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

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Introduction Superparamagnetic iron-oxide (SPIO) particles are increasingly being used to track cells and target specific molecules *in vivo*. SPIO detection and quantification is often challenging because of the sometimes subtle contrast produced by these agents or ambiguity between agent-laden tissue and intrinsic sources of tissue hypointensity. We describe a novel post-processing method, called the Phase map cross-correlation Detection and Quantification (PDQ) method, that automatically identifies and counts localized SPIO accumulations throughout an MRI volume. The PDQ method generates stark positive-contrast images showing the SPIO-labeled cell locations. The method is effective even in low-SNR images. We apply the PDQ method to gel phantom data and to rat heart infiltrated by SPIO-labeled macrophages. In these two examples, apparent dipoles were automatically counted and their distribution visualized in 3D. In the heart, these data were used to calculate a quantitative index of cellular infiltration (i.e., "infiltration index"). The PDQ method requires no special pulse sequences or extra scan time and works on previously acquired data.

Theory An MRI dataset is generally complex-valued, and typically only the magnitude image is displayed while phase angle information is discarded. A voxel's phase is proportional to the local magnetic field. We assume that spherical deposits of SPIO behave like small magnetic dipoles, creating a magnetic susceptibility pattern in the phase component of an MR image that can be described analytically by (1):

$$\phi_{Offset}(r,\theta) \propto \Delta B_Z(r,\theta) = \frac{\Delta\chi B_0}{3}\left(\frac{a}{r}\right)^3 (3\cos^2\theta - 1) \qquad [1]$$

where $\Delta\chi$ is the magnetic susceptibility difference between the spheroid and the surrounding tissue, B_0 is the magnetic field strength, a is the effective spheroid radius, r is distance from its center, and θ is the angular deviation from the direction of B_0. The PDQ method starts with a 3D model of this equation, or dipole template, and then applies a cross-correlation algorithm between the template and the high-resolution MRI phase data to identify occurrences of magnetic dipoles in an MRI phase map. Specifically, cross-correlating a template, T, with a source image, S, gives a resulting image, R, given by (2):

$$R(u,v) = \frac{\sum_{x,y}[S(x,y)-\bar{S}_{u,v}][T(x-u,y-v)-\bar{T}]}{\left[\sum_{x,y}[S(x,y)-\bar{S}_{u,v}]^2 \sum_{x,y}[T(x-u,y-v)-\bar{T}]^2\right]^{1/2}} \qquad [2]$$

where $\bar{T}$ is the mean pixel value of the template, and $\bar{S}_{u,v}$ is the mean of the image patch that is compared with the template. The cross-correlation algorithm systematically overlays the search template onto every template-sized patch across the phase image. The result is a 3D similarity matrix image, containing positive contrast spots indicating apparent dipole locations (i.e., labeled cells or SPIO accumulations) against a null background. Consequently, one can automatically count apparent dipoles in a region of interest or throughout an entire volume. Furthermore, similarity matrix images can be imported into 3D rendering software to visualize dipole distributions within organs and tissues.

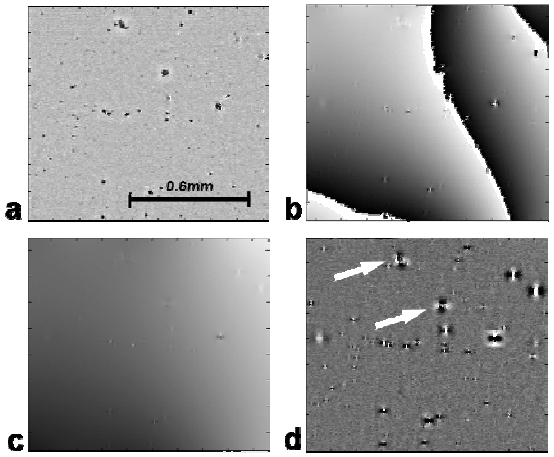

FIG. 1. Four image types of the same gel phantom MR data containing a mixture of MPIO particles, air bubbles, and undissolved agarose crystals. The image types displayed include: (**a**) magnitude image, (**b**) phase image, (**c**) unwrapped phase image, and (**d**) phase offset image. Arrows indicate unidentified diamagnetic dipoles. Each dipole in the magnitude image (**a**) appears as a dark spot against the background while those in the phase offset image (**d**) have a clear dipolar impression.

