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Simultaneous quantification of multiple magnetic nanoparticles

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Abstract

Distinct magnetic nanoparticle designs can have unique spectral responses to an AC magnetic field in a technique called the magnetic spectroscopy of Brownian motion (MSB). The spectra of the particles have been measured using desktop spectrometers and *in vivo* measurements. If multiple particle types are present in a region of interest, the unique spectral signatures allow for the simultaneous quantification of the various particles. We demonstrate such a potential experimentally with up to three particle types. This ability to concurrently detect multiple particles will enable new biomedical applications.

1. Introduction

There is widespread interest across the biomedical realm in an ability to detect or image a plurality of targets simultaneously. Diagnoses of complex diseases are increasingly dependent on knowledge of the patients' biomarker profiles. Rapid clinical based methods for detecting an array of proteomic and genetic markers are being developed (Osterfeld *et al* 2008). Several existing molecular imaging modalities have been utilized for such concurrent detection as this ability can allow for the interactions between distinct processes to be elucidated. Dual-tracer positron emission tomography, PET, has shown increased efficacy over single-tracer imaging in the evaluation of primary and metastatic hepatocellular carcinoma (Ho *et al* 2007). Favorable results have also been seen in the diagnosis of pulmonary lesions and predicting the behavior of colorectal cancer (Tian *et al* 2008, Hui *et al* 2009). The market for multi-tracer imaging is growing, but current techniques face numerous challenges.

The different radioisotopes used in single photon emission computed tomography, SPECT, can have unique emission energies and by using detector energy windows the tracers can be imaged simultaneously. However, challenges exist because the energy resolution of the detectors is typically inadequate to fully separate the different gamma rays, and corrections for this cross-talk are needed (Meikle *et al* 2005). Efforts toward developing dual-tracer PET are hampered by the constant 511 keV energy of detected photons, which precludes energy windowing. Some successful human results have been reported but they require staggered tracer injections, arterial blood sampling, or assumptions about a tracer reaching a steady state distribution (Joshi *et al* 2009, Kudomi *et al* 2007).

A number of promising results have been achieved using fluorescence probes. The spectral signal from numerous passive fluorescent probes can be readily separated, and recent work with fluorescent resonance energy transfer, FRET, has allowed for simultaneous imaging of up to four cellular processes (Piljic and Schultz 2008). These primarily microscopy based

techniques have a very limited ability to function at depth, but efforts are underway to apply these techniques to diffuse optical tomography (Weissleder and Pittet 2008).

Recent progress has been made in the use of molecular MRI for concurrent dual contrast agent detection. Gilad *et al* (2009) simultaneously imaged superparamagnetic iron oxide nanoparticles and the saturation transfer agent poly-L-lysine by utilizing their distinct mechanisms of contrast.

In 2005, Gleich and Weizenecker (2005) introduced a new magnetic nanoparticle based molecular imaging modality termed magnetic particle imaging, MPI. A preclinical version of MPI is jointly being developed by Royal Philips Electronics and Bruker BioSpin and has produced *in vivo* images (Weizenecker *et al* 2009). MPI employs a series of static and alternating magnetic fields to both generate and localize a harmonic signal unique to the nanoparticles. The design of an MPI particle including its material, size, shape, and coating will determine the amplitude of the particle's magnetic moment and the relaxation time constant governing its dynamic response to an alternating field (Rauwerdink and Weaver 2010a). The particle's dynamics can impact the effectiveness of the nanoparticle for MPI. Magnetic spectroscopy of nanoparticle Brownian motion, MSB, is a related technique that has been used to evaluate the nanoparticle microscopic environment including its temperature (Weaver *et al* 2009) and chemical bound state (Rauwerdink and Weaver 2010b).

Quantitative detection of magnetic nanoparticles has also been utilized for the *in vitro* detection of specific analytes (Koh and Josephson 2009). Several methods of multiplex detection have been proposed. Osterfeld *et al* (2008) used an array of giant magnetoresistive sensors, each tagged with an antibody for a specific analyte. Lenglet (2009) used the unique magnetic response of two different micron size particles to quantify each particle for immunoassays without the need for spatial resolution. However, micron-sized particles have limited *in vivo* applications because their large size limits the distribution. Here we show how the unique spectral signature of different nanoparticle designs could enable MPI and MSB to serve as multi-tracer modalities. The signatures for micron-sized particles are significantly different from those of nanoparticles, but we demonstrate that the concentrations of different nanoparticles can be found. Indeed, the differences in the particles' harmonic spectra allowed us to simultaneously quantify up to three nanoparticle types with single measurement.

