Published in final edited form as:

J Magn Magn Mater. 2015 March 15; 378: 267–277. doi:10.1016/j.jmmm.2014.11.049.

Nonlinear Susceptibility Magnitude Imaging of Magnetic Nanoparticles

Bradley W. Ficko*, Paolo Giacometti, and Solomon G. Diamond

Thayer School of Engineering at Dartmouth, Hanover, New Hampshire, USA

Abstract

This study demonstrates a method for improving the resolution of susceptibility magnitude imaging (SMI) using spatial information that arises from the nonlinear magnetization characteristics of magnetic nanoparticles (mNPs). In this proof-of-concept study of nonlinear SMI, a pair of drive coils and several permanent magnets generate applied magnetic fields and a coil is used as a magnetic field sensor. Sinusoidal alternating current (AC) in the drive coils results in linear mNP magnetization responses at primary frequencies, and nonlinear responses at harmonic frequencies and intermodulation frequencies. The spatial information content of the nonlinear responses is evaluated by reconstructing tomographic images with sequentially increasing voxel counts using the combined linear and nonlinear data. Using the linear data alone it is not possible to accurately reconstruct more than 2 voxels with a pair of drive coils and a single sensor. However, nonlinear SMI is found to accurately reconstruct 12 voxels ($R^2 = 0.99$, $CNR = 84.9$) using the same physical configuration. Several time-multiplexing methods are then explored to determine if additional spatial information can be obtained by varying the amplitude, phase and frequency of the applied magnetic fields from the two drive coils. Asynchronous phase modulation, amplitude modulation, intermodulation phase modulation, and frequency modulation all resulted in accurate reconstruction of 6 voxels ($R^2 > 0.9$) indicating that time multiplexing is a valid approach to further increase the resolution of nonlinear SMI. The spatial information content of nonlinear mNP responses and the potential for resolution enhancement with time multiplexing demonstrate the concept and advantages of nonlinear SMI.

1. Introduction

The use of magnetic nanoparticles (mNPs) in medicine is an active area of research with several promising therapies currently under study [1, 2]. One of the most promising uses of mNPs in medicine is as an imaging contrast agent. Prior research by several groups has resulted in the development of methods such as magnetic relaxometry (MRX) [3–11] and AC susceptibility detection [12–17]. Due to the nonlinear magnetic properties of nanoparticles, an entire field has developed around the detection and imaging of harmonic

© 2014 Elsevier B.V. All rights reserved.

*Corresponding author: Bradley.W.Ficko@Dartmouth.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

fields that arise when mNPs magnetically saturate in strong applied magnetic fields [18, 19]. The idea of magnetic particle imaging (MPI) based on harmonic fields was first demonstrated in 2005 [20]. Various important achievements have been reported since that time including three dimensional real-time imaging [21], the use of intermodulation to create harmonics at frequencies other than multiples of the primary field [22, 23], x-space based imaging [24, 25], inclusion of a direct current (DC) field component to create even harmonics [26, 27], improvements in the magnetic characteristics of mNPs [28], the use of field free lines [29–31], a one-sided MPI system [32], an MPI system without a field free point [33], an alternative field free point [34], use in medical applications [21, 35] and a model-based imaging approach [36].

We previously introduced the susceptibility magnitude imaging (SMI) method that achieves mNP imaging with an array of drive coils, fluxgate magnetometers, and compensation coils using linear magnetic susceptibility measurements [37]. SMI scans localize and quantify mNPs with known alternating current (AC) magnetic susceptibility properties within the imaging zone of the system. In the present study we propose a method that uses the nonlinear magnetization response characteristics of mNPs to gain additional spatial information for SMI in a method we call nonlinear SMI. The additional spatial information results from the nonlinear responses of the mNPs in the imaging zone to applied DC and AC magnetic field gradients. Unlike SMI where spatial information is derived from the primary AC frequency, nonlinear SMI also derives spatial information from higher order frequencies that arise due to the magnetic saturation of the mNPs and this additional spatial information leads to higher imaging resolution. Figure 1 illustrates the concept of nonlinear mNP response characteristics that are used in nonlinear SMI. Measuring the nonlinear mNP response is very similar to SMI except that the induced magnetic field measurements are no longer sinusoidal in nature due to the magnetic saturation characteristics of mNPs. The non-sinusoidal response can be quantified by the assessment of the harmonics in the magnetic field measurements.

Magnetic susceptibility imaging relies on the inherent magnetic susceptibility of mNPs to provide imaging contrast. When a magnetically susceptible material is subjected to an external magnetic field $\mathbf{H}$, the resulting magnetic field will be $\mathbf{B} = \mu_0 \mathbf{H} + \mathbf{M}$, where μ_0 is the magnetic permeability in a vacuum, $\mathbf{B}$ is the magnetic induction or B-field, and $\mathbf{M}$ is the magnetization field from the magnetic material. The magnetization field arises from the magnetically susceptible material as $\mathbf{M} = \mathbf{H}\chi_v$, where χ_v is the volume magnetic susceptibility. In an AC magnetic field, the susceptibility will be frequency dependent and have an in-phase component χ'_v and an out-of-phase component χ''_v . For multidomain mNPs in solution, like the ones we used in the present study, AC susceptibility can be modeled as Brownian relaxation [37, 38].

Although the M-field only exists inside of the magnetic material, it gives rise to an additional external B-field that contributes to the magnetic field detected by a sensor. In magnetic saturation methods, the applied magnetic field becomes strong enough that the magnetization resulting from the applied magnetic field is no longer linear. The nonlinear magnetization $\mathbf{M}$ as a function of $\mathbf{H}$ is typically modeled with a Langevin function

$$Lx = \coth x - \frac{1}{x}. \quad (1)$$

A Taylor series expansion of the Langevin function can be used to approximate the magnetization in the nonlinear saturation regime

$$Lx = \frac{1}{3}x - \frac{1}{45}x^3 + \dots \quad (2)$$

The applied magnetic field can then be substituted into the Taylor series to model the susceptibility behavior at AC frequencies. The Taylor series expansion of the Langevin function model of susceptibility has been studied previously [39–44].

The nonlinear AC susceptibility response of mNPs will vary spatially if there are magnetic field strength differences throughout an imaging volume. Several MPI related studies have modeled these effects [36, 45] but not for the purpose of gaining additional spatial encoding information for tomographic imaging. We demonstrate in the present study that by exploiting these spatial variations in nonlinear AC susceptibility it is possible to obtain higher resolution images without the need for a field free point.

