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Sensitive and automated detection of iron-oxide-labeled cells using phase image cross-correlation analysis☆

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Abstract

Superparamagnetic iron oxide (SPIO) nanoparticles are increasingly being used to noninvasively track cells, target specific molecules and monitor gene expression in vivo. Contrast changes that are subtle relative to intrinsic sources of contrast present a significant detection challenge. Here, we describe a postprocessing algorithm, called Phase map cross-correlation Detection and Quantification (PDQ), with the purpose of automating identification and quantification of localized accumulations of SPIO agents. The method is designed to sacrifice little flexibility — it works on previously acquired data and allows the use of conventional high-SNR pulse sequences with no extra scan time. We first investigated the theoretical detection limits of PDQ using a simulated dipole field. This method was then applied to three-dimensional (3D) MRI data sets of agarose gel containing isolated dipoles and ex vivo transplanted allogenic rat hearts infiltrated by numerous iron-oxide-labeled macrophages as a result of organ rejection. A simulated dipole field showed this method to be robust in very low signal-to-noise ratio images. Analysis of agarose gel and allogenic rat heart shows that this method can automatically identify and count dipoles while visualizing their biodistribution in 3D renderings. In the heart, this information was used to calculate a quantitative index that may indicate its degree of cellular infiltration.

Keywords: SPIO; MPIO; Positive contrast; Iron oxide; Superparamagnetic; Cell labeling; Cell tracking; T_2; T_2-weighted imaging

1. Introduction

The detection of superparamagnetic iron oxide (SPIO) contrast agents in vivo often presents significant challenges. The contrast changes produced by these agents may be subtle relative to other sources of intrinsic contrast, especially if the SPIO biodistribution is not known a priori. In this article, we describe a novel postprocessing method that can aid in the automated identification of localized accumulations of SPIO agents. This algorithm, called the Phase map cross-correlation Detection and Quantification (PDQ) method, can be used to determine the quantity of paramagnetic deposits in tissues. We show that the PDQ method can help to reduce false positives and negatives in the analysis of SPIO-laden tissues.

SPIO agents and larger, micron-sized iron oxide (MPIO) particles locally perturb the static magnetic field over length scales on the order of ~50 times the particle's diameter [1]. This inhomogeneity causes nearby protons to rapidly dephase, leading to a dramatic reduction in the T_2 and T_2^* relaxation times. T_2^*-weighted images are particularly sensitive to these effects, and often, regions of agent accumulation show pronounced hypointensity or complete signal dropout. The development of new in vivo imaging methods using SPIO nanoparticles is currently a very active research area. Unmodified SPIO nanoparticles, such as Resovist and Feridex, are used for liver lesion detection and for distinguishing between normal and cancerous lymph nodes [2]. Modified SPIO particles, for example, conjugated

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to targeting moieties, such as peptides or antibodies, have been described [3–7]. Other studies have investigated whether SPIOs have detrimental cellular effects [3,8–10] or have improved methods for intracellular SPIO labeling [11,12]. In addition, the empirical detection limits of SPIO-labeled cells have been investigated [9,13,14]. Pilot studies have taken steps toward clinical translation of MRI cell tracking with SPIO agents, for example, demonstrating dendritic cell tracking in human cancer patients undergoing immunotherapy [15].

Often, a key challenge when using SPIO agents is to distinguish the contrast derived from agent accumulation from intrinsic sources of image hypointensity in T_2- and T_2^*-weighted images. To address this challenge, several imaging methods and pulse sequences have been devised to generate positive contrast images that highlight the presence of SPIOs and other tissue structures that create local magnetic field perturbations due to magnetic susceptibility differences. These techniques include, for example, weighted contrast methods [16,17], spectrally selective excitation [18,19], quantum coherence imaging [20], gradient dephasing [21] and ultrashort TE image subtraction [22]. Effective implementation of positive contrast pulse sequences often requires careful optimization and considerations. For example, positive contrast sequences may require a priori estimates of the field disturbance created by SPIO deposits for a particular experiment or require specific imaging hardware [18,21]. Positive contrast methods tend to diminish the signal-to-noise ratio (SNR) per unit scan time compared to conventional magnitude images in order to reap the benefits of positive contrast.