2. Materials and methods

2.1. Nanoparticles and sample vials

Iron oxide nanoparticles with a single magnetic core and functionalized coating (SHA-25 and SHP-40) were purchased from Ocean NanoTech (Springdale, AR). From Micromod Partikeltechnologie GmbH (Rostock, Germany), we purchased nanoparticles (79-00-501) that had multiple iron oxide crystals embedded in a dextran matrix. For the Micromod particles the weight percentage of iron was approximately 35%. We measured the hydrodynamic size using a Malvern (Worcestershire, UK) ZetaSizer Nano ZS, and the magnetic cores were measured with a FEI Company Tecnai F20 FEG TEM (Hillsboro, Oregon). The particle characteristics are presented in table 1.

We dispensed 10 μ l volumes of the particles in 1.5 mm capillary tubes and sealed them with epoxy. The Ocean NanoTech particles were used at stock concentration, 5 mg Fe ml⁻¹, so each tube contained 50 μ g of iron. The MicroMod particles had a substantially stronger harmonic signal per mass of iron and were diluted so that each vial had 8 μ g of iron.

2.2. Experimental setup

The spectrometer setup used has been described previously (Weaver *et al* 2009). A Stanford Research Systems (Sunnyvale, CA) SR830 Lock-In Amplifier was used to generate the sinusoidal drive field and to analyze the harmonics generated by the particles. In place of a single 2 ml sample vial as used previously, various combinations of the nearly identical particle filled capillary tubes were used. The use of these prefabricated tubes allowed for simple control of the amount of each particle type. We also measured the harmonic response from particles mixed together within one vial to confirm that the technique worked under these more biologically relevant conditions. The third and fifth harmonics were recorded complexly with the phase of the drive field as a reference.

3. Results

Under the influence of an alternating magnetic field, the magnetic moment of a superparamagnetic nanoparticle will attempt to reach an energy equilibrium with the applied field. The magnetization in static equilibrium is well defined by the Langevin function (Cullity and Graham 2008). This static magnetization will influence the amplitude of the harmonics as well as the rate at which the harmonic amplitudes decrease with increasing harmonic number. As the frequency of the excitation field increases the nanoparticles may no longer be capable of achieving the static equilibrium magnetization and a phase lag can develop between the particle magnetization and the drive field. A relaxation time constant influences the extent of this phase lag. Both the static and dynamic influences can be grouped into a differential equation known as the effective field method, which has been shown to model the magnetization accurately (Rauwerdink and Weaver 2010a, Felderhof and Jones 2003). In figure 1, the third and fifth harmonic vectors of three nanoparticle designs are shown. These measurements at a field strength of $13.2 \text{ mT}/\mu_0$ and 1740 Hz show profound differences in the ratio of the fifth to third harmonic amplitude and in the phase of the harmonics. The response of the Micromod and 40 nm Ocean particles are seen to be nearly orthogonal with very different relative fifth harmonic contributions.

The unique spectral response of the different particle types makes concurrent quantification a possibility. If multiple particle types are present within one region of interest a least squares method can be used to solve for the quantity of each particle type. A comparable technique has been implemented with near-infrared tomography to quantify multiple parameters (Srinivasan *et al* 2003). The results of figure 1 are used as a system function containing the harmonic response of a known quantity of each particle design. The system response matrix and general form of the solution are shown in equation (1). The response matrix has units of μV per tube.

$$\begin{bmatrix} 3.006 & 5.606 & 3.842 \\ 1.928 & -2.306 & 1.155 \\ 1.016 & 1.097 & 0.690 \\ 1.509 & -1.071 & 0.301 \end{bmatrix} \begin{bmatrix} M50 \\ O40 \\ O25 \end{bmatrix} = \begin{bmatrix} 3X_{MIX} \\ 3Y_{MIX} \\ 5X_{MIX} \\ 5Y_{MIX} \end{bmatrix} \quad (1)$$

It is also possible to exploit the particles' unique responses to changes in excitation amplitude or frequency. Holding the frequency constant at 880 Hz, we varied the excitation amplitude between 7 and 21 mT/μ_0 , see figure 2. As in figure 1, the MicroMod particles have the highest ratio for a given field strength and the Ocean 25 nm the lowest. The slope of the ratio versus field strength curve is also different for each particle type. Across this same field range, we measured the response of a physical mixture of the MicroMod and Ocean 40 nm particles within one vial. The measured harmonic ratio averaged within 0.80% of the ratio calculated by adding the individual particles' responses. The excitation

frequency also has an important impact on the harmonic response, see figure 3. Across the frequency range used, the third harmonic amplitudes for the Ocean 40 nm and 25 nm particles drop by 52% and 30%, respectively. The MicroMod third harmonic amplitude remains within 2.3% of its initial value and actually increases slightly at the highest frequency. The harmonic ratios tell a similar story, see figure 3(b).

