd harmonics are set forth in EQUATIONS 3 and 4.

$$M_D(t)|_{3rd} \approx \frac{-m_s c}{60} A_H A_L^2 \left(\frac{m_s}{k_B T}\right)^3 \times \cos[2\pi(f_H + 2f_L)t] \quad \text{(Equation 3)}$$

$$M_D(t)|_{5th} \approx \frac{m_s c}{1512} A_H A_L^4 \left(\frac{m_s}{k_B T}\right)^5 \times \cos[2\pi(f_H + 4f_L)t] \quad \text{(Equation 4)}$$

[0058] Amplitudes of induced voltages at the third and fifth harmonics are given by EQUATIONS 5 and 6.

$$u_{3rd} \approx \frac{\sqrt{2}\pi S_0 V m_s c}{60} A_H A_L^2 \times (f_H + 2f_L) \times \left(\frac{m_s \mu_0}{k_B T}\right)^3 \quad \text{(Equation 5)}$$

$$u_{5th} \approx \frac{\sqrt{2}\pi S_0 V m_s c}{1512} A_H A_L^4 \times (f_H + 4f_L) \times \left(\frac{m_s \mu_0}{k_B T}\right)^5 \quad \text{(Equation 6)}$$

[0059] The amplitude ratio (or harmonic ratio) of the third over the fifth harmonics is given by EQUATION 7.

$$R|_{3rd/5th} = \frac{u_{3rd}}{u_{5th}} \approx \frac{\text{constant}}{D^6 M_s^2} \quad \text{(Equation 7)}$$

[0060] Without being bound by theory, the harmonic ratio is inversely proportional to the sixth power of D and the second power of M_s . Another advantage of using this harmonic ratio to characterize magnetic properties of SPIONs is that this parameter is independent of concentration of SPIONs in the sample. While EQUATION 7 provides an inverse relation between the selected harmonic with particular powers of D and M_s , the selected harmonic ratio may also have an inverse relation with other powers of D and M_s , as discussed elsewhere in the disclosure.

[0061] Therefore, example techniques according to the disclosure may include extracting one or both of the phase lag or the harmonic ratio from a signal representing a magnetic response of a sample volume of a plurality nanoparticles, and determining one or more properties such as saturation magnetization, hydrodynamic volume, and core state (single-core or multi-core) of the plurality of magnetic nanoparticles, based on one or both of the phase lag or the harmonic ratio. Example systems described below may be used to subject the sample volume of nanoparticles to excitation fields to stimulate the magnetic response for extracting one or both of the phase lag or the harmonic ratio.

[0062] FIG. 1 is a conceptual and schematic block diagram illustrating an example system 10 including a computing device 20 and a search coil 14 for characterizing a sample volume containing a plurality of magnetic nanoparticles contained in a sample container 12. In FIG. 1, sample container 12 is adjacent to or within search coil 14, and computing device 20 is coupled to search coil 14. For example, computing device 12 may be electrically coupled to search coil 14 by one or more wired or wireless connection capable of carrying one or more signals. Computing device 20 may control search coil 14 to one or both of magnetically stimulate the sample volume and sense a magnetic response of the volume in sample container 12.

[0063] Sample container 12 may include a container, for example, a vial, capable of containing a predetermined volume of a plurality of magnetic nanoparticles. In some examples, sample container 12 may contain a liquid including the plurality of magnetic nanoparticles, for example, as suspension. In some examples, sample container 12 may contain a solid, for example, a frozen liquid including the plurality of magnetic nanoparticles, or a dried or particulate form of the plurality of magnetic nanoparticles.

While sample container 12 may be disposed within a region of search coil 14, for example, as shown in FIG. 1, in other examples, sample container 12 may be outside search coil 14. For example, search coil 14 may be sufficiently proximate to sample container 12 to one or both of magnetically stimulate sample container 12 or sense the magnetic response of the sample volume in sample container 12 while sample container 12 is outside search coil 14.

[0064] Search coil 14 includes an excitation coil 16 configured to subject the sample volume to an excitation field, and a detection coil 18 configured to detect or sense the magnetic response of the sample volume to the excitation field. Excitation coil 16 may include one or more coils. For example, as shown in FIG. 1, excitation coil 16 may include a first coil 16a and a second coil 16b. In some examples, excitation coil 16 may simultaneously subject the sample volume to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L . The frequency f_H is greater (or higher) than the frequency f_L . For example, first coil 16a may be a high frequency coil generating frequency f_H , and second coil 16b may be a low frequency coil generating frequency f_L . One or both of first and second coils 16a and 16b may be wound with an appropriate number of windings of a metal or alloy filament or wire, to generate the respective predetermined frequencies.

[0065] In some examples, f_H may be variable within a predetermined frequency range. For example, f_H may be variable between a predetermined lower frequency and a predetermined higher frequency defining the frequency range. In some examples, first coil 16a may include a plurality of subsets of windings to change the effective generated frequency. For example, a first subset of windings may generate a first frequency and a second subset of windings may generate a second frequency. In some examples, excitation coil 16, for example, first coil 16a, may include a slider or another adjustment mechanism to select a selected subset of windings associated with a particular predetermined frequency from a range of frequencies. In other examples, first coil 16a may be otherwise capable of generating or delivering a variable frequency within the predetermined frequency range. In some examples, excitation coil 16 may deliver a first excitation field having a frequency f_H varying between a lower frequency of about 10 kHz and a higher frequency of about 20 kHz, for example, a frequency that linearly sweeps the frequency range. In other examples, a non-linear sweep may be performed, for example, a power-law, logarithmic, or a sweep based on any non-linear curve passing through end points defined by the lower frequency and the higher frequency.

The amplitude of the first excitation field generated by first coil 16a may be set to any suitable amplitude for generating a detectable magnetic response in the sample volume. For example, the amplitude of the first excitation field may be about 10 Oe.

[0066] In some examples, the frequency f_L of the second excitation field may be a predetermined fixed frequency. For example, second coil 16b include a plurality of windings including a number of windings selected to generate the predetermined fixed frequency. In other examples, second coil 16b may include a variable winding, for example, similar to some examples of first coil 16a, but which may be set to a predetermined fixed frequency selected from the frequency range. In some examples, the frequency f_L may be at least 10 to 100 times lower than the frequency f_H . For example, the second excitation field may have a frequency f_L of about 50 Hz. The amplitude of the second excitation field generated by second coil 16b may be set to any suitable amplitude for generating a detectable magnetic response in the sample volume. In some examples, the amplitude of the second excitation field is about 10 times the amplitude of the first excitation field. For example, the amplitude of the second excitation field may be about 100 Oe.

[0067] Detection coil 18 may be configured to detect a magnetic response of the sample volume subjected to excitation fields from excitation coil 16. For example, detection coil 18 may include a plurality of windings susceptible to a magnetic response from the sample volume. The plurality of windings may generate a signal, for example, an electrical signal, in response to the magnetic response. Detection coil 18 may be disposed about or adjacent the sample volume.

[0068] In some examples, detection coil 18 may include a pair of differentially wound pick-up coils 18a and 18b. For example, pick-up coil 18a may be wound clockwise, while pick-up coil 18b may be wound counterclockwise, with a similar number of windings. In some examples, sample container 12 may be disposed within or adjacent one of the differentially wound pick-up coils, for example, within pick-up coil 18a, as shown in FIG. 1. In some examples, signals generated by pick-ups coils 18a and 18b may be combined, for example, superimposed or added, to generated a combined signal 19 sent to computing device 20. In some examples, a signal generated by pick-up coil 18a may be selected as a signal indicative of a magnetic response of the sample volume to the excitation field, and sent to computing device 20, while a signal generated by pick-up coil 18b may be monitored to detect noise or other phenomena which may be sent to computing device 20 for subsequent processing and analysis. In some examples,

the raw signal or signals generated by detection coil 18 may be passed through a bandstop filter 13 to generate signal 19 sent to computing device 20 from detection coil 10.

[0069] In some examples, search coil 14 may include first coil 16a, second coil 16b, detection coil 18, and sample container 12 in relatively close proximity. For example, second coil 16b may surround first coil 16a, while first coil 16a surrounds detection coil 18, and detection coil 18 surrounds sample container 12. This may allow for a relatively compact assembly of search coil 14. However, one or more components of search coil 14 may be rearranged. For example, first coil 16a may surround second coil 16b, while detection coil 18 may surround one or both of first coil 16a and second coil 16b.

[0070] Computing device 20 may generate an excitation signal 21 and send excitation signal 21 to one or both of first coil 16a and second coil 16b to control the respective first and second excitation fields, for example, by controlling the respective frequencies and the amplitudes. Excitation signal 21 may include respective subsignals respectively sent to first coil 16a and second coil 16b. In some examples, system 100 may optionally include at least one instrument amplifier 15 to amplify excitation signal 21 before it is sent to excitation coil 16. In some examples, excitation signal 21 may optionally be passed through a bandpass filter 17 before it is sent to excitation coil 16. Using bandpass filter 17 may suppress higher harmonics that may be introduced into excitation signal 21 by at least one instrument amplifier 15.

[0071] The plurality of magnetic nanoparticles in the sample volume in sample container 12 may get magnetically stimulated by the excitation fields emitted by excitation coil 16 in response to excitation signal 21. The magnetically stimulated magnetic nanoparticles may generate a magnetic response, for example, a response field, in response to the excitation fields. Detection coil 18 may detect the magnetic response, for example, generating and outputting response signal 19, which is sent to computing device 20. Computing device 20 may use response signal 19 to determine properties of magnetic nanoparticles in the sample volume in sample container 12. For example, computing device 20 may extract one or more of harmonic components, harmonic ratios, or phase lags, for example, phase lag of a predetermined harmonic component, and determine properties of the magnetic nanoparticles based on the one or more extracted parameters.

[0072] FIG. 2 is a conceptual block diagram illustrating an example of computing device 20 illustrated in FIG. 1. In some examples, computing device 20 may include, for example, a desktop computer, a laptop computer, a workstation, a server, a mainframe, a cloud computing system, or the like. In some examples, computing device 20 controls the operation of system 10, including, for example, excitation coil 16 and detection coil 18.

[0073] In the example illustrated in FIG. 2, computing device 20 includes one or more processors 22, one or more input devices 24, one or more communication units 26, one or more output devices 28, and one or more storage devices 32. In some examples, one or more storage devices 32 stores excitation signal generation module 34 and response signal analysis module 36. In other examples, computing device 20 may include additional components or fewer components than those illustrated in FIG. 2.

[0074] One or more processors 22 are configured to implement functionality and/or process instructions for execution within computing device 20. For example, processors 22 may be capable of processing instructions stored by storage device 32. Examples of one or more processors 22 may include, any one or more of a microprocessor, a controller, a digital signal processor (DSP), an application specific integrated circuit (ASIC), a field-programmable gate array (FPGA), or equivalent discrete or integrated logic circuitry.

[0075] One or more storage devices 32 may be configured to store information within computing device 20 during operation. Storage devices 32, in some examples, include a computer-readable storage medium or computer-readable storage device. In some examples, storage devices 32 include a temporary memory, meaning that a primary purpose of storage device 32 is not long-term storage. Storage devices 32, in some examples, include a volatile memory, meaning that storage device 32 does not maintain stored contents when power is not provided to storage device 32. Examples of volatile memories include random access memories (RAM), dynamic random access memories (DRAM), static random access memories (SRAM), and other forms of volatile memories known in the art. In some examples, storage devices 32 are used to store program instructions for execution by processors 22. Storage devices 32, in some examples, are used by software or applications running on computing device 20 to temporarily store information during program execution.

[0076] In some examples, storage devices 32 may further include one or more storage device 32 configured for longer-term storage of information. In some examples, storage

devices 32 include non-volatile storage elements. Examples of such non-volatile storage elements include magnetic hard discs, optical discs, floppy discs, flash memories, or forms of electrically programmable memories (EPROM) or electrically erasable and programmable (EEPROM) memories.

[0077] Computing device 20 may further include one or more communication units 26. Computing device 20 may utilize communication units 26 to communicate with excitation coil 16 and detection coil 18 via one or more networks, such as one or more wired or wireless networks. Communication unit 26 may include a network interface card, such as an Ethernet card, an optical transceiver, a radio frequency transceiver, or any other type of device that can send and receive information. Other examples of such network interfaces may include WiFi radios or Universal Serial Bus (USB). In some examples, computing device 20 utilizes communication units 26 to wirelessly communicate with an external device such as a server.

[0078] Computing device 20 also includes one or more input devices 24. Input devices 24, in some examples, are configured to receive input from a user through tactile, audio, or video sources. Examples of input devices 24 include a mouse, a keyboard, a voice responsive system, video camera, microphone, touchscreen, or any other type of device for detecting a command from a user.

[0079] Computing device 20 may further include one or more output devices 28. Output devices 28, in some examples, are configured to provide output to a user using audio or video media. For example, output devices 28 may include a display, a sound card, a video graphics adapter card, or any other type of device for converting a signal into an appropriate form understandable to humans or machines. In some example, computing device 20 outputs a representation of one or more of excitation signal 21, response signal 19, or one or more properties of the plurality of magnetic nanoparticles in the sample volume, for example, a saturation magnetization, a hydrodynamic volume, a core diameter, or a core state, via output devices 28.

[0080] In some examples, computing device 20 may output a representation of excitation signal 21 or response signal 19, via output devices 28. In some examples, computing device 20 may determine excitation signal 21 based on response signal 19 and send excitation signal 21 to at least one component to control system 10 by adjusting the attributes or parameters of excitation coil 16 or detection coil 18, for example, to adjust a frequency or amplitude at which fields are generated or signals are sensed.

[0081] Computing device 20 also may include excitation signal generation module 32 and response signal analysis module 36 for stimulating a magnetic response from the sample volume and analyzing the magnetic response of the sample volume to the excitation field to determine one or more properties of a plurality of magnetic nanoparticles in the sample volume. Functions performed by excitation signal generation module 32 and response signal analysis module 36 are explained below with reference to the example techniques represented by respective flow diagrams illustrated in FIGS. 3–5.

[0082] Excitation signal generation module 32 and response signal analysis module 36 may be implemented in various ways. For example, excitation signal generation module 32 and response signal analysis module 36 may be implemented as software, such as an executable application or an operating system, or firmware executed by one or more processors 22. In other examples, excitation signal generation module 32 and response signal analysis module 36 may be implemented as part of a hardware unit of computing device 20.

[0083] Computing device 20 may include additional components that, for clarity, are not shown in FIG. 2. For example, computing device 20 may include a power supply to provide power to the components of computing device 20. Similarly, the components of computing device 20 shown in FIG. 2 may not be necessary in every example of computing device 20.

[0084] Examples of system 10 and computing device 20 are described with reference to FIGS. 1 and 2 above, including examples of excitation coil 16 for generating excitation fields to stimulate a magnetic response from the sample volume, and detection coil 18 for detecting response signal 19 indicative of the magnetic response generated by the sample volume. Example techniques for generating the excitation fields and analyzing response signal 19 to determine one or more properties a plurality of magnetic nanoparticles are described with reference to FIGS. 3–5.

[0085] FIG. 3 is a flow diagram illustrating an example technique for determining an average saturation magnetization of a plurality of magnetic nanoparticles based on a harmonic ratio extracted from a magnetic response of the plurality of magnetic nanoparticles to applied excitation fields. The example technique of FIG. 3 includes sending, by computing device 20, excitation signal 21 to excitation coil 16 to simultaneously subject the sample volume to the first and the second excitation fields (40). In some examples, excitation signal generation module 34 may determine or

generate excitation signal 21 based on the predetermined respective frequencies and amplitudes with which the first and the second excitation fields are to be generated. The first and the second excitation fields may cause the sample volume to generate a magnetic response including a plurality of harmonics.

[0086] Detection coil 18 may detect the magnetic response and output response signal 19 based on the magnetic response. In some examples, response signal 19 substantially preserves predetermined information in the detected magnetic response. For example, the response signal may be indicative of a plurality of harmonics of the magnetic response. Computing device 20 may receive response signal 19 from detection coil 18, and analyze response signal 19 (42). In some examples, response signal analysis module 36 of computing device 20 may analyze response signal 19. For example, response signal analysis module 36 may extract predetermined odd harmonics of the plurality of harmonics from response signal 19 (44). In some examples, the odd harmonic components may include at least a third harmonic having a frequency substantially equal to $f_H + 2f_L$ and a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$. In some examples, computing device 20 may use an inverse relationship between the harmonic ratio and the average saturation magnetization to determine the average saturation magnetization based on a determined harmonic ratio. The example technique includes, by computing device 20, determining an average saturation magnetization of a plurality of magnetic nanoparticles in the sample volume based on the predetermined harmonic components (46). For example, computing device 20 may determine a respective amplitude of a predetermined harmonic, for example, one or both of the third harmonic and the fifth harmonic, and use the respective amplitude to determine the average saturation magnetization. In some examples, computing device 20 may determine a harmonic ratio, or a ratio of the third harmonic to the fifth harmonic, and use the harmonic ratio to determine the average saturation magnetization. For example, computing device may use a lookup table, a database, an equation, a numerical approximation, or a simulation, to relate the respective amplitude or the harmonic ratio to an average saturation magnetization. In some examples, the lookup table or database may be determined by solving one or more of EQUATIONS 1–8, or by performing a numerical approximation or a numerical simulation. In some examples, the lookup table or database may include entries determined by determining properties of magnetic nanoparticles using alternative techniques, for example, VSM, TEM, or DSL. In some examples, computing device 20

may also account for an average core diameter in determining the average saturation magnetization. For example, computing device 20 may use a lookup table, a database, an equation, a numerical approximation, or a simulation, to relate both the harmonic ratio and the average core diameter to the average saturation magnetization. In some examples, computing device 20 may use one or more of EQUATIONS 1–8 or variants thereof to determine the average saturation magnetization, for example, by assigning values to mathematically known variables in one or more of EQUATIONS 1–8, and using a linear or non-linear numerical method to solve for one or more mathematically unknown variables in EQUATIONS 1–8, including the average saturation magnetization. Thus, the example technique of FIG. 3 may be used to determine the average saturation magnetization for a plurality of magnetic nanoparticles in the sample volume.