In this study we propose a method for using spatial variations in the nonlinear magnetization responses of mNPs to increase the number of voxels that can be tomographically resolved in SMI. The spatial variations arise from applying both DC and AC magnetic field gradients across the imaging zone to cause spatial variations in the nonlinear susceptibility response of mNPs. We first demonstrate how to measure the nonlinear magnetization responses at harmonic frequencies from a single drive coil to improve SMI. We then demonstrate how intermodulation, resulting from two magnetic fields at different frequencies, can also be used to improve SMI. Combining these two effects we demonstrate additional improvement in SMI resolution. Next, we explore five time-multiplexing methods to determine if the nonlinear SMI imaging resolution can be further improved without changing the hardware configuration. These methods rely on two drive coils being used together to alter the spatial patterns of the magnetic field phase, amplitude or frequency over time. The first time-multiplexing method shifts the phase of the current in one of the drive coils while keeping the phase of the other drive coil constant. The second method scales the amplitude of the current in one or both drive coils. The third method shifts the phase of the current at two different frequencies in one of the drive coils while the phase of the other drive coil remains constant. The fourth method synchronously shifts the phase in both drive coils. Finally, the fifth method varies the frequency of the AC current in the drive coils. These time-multiplexing methods rely on the nonlinear magnetization of mNPs and could potentially be used to further improve the spatial resolution of nonlinear SMI.

2. Methods

2.1 Analog and Digital Systems

The experimental setup had two drive coils and a sense coil as depicted in Figure 2. The drive coils (Jantzen-1257, 0.3 mm diameter wire, 7 mH, 11.8 Ω at DC, 15 mm inner diameter $\times$ 15 mm height $\times$ 26 mm outer diameter, Jantzen, Praestoe, Denmark) were arranged orthogonally to the sense coil (Jantzen-1257) and provide a magnetic gradient field across the imaging zone. All three coils were positioned around an imaging grid with a 20 mm separation between the drive coils and a 2.5 mm separation between the sense coil and the edge of the drive coils. A 3D printed grid was used to place mNP samples in 12 locations (3×4), spaced 5 mm apart. Much of the hardware was previously described [37], this references describes the link between analog and digital processes and the workflow for generating AC currents, measurement and post-processing of acquired magnetization responses. In order to produce higher magnetic fields, LME 49720 (Texas Instruments, Dallas, TX, USA) and LME 49610 (Texas Instruments, Dallas, TX, USA) amplifiers were placed in series to create low noise, high power currents. The current in the coils was between 150 – 200 mA depending on the testing frequency, and produced magnetic fields of approximately 5 mT in the center of a drive coil. A DC magnetic field gradient was also added by positioning 8 neodymium permanent magnets in plane with the drive coils 50 mm from the back of the imaging grid, and one permanent magnet perpendicular to the others to break up the magnetic symmetry in the imaging grid.

2.2 Nanoparticles

Testing was performed using Fe_3O_4 starch coated mNPs with a hydrodynamic diameter of 100 nm (10-00-102, micromod Partikeltechnologie GmbH, Rostock, Germany). Two samples of these mNPs were prepared. The first was a full concentration sample (25 mg Fe/ml) and the second sample was diluted to 12.5 mg Fe/ml. In each case 0.5 ml was measured out into a 0.5 ml Eppendorf tube. The magnetic properties of these mNPs have been previously characterized by [46, 47].

2.3 Model and Imaging

A simplified model is developed here to show the mathematical principles of nonlinear SMI. An illustration of this simplified model of nonlinear SMI is shown in Figure 3. In this model, it is assumed that the imaging system contains one drive coil, one sense coil, and two imaging voxels. As illustrated in Figure 3, the interactions of the applied field gradient with the nonlinear magnetization of the mNPs gives rise to spatially varying harmonic amplitudes that can be used to recover spatial information in SMI. Due to the applied magnetic field gradient, the spectral content of each voxel is unique and will provide spatial information that is linearly dependent on the concentration of mNPs at that location. Additionally, each resolvable spectral component will add new information about the spatial location of the mNPs and therefore the achievable resolution will increase as more spectral content is observed.

We have previously introduced the modeling of AC magnetic fields of the form

$$H_{DV_n} = G_{DV_n} A'' \sin(\omega_0 t + \phi_0) + A' \cos(\omega_0 t + \phi_0), \quad (3)$$

where G_{DV_n} is a geometric factor between the drive coil and voxel, the A coefficients are user-specified gains, ω_0 is the drive coil frequency, and ϕ_0 is an unknown but constant phase lag from the system hardware [37]. The magnetic field in the n th voxel is

$$B_{DV_n} = \mu_0 (H_{DV_n} + M_{DV_n}), \quad (4)$$

where the magnetization of the voxel from the drive coil field is assumed to follow the Langevin function of equation 1 applied to the mNPs

$$M_{DV_n} = mNP_n m_n \left[\coth \left(\frac{m_n H_{DV_n}}{k_B T} \right) - \frac{k_B T}{m_n H_{DV_n}} \right], \quad (5)$$

where T is the temperature, k_B is Boltzmann's constant, mNP_n is the concentration of mNPs,

and m_n is the magnetic moment of the n th voxel. The magnetic moment is $m_n = \frac{1}{6} \pi D_n^3 M_{sn}$, where D is the diameter and M_{sn} is the magnetic saturation of the mNPs in the n th voxel. A simplified version of the Langevin model may be obtained with a Taylor series expansion (equation 2), which is proportional to the mNP concentration in the voxel and the lumped parameter coefficients β_1 and β_3

$$M_{DV_n} = [mNP]_n \beta_1 H_{DV_n} + \beta_3 H_{DV_n}^3. \quad (6)$$

The magnetic field measured by sensor S due to the magnetization of the n th voxel is

$$B_{DV_n S} = G_{V_n S} V_n \mu_0 M_{DV_n}, \quad (7)$$

where $G_{V_n S}$ is a geometric factor from the voxel to the sensor and V_n is the volume of the voxel. Expanding the sensor measurement expression we obtain

$$B_{DV_n S} = G_{V_n S} V_n \mu_0 [mNP]_n \left\{ \left[\frac{3\beta_3 G_{DV_n}^3}{4} A'^2 A'' + A''^3 + \beta_1 G_{DV_n} A'' \right] \sin \omega t + \phi_0 + \phi_1 + \left[\frac{3\beta_3 G_{DV_n}^3}{4} A' A''^2 + A'^3 + \beta_1 G_{DV_n} A' \right] \cos \omega t + \phi_0 \right\}$$

where ϕ_1 and ϕ_3 are additional unknown but constant phase lags due to sensor electronics and the frequency-dependent complex susceptibility response of the magnetic material.