To demonstrate the potential for the concurrent quantification of multiple particles, we measured several combinations of MicroMod and Ocean 25 nm particle tubes. For each particle combination, a single acquisition of the complex third and fifth harmonics was measured at 1740 Hz and 13.2 mT/ μ_0 . We measured zero to four tubes of each individual particle type and all possible combinations for a total of 25 different mixtures. A linear least squares solution accurately quantified all combinations with a mean error of 1.20% and a maximum error of 2.38%. The condition number of the spectral response matrix for the MicroMod and Ocean 25 nm particles was 4.5. For these two particle types the use of additional higher harmonics did not substantially improve the conditioning. As seen in figure 1, the MicroMod and Ocean 40 nm particles are nearly orthogonal and these two particles have a condition number of 1.6 with 1 being ideal.

A single measurement of the complex third and fifth harmonics has four independent variables and in an ideal case could allow for the quantification of up to four particles in one measurement. Through the use of additional excitation frequencies or amplitudes, a greater number of particle types could be quantified. Continuing with a single acquisition of the complex third and fifth harmonics, we measured combinations of all three particle types shown in figure 1. We simulated a scenario of Ocean 25 nm uptake and MicroMod removal in the presence of a stable concentration of Ocean 40 nm, see table 2. The mean error for this set of measurements was 1.44%.

4. Discussion

The unique spectral signature of different nanoparticle designs makes the harmonics based detection of multiple particle types possible. This ability has applications in magnetic particle imaging and also in the spectrometer based detection of particles. To emphasize the potential strength of this technology we have quantified an array of different particle combinations using only a single measurement of the third and fifth harmonics. We have quantified up to three particle types simultaneously, but the potential exists for quantifying larger numbers. The spectral response of different particle designs to changes in drive field amplitude and frequency, see figures 2 and 3, could be exploited to separate additional particle responses. There is a striking difference in the frequency response of the core/shell Ocean Nanotech particles compared to the multi-crystal MicroMod particles. Other particle designs such as rods or hollow spheres could provide an additional signal contrast. For specific applications of this technique, the excitation parameters and the nanoparticle designs would need to be optimized for the situation at hand. In addition, one could choose to tailor the use of additional excitation parameters toward the specific goal of quantifying extra particle types, improving the sensitivity, or decreasing the error in the solution. The mathematical method used to solve for the different particle types could also be modified.

The drift in our spectrometer's signal over time was a significant source of error for our results. The reproducibility of our spectrometer measurements of harmonic amplitude is on the order of 0.1% when measured in rapid succession. Over extended periods of time the amplitude and phase of the harmonics can drift by several per cent. One source of this error is heating of the magnetic coils and electronics, which is not controlled in our current spectrometer. For the mixture results discussed and presented in table 2, we measured the response of each particle type at the beginning of the experiment and used these responses to

solve for subsequent combinations, which were measured over the course of about one hour. If we instead limit the influence of drift by measuring the response of each particle type immediately before measuring the combination we can routinely achieve errors less than 0.5%.

The sensitivity of our spectrometer is also a source of error. We did not utilize harmonics beyond the fifth but with improved sensitivity these higher harmonics could provide further information. The minimum concentration that we measured was $143 \mu\text{mol (Fe) l}^{-1}$, which is 3.6 times the *in vivo* limit (Weizenecker *et al* 2009). The concentrations used here are a fault of our hardware, not of the underlying technique. There is ongoing study of the optimal particle design for MPI and that work has parallels here. The MicroMod particles had a substantially stronger signal per mass of iron than the core/shell particles. Figure 3 shows that there is clearly a dynamic aspect to this, but a thorough study of the impact of the particle size and design on the harmonic signal is needed. The particle design, excitation parameters, solution techniques, and hardware could all be optimized to drive the sensitivity much lower for a specific application.