[0087] FIG. 4 is a flow diagram illustrating an example technique for determining an average hydrodynamic volume of a plurality of magnetic nanoparticles based on a phase lag extracted from a magnetic response of the plurality of magnetic nanoparticles to applied excitation fields. The example technique of FIG. 4 includes sending, by computing device 20, excitation signal 21 to excitation coil 16 to simultaneously subject the sample volume to the first and the second excitation fields (50). In some examples, excitation signal generation module 34 may determine or generate excitation signal 21 based on the predetermined respective frequencies and amplitudes of the first and the second excitation fields.

[0088] The first and the second excitation fields may cause the sample volume to generate a magnetic response including a plurality of harmonics. The magnetic response may be detected by detection coil 18, which may output response signal 19 based on the magnetic response. In some examples, response signal 19 substantially preserves predetermined information in the detected magnetic response. For example, the response signal may be indicative of a phase lag of the magnetic response of the sample volume to the excitation fields. Computing device 20 may receive response signal 19 from detection coil 18, and analyze response signal 19 (52). For example, response signal analysis module 36 of computing device 20 may analyze response signal 19. In some implementations, response signal analysis module 36 may extract a phase lag of the response signal 19 (54). In some examples, the phase lag may be a phase lag for a third harmonic having a frequency substantially equal to $f_H + 2f_L$, or for a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$. In some examples,

computing device 20 may use an inverse relationship between the phase lag and the average hydrodynamic volume to determine the average hydrodynamic volume based on a determined phase lag. The example technique includes, by computing device 20, determining an average hydrodynamic volume of a plurality of magnetic nanoparticles in the sample volume based on the predetermined harmonic components (56). For example, computing device 20 may use a lookup table, a database, an equation, a numerical approximation, or a simulation, to relate the phase lag to an average hydrodynamic volume. In some examples, the lookup table or database may be determined by solving one or more of EQUATIONS 1–8, or by performing a numerical approximation or a numerical simulation. In some examples, the lookup table or database may include entries determined by determining properties of magnetic nanoparticles using alternative techniques, for example, VSM, TEM, or DSL. In some examples, computing device 20 may use one or more of EQUATIONS 1–8 or variants thereof to determine the average hydrodynamic volume, for example, by assigning values to mathematically known variables in one or more of EQUATIONS 1–8, and using a linear or non-linear numerical method to solve for one or more mathematical unknowns in EQUATIONS 1–8, including the average hydrodynamic volume. Thus, the example technique of FIG. 3 may be used to determine the average hydrodynamic volume for a plurality of magnetic nanoparticles in the sample volume.

[0089] FIG. 5 is a flow diagram illustrating an example technique for determining a core state of a plurality of magnetic nanoparticles based on one or both of a phase lag or a harmonic ratio extracted from a magnetic response of the plurality of magnetic nanoparticles to applied excitation fields. The example technique of FIG. 5 includes maintaining the sample volume in one of a liquid state or a frozen state (60), and determining one or both of the predetermined harmonic components or the phase lag (68) in the liquid state and the frozen state, respectively. For example, computing device 20 may determine one or both of the predetermined harmonic components or the phase lag in the liquid state, and again in the solid state. In some examples, the sample volume may be maintained in the liquid state by maintaining a temperature within sample container 12 above a melting point of a composition in sample container 12. In some examples, the sample volume may be maintained in the solid state by maintaining a temperature within sample container 12 below a melting point of the composition in sample container 12. To determine one or both of the predetermined harmonic components or the phase lag, computing device may implement one or more of steps 62

to 66. For example, the example technique of FIG. 5 may include, by computing device 20, sending excitation signal 21 to excitation coil 16 to simultaneously subject the sample volume to the first and the second excitation fields (62). In some examples, excitation signal generation module 34 may determine excitation signal 21 based on the predetermined respective frequencies and amplitudes of the first and the second excitation fields. The first and the second excitation fields may cause the sample volume to generate a magnetic response including a plurality of harmonics. The magnetic response may be detected by detection coil 18, which may generate response signal 19 based on the magnetic response. In some examples, response signal 19 substantially preserves predetermined information in the detected magnetic response. For example, the response signal may be indicative of one or both of a plurality of harmonics or a phase lag of the magnetic response. Computing device 20 may receive response signal 19 from detection coil 18, and analyze response signal 19 (64). In some examples, response signal analysis module 36 of computing device 20 may analyze response signal 19. For example, response signal analysis module 36 may extract one or both of the phase lag and the harmonic components of the response signal 19 (54). In some examples, the phase lag may be a phase lag for a third harmonic having a frequency substantially equal to $f_H + 2f_L$, or for a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$. In some examples, response signal analysis module 36 may extract predetermined odd harmonics of the plurality of harmonics from response signal 19 (66). In some examples, the odd harmonic components may include at least a third harmonic having a frequency substantially equal to $f_H + 2f_L$ and a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$. The example technique may include, by computing device 20, determining a core state of a plurality of magnetic nanoparticles in the sample volume based on the predetermined harmonic components (68). For example, computing device 20 may determine a respective amplitude of a predetermined harmonic, for example, one or both of the third harmonic and the fifth harmonic, and use the respective amplitude to determine the core state. In some examples, computing device 20 may determine a harmonic ratio, or a ratio of the third harmonic to the fifth harmonic, and use the harmonic ratio to determine the core state. The example technique includes, by computing device 20, determining core state of a plurality of magnetic nanoparticles in the sample volume based on the predetermined harmonic components (68). For example, computing device 20 may use a lookup table, a database, an equation, a numerical approximation, or a simulation, to relate one or

both of the phase lag and the harmonic component or ratio to an average hydrodynamic volume. In some examples, the lookup table or database may be determined by solving one or more of EQUATIONS 1–8, or by performing a numerical approximation or a numerical simulation. In some examples, the lookup table or database may include entries determined by determining properties of magnetic nanoparticles using alternative techniques, for example, VSM, TEM, or DSL. In some examples, computing device 20 may use one or more of EQUATIONS 1–8 or variants thereof to determine the core state, for example, by assigning values to mathematically known variables in one or more of EQUATIONS 1–8, and using a linear or non-linear numerical method to solve for one or more mathematically unknown variables in EQUATIONS 1–8. Computing device 20 may compare the mathematically unknown variables derived in a liquid state of the sample volume with the mathematically known variables derived in a frozen state of the sample volume, and on the basis of the comparison, determine whether the core state includes single-core nanoparticles or multi-core nanoparticles.

[0090] In some examples, a reduction in an amplitude of a predetermined harmonic component that is greater than a predetermined threshold may be indicative of a core state associated with single-core nanoparticles. For example, a reduction in the amplitude of the third harmonic by over 90%, or over about 98%, between the frozen and liquid states may be indicative of single-core nanoparticles. In contrast, a change in the amplitude of the predetermined harmonic component of less than a predetermined threshold may be indicative of multi-core nanoparticles. For example, a maximum change in the amplitude of the third harmonic of less than about 10%, for example, less than about 5%, or about 4%, between the liquid and the frozen states may be indicative of a multi-core state.

[0091] In some examples, a reduction in an harmonic ratio that is greater than a predetermined threshold may be indicative of a core state associated with single-core nanoparticles. For example, a reduction in the harmonic ratio of the third harmonic to the fifth harmonic by over 1, or over 2, or over 3 may be indicative of a single-core state. In contrast, a change in the amplitude of the predetermined harmonic component of less than a predetermined threshold may be indicative of a multi-core state. For example, a change in the amplitude of the third harmonic of less than about 1 unit, or about 0.25 units between the liquid and the frozen states may be indicative of a multi-core state.

[0092] In some examples, a comparison of the phase lag between the liquid and the frozen state may be used in addition to or in place of the comparison of the harmonic ratio. For example, a substantially similar phase lag in the frozen and liquid states may be indicative of a multi-core state, while a difference in the phase lag, for example, at least about 5°, or at least about 10°, or at least about 20°, between the frozen and liquid states may be indicative of a single-core state. Thus, the example technique of FIG. 3 may be used to determine the core state for a plurality of magnetic nanoparticles in the sample volume.

[0093] Thus, example systems and techniques according to the disclosure may be used to conduct search coil based frequency mixing to characterize properties such as saturation magnetizations, hydrodynamic volumes, and core states, of MNPs, for example, SPIONs. Single- and multi-core SPIONs may be distinguished by comparing their harmonic ratio and phase information in liquid and frozen states. Analyzing the harmonic signals may also be used to determine the relaxation process that dominates under particular conditions.

[0094] The techniques described in this disclosure may be implemented, at least in part, in hardware, software, firmware, or any combination thereof. For example, various aspects of the described techniques may be implemented within one or more processors, including one or more microprocessors, digital signal processors (DSPs), application specific integrated circuits (ASICs), field programmable gate arrays (FPGAs), or any other equivalent integrated or discrete logic circuitry, as well as any combinations of such components. The term “processor” or “processing circuitry” may generally refer to any of the foregoing logic circuitry, alone or in combination with other logic circuitry, or any other equivalent circuitry. A control unit including hardware may also perform one or more of the techniques of this disclosure.

[0095] Such hardware, software, and firmware may be implemented within the same device or within separate devices to support the various techniques described in this disclosure. In addition, any of the described units, modules or components may be implemented together or separately as discrete but interoperable logic devices.

Depiction of different features as modules or units is intended to highlight different functional aspects and does not necessarily imply that such modules or units must be realized by separate hardware, firmware, or software components. Rather, functionality associated with one or more modules or units may be performed by separate hardware,

firmware, or software components, or integrated within common or separate hardware, firmware, or software components.

[0096] The techniques described in this disclosure may also be embodied or encoded in a computer system-readable medium, such as a computer system-readable storage medium, containing instructions. Instructions embedded or encoded in a computer system-readable medium, including a computer system-readable storage medium, may cause one or more programmable processors, or other processors, to implement one or more of the techniques described herein, such as when instructions included or encoded in the computer system-readable medium are executed by the one or more processors. Computer system readable storage media may include random access memory (RAM), read only memory (ROM), programmable read only memory (PROM), erasable programmable read only memory (EPROM), electronically erasable programmable read only memory (EEPROM), flash memory, a hard disk, a compact disc ROM (CD-ROM), a floppy disk, a cassette, magnetic media, optical media, or other computer system readable media. In some examples, an article of manufacture may comprise one or more computer system-readable storage media.

[0097] The present disclosure will be illustrated by the following non-limiting examples.

EXAMPLES

Example 1

[0098] Numerical simulations conforming to our experimental design were performed to reveal the correlation between the harmonic ratio and M_s as well as D . A SPION system following a log-normal size distribution with average D of 30 nm, standard deviation of 10 nm, surface polymer coating thickness of 4 nm, concentration of 300 pmole/ml was assumed and tested at a simulation room temperature of $T = 300\text{ K}$. Two simulated sinusoidal fields are applied to the simulated SPION system: one with amplitude of 100 Oe and frequency of 50 Hz, the other with amplitude of 10 Oe and its frequency was swept from 8 kHz to 22 kHz. White noise and $1/f$ noise (also referred to as pink noise) were added in the simulation. Due to crystal asymmetry on the surface of MNPs (spin-canting effect), a smaller M_s and a larger effective anisotropy constant K_{eff} are expected in SPIONs compared to bulk magnetite. Herein, we vary M_s from 20 emu/g to 90 emu/g in our simulation. Data was collected during 0.1 s with a

sampling rate of 1 MHz, a discrete Fourier Transform (DFT) was performed on these discrete-time harmonic signals by applying a Hanning window. FIG. 6A is a chart presenting harmonic ratio as a function of frequency for simulated nanoparticles having different magnetizations. The harmonic ratios are plotted in FIG. 6A as function of frequency for 30 nm SPIONs with different M_s . FIG. 6B is a chart presenting averaged harmonic ratio as a function of saturation magnetization for simulated nanoparticles. Curve fitting to the averaged harmonic ratios vs. M_s in FIG. 6B shows that the harmonic ratios are inversely proportional to the 0.55th power of M_s . From the simulation results, it was concluded that for MNPs with identical core diameters, a smaller harmonic ratio corresponds to a higher M_s .

[0099] In the second part of the simulation, $M_s = 50$ emu/g was set as a constant, and core size D was varied from 15 nm to 35 nm. FIG. 6C is a chart presenting harmonic ratio as a function of frequency for simulated nanoparticles having different core diameters. The harmonic ratios from different core sizes are summarized in FIG. 6C as function of frequency. FIG. 6D is a chart presenting averaged harmonic ratio as a function of core diameter for simulated nanoparticles. Curve fitting in FIG. 6D shows that harmonic ratio is inversely proportional to the 1.58th power of D . Therefore, for MNPs with identical M_s , a smaller core size yields a larger harmonic ratio. The simulation results yielded a simplified formula of harmonic ratio provided by EQUATION 8.

$$[0100] R|_{3rd/5th} \sim M_s^{-0.55} \times D^{-1.58} \quad (\text{Equation 8}).$$

Example 2

The relative contribution of Brownian relaxation and Néel relaxation to the effective relaxation was evaluated by simulation. For single-core MNPs, small SPIONs relax via Néel process whereas larger SPIONs relax via Brownian process. The cut off size between these two processes for single-core SPIONs is about 12 nm. If, instead the SPIONs are embedded in a matrix with overall size of 50 nm, then the cut off size for multi-core is around 15 nm. If single-core SPIONs with core diameters larger than 20 nm are used, Brownian process will dominate and phase lag is proportional to hydrodynamic volume. For multi-core beads (SPIONs imbedded in a matrix, such as MACS microbeads), SPIONs will go through Néel process. FIG. 7A is a conceptual and schematic diagram illustrating predominance of Néel relaxation exhibited by multi-

core nanoparticles. FIG. 7B is a conceptual and schematic diagram illustrating predominance of Brownian relaxation exhibited by single-core nanoparticles.

[0101] The effect of core diameters on relaxation times was simulated. FIG. 8A is a chart presenting the relative contribution of Néel relaxation and Brownian relaxation to effective relaxation time as a function of core diameter for sample single-core nanoparticles. FIG. 8A shows that small SPIONs relax via Néel process whereas larger SPIONs relax via Brownian process. The cut off size of single-core SPIONs is about 12 nm. FIG. 8B is a chart presenting the relative contribution of Néel relaxation and Brownian relaxation to effective relaxation time as a function of core diameter for sample multi-core nanoparticles. If instead the SPIONs are placed in a matrix with diameter of 50 nm the cut off size in this case shown in FIG. 8B is about 15 nm. Here, single-core SPIONs with core diameters larger than 20 nm are used, thus Brownian process will dominate and phase lag is dependent on hydrodynamic volumes. For those multi-core SPIONs imbedded in a matrix (such as MACS microbeads), SPIONs will probably go through Néel process although beads have an overall diameter of 50 nm.

Example 3

[0102] Four commercially available SPION samples (Ocean NanoTech Inc., Springdale, AZ, and Miltenyi Biotec, Bergisch Gladbach, Germany) were measured using a search coil system and compared with standard methods. These four samples are: SHP25 (SPIONs with average core size of 25 nm coated with approximately 4 nm of oleic acid and amphiphilic polymer shells, dispersed in 0.02% sodium azide, 290 pmole/ml); SMG30-II (SPIONs with average core size of 30 nm coated with approximately 6 nm of amphiphilic polymer and PEG shells, dispersed in 0.02% sodium azide, 34 pmole/ml); SMG30-I are aged SMG30-II; MACS (small SPIONs embedded in matrix, the average overall size is 50 nm, coated with streptavidin, dispersed in 0.05% sodium azide, 3.14 pmole/ml). The concentrations of these SPION samples are below 290 pmole/ml and the volume concentration is calculated to be less than 0.13 vol %, which is low enough to safely rule out the dipolar interactions and can be treated as a non-interacting system. Amplitudes and phases of the 3rd and the 5th harmonics were measured from four SPION samples. The magnetic and physical properties of SPIONs were determined and compared with standard characterization methods: Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Vibrating Sample Magnetometer (VSM), X-ray Diffractometer (XRD), and high-angle annular dark field-

scanning transmission electron microscope-energy dispersive X-ray spectroscopy (HAADF-STEM-EDS).

[0103] A system was set up for subjecting samples to excitation fields and detecting the magnetic response. A PC with a data acquisition card (NI USB-6289, National Instruments, Austin, TX) generated two sinusoidal waves, amplified by two instrument amplifiers (HP 6824A, Hewlett-Packard, Palo Alto, CA). Band-pass-filters (BPFs) are used to suppress higher harmonics introduced by IAs. The filtered waves drive excitatory coils to generate oscillating fields: the amplitude of higher frequency field is 10 Oe and its frequency f_H varies from 10 kHz to 20 kHz, while the amplitude of lower frequency field $f_L = 50\text{ Hz}$ is 100 Oe. The response signals at combinatorial frequencies $f_H + 2f_L$ and $f_H + 4f_L$ were collected. One pair of differentially wound pick-up coils (600 windings in clock-wise and counter-clock-wise, respectively) collected induced voltage and phase signals from ferrofluid samples and send back to a band-stop filter before being digitalized on the DAQ. FIG. 9A is photograph illustrating an example search coil assembly including a sample vial, a detection coil, a low frequency coil, and a high frequency coil. FIG. 9B is photograph illustrating the sample vial, the detection coil, the low frequency coil, and the high frequency coil of the search coil assembly of FIG. 9A. FIG. 10A is schematic and conceptual drawing illustrating the structure and dimensions of the sample vial, the detection coil, the low frequency coil, and the high frequency coil of FIG. 9B. FIG. 10B is schematic and conceptual drawing illustrating the structure and dimensions of the sample vial of FIG. 9B.

[0104] The relation between harmonic ratio and frequency, and phase lag and frequency, for different nanoparticles having different particle sizes and clustering was evaluated. Each SPION sample was sonicated at room temperature for 1 hour to ensure uniformity. 50 μL of aqueous sample was drawn from each SPION sample by pipette and transferred to our specially designed plastic vial. In each test, the background noise is collected for 10 seconds; the vial is then inserted into a pair of differentially wound pick-up coils and the signal is collected for another 10 seconds. The low frequency coil generates a sinusoidal magnetic field of 100 Oe and frequency of 50 Hz. The high frequency coil generates a sinusoidal magnetic field of 10 Oe and the frequency is varied from 10 kHz to 20 kHz.

