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Increasing the sensitivity for stem cell monitoring in system-function based magnetic particle imaging

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Abstract
The use of superparamagnetic iron oxide nanoparticles (SPIONs) has provided new possibilities in biophysics and biomedical imaging technologies. The magnetization dynamics of SPIONs, which can be influenced by the environment, are of central interest. In this work, different biological SPION environments are used to investigate three different calibration methods for stem cell monitoring in magnetic particle imaging. It is shown that calibrating using SPIONs immobilized via agarose gel or intracellular uptake results in superior stem cell image quality compared to mobile SPIONs in saline. This superior image quality enables more sensitive localization and identification of a significantly smaller number of magnetically labeled stem cells. The results are important for cell tracking and monitoring of future SPION based therapies such as hyperthermia based cancer therapies, targeted drug delivery, or tissue regeneration approaches where it is crucial to image a sufficiently small number of SPIONs interacting with biological matter.

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(Some figures may appear in colour only in the online journal)

Superparamagnetic iron oxide nanoparticles (SPIONs) are nanoscale magnets which can be biologically functionalized for a variety of biomedical applications, e.g. magnetic cell labeling. When cell cultures are incubated with SPIONs, most adherent growing cells such as macrophages, mesenchymal stem cells (MSC), or most cancer cell lines, SPIONs are phagocytized and clustered in a membrane-bound vesicles called phagosomes. Stem cell therapies to accelerate tissue repair (Gupta et al 2012, Longo et al 2012, Li et al 2013), magnetic fluid hyperthermia for cancer therapy, and targeted drug delivery based on SPIONs require fast and sensitive visualization of the SPION distribution. The identification and improvement of suitable biomedical imaging modalities for stem cell tracking and SPION monitoring is vital for a comprehensive understanding of the migration and distribution of cells and functionalized SPIONs as targeted drugs. Several different methods for stem cell tracking have been presented using bioluminescence imaging (Sheyn et al 2011, Wolbank et al 2011), single photon emission computed tomography (Bindslev et al 2006), positron emission tomography (Ma et al 2005), fluorescence imaging (Baba and Nishida 2012), x-ray based computed tomography (Gildehaus et al 2011), and magnetic resonance imaging (MRI) (Zhang et al 2012). Investigating SPION labeled stem cells via MRI provides high spatial resolution, long term monitoring up to several days after application, and a general absence of ionizing radiation (Li et al 2013). However, a key challenge of imaging SPION labeled stem cells with T2 or T2*MRI is clear discrimination of tissue structures with genuine low MR signal, such as iron rich tissues (liver, spleen), blood vessels (flow voids), dense connective tissue, bone, or air. It is especially problematic when small SPION labeled cell clusters or even single cells are investigated such that the gray value within an image voxel is only partially affected. Therefore, a suitable ionizing radiation and background-free device for SPION detection (favorable with positive contrast and high temporal and spatial distribution) is desirable for future biomedical applications.

In 2005, a new method for tomographic SPION imaging was introduced, named magnetic particle imaging (MPI). MPI takes advantage of nonlinear SPION magnetization behavior and allows direct imaging of the SPION distribution with positive contrast as well as high temporal and spatial resolution. Since tissue does not generate an MPI signal, this method is highly sensitive with low background noise. A theoretical estimation (Knopp and Buzug 2012) predicts that MPI is potentially sensitive enough to image 1 pg Fe, which means a sensitivity to detected single stem cells. First MPI images of labeled stem cells were shown in Saritas et al (2013), where a detection limit of about 10^4 cells was reached. A SPION labeled stem cell study in a rat was reported in Zheng et al (2015), where a detection limit of approximately 200 cells with 27.0 ± 3.3 pg (Fe)/cell was reported. However, localization and identification of SPION concentrations in MPI low enough for medical applications is still challenging due to artifacts.