Nanoparticles for use with this technique would need to be tuned to account for or exploit the impact of the nanoparticle environment on the harmonic spectrum. We have shown previously that molecular binding to the nanoparticle surface can have a pronounced impact on the spectral response (Rauwerdink and Weaver 2010b). Viscous and temperature effects have also been detailed (Rauwerdink and Weaver 2010a, Weaver *et al* 2009). To quantify the nanoparticle concentration these influences would need to be avoided or corrections made. The spectral response of the nanoparticles in each bound state is sufficiently different to suggest that the bound state and the size of each nanoparticle population can both be found, however, further study is clearly required. This would allow for multiparameter detection instead of just multiparticle detection. By exploiting the potential influence of nanoparticle dipole interactions on the harmonic spectra, a magnetic version of FRET might even be possible.

The design of nanoparticles for this technique would also have to take into account non-specific binding. Non-specific binding is known to complicate the biological use of nanoparticles both *in vivo* and *in vitro* (Chalmers *et al* 2010, Davis *et al* 2008). The surface functionalization of the particles must limit the non-specific binding to unwanted biological targets and unwanted binding to other nanoparticles. Though most of our mixture results were acquired with the different particle types isolated in separate tubes, the results of figure 2 suggest that this technique can be used with physical mixtures of the particles. The error between the calculated and measured mixture response was comparable to the error between different samples from one particle batch. The response from the mixture sample also showed minimal change after 24 h suggesting negligible interaction between the two particle types.

As with other techniques demonstrated with MSB, the incorporation of this technique into MPI would have distinct requirements of the acquisition sequence. MPI relies on a measured or modeled system function that maps the harmonic response of a point source of particles across the field of view. For multiparticle detection this system function would be needed for each particle type, and if using the techniques described here, the individual harmonic amplitudes and phases would need to be resolved. The hardware and software of MRI have proven to be highly adaptable over the past few decades and MPI should have a similar potential. Though the sub-cellular resolution of fluorescence techniques might be unachievable with these harmonics based methods, MPI does offer an ability to overcome some of the challenges inherent in translating multi-tracer fluorescence techniques into the tomographic imaging realm. Outside of imaging, single point measurements of the MSB

signal could be acquired at depth by exploiting the geometric sensitivities of the detection or excitation coils, or through the use of a static gradient and field free point as used in the first generation MPI (Gleich and Weizenecker 2005).

5. Conclusion

The unique spectral response of different nanoparticle designs makes multiparticle detection a possibility. We have demonstrated an ability to accurately quantify up to three nanoparticle designs with a single measurement of the harmonic spectrum. This could allow for MPI to monitor a plurality of molecular targets simultaneously or provide new applications for nanoparticle spectrometer systems.

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Figure 1. Third (solid lines) and fifth (dashed lines) harmonic responses for different nanoparticles at 1740 Hz and 13.2 mT/ μ_0 . Third harmonic amplitudes were normalized to a value of 1.

Figure 2.

The harmonic ratio of two individual particle types and a comparison of the calculated and actual ratio when they are physically mixed. The excitation frequency was 880 Hz and the field strength was varied between 7 and 21 mT/μ₀. The average error between the calculated and actual ratio was 0.80%.

Figure 3.

(a) The third harmonic amplitude per cycle of the excitation field as a function of frequency at $10 \text{ mT}/\mu_0$. The harmonic amplitudes were normalized to the third harmonic amplitude at the lowest frequency. (b) The ratio of the fifth and third harmonic amplitudes at the same frequencies.

Table 1

Particle characteristics.

Particle type	Particle design	Core diameter (nm)	Hydrodynamic size (nm)	Surface coating
Ocean Nanotech SHA-25	Core/shell	25.1 ± 1.6	42.0 ± 13.8	PEG/amine
Ocean Nanotech SHP-40	Core/shell	41.3 ± 5.8	49.3 ± 11.5	Carboxylic acid
MicroMod 79-00-501	Multi-crystal	12.0 ± 7.4	95.9 ± 49.7	Dextran

Table 2

Solution for combinations of three particle types.

Particle combinations (calculated/actual)						
A	B	C	D	E	F	
Ocean 40	1.985/2	1.976/2	2.087/2	1.997/2	2.070/2	2.034/2
Ocean 25	0.038/0	0.997/1	1.935/2	3.019/3	4.092/4	4.060/4
MicroMod 50	4.045/4	3.978/4	3.010/3	2.007/2	0.989/1	0.005/0
Total Error	1.63%	0.70%	2.30%	0.42%	2.46%	1.63%