Using reference sinusoids

$$R_1' = \cos(\omega t), \quad R_1'' = \sin(\omega t), \quad R_3' = \cos(3\omega t), \quad R_3'' = \sin(3\omega t), \quad (9)$$

multiplying the sensor measurement by each of the references and taking a time average, we obtain the expected values

$$\langle B_{DV_nS} R'_1 \rangle = G_{V_nS} V_n \mu_0 [mNP]_n \left\{ \left[\frac{3\beta_3 G_{DV_n}^3}{4} A'^2 A'' + A''^3 + \beta_1 G_{DV_n} A'' \right] \sin \phi_0 + \phi_1 + \left[\frac{3\beta_3 G_{DV_n}^3}{4} A' A''^2 + A'^3 + \beta_1 G_{DV_n} A' \right] \cos \phi_0 + \phi_1 \right\} \quad (10)$$

$$\langle B_{DV_nS} R''_1 \rangle = G_{V_nS} V_n \mu_0 [mNP]_n \left\{ \left[\frac{3\beta_3 G_{DV_n}^3}{4} A'^2 A'' + A''^3 + \beta_1 G_{DV_n} A'' \right] \cos \phi_0 + \phi_1 - \left[\frac{3\beta_3 G_{DV_n}^3}{4} A' A''^2 + A'^3 + \beta_1 G_{DV_n} A' \right] \sin \phi_0 + \phi_1 \right\} \quad (11)$$

$$\langle B_{DV_nS} R'_3 \rangle = G_{V_nS} V_n \mu_0 [mNP]_n \left\{ \frac{3\beta_3 G_{DV_n}^3}{4} A'^2 A'' - A''^3 \sin \phi_0 + \phi_3 + \frac{3\beta_3 G_{DV_n}^3}{4} A'^3 - A' A''^2 \cos \phi_0 + \phi_3 \right\}, \quad (12)$$

$$\langle B_{DV_nS} R''_3 \rangle = G_{V_nS} V_n \mu_0 [mNP]_n \left\{ \frac{3\beta_3 G_{DV_n}^3}{4} A'^2 A'' - A''^3 \cos \phi_0 + \phi_3 - \frac{3\beta_3 G_{DV_n}^3}{4} A'^3 - A' A''^2 \sin \phi_0 + \phi_3 \right\}. \quad (13)$$

Assuming we have only 2 voxels and one harmonic response, these equations can be arranged into a matrix form

$$\begin{bmatrix} \langle B_{DV_nS} R'_1 \rangle \\ \langle B_{DV_nS} R''_1 \rangle \\ \langle B_{DV_nS} R'_3 \rangle \\ \langle B_{DV_nS} R''_3 \rangle \end{bmatrix} = \mu_0 V_n \begin{bmatrix} G_{V_1S} K'_{1V_1} & G_{V_2S} K'_{1V_2} \\ G_{V_1S} K''_{1V_1} & G_{V_2S} K''_{1V_2} \\ G_{V_1S} K'_{3V_1} & G_{V_2S} K'_{3V_2} \\ G_{V_1S} K''_{3V_1} & G_{V_2S} K''_{3V_2} \end{bmatrix} \begin{bmatrix} [mNP]_1 \\ [mNP]_2 \end{bmatrix} \quad (14)$$

where the K' constants represent all of the terms inside the curly brackets of the in-phase amplitudes of equations 10 and 12 and the K'' constants represent all of the terms inside the curly brackets of the out-of-phase amplitudes of equations 11 and 13. Equation 14 allows for the simultaneous best fit solution of the mNP concentrations in voxels 1 and 2 from the measurements made by a single drive coil and single sensor as illustrated qualitatively in Figure 3. The inverse method that we applied throughout the present study was the Moore-Penrose pseudo-inverse method without any regularization, which corresponds to a best fit by minimizing the sum of squared residuals.

The forward model given above can be obtained empirically from a calibration data set without any prior knowledge of the mNP properties [37]. This approach to nonlinear SMI can also be extended to the case where intermodulation is created or when a DC magnetic field is applied. Some of the mathematical modeling for intermodulation has been developed in a previous study exploring the use of intermodulation for MPI [45].

The model shown in Equation 14 can be expanded to include additional terms from higher order harmonics and intermodulation frequencies. To recover the concentrations of mNPs in nonlinear SMI, an inverse method such as the pseudo-inverse can be applied to the entire system matrix.

2.4 Data Processing

The data processing and imaging workflow have been previously detailed for SMI [37]. Nonlinear SMI uses the same processing principles except that in the present study, the data processing is simplified by the use of fewer coils, fewer sensors and the absence of compensation coils. However, due to the need for harmonic and intermodulation frequency measurements, additional data was collected in the present study at frequencies other than the primary frequencies. A lock-in amplifier measurement was used to process these harmonic and intermodulation components just as was done with the primary frequencies. The lock-in amplifier measurements were performed digitally. A reference waveform for the lock-in amplification was digitized at the primary frequency and raised to a sufficient power to create a harmonic reference at the appropriate frequency. The digitally created reference waveform was then mixed with the data stream to compute the in-phase and out-of-phase components.