There are different calibration methods available in MPI which can be used for image reconstruction. In measurement based calibrations, a small sample of SPIONs (‘δ-sample’) is shifted through a certain region in the scanner tube and measured at certain positions via external magnetic fields to obtain the system characteristics. The degree of freedom of mechanical rotation of the SPIONs is restricted depending on the viscosity of the binding of the SPION shell to the environment (e.g. proteins or lipids). Therefore, in different biological environments SPIONs respond differently to oscillating external magnetic fields, which has been

One issue which has not been investigated thus far is the influence of different SPION environments on calibration methods for stem cell imaging. This is especially relevant for biomedical applications such as cell tracking, hyperthermia, or targeted drug delivery where the SPIONs’ rotational degree of freedom is restricted due to binding to biological matter such as cell membranes, proteins, or lipids, or due to clustering in intracellular vesicles. The measurement based calibration procedure is widely used for phantom (Knopp et al 2010b) and in vivo (Weizenecker et al 2009, Rahmer et al 2013, Kaul et al 2015) experiments. Currently, image reconstruction in MPI is usually performed using a calibration matrix measured using SPIONs in a watery environment which is very inviscid. It has not yet been investigated whether this has a negative influence on the reconstruction result when biologically bounded SPIONs are imaged.

The goal of this work is to show that a mismatch between the SPION rotational degree of freedom in the δ-sample and the investigated object causes image artifacts that reduce the sensitivity of MPI. Using a matching calibration method can reduce artifacts, leading to an increase in sensitivity such that smaller amounts of labeled stem cells can be localized and identified.

The external magnetic field which acts on the SPIONs in MPI consists of a static magnetic gradient field (‘selection field’) and an oscillating magnetic field (‘drive field’) which are detected in signal receiver coils. The selection field provides a single field-free point (FFP) which is moved through space by the drive field. The gradient strength of the selection field and the drive field amplitude determine the field of view (FOV) in which the trajectory of the FFP is located. Detailed descriptions of the MPI principle have been published in Gleich and Weizenecker (2005), Knopp et al (2010a) and (2010c). In this work we will focus on fast imaging sequences up to 46 volumes/s using Lissajous trajectories (Knopp et al 2009) for the FFP.

Assuming a linear relation (Lu et al 2013) between the SPION distribution $\mathbf{c} \in \mathbb{R}^n$ (which provides the image) and the measurement signal $\hat{\mathbf{u}} \in \mathbb{C}^m$, the calibration method yields the complex $m \times n$ system matrix $\mathbf{S} \in \mathbb{M}_{mn}(\mathbb{C})$ of the linear system

$$\mathbf{Se} = \hat{\mathbf{u}},$$

(1)

column by column. The vector entries (coefficients) $\hat{u}_k, k = 1, ..., m$ of $\hat{\mathbf{u}}$ in equation (1) are complex amplitudes of the Fourier transformed signal which is detected in the receiver coils and related to the magnetization dynamics of the SPIONs. The index $k$ represents the index of the time-harmonic of the drive field. They are obtained by calculating

$$\hat{u}_k = -\hat{a}_k \frac{\mu_0}{T} \int_{\Omega_1} \int_0^T \left( \frac{\partial}{\partial t} \mathbf{M}(r, t) \right) \cdot \mathbf{p}_l(r) e^{2\pi i k t/T} dr dt,$$

(2)