[0105] FIG. 11A is a chart presenting harmonic ratio as a function of frequency for sample nanoparticles having different core states. FIG. 11A shows the measured harmonic ratios of the four SPION samples using our search coil system. From the

measured harmonic ratios, it was anticipated that SMG30-II would have the highest M_s while SMG30-I would have the lowest. SMG30-I, -II, and SHP25 are single-core SPIONs that go through Brownian relaxation; therefore, their phase lag is directly related to their relative hydrodynamic volumes. FIG. 11B is a chart presenting phase lag as a function of frequency for sample nanoparticles having different core states. The legend shown in FIG. 11A noting the types of data points is also applicable to the chart of FIG. 11B. In FIG. 11B, SMG30-I and -II have almost the same hydrodynamic volumes and SHP25 comes in second. The phase lag may indicate that MACS has the smallest hydrodynamic volume; this however, is not the case. Because MACS beads are composed of smaller SPIONs embedded in a matrix, this phase lag is due to Néel process. By freezing up the aqueous SPION samples, we can effectively block the Brownian rotation, and thus, all the SPIONs will display Néel relaxation exclusively. In this way, the multi-core magnetic beads would have similar magnetic response both in liquid and frozen states while the single-core MNPs would have completely different magnetic responses between these two states.

Example 4

[0106] The harmonic ratio and phase lag for single-core particles was compared with multi-core particles in a frozen state and a liquid state. A plastic vial containing 50 μL of liquid MACS sample was measured from 10 kHz to 20 kHz. The amplitude and phase lag of the 3rd harmonic signal, and the harmonic ratio of the 3rd over the 5th harmonics were calculated using phasor theory. Then the vial was stored at -20 °C for 3 hours to ensure complete freezing. This vial containing the same MACS sample (except that it is in frozen state) was measured for a second time. FIG. 10A is a chart presenting the difference in amplitudes of the third harmonic for multi-core nanoparticles in liquid and frozen states. FIG. 12A shows the amplitude difference of the 3rd harmonic signal between liquid and frozen states for MACS sample. Overall, there was little difference (~4%) in the amplitude of the 3rd harmonic signal for MACS sample in frozen and liquid states. That is because the microbeads in MACS sample are multi-core beads, and the tiny SPIONs are imbedded in a polymer matrix, which blocks the physical rotation process (namely, Brownian relaxation is blocked). The magnetic moment flipping upon AC field is accomplished by Néel relaxation process. Microbeads are immobilized upon frozen, while for these tiny SPIONs, the dominating relaxation process is always Néel relaxation. Thus, Néel relaxation dominates in both liquid and frozen states for

MACS sample. This gave rise to the identical phase lags of the 3rd harmonic signal from liquid and frozen states. The relatively small difference in the amplitudes of the 3rd harmonic (FIG. 12A) between liquid and frozen MACS samples was due to the difference in temperature: liquid MACS sample is measure at room temperature (~20°C) while frozen MACS sample is measured at 0°C.

[0107] Similarly, liquid and frozen SMG30-II samples were prepared in the same way and collected the amplitude and phase lag of the 3rd harmonic signal, and the harmonic ratio of the 3rd over the 5th harmonics accordingly. FIG. 12B is a chart presenting the difference in amplitudes of the third harmonic for single-core nanoparticles in liquid and frozen states. FIG. 12B shows the amplitude difference of the 3rd harmonic signal between liquid and frozen states for SMG30-II sample. Overall, the harmonic signal strength decreased by ~98% in frozen state compared to liquid state. That is because the SPIONs in SMG30-II sample are single-core particles and Brownian relaxation plays the major role when dispersed in liquid solution. Although Brownian and Néel relaxation processes go in parallel in an alternating field, the faster process dominates macroscopically. Herein, for SMG30-II, the Brownian relaxation dominates in liquid state. While for SMG30-II in frozen state, SPIONs are fixed and Brownian relaxation is blocked, which, as a result, Néel relaxation starts to play its role.

[0108] Since Néel process is much slower than Brownian process for SMG30-II SPIONs, a larger phase lag in frozen state is anticipated. The strength of harmonic signals is attenuated due to larger phase lag to the driving fields, accounting for a 98% drop in the 3rd harmonic signal from frozen SMG30-II compared to liquid.

[0109] For MACS beads, SPIONs were immobilized in a matrix and Néel relaxation dominates regardless which state it was in. In contrast, SMG30-II primarily exhibited Brownian relaxation in the liquid state and Néel relaxation in the frozen state. Thus, due to the great differences in phase lags and harmonic ratios between liquid and frozen states shown in FIGS. 13A–13D, single- and multi-core SPIONs can be distinguished. FIG. 13A is a chart presenting harmonic ratio as a function of frequency for sample single-core nanoparticles in liquid and frozen states. FIG. 13A presents a comparison of the harmonic ratios and phase lag of MACS in liquid and frozen states. FIG. 13B is a chart presenting phase lag as a function of frequency for sample multi-core nanoparticles in liquid and frozen states. The legend shown in FIG. 13A noting the types of data points is also applicable to the chart of FIG. 13B. The harmonic ratio and phase lag of the 3rd harmonic signal between frozen and liquid states are compared in

FIGS. 13A and 13B. IG. 13C is a chart presenting harmonic ratio as a function of frequency for sample multi-core nanoparticles in liquid and frozen states. FIG. 13C presents a comparison of the harmonic ratios and phase lag of SMG30-II in liquid and frozen states. FIG. 13D is a chart presenting phase lag as a function of frequency for sample multi-core nanoparticles in liquid and frozen states. The legend shown in FIG. 13C noting the types of data points is also applicable to the chart of FIG. 13D. The harmonic ratio and phase lag of the 3rd harmonic signal between frozen and liquid states are compared in FIGS. 13C and 13D.

[0110] Based on experimental results shown in FIGS. 12A, 12B, and 13A–13D, the magnetic and physical properties of these samples can be estimated using a search coil system. In summary, SMG30-II would have the highest M_s , followed by SHP25, and SMG30-I to have the lowest M_s . Samples SMG30-I and -II are expected to have similar hydrodynamic volumes and are larger than SHP25. The aforementioned SPION samples (SHP25, SMG30-I, and -II) were also determined to be single-cored by measuring their harmonic signals in liquid and frozen states. The MACS sample however, is expected to be multi-cored with average core diameters of 10 nm. Because the harmonic ratio is inversely proportional to the 0.55th power of M_s , MACS is expected to have lower M_s than SMG30-II and SHP25. Thus, the M_s of these single-core SPION samples from highest to lowest are: SMG30-II > SHP25 > SMG30-I > MACS. The average hydrodynamic sizes followed in descending orders are: SMG30-II $\approx$ SMG30-I > SHP25.

Example 5

[0111] SPION samples were imaged using TEM. SPION samples were drop-cast onto supporting carbon grids and air dried prior to investigation by TEM. FIGS. 14A–14D are photographs illustrating bright-field transmission electron microscopy micrographs of sample nanoparticle compositions collected using dynamic light scattering. The bright-field TEM micrographs confirmed the spherical morphology in our samples (FIGS. 14A–14D), and further confirmed that SHP25, SMG30-I, and SMG30-II are single-core particles. FIG. 14D shows that each MACS bead is composed of smaller magnetic nanoparticles embedded in a large matrix. The hydrodynamic sizes of these SPION samples in liquid states are tested by DLS. FIGS. 15A–15D are charts presenting statistical hydrodynamic size distributions for the sample nanoparticle compositions shown in FIGS. 14A–14D. Statistic results from FIGS. 15A–15D yielded

the mean hydrodynamic sizes of SHP25, SMG30-I, SMG30-II, and MACS: 36.90 nm, 40.20 nm, 40.06 nm, and 61.92 nm, respectively. The results from DLS are in good agreement with the search coil based results and analysis. It is worth mentioning that the DLS result of MACS in FIG. 15D differs from TEM image in FIG. 14D, due to the flattening of matrix during TEM specimen preparation. The corresponding core diameters were determined by fitting TEM results to a log-normal distribution curve, as shown in FIGS. 16A–16D. FIGS. 16A–16D are charts presenting core diameter distributions for sample single-core and multi-core nanoparticles shown in FIGS. 14A–14D.

Example 6

[0112] The crystalline structure of these SPION samples was characterized by D8 X-ray Diffractometer using Cu-K α_1 radiation ($\lambda=0.15406$ nm) at a rate of 2°/min. FIG. 17A is a chart presenting x-ray diffraction spectra for sample nanoparticles. All diffraction peaks in FIG. 17A are consistent with the standard XRD pattern of magnetite for SHP25, SMG30-I, and SMG30-II. The XRD pattern of MACS shows that the particles from these beads are mainly composed of α -Fe₂O₃ and Fe₃O₄, which well explained that MACS has the lowest M_s . FIG. 17B is a chart presenting magnetization curves for the sample nanoparticles of FIG. 14A. As seen in FIG. 17B, all of these SPION samples show superparamagnetism and the saturation magnetization from highest to lowest are: SMG30-II, SHP25, SMG30-I, MACS. This agrees well with the search coil based experiments and analysis.

[0113] Various examples have been described. These and other examples are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A system comprising:
 - an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , wherein the first and the second excitation fields are configured to generate a magnetic response comprising a plurality of harmonics from the sample volume;
 - a detection coil configured to sense the magnetic response and output a response signal in response to the magnetic response, wherein the response signal is indicative of the plurality of harmonics; and
 - a computing device configured to:
 - receive the response signal from the detection coil;
 - extract predetermined harmonic components of the plurality of harmonics from the response signal; and
 - determine an average saturation magnetization of the plurality of magnetic nanoparticles based on the predetermined harmonic components.
2. The system of claim 1, wherein the predetermined harmonic components comprise odd harmonic components.
3. The system of claim 2, wherein the odd harmonic components include at least a third harmonic having a frequency substantially equal to $f_H + 2f_L$ and a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$.
4. The system of claim 3, wherein determining the average saturation magnetization comprises determining a harmonic ratio of the third harmonic to the fifth harmonic.
5. The system of any one of claims 1 to 4, wherein f_H is variable within a predetermined frequency range, and wherein f_L is a predetermined fixed frequency.
6. The system of any one of claims 1 to 5, wherein the computing device is further configured to send an excitation signal to the excitation coil to generate the first excitation field and the second excitation field.

7. The system of claim 6, wherein the excitation signal is configured to sweep f_H between a predetermined lower frequency and a predetermined higher frequency defining the frequency range, and wherein the excitation signal is configured to hold f_L at about the predetermined fixed frequency.
8. The system of any one of claims 1 to 7, wherein the excitation coil comprises a first coil configured to generate the first excitation field and a second coil configured to generate the second excitation field.
9. The system of claim 8, wherein the second coil surrounds the first coil, wherein the first coil surrounds the detection coil, and wherein the detection coil surrounds the sample volume.
10. The system of any one of claims 1 to 9, wherein the detection coil comprises a pair of differentially wound pick-up coils.
11. A method comprising, by a computing device:
 - sending an excitation signal to an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , wherein the first and the second excitation fields are configured to generate a magnetic response comprising a plurality of harmonics from the sample volume;
 - receiving a response signal indicative of the plurality of harmonics;
 - extracting predetermined harmonic components of the plurality of harmonics from the response signal; and
 - determining an average saturation magnetization of the plurality of magnetic nanoparticles based on the predetermined harmonic components.
12. The method of claim 11, wherein the predetermined harmonic components comprise odd harmonic components.

13. The method of claim 12, wherein the odd harmonic components include at least a third harmonic having a frequency substantially equal to $f_H + 2f_L$ and a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$.
14. The method of claim 13, wherein determining the saturation magnetization comprises determining a harmonic ratio of the third harmonic to the fifth harmonic.
15. The method of any one of claims 11 to 14, wherein f_H is variable within a predetermined frequency range, and wherein f_L is a predetermined fixed frequency.
16. The method of any one of claims 11 to 15, wherein the excitation signal is configured to sweep f_H between a predetermined lower frequency and a predetermined higher frequency defining the frequency range, and wherein the excitation signal is configured to hold f_L at the predetermined fixed frequency.
17. A system comprising:
an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , wherein the first and the second excitation fields are configured to generate a magnetic response having a phase lag relative to the first and the second excitation fields from the sample volume;
a detection coil configured to sense the magnetic response and output a response signal in response to the magnetic response, wherein the response signal is indicative of the phase lag; and
a computing device configured to:
receive the response signal from the detection coil;
extract the phase lag from the response signal; and
determine an average hydrodynamic volume of the plurality of magnetic nanoparticles based on the phase lag.
18. The system of claim 17, wherein the phase lag is determined at a third harmonic having a frequency substantially equal to $f_H + 2f_L$ of the magnetic response.

19. The system of claim 17 or 18, wherein f_H is variable within a predetermined frequency range, and wherein f_L is a predetermined fixed frequency.

20. The system of any one of claims 17 to 19, wherein the computing device is further configured to send an excitation signal to the excitation coil to generate the first excitation field and the second excitation field.

21. The system of claim 20, wherein the excitation signal is configured to sweep f_H between a predetermined lower frequency and a predetermined higher frequency defining the frequency range, and wherein the excitation signal is configured to hold f_L at the predetermined fixed frequency.

22. The system of any one of claims 17 to 21, wherein the excitation coil comprises a first coil configured to generate the first excitation field and a second coil configured to generate the second excitation field.

23. The system of claim 22, wherein the second coil surrounds the first coil, wherein the first coil surrounds the detection coil, and wherein the detection coil surrounds the sample volume.

24. The system of any one of claims 17 to 23, wherein the detection coil comprises a pair of differentially wound pick-up coils.

25. A method comprising, by a computing device:
sending an excitation signal to an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , wherein the first and the second excitation fields are configured to generate a magnetic response having a phase lag relative to the first and second excitation fields from the sample volume;

receiving a response signal indicative of the phase lag;

extracting the phase lag from the response signal; and

determining an average hydrodynamic volume of the plurality of magnetic nanoparticles based on the phase lag.

26. The method of claim 25, wherein the phase lag is determined at a third harmonic having a frequency substantially equal to $f_H + 2f_L$ of the magnetic response.

27. The method of claim 25 or 26, wherein f_H is variable within a predetermined frequency range, and wherein f_L is a predetermined fixed frequency.

28. The method of any one of claims 25 to 27, wherein the excitation signal is configured to sweep f_H between a predetermined lower frequency and a predetermined higher frequency defining the frequency range, and wherein the excitation signal is configured to hold f_L at the predetermined fixed frequency.

29. A system comprising:

an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , wherein the first and the second excitation fields are configured to generate a magnetic response comprising a plurality of harmonics from the sample volume, wherein the magnetic response has a phase lag relative to the first and second excitation fields;

a detection coil configured to sense the magnetic response and output a response signal in response to the magnetic response, wherein the response signal is indicative of one or both of the plurality of harmonics or the phase lag; and

a computing device configured to:

- receive the response signal from the detection coil;
- extract one or both of the phase lag or predetermined harmonic components of the plurality of harmonics from the response signal; and
- determine a core state of the plurality of magnetic nanoparticles based on one or both of the phase lag or the predetermined harmonic components, wherein the core state is one of a single-core state or a multi-core state.

30. The system of claim 29, wherein the computing device is configured to receive the response signal from the detection coil and extract one or both of the phase lag or predetermined harmonic components of the plurality of harmonics from the response signal in a liquid state of the sample volume and in a frozen state of the sample volume.

31. The system of claim 30, wherein the computing device determines the core state by comparing one or both of the phase lag and the predetermined harmonic components in the liquid state with one or both of the phase lag and the predetermined harmonic components in the frozen state.

32. The system of any one of claims 29 to 31, wherein the predetermined harmonic components comprise odd harmonic components.

33. The system of claim 32, wherein the odd harmonic components include at least a third harmonic having a frequency substantially equal to $f_H + 2f_L$ and a fifth harmonic having a frequency substantially equal to $f_H + 4f_L$.

34. The system of claim 33, wherein determining the core state comprises determining a harmonic ratio of the third harmonic to the fifth harmonic.

35. The system of any one of claims 29 to 34, wherein the phase lag is determined at a third harmonic having a frequency substantially equal to $f_H + 2f_L$ of the magnetic response.

36. The system of any one of claims 29 to 35, wherein f_H is variable within a predetermined frequency range, and wherein f_L is a predetermined fixed frequency.

37. The system of any one of claims 29 to 36, wherein the computing device is further configured to send an excitation signal to the excitation coil to generate the first excitation field and the second excitation field.

38. The system of claim 37, wherein the excitation signal is configured to sweep f_H between a predetermined lower frequency and a predetermined higher frequency defining the frequency range, and wherein the excitation signal is configured to hold f_L at the predetermined fixed frequency.

39. The system of any one of claims 29 to 38, wherein the excitation coil comprises a first coil configured to generate the first excitation field and a second coil configured to generate the second excitation field.

40. The system of claim 39, wherein the second coil surrounds the first coil, wherein the first coil surrounds the detection coil, and wherein the detection coil surrounds the sample volume.

41. The system of any one of claims 29 to 40, wherein the detection coil comprises a pair of differentially wound pick-up coils.

42. A method comprising, by a computing device:
- sending an excitation signal to an excitation coil configured to simultaneously subject a sample volume comprising a plurality of magnetic nanoparticles to a first excitation field having a frequency f_H and a second excitation field having a frequency f_L , wherein the first and the second excitation fields are configured to generate a magnetic response comprising a plurality of harmonics from the sample volume, wherein the magnetic response has a phase lag relative to the first and second excitation fields;
 - receiving a response signal indicative of one or both of the plurality of harmonics or the phase lag;
 - extracting one or both of the phase lag or predetermined harmonic components of the plurality of harmonics from the response signal; and
 - determining a core state of the plurality of magnetic nanoparticles based on one or both of the phase lag or the predetermined harmonic components, wherein the core state is one of a single-core state or a multi-core state.
43. The method of claim 42, further comprising sending the excitation signal, receiving the response signal, and extracting one or both of the phase lag or predetermined harmonic components of the plurality of harmonics in a liquid state of the sample volume and in a frozen state of the sample volume.
44. The method of claim 43, wherein determining the core state comprises comparing one or both of the phase lag and the predetermined harmonic components in the liquid state with one or both of the phase lag and the predetermined harmonic components in the frozen state.
45. The method of any one of claims 42 to 44, wherein the predetermined harmonic components comprise odd harmonic components.
46. The method of claim 45, wherein the odd harmonic components include at least a third harmonic having a frequency $f_H + 2f_L$ and a fifth harmonic having a frequency $f_H + 4f_L$.