2.5 Experimental Setup

To demonstrate nonlinear SMI using the additional spatial information obtained from nonlinear interactions between the applied magnetic field and mNPs, we conducted an experiment using a 3×4 voxel imaging grid (Figure 2). Several data analysis approaches were then used to demonstrate spatial encoding using each of the nonlinear effects listed in the introduction. Initially, a calibration was performed by placing the 25 mg Fe/ml mNP sample into each of the 12-voxel locations for 15 s using a frequency of 325 Hz in the first drive coil and 440 Hz in the second drive coil. A calibration matrix was constructed according to equation 14 and as detailed in our previous report on SMI [37]. Once the calibration was complete, the 25 mg Fe/ml sample was re-tested in each of the 12-voxel locations for 15 s. Nonlinear SMI was first performed using only data at the primary frequency and harmonics of drive coil 1, then using data at the primary frequency and harmonics of drive coil 2, and then using data at the primary frequencies of drive coils 1 and 2 and all intermodulation frequencies. This resulted in separate nonlinear SMI reconstructions for each of these three conditions. In each image, the number of voxels reconstructed was sequentially increased from 1 to 12 and coefficients of determination R^2 values were computed. In these images, the voxel sizes are calibrated to be the same size as the Eppendorf tube in which the mNP samples are located. If the nonlinear signals do not contain additional spatial information then the images are expected to have high R^2 values for 2 voxel images and then to decay in quality rapidly. High R^2 values for 3 or more voxels indicates the presence of additional spatial information in the nonlinear signals. In the presence of additional spatial information, we expect that the number of imaging voxels to be proportional to the number of harmonic or intermodulation frequencies contained in the reconstruction. For instance, with 6 harmonic frequencies we expect six additional voxels in reconstruction. As was observed during testing, in-phase and out-of-phase data can contribute different spatial information and the number of expected voxels will increase further. A final step was to combine all the harmonic and intermodulation data together to produce a 12-voxel nonlinear SMI. In addition to computing a coefficient of determination, an estimate of contrast-to-noise (CNR) was also obtained. CNR was determined by dividing the average of the reconstructed voxel intensities on the diagonal (sample locations) by the standard deviation of all of the off-diagonal locations (noise).

To demonstrate multiple-sample tomographic imaging, nonlinear SMI was performed with the 25 mg Fe/ml and 12.5 mg/ml nMP samples located simultaneously in the imaging grid. The two samples were placed in the grid and then one sample was moved to a new location every 20 s. Due to the size of the Eppendorf tubes, it was not possible to place the two samples in immediately adjacent positions. All nonlinear SMIs were reconstructed using a noise-weighted non-negative least squares (lsqnonneg) function in Matlab (The MathWorks Inc., Natick, MA, USA). This non-negative least squares function is effective for image reconstruction even with ill-posed problems as occurred during our testing where some images were reconstructed without sufficient observational data to match the number of imaging voxels.

During testing it was noted that there was a difference between the spatial information contained in the in-phase and the out-of-phase imaging data that was suggested from the modeling development in Section 2.3. This led to an investigation into time multiplexing methods that could lead to additional spatial information beyond the use of harmonic and intermodulation frequencies for nonlinear SMI. A control condition and five time-multiplexing methods were tested. Previous testing treated each drive coil independently; however these methods used both drive coils together such that a spatial variation in phase, amplitude or frequency could be created over time. Due to the nonlinear magnetization of mNPs, the variations in phase, amplitude and frequency of the drive coils create patterns that potentially produce different nonlinear magnetization at each test condition and contribute new spatial information for image reconstruction.

The time-multiplexing methods are illustrated in Figure 4 and were: A. Control condition, B. asynchronous phase modulation, C. amplitude modulation, D. synchronous phase modulation, E. intermodulation phase modulation, and F. frequency modulation. In each method, one parameter was varied over four conditions and the data from each condition was aggregated to reconstruct nonlinear SMI. The control method A used the full AC current amplitude and a 0° phase shift in each coil for each of the four conditions. In method B, the left coil was driven with a 0° phase shift while the right coil was given a phase shift of 90° per condition. In method C, the amplitude of the right and left coils were varied to be either 100% or 75% of the maximum amplitude. In method D, the phase of the left and the right drive coil were shifted synchronously by 90° per condition. In method E, intermodulation was created by using two frequencies in each coil. The left coil was given a constant phase while the phase of the frequencies in the right coil was shifted by 90° per condition. Finally in method F, the frequency of the left and right coil was changed in each condition. In tests A-D, the frequency in each drive coil was 325 Hz, while in test E the frequency in the left coil was 325 Hz while the frequency in the right coil was 440 Hz and in test F the frequency stepped through 210 Hz, 325 Hz, 440 Hz and 620 Hz.

Testing of the time-multiplexing methods was performed by first creating a calibration data set by placing the 25 mg Fe/ml sample in each of the first six voxel locations for 11 s and then testing under the same conditions. Once the calibration and test was completed, the next condition was applied and the same procedure repeated. In each method, only the 3rd harmonic frequency or the first intermodulation frequency was used for SMI reconstruction. This data reduction allowed for a smaller test grid to be used. In each case, eight

experiments were run. The first experiment was a calibration of the new condition followed by an experimental test. Once the data was collected for that condition, the next condition was run. After all conditions were completed, the data was aggregated such that a calibration matrix could be built and an experimental matrix could also be built. Using the aggregated data, nonlinear SMI was performed and the results presented as a series of reconstructions to determine the additional spatial information gained through the variation of different input conditions.

3. Results

A demonstration of the use of nonlinear mNP magnetization to add spatial information to SMI is shown in Figure 5 through Figure 12. In these figures, a voxel index is shown on the y-axis and an image is created at each x-axis position. The sample was moved through the 3×4 voxel grid at regular time intervals and images were reconstructed at each sample position. Perfect image reconstruction would appear as a 1 in the sample position and 0 values in the other voxels. In each case the sample was moved sequentially from voxel 1 to voxel 2, to voxel 3 and so on. In Figure 5 the background subtracted amplitude of the mNP magnetization response is shown for the primary frequency and harmonics 2 – 6. This amplitude was computed with a digital lock-in amplifier at a downsampled rate of 2 Hz. The mNP sample was moved through 12 positions in the imaging grid at periodic intervals starting at voxel 1 and the magnetization response changes for each harmonic depending on the location of the mNP sample as predicted by the modeling of Section 2.3.

In Figure 6, the primary frequency and all of the harmonics measured from drive coil 1 were used to reconstruct between 1 and 12 voxel images. For coil 1, the primary frequency along with harmonics 2–6 were used in the reconstructions. The voxel positions in the imaging grid are shown in Figure 2. The R^2 values are shown above each image. In conventional linear SMI, only a single voxel can be imaged using one drive coil and one sense coil so any position information above this threshold is evidence that increased spatial information is available from the harmonics that arise from the nonlinear mNP magnetization response. The primary frequency and all of the harmonics measured from drive coil 2 were also used to reconstruct between 1 and 12 voxel images. For coil 2, the primary frequency along with harmonics 2–5 were used in the reconstructions. The results are similar to Figure 6 and not presented here.

