Magnetoacoustic imaging of magnetic iron oxide nanoparticles embedded in biological tissues with microsecond magnetic stimulation

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We present an experimental study on magnetoacoustic imaging of superparamagnetic iron oxide (SPIO) nanoparticles embedded in biological tissues. In experiments, a large-current-carrying coil is used to deliver microsecond pulsed magnetic stimulation to samples. The ultrasound signals induced by magnetic forces on SPIO nanoparticles are measured by a rotating transducer. The distribution of nanoparticles is reconstructed by a back-projection imaging algorithm. The results demonstrated the feasibility to obtain cross-sectional image of magnetic nanoparticle targets with faithful dimensional and positional information, which suggests a promising tool for tomographic reconstruction of magnetic nanoparticle-labeled diseased tissues (e.g., cancerous tumor) in molecular or clinic imaging. © 2012 American Institute of Physics. [doi:10.1063/1.3675457]

In the past years, magnetic nanoparticles (MNPs) have been widely used as contrast agents in a variety of molecular or clinical imaging modalities as they are biocompatible with controllable size and can be easily conjugated to functional groups or ligands in biological systems. Magnetic resonance imaging (MRI) of magnetic nanoparticle-labeled molecular targets has been demonstrated to be able to provide enhanced imaging contrast because the nature and the property of the magnetic nanoparticles can significantly shorten T2 relaxation time.¹ Several emerging imaging techniques adopt other contrast mechanism termed magnetomotive effect to actuate those embedded magnetic nanoparticles to move periodically inside tissues by applying a controllable external time-varying magnetic field. These tiny mechanical movements can be measured by the sensitive imaging system to form images. Relevant magnetomotive-based imaging techniques include magnetomotive optical coherent tomography (MM-OCT) which uses optical measurements to dynamically monitor nanometer scale movements *in vivo* with high spatial resolution.^{2,3} However, MM-OCT has limited detection depth due to the strong scattering of light in tissues. Alternatively, magnetomotive ultrasound (MM-US) imaging technique uses B-mode or M-mode ultrasound measurements of magnetic nanoparticles for deep tissue imaging.⁴⁻⁶ Previous magnetomotive ultrasound imaging system either applies continuous harmonic magnetic field or applies millisecond pulsed magnetic field to stimulate samples, which can generate tissue motion or tissue displacement images. In the present study, we launch very short (1.0 μs) pulsed magnetic field stimulation to samples, which induces mega hertz mechanical vibrations in tissues. By collecting magnetomotive ultrasound signals in a circular configuration, we are able to reconstruct tomographic acoustic source images reflecting the dimensional and positional information of the magnetic nanoparticle targets. The image reconstruction in this study is based on the use of the time reversal method derived from the wave propagation equation in the medium.

In our imaging experiments, a large-current-carrying excitation coil is employed to send out a microsecond pulsed magnetic stimulation **B** to a nearby tissue sample with embedded magnetic nanoparticles as shown in Fig. 1(a). In biomedical application, the nanoparticles are usually diluted and suspended in a weakly magnetic background medium (e.g., biological tissues with magnetic susceptibility $\chi \approx 0$). These nanoparticles exposed to the magnetic stimulation **B** will experience magnetic forces. If we only consider **B** field gradient in the coil axis direction, the magnetic translational force on nanoparticles per unit volume is⁷

$$\mathbf{F}_m = \frac{\chi_{np}}{2\mu_0} \nabla(B^2), \quad (1)$$

where χ_{np} is the magnetic susceptibility of the nanoparticles and μ_0 is the permeability of free space. Assuming biological tissue an incompressible inviscid medium, we have the

FIG. 1. (Color online) (a) Schematic diagram for magnetic nanoparticle imaging. (b) Recorded coil current waveform with charging voltage setting at 24 kV in the magnetic stimulator. (c) A transmission electron microscopy image of iron oxide magnetic nanoparticles in the EMG 304 ferrofluid.