47. The method of claim 46, wherein determining the core state comprises determining a harmonic ratio of the third harmonic to the fifth harmonic.
48. The method of any one of claims 42 to 47, wherein the phase lag is determined at a third harmonic having a frequency $f_H + 2f_L$ of the magnetic response.
49. The method of any one of claims 42 to 48, wherein f_H is variable within a predetermined frequency range, and wherein f_L is a predetermined fixed frequency.
50. The method of any one of claims 42 to 49, wherein the excitation signal is configured to sweep f_H between a predetermined lower frequency and a predetermined higher frequency defining the frequency range, and wherein the excitation signal is configured to hold f_L at the predetermined fixed frequency.

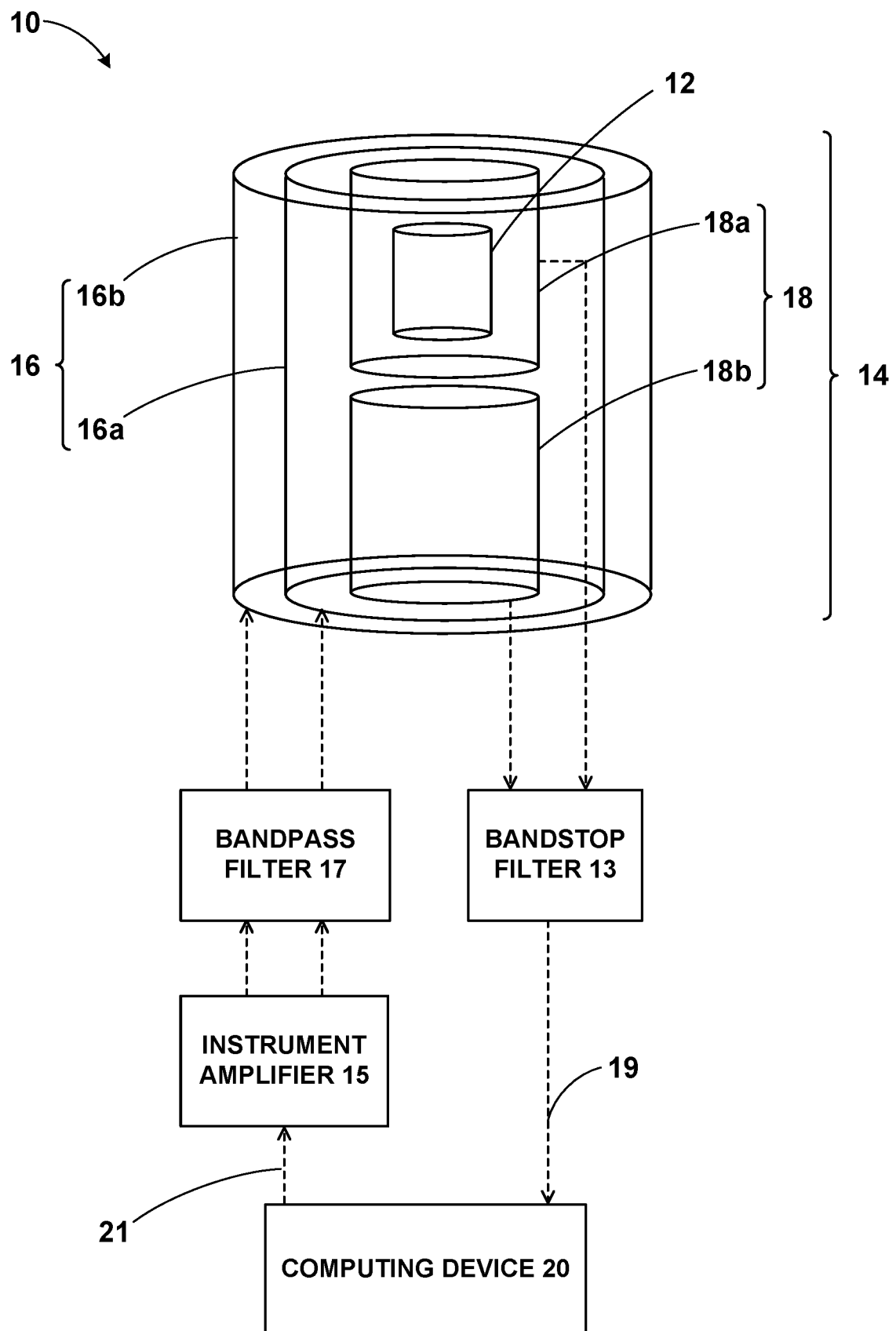


FIG. 1

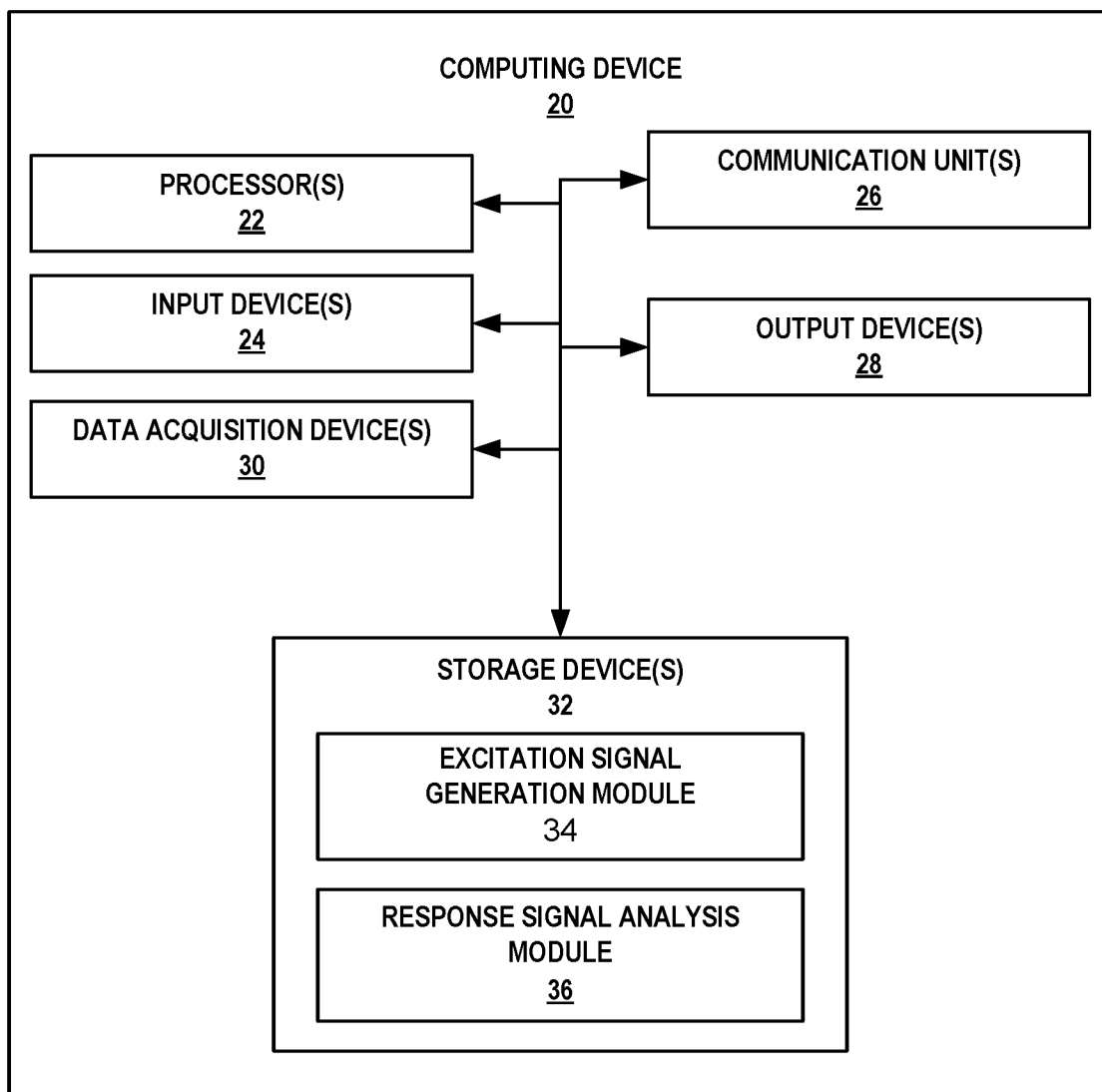


FIG. 2

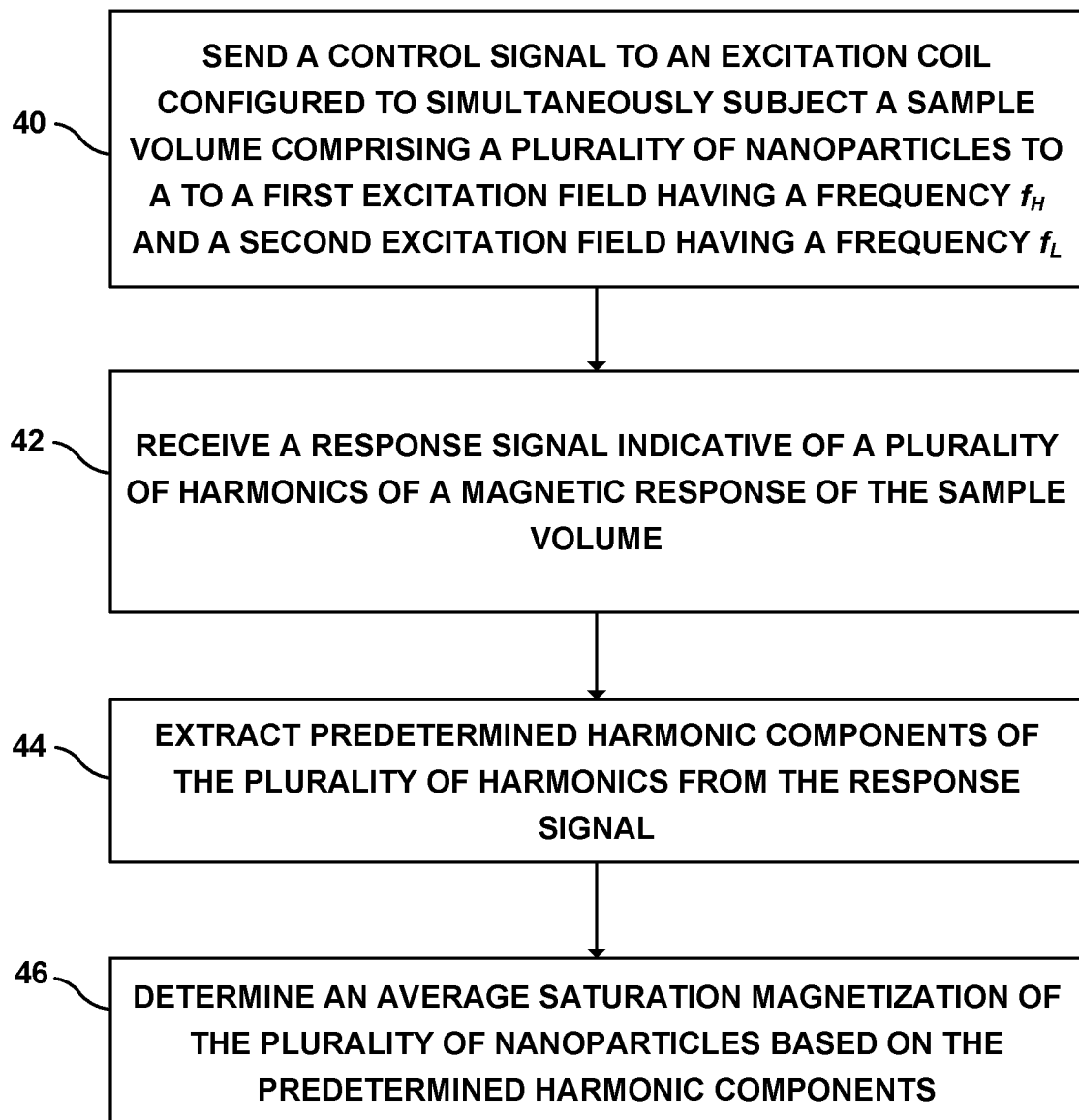


FIG. 3

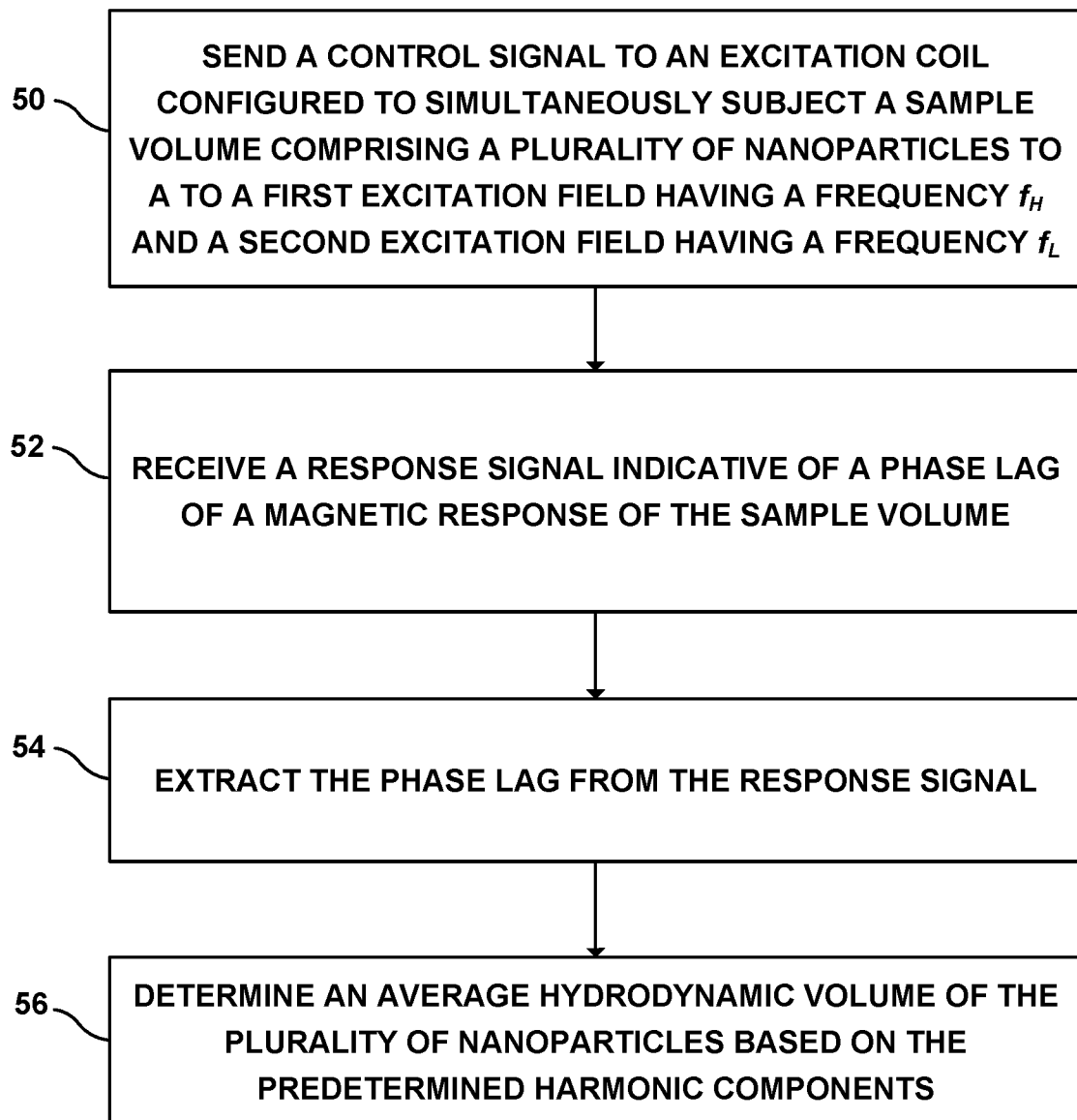


FIG. 4

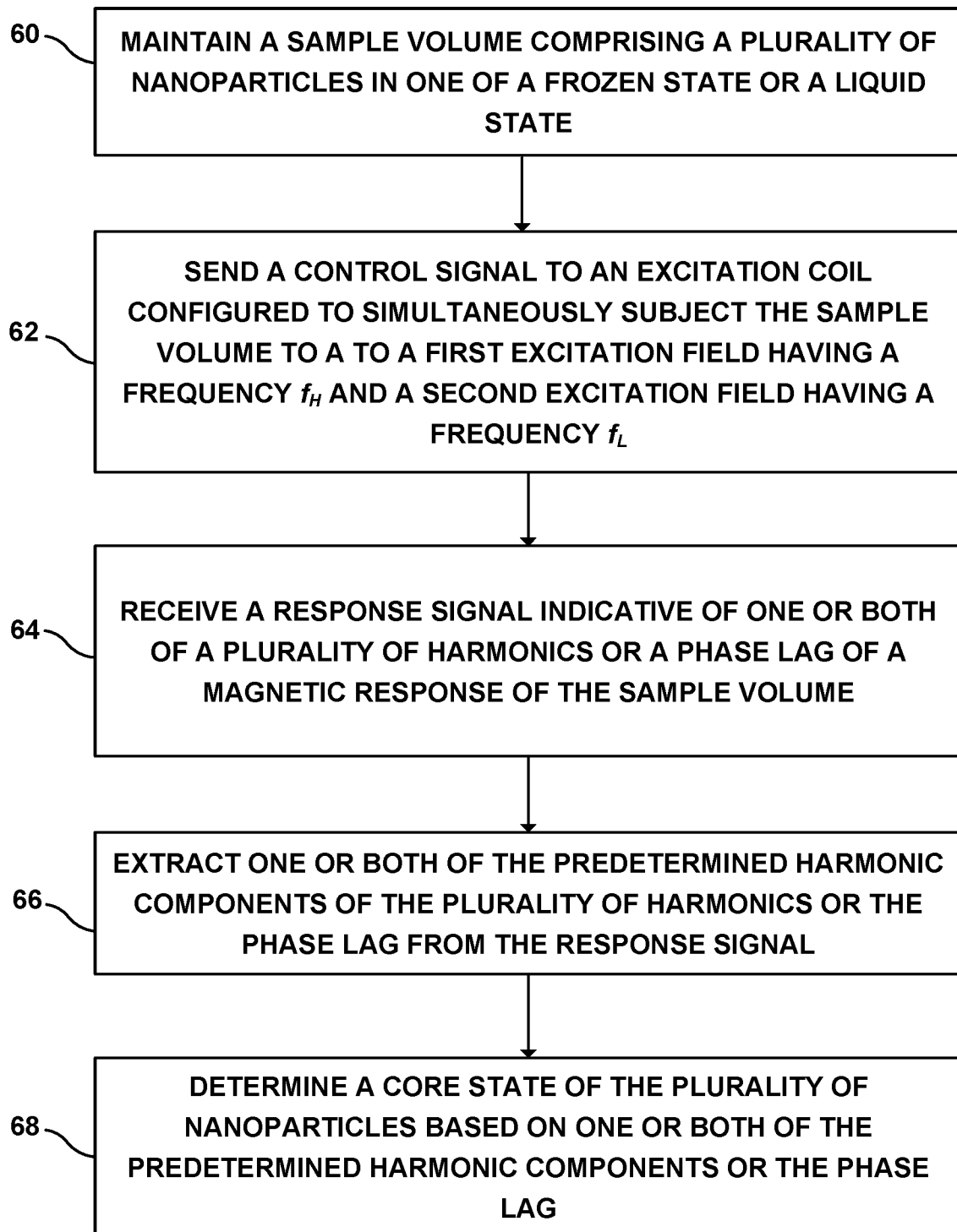


FIG. 5

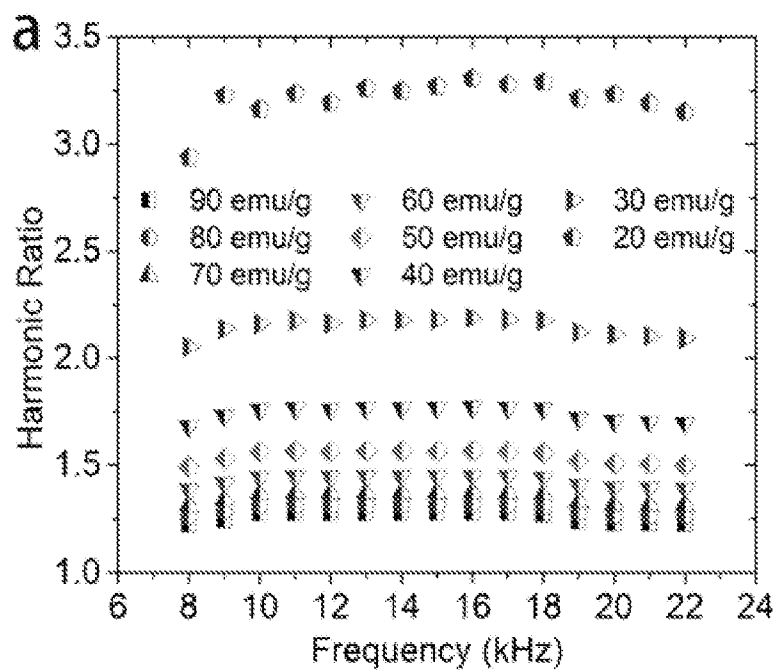


FIG. 6A

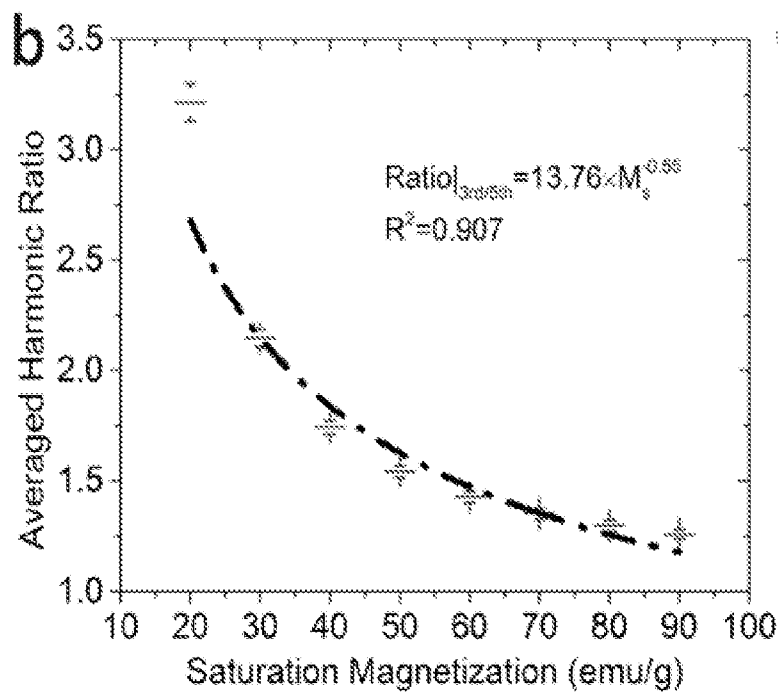