In Figure 7, data at the primary frequencies of drive coils 1 and 2 as well as data at the intermodulation frequencies were used to reconstruct between 1–12 voxel images. The observed intermodulation frequencies were $\omega_1 + \omega_2$, $\omega_1 + 2\omega_2$, $\omega_1 + 3\omega_2$, $\omega_2 + 2\omega_1$ and $\omega_2 + 3\omega_1$. Conventional linear SMI with two drive coils and one sense coil can reconstruct only two voxels so any position information above this threshold is evidence that increased spatial information is available from the intermodulation that arises from the nonlinear mNP response.

During the testing of spatial information in harmonics (Figure 6) and intermodulation (Figure 7), an interesting observation was noted. If only the in-phase or out-of-phase data were used for image reconstruction, the R^2 values would degrade more rapidly. However, if

the in-phase and out-of-phase data were used together, the R^2 values remained high for more voxels than expected. This is illustrated in Figure 8 for the image reconstructions using data at primary frequencies and intermodulation frequencies. In Figure 8, the R^2 values fall rapidly after 6 voxels for in-phase and out-of-phase image reconstructions when carried out separately but remain high for all 12 voxels when both measurement sets are used together.

To demonstrate the combined effects of using data at the primary frequencies, harmonic frequencies and intermodulation frequencies on SMI reconstruction, all data were combined to produce a 12 voxel nonlinear SMI (Figure 9). In this case R^2 was 0.996 and the CNR was 84.9.

Nonlinear SMI was conducted with two samples placed simultaneously in the imaging grid (Figure 10) to illustrate that superposition imaging can be performed with this method. In this case one of the samples was full concentration mNP solution and the other was a half concentration mNP solution. Due to the size of the Eppendorf tubes relative to the grid, it was not possible to place the samples immediately adjacent to one another in the grid. A sample grid is shown at the top of the figure to illustrate where the samples were located at each of the seven locations used in the reconstruction.

The results of a control condition test and five time-multiplexed methods are shown in Figure 11. Between 2 and 6 voxels were reconstructed using only the 3rd harmonic or the first intermodulation frequency of the data. R^2 values are shown for each of the six tests performed. Of these methods, four of the five showed an improvement in R^2 values compared with the control test. The control condition (A) fails with more than 3 voxels rather than 2 voxels (as expected) because of the nonnegative reconstruction constraint. The asynchronous phase shifting method (B) showed the very high R^2 values up to 6 voxels. Amplitude modulation (C) remained above $R^2 = 0.97$ except for 6 voxel reconstruction where it fell to $R^2 = 0.91$. The synchronous phase shifting method (D) performed no better than the control test over the imaging conditions. The asynchronous phase shifting method (E) showed the highest R^2 values up to 6 voxels of all the imaging methods tested. The frequency shifting method (F) showed moderately high R^2 values.

Figure 12, shows a summary of the R^2 values for the control test and the five time-multiplexed methods. Four of the five methods remain above $R^2 > 0.9$ for up to six voxel reconstruction while the synchronous phase shifting method has a poor R^2 value after two voxel reconstruction.

4. Discussion and Conclusion

In this study we demonstrated a method of using the nonlinear magnetization response of mNPs to gain additional spatial information for SMI. We demonstrated how to use the magnetization of mNPs at harmonic frequencies from a single input coil to gain additional spatial information and demonstrated how intermodulation frequencies resulting from two different applied magnetic fields at different AC frequencies can also be used to gain spatial information. We then combined these effects to demonstrate how they can be used together to further improve SMI resolution. Drawing on an observation during this experimentation

on the benefits of combining in-phase and out-of-phase data, we explored five time-multiplexed methods to gain spatial information beyond the straightforward use of nonlinear mNP magnetization behavior. Using two drive coils these methods were able to create shifts in the magnetic field phase, amplitude and frequency over time. Four of the five methods yielded R^2 values higher than the control experiment. These were asynchronous phase modulation, amplitude modulation, intermodulation phase modulation, and frequency modulation (methods B, C, E and F in Figure 4). These methods could potentially be used to extract additional spatial information for nonlinear SMI.

In our previous report, we demonstrated 6-voxel SMI [37]. This level of resolution was achieved by combining linear magnetization response data resulting from three drive coils and two fluxgate sensors. In the present study, we used two drive coils and one sense coil to reconstruct 12 voxels, which represents a six-fold improvement over our prior work. This resolution improvement was also accomplished with a limited amount of harmonic and intermodulation data. In our reconstructions we were able to see a strong response from the first 4 harmonic frequencies, a limited response from 5th and 6th harmonics, and a strong response at 5 intermodulation frequencies. In comparison, a recent report was able to measure 100 harmonic frequencies with a 50 mT applied magnetic field [48] whereas our measurement field was approximately 5 mT. With an increased applied magnetic field strength it may be possible to see an improvement by up to a factor of 100 over our original version of SMI. In our previous report we used linear susceptibility and weaker magnetic fields but required more hardware complexity with additional coils and sensors. In this report we have reduced the hardware complexity but increased the field strengths in order to reconstruct more voxels. In future work, the complexity of nonlinear SMI will increase as the field strengths increase and more drive and sense coils are added. The limits of resolution for nonlinear SMI depend on many factors including the magnetic field strength, the frequencies used, the number of drive and sense coils, coil size, location and geometry and the configuration of DC magnetic field gradients. Additional imaging specifications of importance are the sensitivity and depth needed for various applications. These two specifications can limit the achievable resolution because as sensitivity and depth requirements increase, the number of harmonics and intermodulation frequencies above from the noise floor decreases. Overall, it is clear that the performance limits of nonlinear SMI have not been reached in the present study and that a follow-up modeling study is necessary to study in more depth the factors that determine imaging resolution. It is anticipated, however, that further exploiting the nonlinear effects in a higher density imaging array could increase the resolution of the reconstructed images by a factor of 10 and that implementing time-multiplexed methods could further improve resolution by another factor of 5. In a small imaging application, with a total of 5 drive coils and 4 sense coils we anticipate the imaging limits to be $5 \times 4 \times 10 \times 5 = 1000$ voxels. For a field of view of $3 \text{ cm} \times 3 \text{ cm} \times 3 \text{ cm}$, this would correspond to 3 mm isotropic voxels.