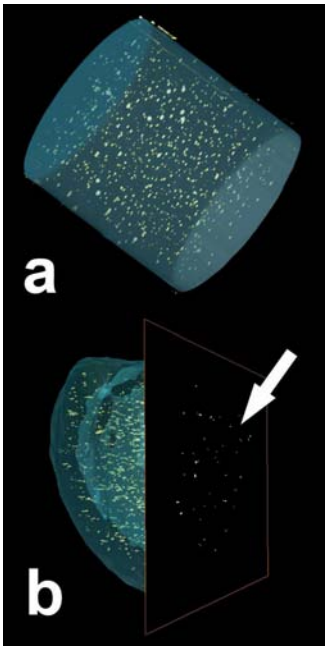

FIG. 2. 3D renderings of PDQ-detected dipoles. Shown is (**a**) gel phantom and (**b**) allograft rat heart infiltrated by MPIO-labeled macrophages. Gel and heart volumes are outlined in translucent blue, while dipoles are rendered as yellow spots. The arrow denotes dipoles found in a typical tissue slice. All dipole marks in the gel phantom appear spherical, but a fraction of marked areas in the heart tissue have linear shapes that may indicate curvilinear distributions of labeled macrophages or blood vessels with trajectory components parallel to B_0.

Methods As an initial test of the PDQ method, 3D MRI data were acquired in an agarose gel phantom that was lightly doped with micron-sized SPIO (MPIO) particles using a gradient-echo sequence with TE/TR=7/1500 ms and an isotropic resolution of 40 μm. The resulting MR phase data were unwrapped using an open-source unwrapping algorithm (3). The data were then high-pass filtered to remove the phase angle contribution from the background magnetic field. 3D templates were generated from Eq. [1], and the cross-correlation similarity operation in Eq. [2] was applied. The resulting similarity matrix image (R) was thresholded so that dipoles could be counted automatically by computer, and their spatial biodistribution visualized using 3D renderings. For an MRI slice to be analyzed using this technique, its in-plane orientation must have a component parallel to the applied magnetic field (B_0), as required by Eq. [1]. These methods were also applied to 3D MRI data of a transplanted allogenic rat heart *ex vivo* using similar imaging parameters as above. The hearts were infiltrated with macrophages as a result of acute organ rejection, where macrophages were labeled *in situ* with MPIO using techniques described elsewhere (4). A control isogeneic transplanted heart was also imaged. The dipole density in the rejecting heart and isograft was computed.

Results/Conclusions Fig. 1 displays PDQ results in a gel phantom slice, including several intermediate algorithm steps. Fig. 2 shows 3D volume renderings for the gel phantom (Fig. 2a) and in the myocardium undergoing rejection (Fig. 2b). Representative sub-volumes of the agarose phantom and heart allograft tissue were analyzed to characterize the method's sensitivity and accuracy. In the agarose, the PDQ method detected ~94% of the dipoles that were apparent via manual inspection, with a false positive rate of ~8%. In the heart allograft tissue, the PDQ method detected ~79% of the dipoles and had a false positive rate of ~15%. In the allograft heart, the dipole density, or infiltration index, was calculated to be 7.36 dipoles/mm^3, and we propose that this represents a quantitative marker of macrophage infiltration and thus rejection. Overall, the PDQ method generates positive contrast images that can be overlaid onto conventional magnitude images to selectively identify and quantify putative deposits of superparamagnetic agents in tissue. Importantly, the PDQ method can be used with conventional imaging sequences with no loss in SNR.

References

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