FIG. 6B

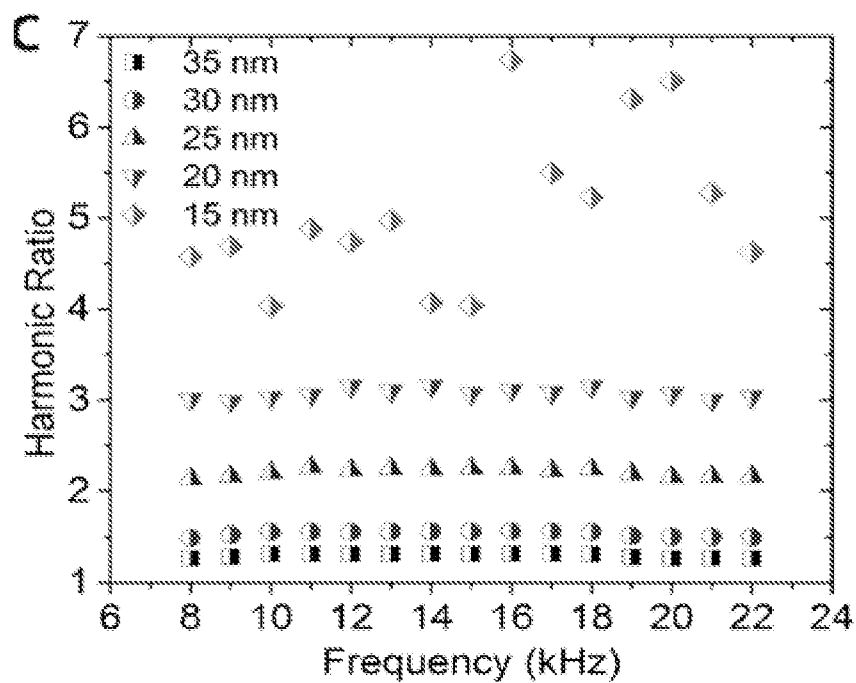


FIG. 6C

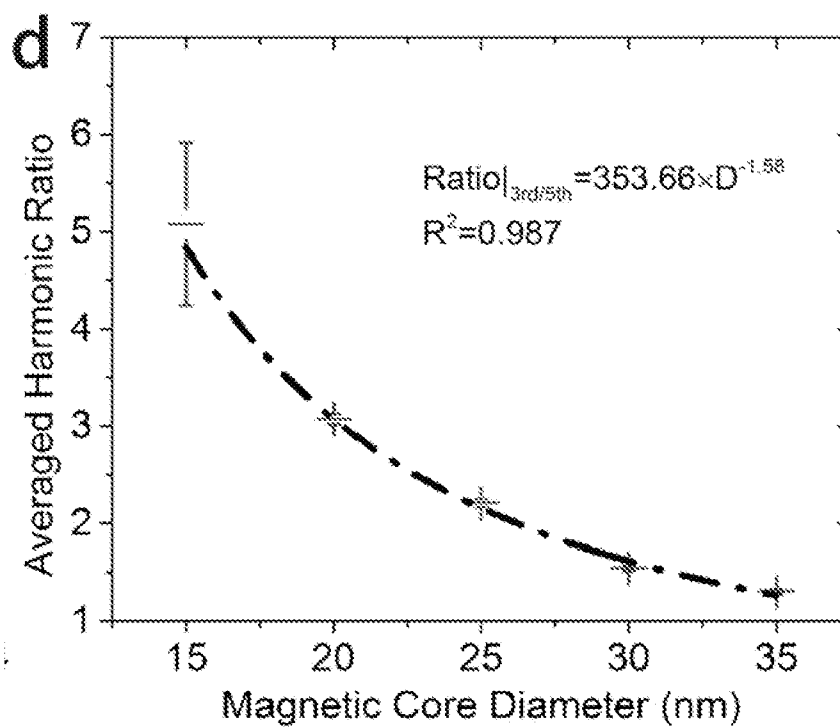


FIG. 6D

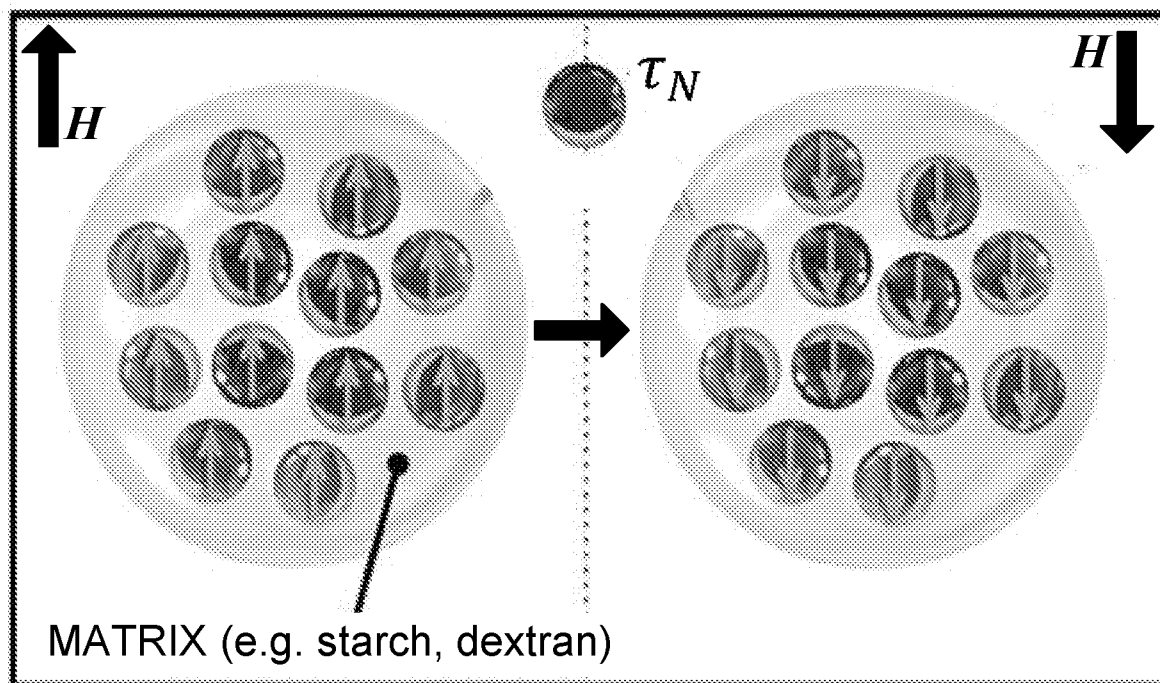


FIG. 7A

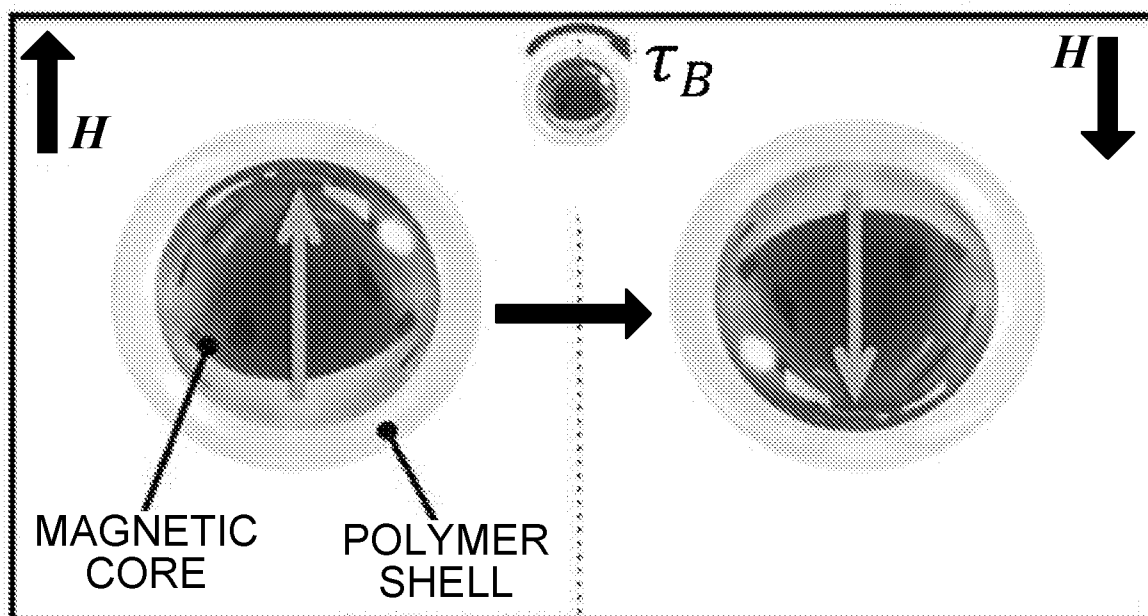


FIG. 7B

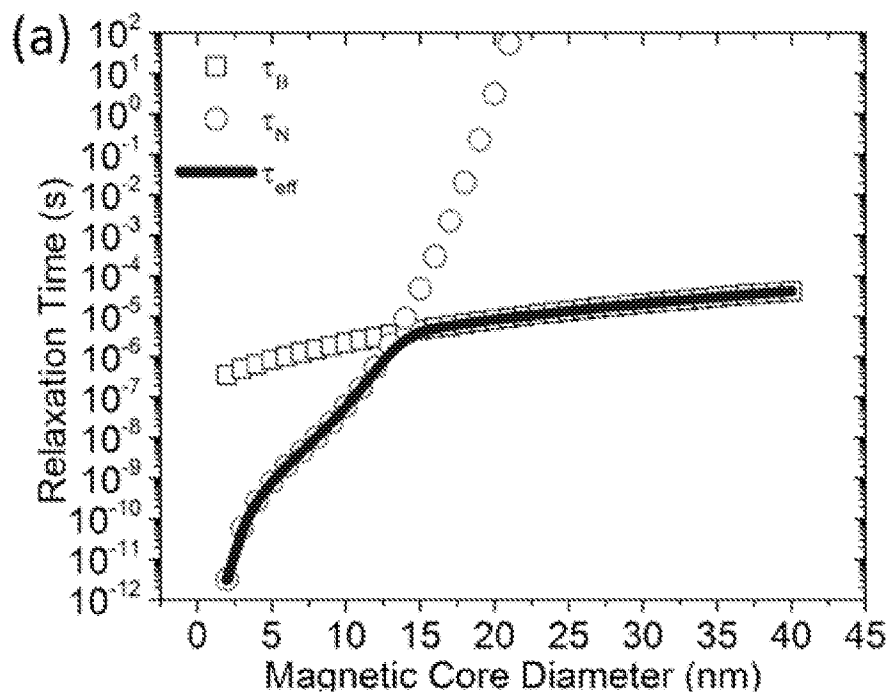


FIG. 8A

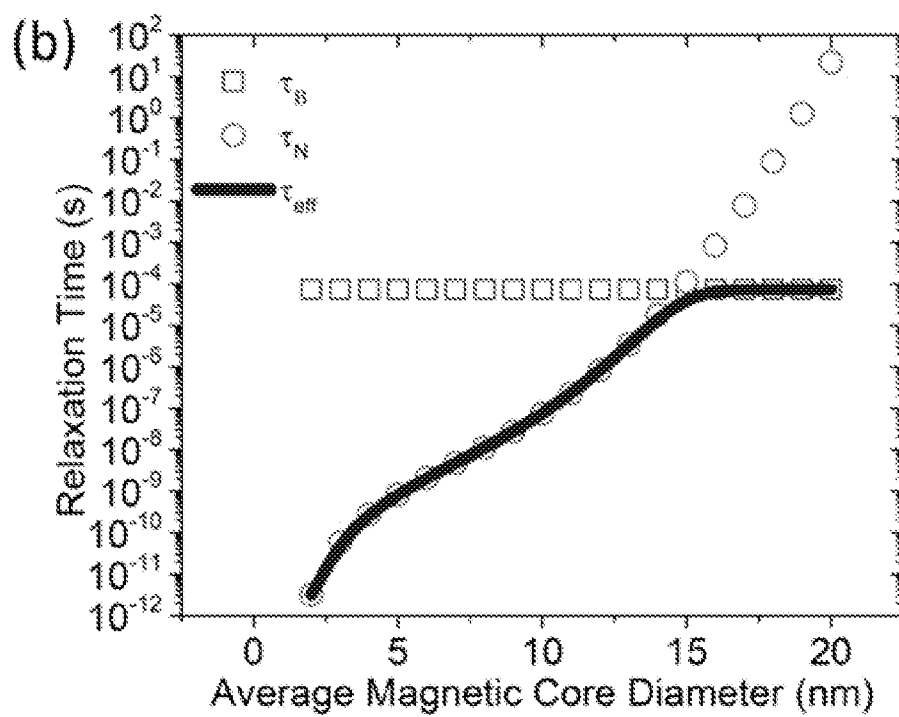


FIG. 8B



FIG. 9B

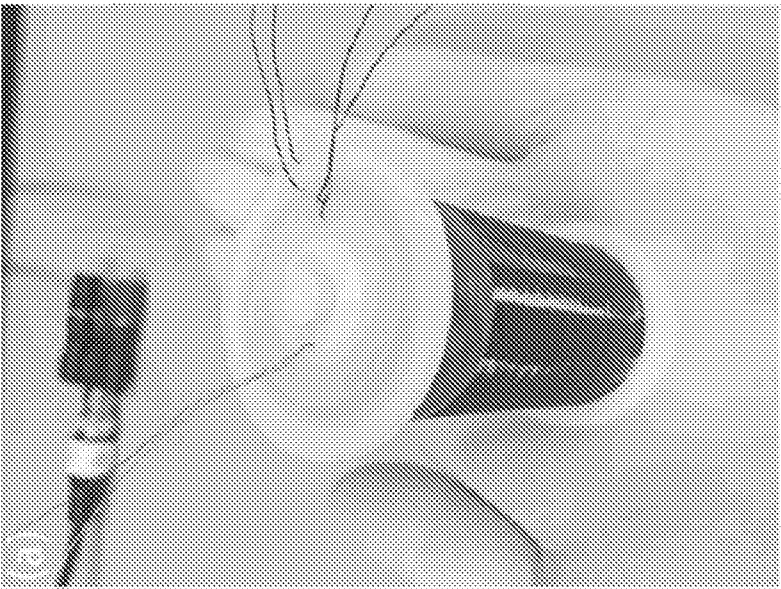


FIG. 9A

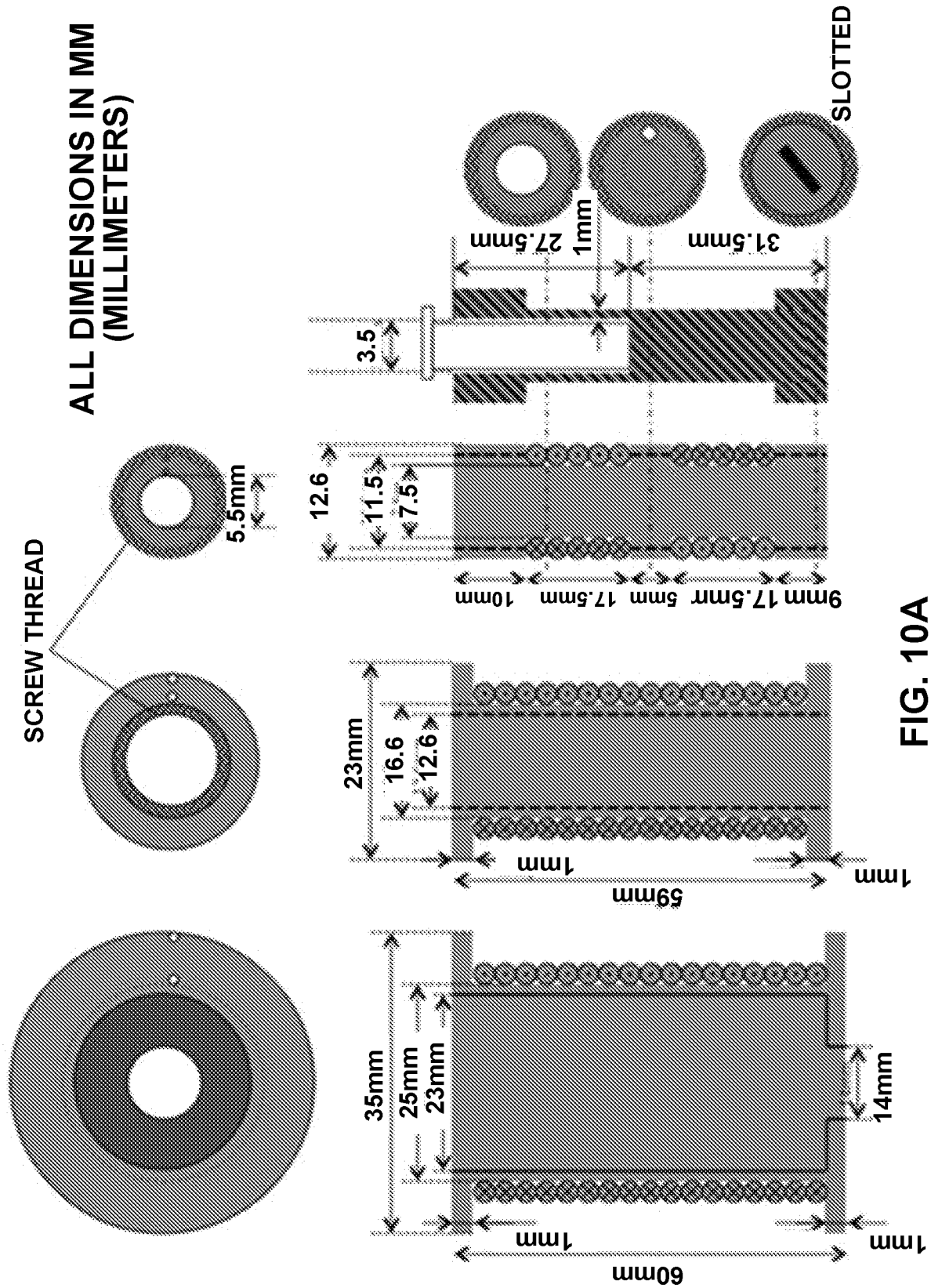


FIG. 10A

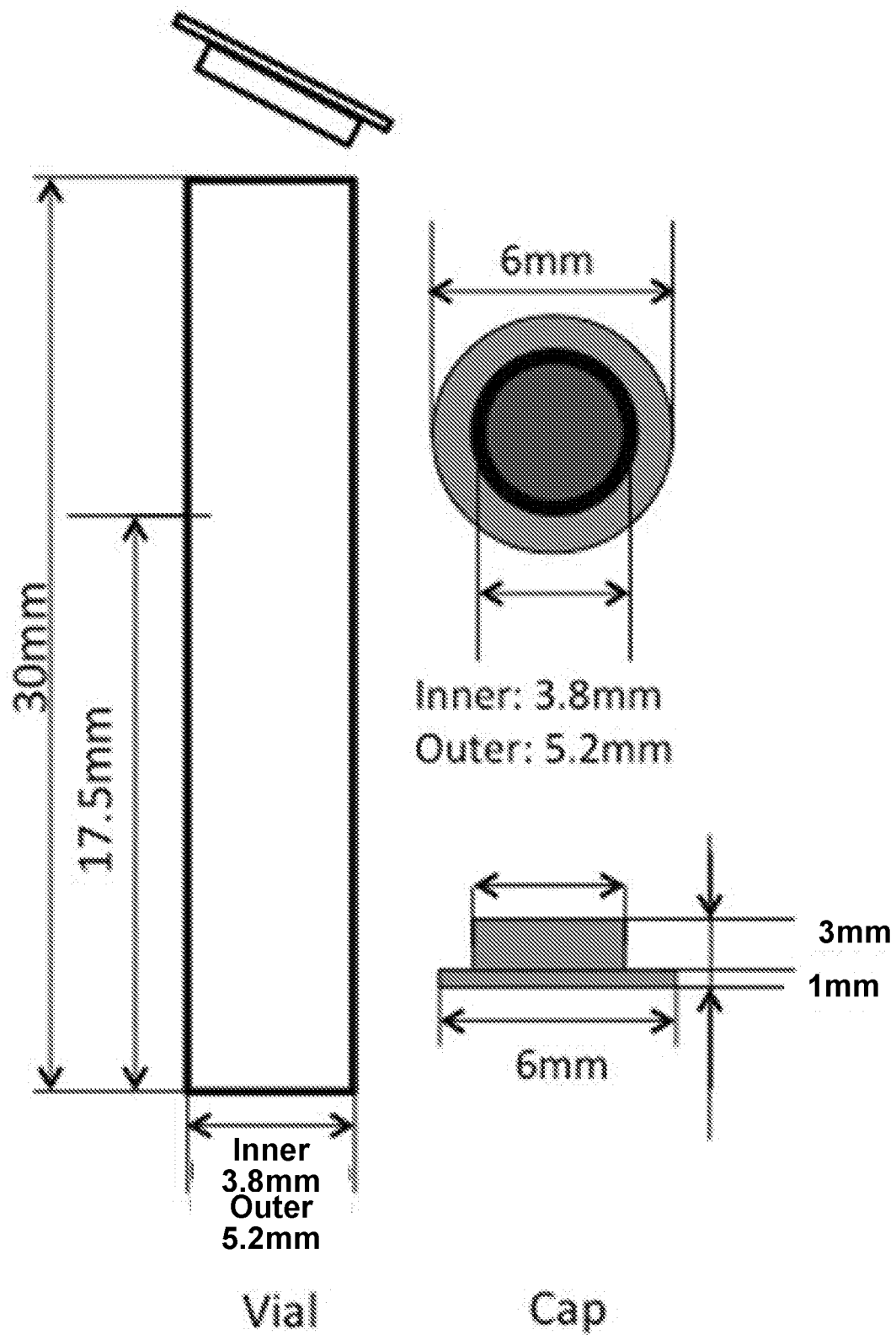
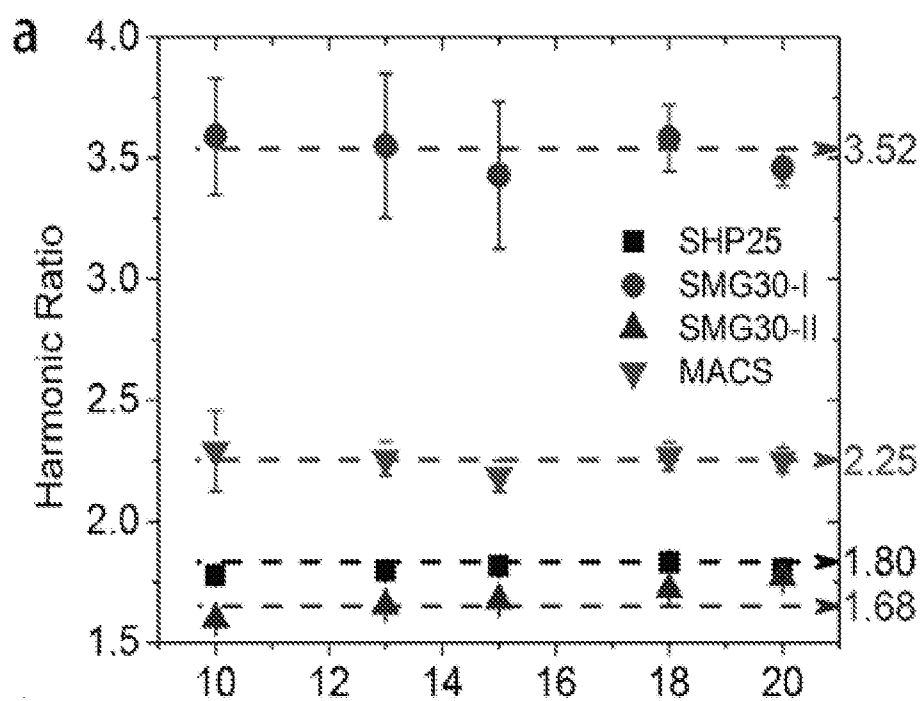
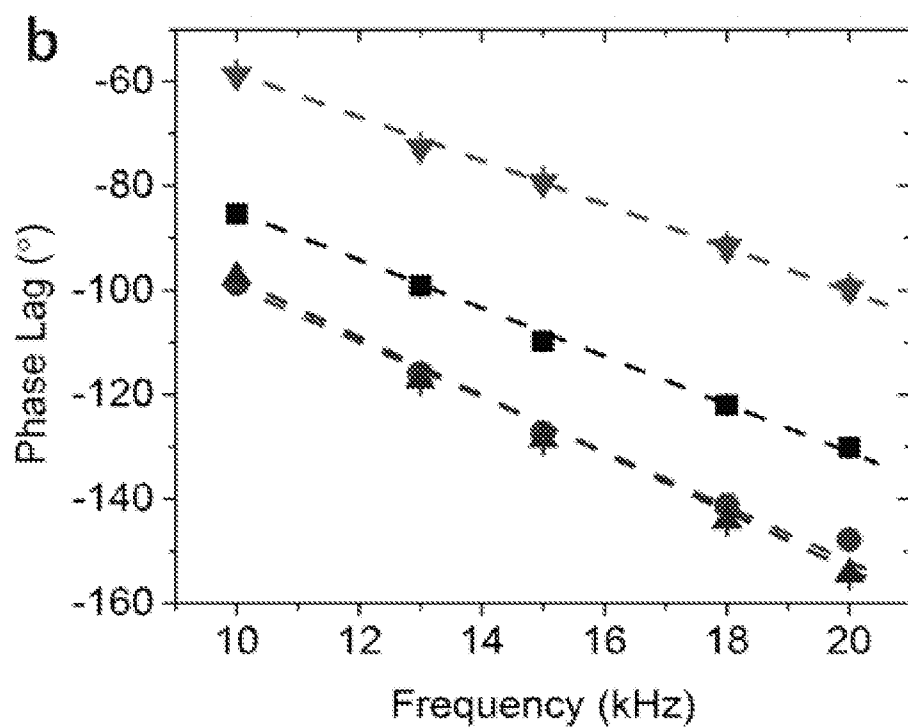