As was shown in Figure 8 and seen in equations 10–13, the characteristic phase response of mNPs gives rise to different spatial information in the in-phase and the out-of-phase amplitudes. This means that the in-phase and out-of-phase data contribute complementary information to the spatial reconstruction. In addition to exploring the use of the nonlinear magnetization for SMI, we also explored time-multiplexed methods to improve upon the

results obtained in nonlinear SMI. These methods included, phase, frequency and amplitude modulation of the drive coils. These tests were performed in a time-multiplexed manner so it took longer to acquire all of the imaging data. Asynchronous phase modulation methods yielded R^2 values of 1.00 for up to six voxels while the control condition could only reconstruct up to three voxels. Both the frequency and amplitude modulation were also able to reconstruct up to six voxels up with slightly lower R^2 values. This reduction was primarily due to the inadequate strength of the applied magnetic fields, which were not strong enough to induce clear harmonics from all voxels. However, these methods show that it is possible to get additional spatial information by varying the amplitude, phase and frequency of the drive coils if longer data acquisition times can be tolerated.

The coil and sensor configuration used in the present study is most likely not optimal for nonlinear SMI. Several improvements could be made beyond increasing the applied magnetic field strength. Larger diameter coils could be used to increase imaging depth and an array of sensing coils and drive coils could be used to increase the imaging resolution beyond the added benefits of nonlinear effects. In addition, MPI uses frequencies in the kilohertz range, which reduces noise in the sensing coils. Adopting the use of frequencies in this range could help improve the signal to noise ratio.

Another potential application of the nonlinear SMI method is to improve MPI resolution. Currently, the MPI method generally uses a field free point (FFP) to localize mNPs. An FFP is difficult to create because strong gradient fields are needed to generate a small FFP for high-resolution imaging. Nonlinear SMI could be incorporated into MPI to eliminate the FFP or to use the spatial information of harmonics within a broader FFP to improve resolution. In a 3 dimensional FFP, doubling the resolution would require 8 voxels, less than the number demonstrated in this study. This would however involve some tradeoff in mNP sensitivity because the use of harmonic averaging would have to be reduced.

In this study we have demonstrated a method for nonlinear SMI. Previously we demonstrated linear SMI using an array of drive coils and fluxgate sensors that measured the linear susceptibility of mNPs. Now by introducing the nonlinear magnetization effects it is possible to significantly improve the imaging resolution of SMI. The present study also provides a clear path for the development of a higher density SMI system that could have applications in medicine.

Acknowledgements

This work was supported in part by the Dartmouth Center of Cancer Nanotechnology Excellence (DCCNE) NIH NCI U54-CA151662-04, the Center for the Translation of Rehabilitation Engineering Advances and Technology (TREAT) 5-R24-HD065703-04, and NIH NIBIB 1R21EB016241-01A1.

Bibliography

1. Pankhurst QA, et al. Applications of magnetic nanoparticles in biomedicine. *Journal of Physics D-Applied Physics*. 2003; 36(13):R167–R181.
2. Pankhurst QA, et al. Progress in applications of magnetic nanoparticles in biomedicine. *Journal of Physics D-Applied Physics*. 2009; 42(22):224001.

3. Steinhoff, U., et al. Spatially Resolved Measurement of Magnetic Nanoparticles Using Inhomogeneous Excitation Fields in the Linear Susceptibility Range ($<1\text{mT}$). In: Buzug, TM.; Borgert, J., editors. *Magnetic Particle Imaging*. Berlin Heidelberg: Springer; 2012. p. 295-300.
4. Adolphi NL, et al. Imaging of Her2-targeted magnetic nanoparticles for breast cancer detection: comparison of SQUID-detected magnetic relaxometry and MRI. *Contrast Media & Molecular Imaging*. 2012; 7(3):308–319. [PubMed: 22539401]
5. Enpuku K, et al. Characterization of Magnetic Markers for Liquid-Phase Immunoassays Using Brownian Relaxation. *Japanese Journal of Applied Physics*. 2012; 51(2):3002.
6. Janosek M, Ripka P, Platil A. Magnetic markers detection using PCB fluxgate array. *Journal of Applied Physics*. 2009; 105(7):07E717.
7. Ludwig F, et al. Magnetorelaxometry of magnetic nanoparticles with fluxgate magnetometers for the analysis of biological targets. *Journal of Magnetism and Magnetic Materials*. 2005; 293(1):690–695.
8. Richter H, et al. Magnetorelaxometry for localization and quantification of magnetic nanoparticles for thermal ablation studies. *Physics in Medicine and Biology*. 2010; 55(3):623–633. [PubMed: 20071755]
9. Romanus E, et al. Magnetic nanoparticle relaxation measurement as a novel tool for in vivo diagnostics. *Journal of Magnetism and Magnetic Materials*. 2002; 252(1–3):387–389.
10. Sarangi S, Tan IC, Brazdeikis A. Brownian relaxation of interacting magnetic nanoparticles in a colloid subjected to a pulsatile magnetic field. *Journal of Nanoscience and Nanotechnology*. 2011; 11(5):4136–4141. [PubMed: 21780417]
11. Yoshida T, et al. AC susceptibility of magnetic fluid in nonlinear brownian relaxation region: experiment and comparison with numerical simulation. *Japanese Journal of Applied Physics*. 2010; 49(5):053001.
12. Chieh JJ, Hong CY. Non-invasive and high-sensitivity scanning detection of magnetic nanoparticles in animals using high-T-c scanning superconducting-quantum-interference-device biosusceptometry. *Review of Scientific Instruments*. 2011; 82(8):084301. [PubMed: 21895259]
13. Dieckhoff JH, et al. Homogeneous Bioassays Based on the Manipulation of Magnetic Nanoparticles by Rotating and Alternating Magnetic Fields-A Comparison. *IEEE Transactions on Magnetics*. 2012; 48(11):3792–3795.
14. Enpuku K, et al. AC susceptibility measurement of magnetic markers in suspension for liquid phase immunoassay. *Journal of Applied Physics*. 2010; 108(3):4701.
15. Herrera AP, et al. Monitoring colloidal stability of polymer-coated magnetic nanoparticles using AC susceptibility measurements. *Journal of Colloid and Interface Science*. 2010; 342(2):540–549. [PubMed: 19948339]
16. Oisjoen F, et al. A new approach for bioassays based on frequency- and time-domain measurements of magnetic nanoparticles. *Biosensors & bioelectronics*. 2010; 25(5):1008–1013. [PubMed: 19822413]
17. Yang TQ, et al. Detection of magnetic nanoparticles with ac susceptibility measurement. *Physica C-Superconductivity and Its Applications*. 2004; 412:1496–1500.
18. Borgert, J.; Gleich, B.; Buzug, TM. *Springer Handbook of Medical Technology*. Springer; 2011. Magnetic particle imaging; p. 461-476.
19. Knopp, T.; Buzug, TM. *Magnetic Particle Imaging: An Introduction to Imaging Principles and Scanner Instrumentation*. Springer; 2012.
20. Gleich B, Weizenecker R. Tomographic imaging using the nonlinear response of magnetic particles. *Nature*. 2005; 435(7046):1214–1217. [PubMed: 15988521]
21. Weizenecker J, et al. Three-dimensional real-time in vivo magnetic particle imaging. *Physics in Medicine and Biology*. 2009; 54(5):L1–L10. [PubMed: 19204385]
22. Nikitin MP, et al. Highly sensitive room-temperature method of non-invasive in vivo detection of magnetic nanoparticles. *Journal of Magnetism and Magnetic Materials*. 2009; 321(10):1658–1661.
23. Nikitin PI, Vetoshko PM, Ksenevich TI. New type of biosensor based on magnetic nanoparticle detection. *Journal of Magnetism and Magnetic Materials*. 2007; 311(1):445–449.
24. Goodwill PW, et al. X-Space MPI: Magnetic nanoparticles for safe medical imaging. *Advanced Materials*. 2012; 24(28):3870–3877. [PubMed: 22988557]