where $\hat{a}_k$ is the transfer function (Knopp et al 2010a), $\mu_0$ the vacuum permeability, $\mathbf{p}_l(r)$ the sensitivity of coil $l$, and $T$ is the time interval used for a time-average of the signal. The transfer function depends on electronic hardware components build into the scanner while the coil sensitivity $\mathbf{p}_l(r)$ characterizes the field-generating properties of the coil and depends on the geometry of the coil. The subset $\Omega \subset \mathbb{R}^3$ contains all possible locations of the SPIONs within the scanner tube. The most important expression on the right side of equation (2) is the dynamics of the magnetic field $\mathbf{M}(r, t)$ generated by the SPIONs, which are driven by an oscillating external magnetic field. Basically, there are two mechanisms that can change the magnetic field direction $\mathbf{M}(r, t)$ of the SPIONs. First, there is the mechanical rotation of the particle (called ‘Brownian rotation’ or ‘Brownian relaxation’), which is mainly dominated by the force of the external magnetic field on the magnetic dipole moment, the hydrodynamic diameter of
the SPION, the electrical interaction strength between the outer SPION shell, the molecules in the environment (e.g. proteins, or lipids), and the viscosity of the environment (figure 1). Detailed descriptions for lysosomal metabolism of dextran coated SPIONs can be found in Arbab et al (2005). The other possibility for a change in $\mathbf{M}(\mathbf{r}, t)$ is a change in the internal magnetization dynamics of the $\text{Fe}^{2+}$ and $\text{Fe}^{3+}$ electron spins relative to the crystal structure (in an MPI context usually called ‘Neel rotation’ or ‘Neel relaxation’). The internal magnetization dynamics of the electron spins is mainly dominated by the Heisenberg exchange interaction between the $\text{Fe}^{2+}$ and $\text{Fe}^{3+}$ ions and the anisotropy energies (Them 2014a, 2014b, Them et al 2013, 2016) of the iron oxide nanostructures. Thus, a change in the viscosity of the environment or a change in the electrical binding properties between the SPION shell and the environment changes the Brownian rotation but does not affect the internal magnetization dynamics of the SPIONs. The magnetic stem cell labeling and gel phantom preparation for this work is described in the supplementary materials.

Figure 2(a) shows amplitudes at 177.65 kHz for phantoms with doubling SPION content in saline, gel, and MSC. The frequency with 177.65 kHz was chosen because of its high signal strength far above the background noise. It is the 3828th frequency in the spectrum. The SPION content in saline and gel agrees pairwise, starting at 11 $\mu$g Fe. The phantom with the lowest number of approximately 14 375 MSC contains a total of 8.8 $\mu$g Fe, which was estimated via atomic absorption spectroscopy (AAS). Thus, the Fe data on the x-axis corresponds to the Fe content in saline and gel. In all cases it can be seen that the immobilization of SPIONs causes a signal reduction in the amplitudes, as investigated using spectroscopic measurements in Arami et al (2013), Tu et al (2011) and Weaver and Kuehlert (2012).

Furthermore, it can be seen that in all three cases the signal strength approximates a linear dependence on Fe concentration. This linearity holds down to the smallest numbers of stem cells, meaning that the corresponding MSC phantom can be clearly identified at this frequency. This can be seen as an experimental indication for an absence of magnetic interactions between different SPIONs. The signal from the SPION labeled MSC look similar to the signal from the agarose gel bounded SPIONs. It is assumed that the signal drop is caused by a failure in the signal originating from the degree of freedom of mechanical rotation. Assuming that the immobilized SPIONs in agarose gel generate the same MPI signal as MSC bounded SPIONs, an average iron content per cell can be derived from the MPI raw data. The average across all 7 MPI measurements is 674 pg Fe/cell. AAS investigations of the labeled MSC give 615 pg Fe/cell, a roughly 10% deviation between the two methods.

However, figure 2(b) shows that the similarity between the MPI signals from agarose gel and from MSC is not valid for all frequencies. This can be seen as an experimental indicator that SPIONs like ferucarbotran (Resovist®, Bayer, Germany) possess a different mechanical
rotation in gel, MSC, or saline. The amplitudes in (b) are ranging between 0 and 20,000. All amplitudes in (b) are from the samples with highest amount of Fe, which are the same samples as for the most right amplitudes in figure 2(a). It can be seen that all amplitudes of the frequencies in (b) are of much smaller signal strength than the amplitudes in a) which ranges between 131,000 and 530,000. In (b) it can also be seen that the signal reduction is not given by the same factor for all frequencies. There are frequencies for which the MSC and gel signal reduction is very similar and there are frequencies for which the MSC signal reduction is not proportional to the gel signal reduction. In general figures 2(a) and (b) provides that there are
more similarities between signals of SPIONs in MSC and SPIONs in gel than for SPIONs in MSC and SPIONs in saline.