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equation of motion with magnetic translational force acting as the body force

$$\rho \left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \nabla \mathbf{v} \right) = -\nabla p + \mathbf{F}_m, \quad (2)$$

where ρ is the medium density, p is the pressure, and $\mathbf{v}$ is the flow velocity. Because of the short duration of the magnetic stimulation $\mathbf{B}$, the induced magnetic translational force $\mathbf{F}_m$ is also very short which can be approximated as the product of a pure spatial function $\mathbf{F}_m(\mathbf{r})$ and a delta function $\delta(t)$. Assuming that the medium is acoustically homogeneous, the pressure distribution can be derived as⁸

$$\nabla^2 p(\mathbf{r}', t) - \frac{1}{c^2} \frac{\partial^2}{\partial t^2} p(\mathbf{r}', t) = \nabla \cdot [\mathbf{F}_m(\mathbf{r})] \cdot \delta(t), \quad (3)$$

where the c is the acoustic speed of ~ 1.5 mm/ μ s for tissues. Equation (3) has a similar form to the equation used in those Lorentz force-based bioimpedance imaging techniques, in which the divergence of the Lorentz force acts as the acoustic source term.^{9–17} Hence, the acoustic source distribution $\nabla \cdot [\mathbf{F}_m(\mathbf{r})]$ can be computed using the time reversal method.^{11,18}

$$\nabla \cdot [\mathbf{F}_m(\mathbf{r})] \approx -\frac{1}{2\pi c^3} \iint_{\Sigma} dS_d \frac{\mathbf{n} \cdot (\mathbf{r}_d - \mathbf{r})}{|\mathbf{r}_d - \mathbf{r}|^2} p''(\mathbf{r}_d, |\mathbf{r}_d - \mathbf{r}|/c), \quad (4)$$

where $\mathbf{r}_d$ is the detection point on the surface Σ , p'' is the second time derivative of the pressure.

In the experiments, both the sample and the transducer were immersed under water. The unfocused transducer was driven for mechanical rotation in the XY plane. This transducer had a peak sensitivity frequency at 0.5 MHz (TRS Ceramics, PA, USA). A customized magnetic stimulator was used to deliver microsecond duration pulsed magnetic stimulation to the sample. The five-turn stimulating coil was driven by a coil driver equipped with an adjustable high-voltage source (0–24 kV). The actual current in the coil could be monitored with a built-in calibration terminator. At 24 kV setting, the maximum coil current could reach 780 A as shown in Fig. 1(b). This coil induced an electrical field around 550 V/m in the sample, which was comparable to the stimulating strength in the widely used transcranial magnetic stimulation (TMS).¹⁹ However, the total energy applied to the samples in this study was much lower than TMS because our pulsed stimulation lasts only 1 μ s instead of millisecond level in TMS. A data acquisition system (CS8324, Gage Applied, Canada) with a 5 MHz sampling rate was used to collect data for each channel. In this study, we used commercially available, water soluble ferrofluid superparamagnetic nanoparticles EMG 304 (Ferrotec, NH, USA). The EMG 304 ($\chi \approx 5.0$) is a mixture of Fe_2O_3 and Fe_3O_4 with particle diameter of 5–15 nm in the suspension. The acquired transmission electron microscopy (FEI Tecnai Spirit BioTWIN, OR, USA) image is shown in Fig. 1(c). The iron content in this ferrofluid was determined to be ~ 170 mg/ml based on the nanoparticle characterization protocol.²⁰

Experiments on tissue-equivalent gelatin phantom were conducted. We built a mold with a 22-mm-diameter hole

surrounded by 5% cooled gelatin solution filling a thin-wall container (50 mm diameter, 20 mm height). The EMG 304 suspension was poured into the hole to finalize the phantom. In the imaging experiment, this phantom was placed in a tank filled with distilled water. The detection radius was around 287 mm and the charging voltage was set at 8 kV. The ultrasound data were collected with 70 dB gain and angular step of 2°. At each channel, 10 times data averaging was used. In this test, we estimated $|\nabla \mathbf{B}|^2 = 0.013$ T²/m at the center of the sample in the Z direction. The magnetic force per particle was calculated to be $\sim 1.3 \times 10^{-20}$ N. The estimated peak pressure for this sample was ~ 4.4 Pa by using previously reported method.¹¹ From the measured magnetoacoustic signals, it could be calculated that the acquired acoustic pressure at the transducer surface was ~ 0.10 Pa. Figure 2(a) shows a channel waveform obtained from the phantom in Fig. 2(b). Two bipolar-structure signals with a delay of 14.4 μ s are from the two gelatin-to-ferrofluid interfaces roughly spacing by 22 mm by considering the acoustic speed of 1.5 mm/ μ s in the medium. This waveform is consistent with the theoretical calculation in Eq. (3) which predicts induced ultrasound signals will come mainly from the regions that has intense changes in magnetic force.