25. Goodwill PW, et al. Narrowband magnetic particle imaging. *IEEE Transactions on Medical Imaging*. 2009; 28(8):1231–1237. [PubMed: 19211340]
26. Rauwerdink AM, Hansen EW, Weaver JB. Nanoparticle temperature estimation in combined ac and dc magnetic fields. *Physics in Medicine and Biology*. 2009; 54(19):L51–L55. [PubMed: 19741275]
27. Weaver JB, et al. Frequency distribution of the nanoparticle magnetization in the presence of a static as well as a harmonic magnetic field. *Medical Physics*. 2008; 35(5):1988–1994. [PubMed: 18561675]
28. Ferguson RM, Minard KR, Krishnan KM. Optimization of nanoparticle core size for magnetic particle imaging. *Journal of Magnetism and Magnetic Materials*. 2009; 321(10):1548–1551. [PubMed: 19606261]
29. Erbe, M., et al. Enhancing the Efficiency of a Field Free Line Scanning Device for Magnetic Particle Imaging; *IEEE Nuclear Science Symposium and Medical Imaging Conference Record (NSS/MIC)*; 2012. p. 2566-2568.
30. Knopp T, et al. Field-free line formation in a magnetic field. *Journal of Physics A-Mathematical and Theoretical*. 2010; 43(1):012002.
31. Weizenecker J, Gleich B, Borgert J. Magnetic particle imaging using a field free line. *Journal of Physics D-Applied Physics*. 2008; 41(10):105009.
32. Sattel TF, et al. Single-sided device for magnetic particle imaging. *Journal of Physics D-Applied Physics*. 2009; 42(2):022001.
33. Hong H, et al. Magnetic particle imaging with a planar frequency mixing magnetic detection scanner. *Review of Scientific Instruments*. 2014; 85(1):013705. [PubMed: 24517773]
34. Vogel P, et al. Traveling Wave Magnetic Particle Imaging. *Ieee Transactions on Medical Imaging*. 2014; 33(2):400–407. [PubMed: 24132006]
35. Sattel TF, et al. Single-Sided Magnetic Particle Imaging Device for the Sentinel Lymph Node Biopsy Scenario. *Medical Imaging 2012: Biomedical Applications in Molecular, Structural, and Functional Imaging*. 2012; 8317
36. Knopp T, et al. Model-Based Reconstruction for Magnetic Particle Imaging. *IEEE Transactions on Medical Imaging*. 2010; 29(1):12–18. [PubMed: 19435678]
37. Ficko BW, et al. Development of a magnetic nanoparticle susceptibility magnitude imaging array. *Physics in Medicine and Biology*. 2014; 59(4):1047. [PubMed: 24504184]
38. Rauwerdink AM, Weaver JB. Concurrent quantification of multiple nanoparticle bound states. *Medical Physics*. 2011; 38(3):1136–1140. [PubMed: 21520825]
39. Garcia-Palacios JL, Jonsson P, Svedlindh P. Nonlinear susceptibility of superparamagnets with a general anisotropy energy. *Physical Review B*. 2000; 61(10):6726–6733.
40. Garcia-Palacios JL, Svedlindh P. Large nonlinear dynamical response of superparamagnets: Interplay between precession and thermoactivation in the stochastic Landau-Lifshitz equation. *Physical Review Letters*. 2000; 85(17):3724–3727. [PubMed: 11030991]
41. Jonsson P, et al. Nonlinear dynamic susceptibilities of interacting and noninteracting magnetic nanoparticles. *Journal of Magnetism and Magnetic Materials*. 2000; 222(1–2):219–226.
42. Lopez-Ruiz R, et al. Nonlinear response of single-molecule nanomagnets: Equilibrium and dynamical. *Physical Review B*. 2005; 72(22):224433.
43. Raikher YL, Stepanov VI. Linear and nonlinear superparamagnetic relaxation at high anisotropy barriers. *Physical Review B*. 2002; 66(21):214406.
44. Stepanov VI, Raikher YL. Nonlinear magnetodynamics of a single-domain particle: the high-barrier approximation. *Journal of Magnetism and Magnetic Materials*. 2004; 272:E1277–E1278.
45. Krause HJ, et al. Magnetic particle detection by frequency mixing for immunoassay applications. *Journal of Magnetism and Magnetic Materials*. 2007; 311(1):436–444.
46. Gruttner C, et al. Synthesis and antibody conjugation of magnetic nanoparticles with improved specific power absorption rates for alternating magnetic field cancer therapy. *Journal of Magnetism and Magnetic Materials*. 2007; 311(1):181–186.