In Zheng et al (2015) it was found that the resolution of x-space MPI images worsened when SPIONs were inside the cells. Two potential reasons were listed for that, which are either the viscosity of the environment inside the cell which is causing increased relaxation effects, or the cell selectively uptakes smaller nanoparticles. While simulations using Fokker–Planck equations indeed showed an increasing relaxation time for SPIONs bounded to a viscous environment, a preferred uptake of smaller SPIONs could not be verified in our research studies. It was found that SPIONs with hydrodynamic diameter in the micrometer regime were also absorbed into MSC (Peldschus et al 2013, Salamon et al 2014). Additional experiments to the present work showed that for higher numbers of MSC the worsen of resolution in MSC images mainly occurred when mobile SPIONs were used for calibration. For higher amounts of labeled MSC and a calibration using immobilized SPIONs no strong loss of resolution was verified. One concludes that a loss of resolution due to a different relaxation dynamics can be compensated by using a similar relaxation dynamics in the calibration. However, the spatial resolution in MSC images becomes more worse than the resolution in images using mobile SPIONs for calibration and for the sample when the amount of iron is reduced in the samples. A reason for that is the signal loss (reduced amplitudes) of bounded SPIONs due to the restricted rotational degree of freedom. For low amounts of bounded SPIONs several frequencies immerse into the noise-level due to the signal loss and are therefore useless for reconstruction. Therefore, the images have to be reconstructed using much less suitable frequencies which reduces the resolution.

In a further step, we investigated the extent to which system functions $S^g$ and $S^c$ made by SPIONs in gel and MSC respectively are better suited to identifying MSC phantoms in reconstructed images than a system function $S^s$ made by SPIONs in saline. A typical sample of labeled MSC that was used for experiments is shown in figure 3.

The three different system functions (see supplementary materials) correspond to three different calibration methods for the MPI scanner.

Figure 4 shows reconstructed images obtained by solving $S^c = u^c$ (left side), $S^c = u^c$ (middle) and $S^s = u^s$ (right side), using the iterative Kaczmarz method (Kaczmarz 1937) see the appendix.

For each calibration method, stem cell phantoms containing approximately 57 500, 28 750, and 14 375 (8.8 $\mu$g Fe) magnetically labeled MSC were reconstructed. $S^s$ denotes a system function obtained by using approximately 920 000 magnetically labeled MSC with a total of
566 μg Fe, while $S^g$ and $S^s$ denotes SPIONs with 698 μg Fe in gel and saline, respectively. In all cases, $S^g$ is responsible for the emergence of artifacts. Thus, the artifacts originate from a mismatch in the magnetization dynamics between SPIONs the investigated sample and the SPIONs used for calibration. For 14 375 MSC, artifacts and MSC phantom marked by red arrows have similar signal strength, which makes an identification of the SPION labeled MSC phantom impossible.

The artifacts in the image with 57 500 MSC are of much lower strength or only occur on the boundary of the FOV, far from the phantom’s position. Reconstructing the MSC phantom with the gel system function provided images with negligible artifacts for 57 500 MSC. The reconstruction with the MSC system function leads to artifacts that only occur far away from the MSC phantom.

No reconstruction parameters for solving $S^c = u^c$ were found for which a clear identification of the MSC phantom with 14 375 MSC was possible. Using immobilized SPIONs for the calibration, the MSC phantom with 14 375 MSC can be identified in the reconstructed images obtained by solving $S^c = u^c$ or $S^c = u^e$. Since also immobilized, extracellular SPIONs contribute to the signal (see figure A1) it must be excluded that these extracellular SPIONs are alone responsible for this sensitivity enhancement. This exclusion was indirectly verified by an estimation of the factor for the sensitivity enhancement when immobilized, extracellular SPIONs alone are reconstructed. Since purely extracellular SPIONs and extracellular in combination with intracellular SPIONs enhance the sensitivity by the same factor it is evident that intracellular SPIONs enhance the sensitivity in a similar fashion as the immobilized, extracellular SPIONs. Since most frequencies of the phantoms containing 29 000 MSC and
14,500 MSC are immersed into the background signal and a background subtraction prior image reconstruction worsens image quality a quantification can only be performed using raw data. This surprising fact which is in contrast to the mobile SPIONs investigated in Them et al (2016) still needs to be explained. In analogy to the SNR defined in Them et al (2016), the SNR of the images from the 14,375 MSC phantom are 2.4 for the MSC SF, 1.8 for the gel SF and 1 for the watery SF.