FIG. 10B

**FIG. 11A****FIG. 11B**

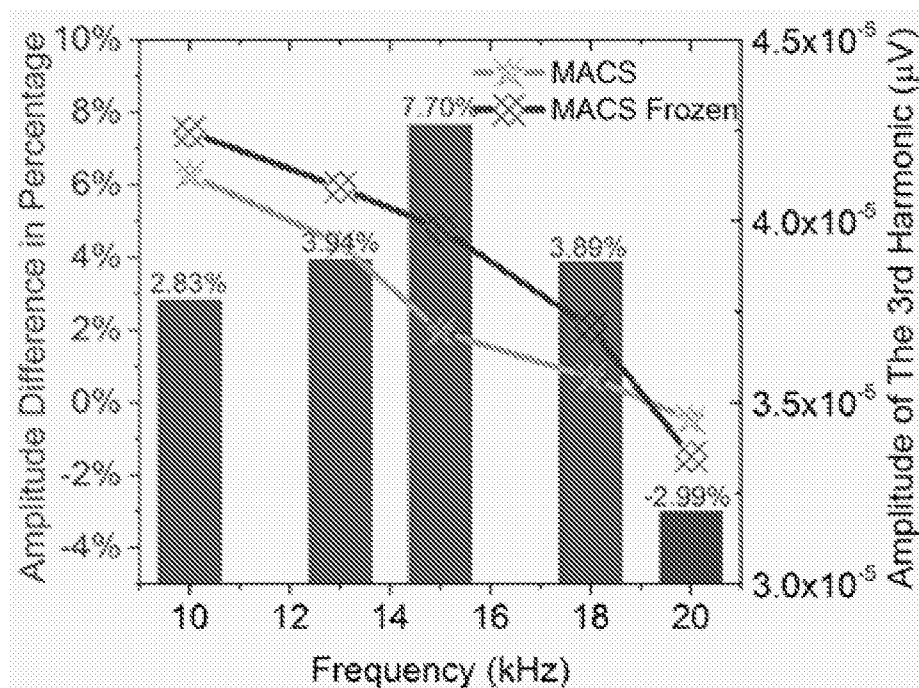


FIG. 12A

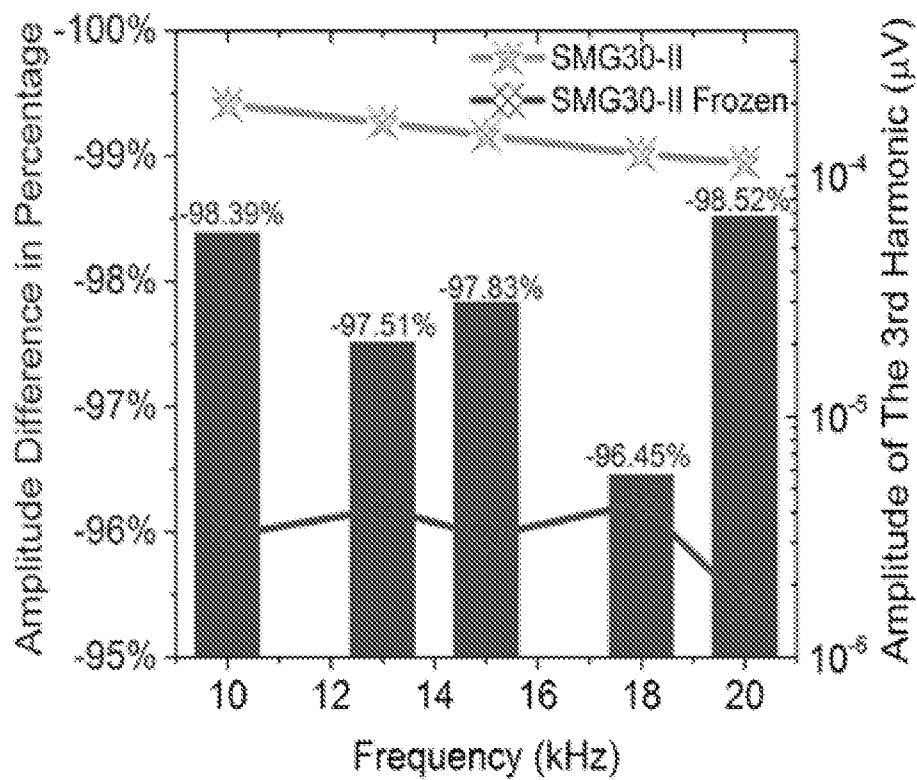


FIG. 12B

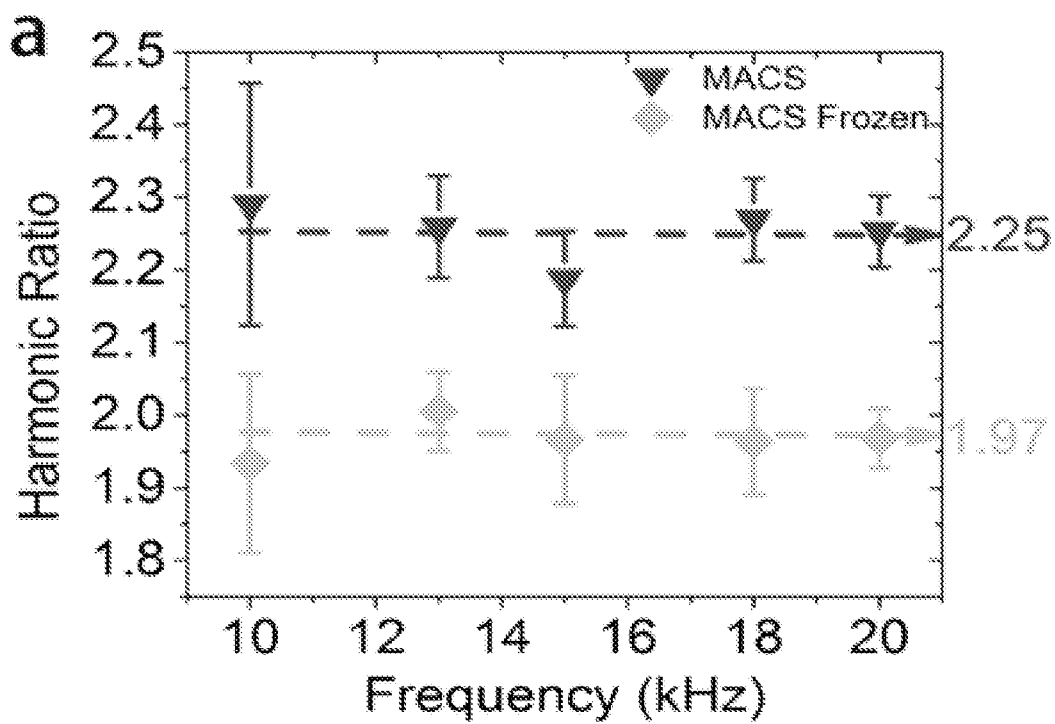


FIG. 13A

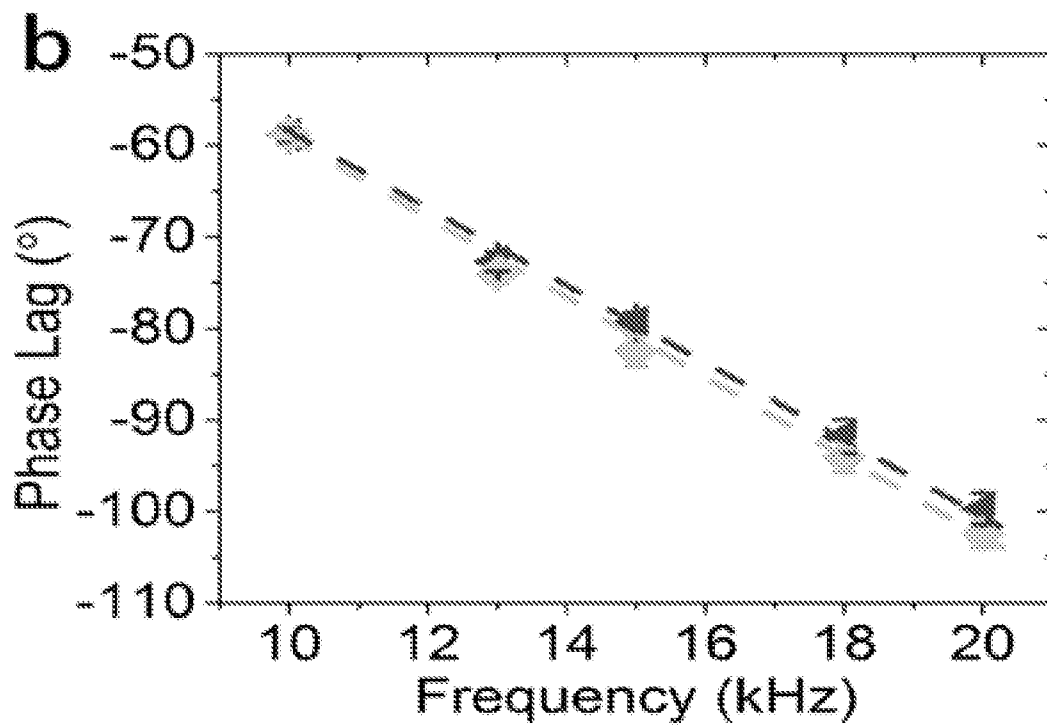


FIG. 13B

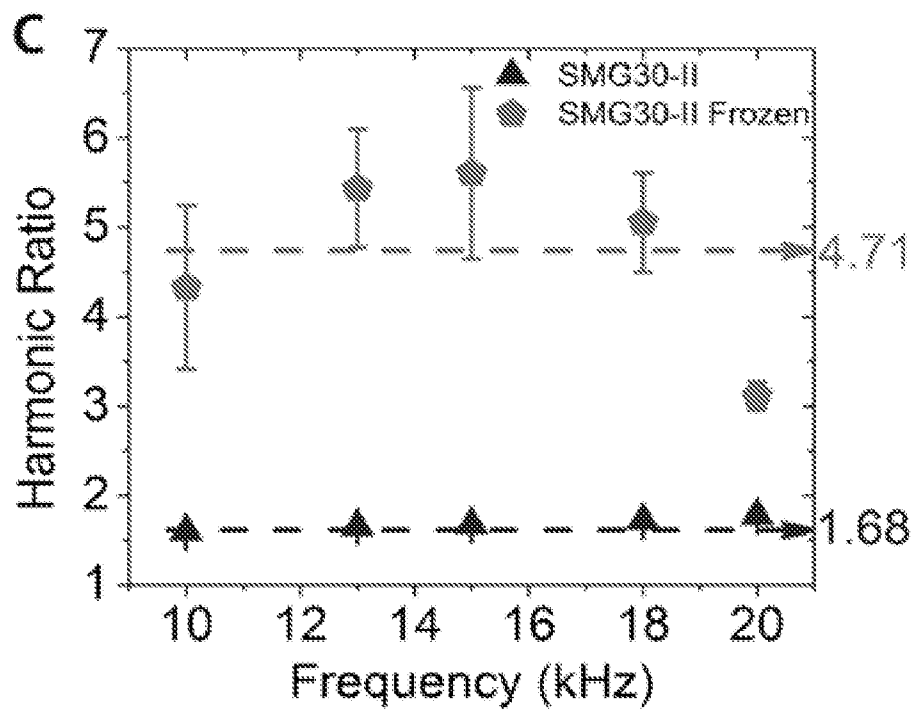


FIG. 13C

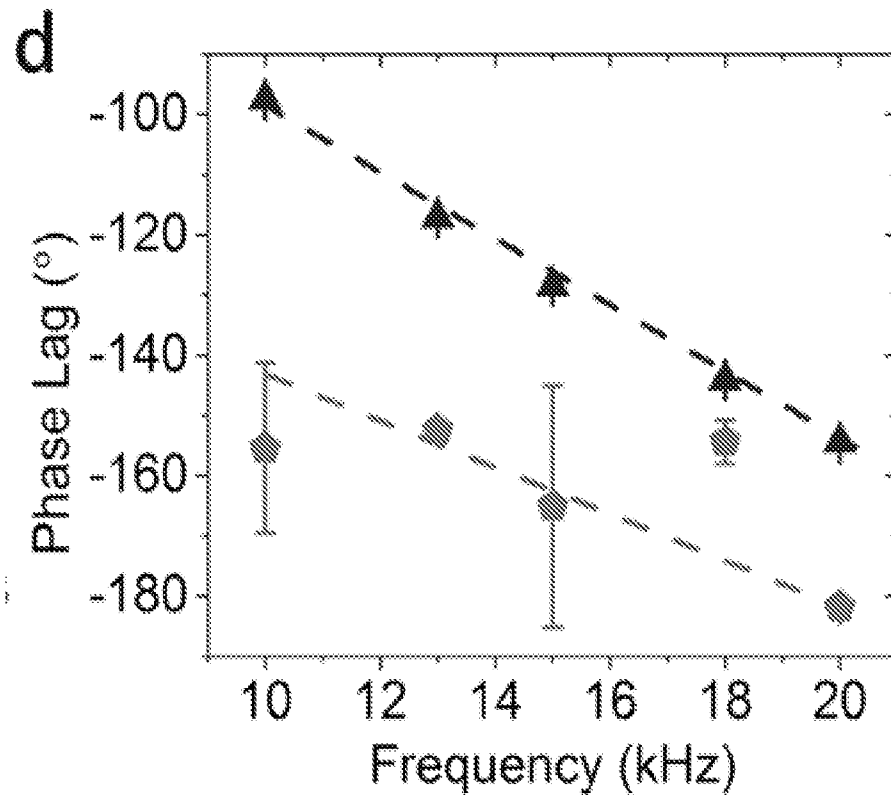
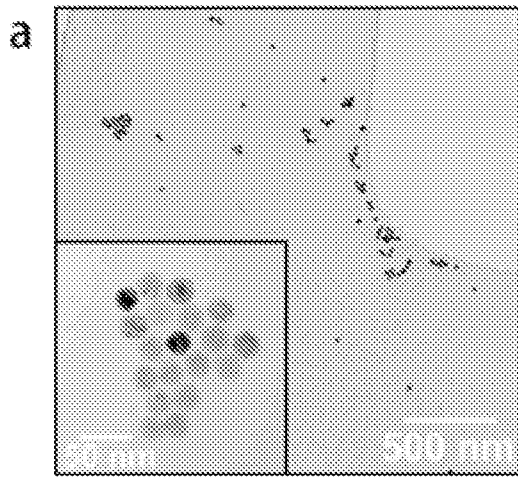
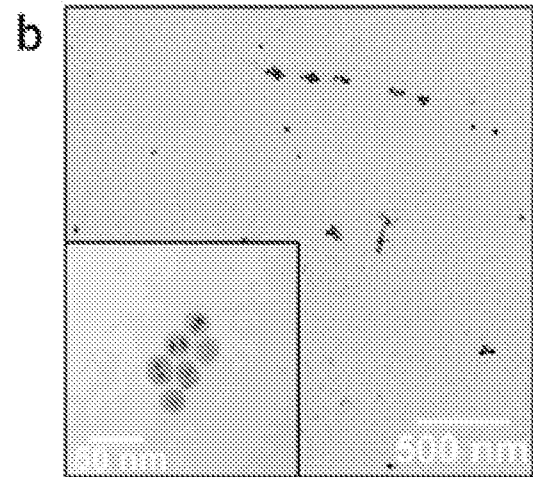
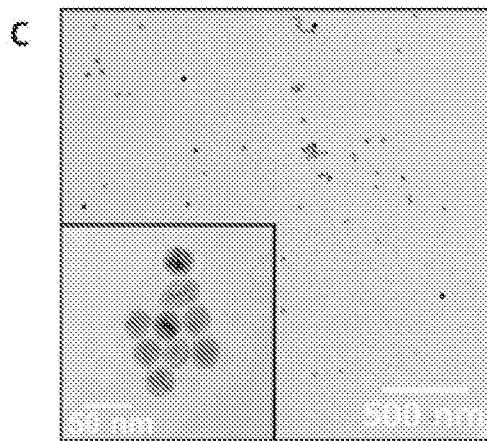
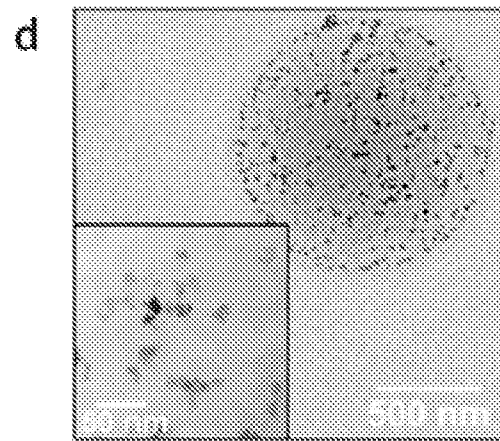