47. Nakamura K, et al. Self-Heating Temperature and AC Hysteresis of Magnetic Iron Oxide Nanoparticles and Their Dependence on Secondary Particle Size. *IEEE Transactions on Magnetics*. 2013; 49(1):240–243.
48. Biederer S, et al. Magnetization response spectroscopy of superparamagnetic nanoparticles for magnetic particle imaging. *Journal of Physics D-Applied Physics*. 2009; 42(20):205007.

Highlights

- Development of a nonlinear susceptibility magnitude imaging model
- Demonstration of nonlinear SMI with primary and harmonic frequencies
- Demonstration of nonlinear SMI with primary and intermodulation frequencies
- Demonstration of a six-fold improvement in imaging resolution using nonlinear SMI
- Demonstration of time-multiplexing imaging resolution improvements

Figure 1.

Measurement of the nonlinear mNP response to an applied AC field. An AC current creates an applied magnetic field in a biological medium that interacts with mNPs and the resulting magnetization is measured by a sense coil. The induced mNP magnetization is no longer sinusoidal due to the magnetic saturation characteristics of mNPs.

Figure 2.

Experimental setup showing the configuration of AC drive coils for generating the applied magnetic field, permanent magnets to create a DC field, sense coil for magnetic field sensing, and a 0.5 cm grid in the imaging zone for positioning a test sample of mNPs.

Figure 3.

Imaging method illustration for a single coil and sensor. A) A depiction of the experimental setup with a single drive coil, sense coil and two voxels containing equivalent mNP samples. B) The input waveform to the drive coil illustrating an AC current. C) The applied magnetic field at voxel 1 and voxel 2. Voxel 2 has a larger applied magnetic field due to its closer proximity to the drive coil. D) The mNP response to an applied magnetic field modeled as a Langevin function. If the applied magnetic field is weak, the AC magnetization of the mNPs is approximately sinusoidal. However, if the applied magnetic field is strong and enters the

saturation regime, the sinusoidal AC magnetization will become distorted and contain broader spectral content. E) The receive coil will detect the superimposed magnetic fields from the mNPs in voxels 1 and 2. The detected field waveforms from each voxel are different because of the nonlinear mNP response to the applied magnetic field. F) The power spectrum of the detected field contributions from voxels 1 and 2. The differences in the power spectra encode spatial information about the mNP concentrations in voxels 1 and 2.

Figure 4.

Time-multiplexed methods. The time-multiplexed methods tested to gain additional spatial information are shown A–F. Each of the four test conditions is shown with a left and a right drive coil waveform. A) Control, with no variations. B) Phase modulation of 90° per condition on the right drive coil. C) Amplitude modulation of both the left and right drive coil. D) Synchronized phase modulation of the left and right drive coils. E) Intermodulation phase shift of 90° per condition on the right drive coil. F) Frequency modulation on the left and right drive coil.

Figure 5.

Amplitude of the magnetization response for the nonlinear SMI of Figure 6. A lock-in amplifier was used to measure the amplitude of the primary frequency and each of the harmonics 2 – 6 and then the mean background signals were subtracted so that all harmonic responses could be superimposed. The response of each harmonic varies with the position of the mNP sample as it moves through the grid starting at voxel 1 and moving through voxel 12.

Figure 6.

Image reconstructions for drive coil 1 using data at a primary frequency of 325 Hz and incorporating signal data for the primary frequency and harmonics 2–6. The mNP sample was moved through the imaging grid in a sequence starting at voxel index 1 and ending at voxel index 12. Images were reconstructed starting with 1 voxel and working up to 12 voxels to determine if additional information is contained in the harmonics measured from drive coil 1. The reconstructions of 1–12 voxels demonstrate that mNP harmonics add spatial information to SMI.

Figure 7.

Image reconstructions using data at primary frequencies of 325 and 420 Hz and incorporating signal data for the intermodulation frequencies of drive coils 1 and 2. The mNP sample was moved through the imaging grid in a sequence starting at voxel index 1 and ending at voxel index 12. Images were reconstructed starting with 1 voxel and working up to 12 voxels to determine if additional information is contained in the intermodulation harmonics measured from drive coils 1 and 2. The reconstructions of 1–12 voxels demonstrate the ability of mNP intermodulation harmonics to add additional spatial information to SMI.

Figure 8. R^2 statistics for image reconstruction of Figure 7 using various data sets of primary and intermodulation frequencies from coils 1 and 2. The figure shows R^2 values as a function of the number of reconstructed voxels for in-phase data, out-of-phase data, and combined data sets.

Figure 9.

Nonlinear SMI reconstruction using data at the primary frequencies (325 Hz and 420 Hz), harmonic frequencies, intermodulation frequencies of drive coils 1 and 2 ($R^2 = 0.996$, CNR = 84.9). The experimental data presented is a combination of the data presented in Figure 6 and Figure 7 and a data set from coil 2 that was not presented but similar to Figure 6. The resulting image combines all data to fully demonstrate the spatial gains achieved with harmonic and intermodulation frequencies over the use of primary frequencies alone.

Figure 10.

Nonlinear SMI reconstruction with two samples located simultaneously in the imaging grid. In this image set a full concentration and a half concentration sample were imaged together by moving one of the samples to a new location every 20 s. A schematic of the position of each sample over time is shown above the image. The reconstruction used all of the available nonlinear data (harmonics and intermodulation) for the reconstruction.

Figure 11.

Image reconstruction for the control test and each of the five time-multiplexed methods. The mNP sample was moved through the imaging grid in a sequence starting at voxel index 1 and ending at voxel index 6. Images were reconstructed starting with 1 voxel and working up to 6 voxels to determine if additional spatial information is contained in the time multiplexed shifting of amplitude, phase and frequency of the applied magnetic fields in drive coils 1 and 2. A) Control condition with no parameter variation over the four test conditions. B) Asynchronous phase shifting of one of the right drive coil. C) Amplitude

modulation of the drive current in one or both coils. D) Synchronous phase shift for both coils. E) Intermodulation asynchronous phase shifting. F) Frequency shifting.

Figure 12.

R^2 values taken from the six methods shown in Figure 11. In every method except the control and synchronous phase shifting, the R^2 values stay above 0.9 for up to 6 voxels indicating an increase in the spatial information contained in these methods relative to the control.