Figure 5. A phantom containing approximately 14,375 MSC is located at two different positions: 1 (down) and 2 (up). The images are reconstructed with a SPION-in-gel SF (left side), an MSC SF (middle) and a watery SF (right side). The identification of the MSC phantom is only possible when the MPI scanner is calibrated with immobilized SPIONs (left and middle cases). A large artifact (marked by the red arrow) occurs when using the watery SF. The image size is 33 × 33 mm and the scan duration is 115 ms.

Figure 6. For a comparison reconstructed images of empty measurements are shown, when no SPIONs are located in the scanner. The same empty measurement is reconstructed with the same gel (left), MSC (middle) and watery (right) system functions as used for the phantom image reconstruction. The image size is 33 × 33 mm and the scan duration is 115 ms.

In figure 5, two different positions (1 (down) and 2 (up)) of a SPION labeled stem cell phantom with approximately 8.8 μg Fe are reconstructed using the same three system functions as described above. Using either the gel or the MSC system function for calibration, the MSC phantom can be identified correctly at the two different positions. Using the SPION in saline system function for calibration, where large artifacts (marked by a red arrow) occur, led to incorrect SPION-MSC localization in case 1. For the SPION in saline system function, no reconstruction parameters were found for which the MSC phantom could be clearly identified at both positions. A comparison to images of an empty measurement, when no SPIONs are located in the scanner is provided by figure 6.
This study can be seen as a proof of principle for an increase in sensitivity and image quality for stem cell reconstruction in MPI. However, as well as intracellular SPIONs, the study was also conducted with extracellular SPIONs, which lead to a very high ratio of pg (Fe)/cell. The extracellular SPIONs did not change the results, because they are immobilized by gel. But they have to be removed by washing for in vivo cell-tracking experiments.

In conclusion, it was shown that calibrating using rotationally immobilized SPIONs provides superior image quality for SPION-labeled stem cells with reduced artifacts compared to images obtained using rotationally mobile SPIONs (figure 4). The reduction in artifacts is valid for small and large amounts of magnetically labeled MSC. The increase in image quality allows identification of a significantly smaller number of SPION-MSC in MPI images (figure 5) and prevents false localization of SPION-MSC. Thus, in our dilution series the sensitivity limit was reduced from $\approx 29\,000$ MSC to $\approx 14\,500$ MSC (totally 8.8 $\mu g$ Fe) by switching from a calibration with mobile SPIONs to a calibration with immobilized SPIONs. The best MSC images were obtained using the gel SF. The reason for that should be the more homogeneous SPION distribution compared to the MSC SF resulting in reduced magnetic interactions between SPIONs.

The corresponding enhancement in sensitivity reduces the detection limit of low concentrated SPION distributions required for future clinical applications. Compared to other technologies for stem cell monitoring, MPI has the advantage of very fast temporal and good spatial resolution with positive contrast. However, the number of stem cells which can be imaged by MPI is still too high for several biomedical applications.

The method presented in this work to increase sensitivity and reduce artifacts in stem cell imaging brings MPI closer to the required number of stem cells that need to be imaged for clinical applications.