FIG. 13D

**FIG. 14A****FIG. 14B****FIG. 14C****FIG. 14D**

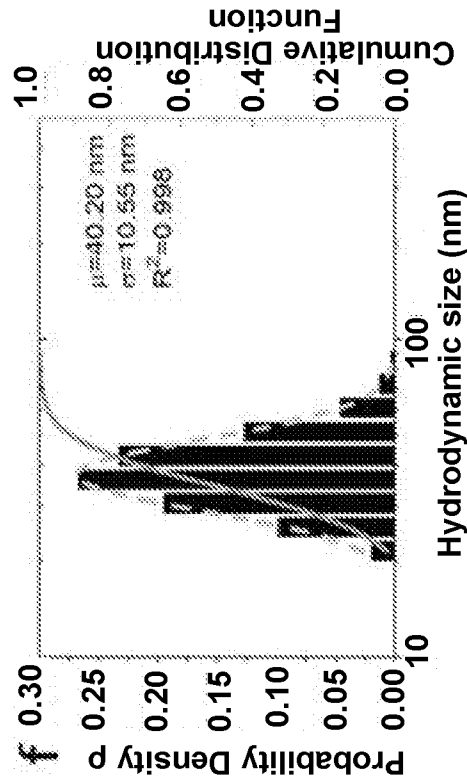


FIG. 15B

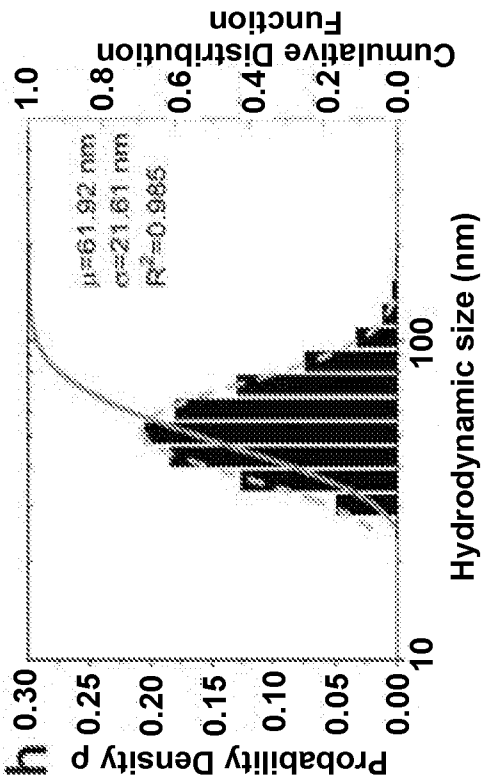


FIG. 15D

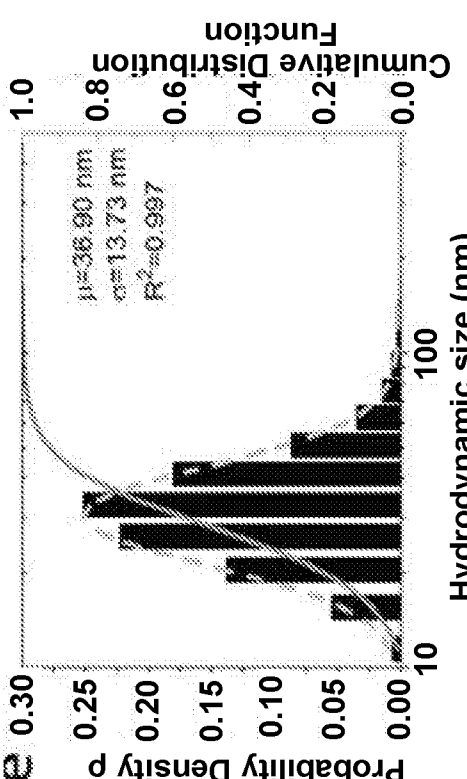


FIG. 15A

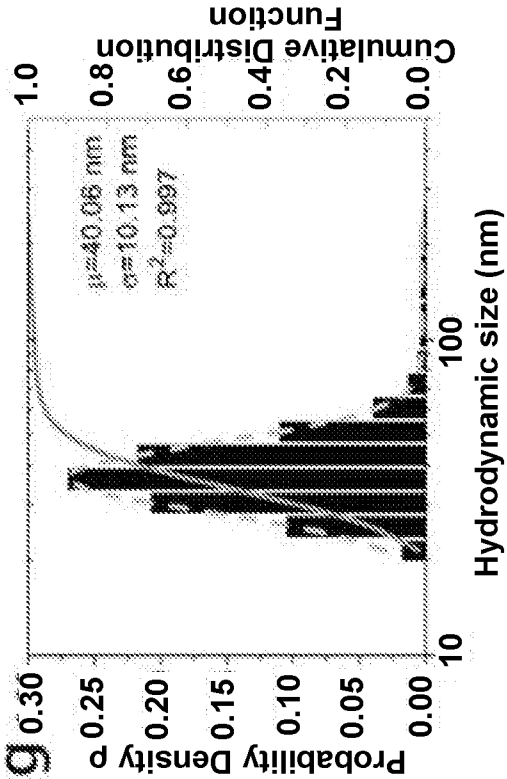


FIG. 15C

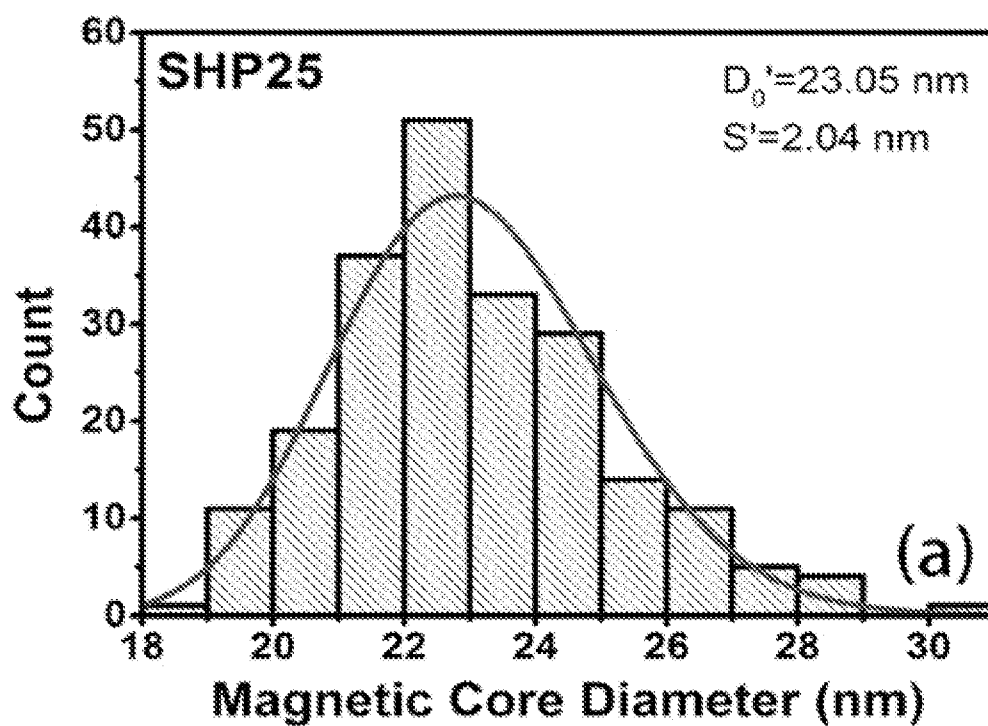


FIG. 16A

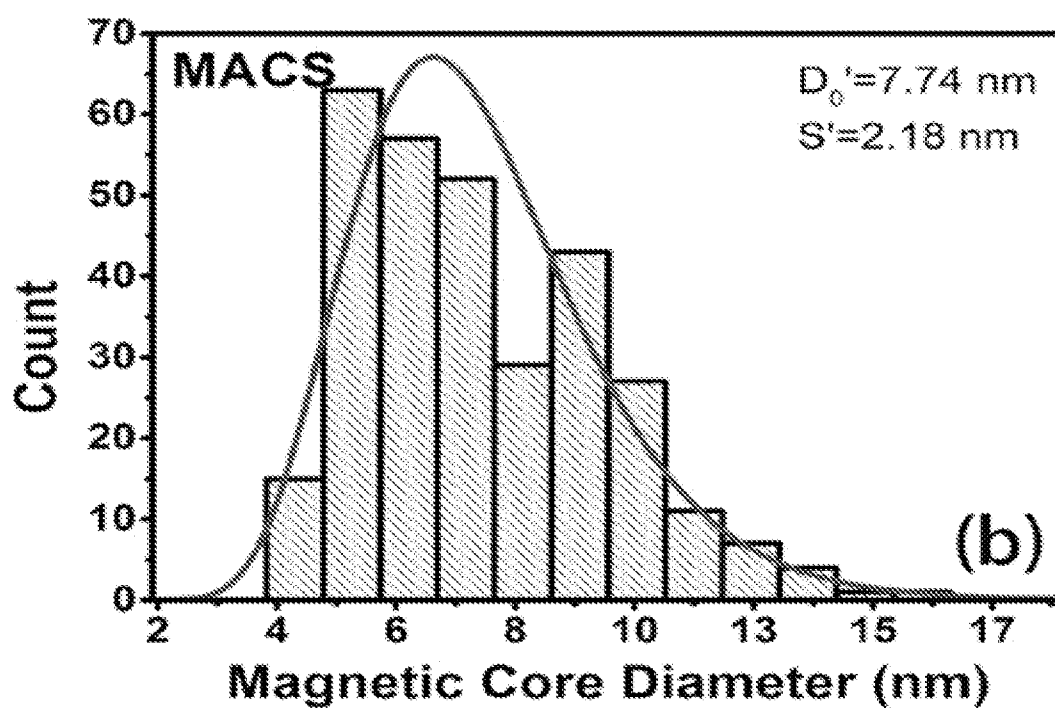


FIG. 16B

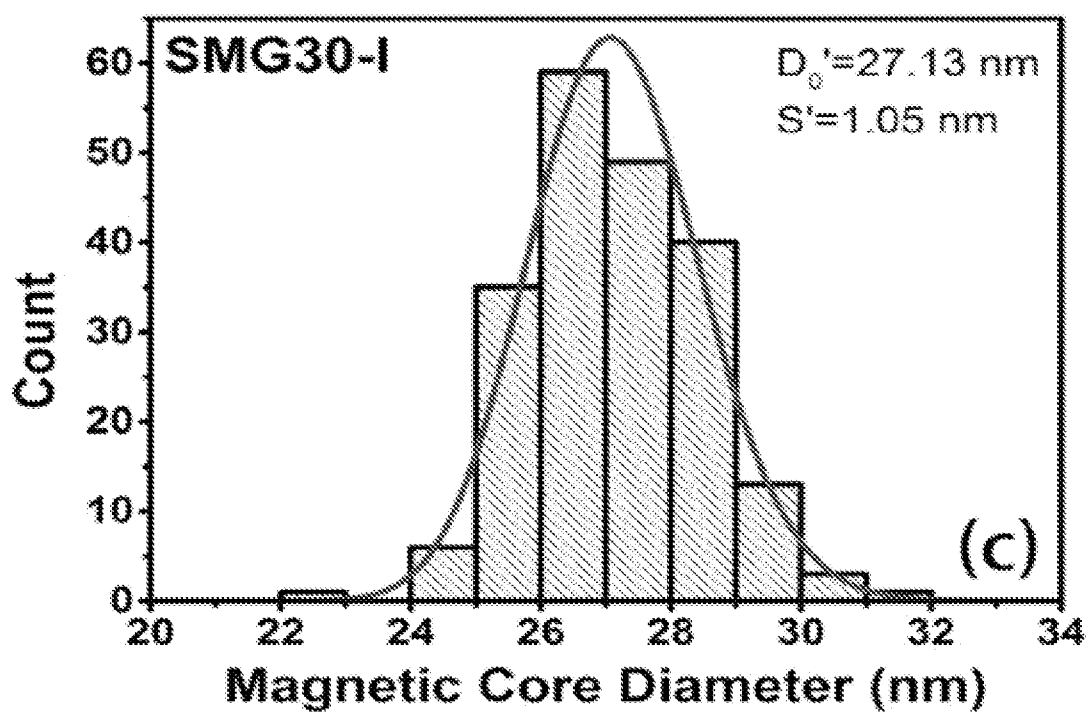


FIG. 16C

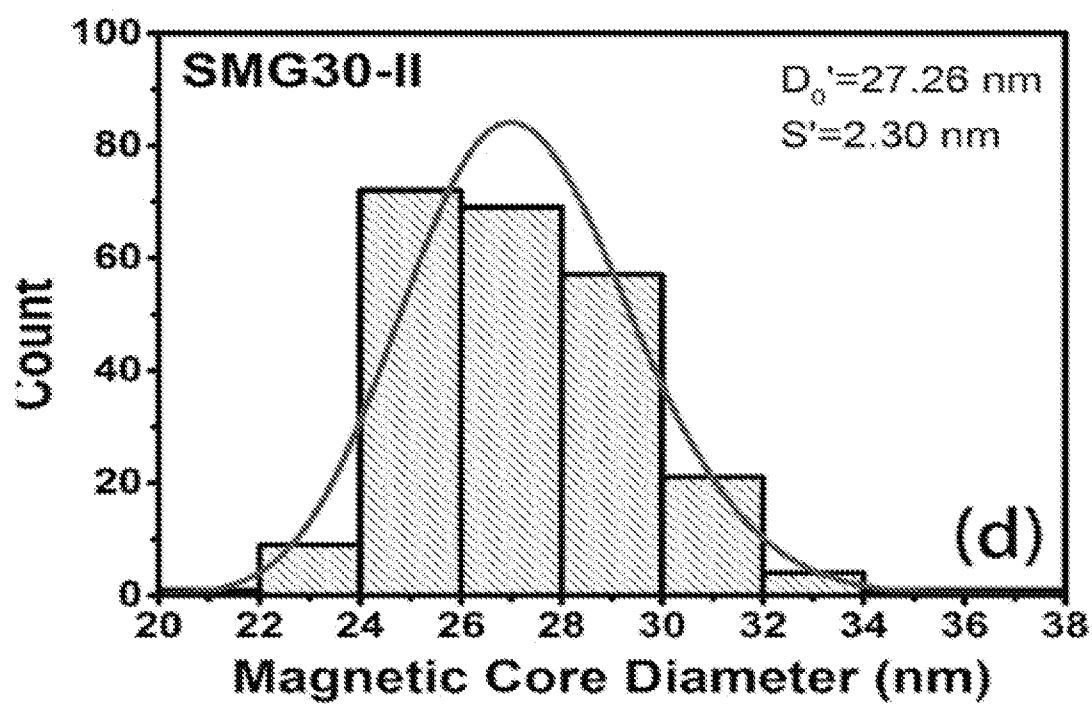


FIG. 16D

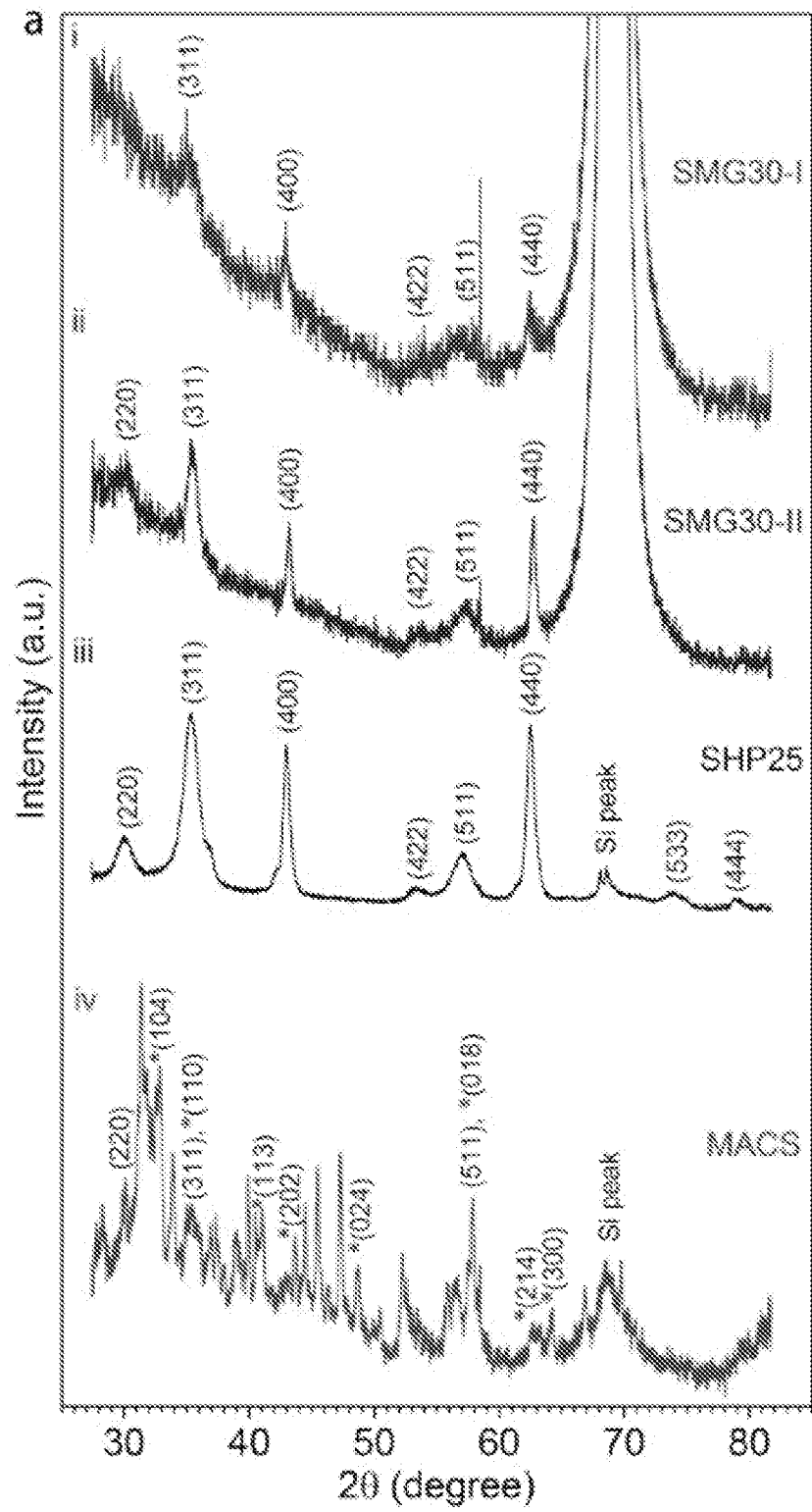


FIG. 17A

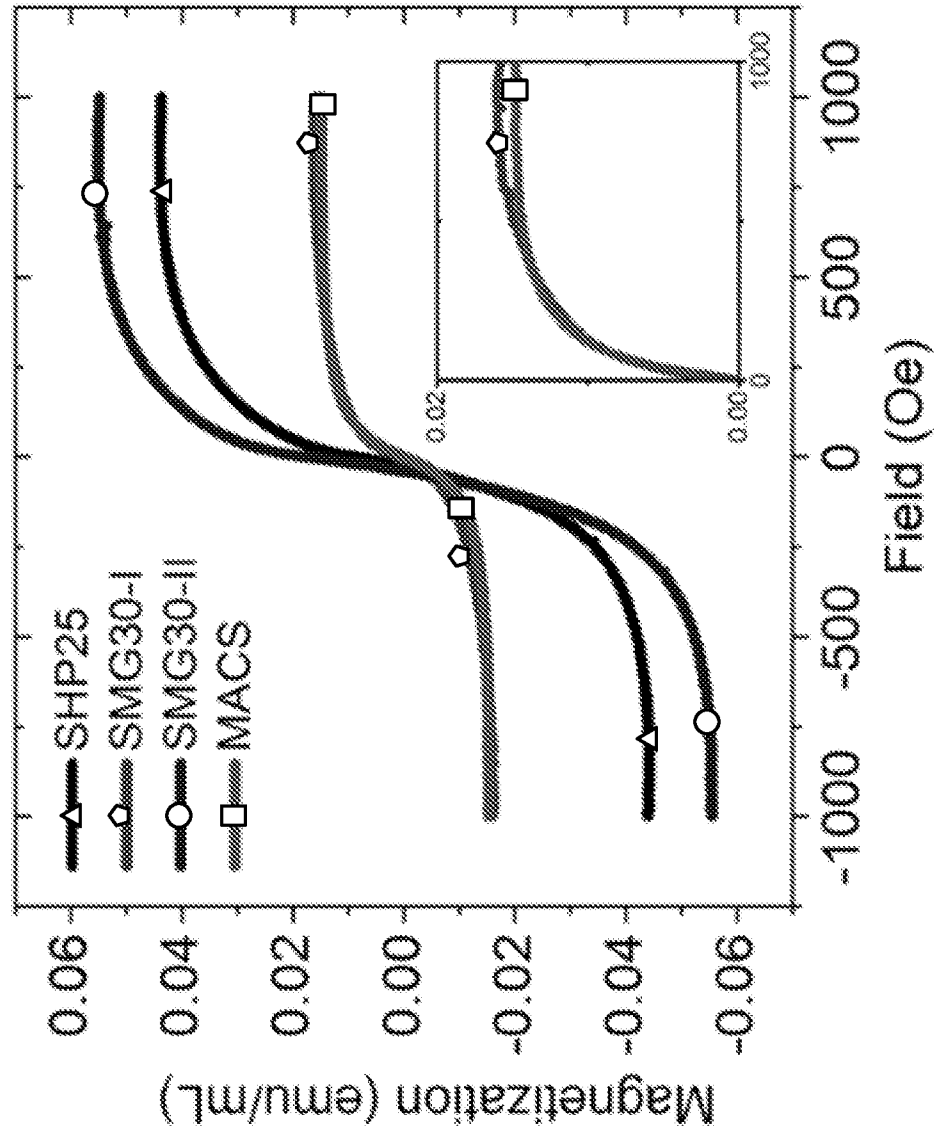


FIG. 17B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/016233**A. CLASSIFICATION OF SUBJECT MATTER****H01F 1/00(2006.01)i, H01F 27/28(2006.01)i, H01F 27/40(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

H01F 1/00; A61B 5/05; A61B 5/055; G01N 27/72; G01R 33/385; G01R 33/12; A61B 5/06; H01F 27/28; H01F 27/40

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: magnetic nanoparticle, coil, frequency, harmonics, excitation, detection

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014-0097829 A1 (WANG et al.) 10 April 2014	1, 5, 11, 15, 17-19
Y	See paragraphs [0032]-[0056]; claims 1-3, 8, 14; and figures 1-5.	, 25-27, 29-31, 42-44
Y	US 2011-0098558 A1 (WEAVER et al.) 28 April 2011	2-4, 12-14, 32-34
	See paragraph [0053]; and figure 3.	, 45-47
A	WO 2006-035359 A2 (PHILIPS INTELLECTUAL PROPERTY & STANDARDS GMBH et al.)	1-5, 11-15, 17-19
	06 April 2006	, 25-27, 29-34, 42-47
	See claims 1-12; and figures 1-11.	
A	US 2015-0015247 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA)	1-5, 11-15, 17-19
	15 January 2015	, 25-27, 29-34, 42-47
	See claims 30-58; and figures 1a-1c, 2a-2c, 3a-3c, 4-5, 6a-6b.	
A	US 2014-0320132 A1 (KONINKLIJKE PHILIPS N.V.) 30 October 2014	1-5, 11-15, 17-19
	See claims 1-15; and figures 1-3, 4A-4B.	, 25-27, 29-34, 42-47



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 May 2018 (11.05.2018)

Date of mailing of the international search report

11 May 2018 (11.05.2018)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/016233

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 7, 9, 21, 23, 38, 40
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 7, 9, 21, 23, 38 and 40 are regarded to be not clear because they refer to claims 6, 8, 20, 22, 37 and 39 respectively, which do not comply with PCT Rule 6.4(a).

3. ☒ Claims Nos.: 6, 8, 10, 16, 20, 22, 24, 28, 35-37, 39, 41, 48-50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2018/016233

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2018/016233

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		IN 4518CHN2014 A	11/09/2015
		JP 2015-502213 A	22/01/2015
		JP 6106184 B2	29/03/2017
		MX 2014006380 A	09/07/2014
		US 9759789 B2	12/09/2017
		WO 2013-080145 A1	06/06/2013