In this article, we describe novel postprocessing methods that can aid in the automated identification and quantification of localized accumulations of SPIO agents. The PDQ algorithm can help to reduce false-positive and false-negative results when SPIO deposits are localized, can be used in conjunction with conventional high-SNR imaging pulse sequences, requires no extra scan time and can be applied retrospectively to previously acquired data. A preliminary account of the PDQ method has been described elsewhere [23]. The PDQ method applies an image cross-correlation algorithm to high-resolution MRI data to identify occurrences of the characteristic magnetic susceptibility pattern created by magnetic dipoles in an MRI phase map. A similarity matrix is calculated showing the location and number of dipole patterns, indicative of localized, spheriodal SPIO deposits. We investigated theoretical detection limits using a simulated dipole field, which demonstrated the robustness of the PDQ algorithm in very low SNR images (<4). At all levels of SNR tested in the simulation, the PDQ method was ~90% accurate. As a further test, three-dimensional (3D) data were acquired in an agarose gel phantom lightly doped with MPIO. When analyzing this homogeneous agarose phantom, the PDQ method found 94% of the dipoles that were identified by visual inspection of MR phase-offset images. Next, we analyzed ex vivo 3D MRI data from transplanted allogenic rat heart specimens that were infiltrated with macrophages as a result of organ rejection; the macrophages were in situ labeled with MPIO nanoparticles using techniques described elsewhere [24]. The resulting 3D positive contrast images starkly highlighted labeled cells and other magnetic dipoles. Dipoles were automatically counted by computer, and their spatial biodistribution was visualized using 3D renderings. In the heart data, this information was used to calculate a quantitative index of MPIO accumulation that potentially reflects the severity of immune cell infiltration (i.e., an "infiltration index") and the stage of organ rejection. Overall, when analyzing heterogeneous heart tissue, the PDQ method found 79% of the dipoles that were also observed by visual inspection of MR phase-offset images.

2. Background

Raw MRI data are generally recorded in a complex form, where a voxel of intensity, I, has real and imaginary components (i.e., $I=a+bi$, $i^2=-1$). In conventional MRI, the magnitude image, given by $|I|=\sqrt{a^2+b^2}$ is typically displayed, and the phase angle information, $I=|I|e^{i\phi}$ where $\phi=\arctan(b/a)$, is typically discarded. Phase MRI maps have been studied extensively [17,25–27]. Generally, a voxel's phase is proportional to the local magnetic field; in our analysis, we exploit this property to analyze the magnetic field distortions caused by localized spheriodal deposits of paramagnetic agents in tissue. We decompose the phase contributions in each voxel as

$$\phi = \phi_0 + \phi_{\mathrm{INT}} + \phi_{\mathrm{MAT}} + \phi_{\mathrm{IOX}} \quad [-\pi \le \phi \le \pi] \tag{1}$$

where ϕ is the measured phase angle, ϕ_0 is the primary phase angle induced by the external magnetic field, ϕ_{INT} is the phase contribution due to the magnetic moments of nearby tissue–fluid interfaces, ϕ_{MAT} is the phase contribution due to the magnetization of a homogeneous tissue or fluid and ϕ_{IOX} is the phase contribution due to magnetic field perturbations of nearby paramagnetic deposits. The uncertainty in the measured phase angle is given by [28]

$$\sigma_\phi = 1/\mathrm{SNR}_{\mathrm{ROI}} \tag{2}$$

where σ_ϕ is the standard deviation in the phase angle in a region of interest (ROI) and $\mathrm{SNR}_{\mathrm{ROI}}$ is the SNR in the conventional magnitude image in the same ROI. In phase images, we define the phase contrast-to-noise ratio (CNR_ϕ) between an ROI and its background as

$$\begin{aligned}\mathrm{CNR}_\phi &= (\phi_{\mathrm{ROI}} - \phi_{\mathrm{BKG}})/\sigma_{\phi,\mathrm{ROI}} \\ &= \mathrm{SNR}_{\mathrm{ROI}}(\phi_{\mathrm{ROI}} - \phi_{\mathrm{BKG}})\end{aligned} \tag{3}$$

where ϕ_{ROI} is the measured phase angle for our ROI and ϕ_{BKG} is the background phase angle near the ROI containing no paramagnetic deposits. The phase map is constrained to the range from $-\pi$ to π and rolls through this range