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Appendix. Supplementary

A.1. MPI measurements and reconstruction details

Experiments were carried out using the Philips preclinical MPI package with a Bruker preclinical MPI system. All experiments used ferucarbotran (Resovist®, Bayer Healthcare, Leverkusen, Germany) as the SPION source. The measurement based system matrices containing SPIONs in low melting agarose gel or saline (both 250 mmol (Fe)/l) were obtained using a cubic $\delta$-sample of size $3 \times 3 \times 3$ mm. The SPION loaded MSC system function was obtained using a 250 $\mu l$ Eppendorf tube as $\delta$-sample of similar size and shape. These $\delta$-samples were shifted to 25 equispaced positions in the $x$-direction, 25 equispaced positions in the $y$-direction and 25 equispaced positions in the $z$-direction in an area of size $50 \times 50 \times 25$ mm. The drive field FOV of $37 \times 37 \times 19$ mm was generated using a selection field with a gradient of 1.5 Tm$^{-1}$ in $z$-direction, 0.75 Tm$^{-1}$ in $x$- and $y$-direction and a drive field amplitude of 14 mT. The drive field frequencies are 24 509.8 Hz in $x$-, 26 041.7 Hz in $y$- and 25 252.5 Hz in $z$-direction. To ensure reduced fluctuations, 30 averages were chosen for the measurements. The SPIONs in saline sample phantoms consisted of 50 $\mu l$ drops of halving Fe concentrations.
The SPIONs in gel sample phantoms consisted of 25 μl drops of halving Fe concentrations plus 25 μl gel. The preparations of the gel and the SPIONs labeled MSC phantoms are described below. All reconstructed images were obtained using the iterative Kaczmarz method with 7 iterations and $\lambda = 0.05$, with a chosen SNR-threshold of 4.0.

### A.2. Culturing and SPION labeling of mesenchymal stem cells

The human mesenchymal stem cells (MSC) were harvested from bone marrow of adult humans as described in Lange \textit{et al} (2005) and grown adherent in standard T 75 culture flasks for more than 10 passages to confirm phenotype and purity using DMEM/Ham’s F-12 medium (Biochrom, Berlin, Germany) supplemented with 20% fetal calf serum (Gibco, Karlsruhe, Germany), 2 mol L$^{-1}$ L-glutamine, 100 U ml$^{-1}$ penicillin and 100 mg ml$^{-1}$ streptomycin (Gibco, Karlsruhe, Germany) as culture medium. MSC were incubated with SPION contrast agent ferucarbotran (Resovist®, Bayer Healthcare, Leverkusen, Germany) for 24 h at an incubation concentration of 560 mg Fe ml$^{-1}$ following the regime described in Ittrich \textit{et al} (2005).

After incubation, three washing steps with phosphate buffered saline (PBS) were performed to reduce extracellular SPIONs. Intracellular iron content was quantified by atomic absorption spectrometry (AAS) with a Perkin Elmer spectrophotometer 2100 (Norwalk, USA) using the fact that the absorption of element-specific (Fe) monochromatic light (wavelength 248.3 nm) during atomization of the probe is proportional to the iron mass of the sample. A concentration series was prepared resulting in 920 000, 460 000, 230 000, 115 000, 57 500, 28 750, and 14 375 cells, respectively. The cells were counted by a standard procedure using a ‘Neubauer counting chamber’ resulting in a homogeneous stock solution of 2.3 mio MSC in 1 ml volume. Then a certain part was taking off from this stock solution with a pipette and transferred into the phantoms. Imaging phantoms of cell samples were prepared in 250 μl Eppendorf tubes. Cells were pelleted by centrifugation and covered with a layer of 10% low melting agarose gel.

![Figure A1. The distribution of the SPIONs in the cell investigated by light microscopy.](image-url)
The SPION-gel concentration series was prepared by intermixing 25 μl (diluted) ferucarbotran with 25 μl gelantine (8%, 180 Bloom, Carl Roth GmbH, Karlsruhe) 2 : 1 in PBS. To avoid sedimentation, the probe was instantly cooled down and rigidified.

A.3. Stem cell images from light microscopy

Light microscopy of MSC (200 × prussian blue dye) produced an example of two cells with high perinuclear SPION accumulation after incubation with ferucarbotran (Resovist). Compared to previous studies, the intracellular accumulation is considerably higher. It can also be seen that extracellular SPION agglomerates are present, which are immobilized by gel.

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