FIG. 2. (Color online) (a) Temporal induced ultrasound signal recorded by piezoelectric transducer. (b) Photograph of the gelatin phantom containing one magnetic nanoparticle target with cylindrical geometry (c) Reconstructed image of the phantom shown in (b). (d) Photograph of the gelatin phantom containing two magnetic nanoparticle targets with cylindrical geometry and rectangular geometry. (e) Reconstructed image of the phantom shown in (d).

Figure 2(c) is the reconstructed image based on Eq. (4). The circular boundary of the nanoparticle suspension is clearly imaged as compared with the original phantom. In the second experiment, we built a phantom with a relatively complex structure, which included a cylindrical hole (12 mm diameter, 20 mm height) and a rectangular hole (18 mm $\times$ 10 mm $\times$ 20 mm) filled with EMG 304 ferrofluid. The horizontal distance between the two holes was about 11 mm. Figure 2(d) shows the phantom photograph with two targets with different shapes to be imaged. The reconstructed acoustic source image in Fig. 2(e) agrees well with the cross section of the original phantom in terms of size and relative location of each target boundary.

We also conducted imaging experiments on a well-controlled biological tissue phantom, which was used to simulate a small nanoparticle-labeled tumor target surrounded by healthy tissues. A piece of fresh pork fat tissue purchased from a local food store was cut into a base with a dimension of 18 mm $\times$ 19 mm $\times$ 24 mm. We carefully made a small hole with an approximate diameter of 7.5 mm on this base. The hole was infused with EMG 304 ferrofluid. The tissue was fixed in the center of the container by filling with 5% cooled gelatin to build the sample shown in Fig. 3(a). In this experiment, scanning with angular step of 2° was done with 20 times signal averaging to suppress random noise. The charging voltage was set at 16 kV in order to improve signal intensity. Figure 3(b) illustrates the reconstructed acoustic source image. The square pork tissue base is invisible in Fig. 3(b) because the tissue magnetic susceptibility ($|\chi_{\text{pork}}| < 10^{-5}$) is too small to generate measurable magnetoacoustic signals. However,

the round magnetic nanoparticle target with 7.5 mm diameter is clearly resolved in the reconstructed image. The expected image spatial resolution is estimated to be ~ 1.5 mm, which is the half wavelength of 0.5 MHz ultrasound wave with 1.5 mm/ μ s acoustic speed in soft tissues. We have also tested the imaging sensitivity on a low-concentration nanoparticle sample. In this experiment, the EMG 304 suspension was diluted 25 times into actual iron content at ~ 6.8 mg/ml, which is comparable to FDA-approved ferumoxides suspension Feridex IV with an iron content of 11.2 mg/ml for human liver lesion magnetic resonance imaging. The diluted suspension was injected into a 12 mm diameter hole surrounded by 5% gelatin shown in Fig. 3(c). Figure 3(d) depicts the reconstructed image showing the visible target boundary in agreement with the phantom photograph. The contrast to noise ratio, defined as the ratio of the dynamic range of the reconstructed image to the standard deviation of the background noise, is used to estimate the image sensitivity. The calculated contrast to noise ratio from Fig. 3(d) is around 10.5, demonstrating the potential for low-concentration magnetic nanoparticle imaging for *in vivo* application.

In summary, we report that our imaging technique is able to obtain boundary images of magnetic nanoparticles embedded in biological tissues with good imaging sensitivity and resolution by using microsecond pulse magnetic stimulation. The extension of this study would be developing high-resolution high-sensitivity imaging system, which can provide three-dimensional (3D) images of *in vivo* magnetic nanoparticle distribution.

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FIG. 3. (Color online) (a) Photograph of a pork fat phantom, which contains magnetic nanoparticle target with 7.5 mm diameter. (b) Reconstructed image of the sample shown in (a). (c) Photograph of gelatin phantom, which contains magnetic nanoparticles with diluted iron content of ~ 6.8 mg/ml. (d) Reconstructed image of the phantom shown in (c).

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