Sensitive positive contrast imaging of paramagnetic contrast agent distributions by visualizing phase gradients

P. H. Mills[1,2], and E. T. Ahrens[1,2]

[1]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Pittsburgh NMR Center for Biomedical Research, Pittsburgh, PA, United States

Introduction Superparamagnetic contrast agents (e.g., SPIO) are increasingly being used to track cells, target specific molecules, and monitor gene expression *in vivo*. The contrast produced by these agents is often subtle in T_2- and T_2^*-weighted images, making their detection challenging if agent biodistribution is not known *a priori.* To reduce detection ambiguity between agent-laden tissue and intrinsic sources of tissue hypointensity, we present a novel post-processing method that significantly enhances contrast and generates positive contrast images. This method detects the magnetic field perturbations produced by these agents via the phase component of the MR image. Importantly, the method performs all calculations in Cartesian image space rather than the Fourier domain, which simplifies the algorithm implementation. As an example of its effectiveness, we apply the method to a mouse brain that was locally inoculated with a recombinant, replication-defective adenovirus (AdV) carrying the human transferrin receptor (TfR-1) gene. Following virus transduction, cells over-express the iron storage protein ferritin, which has been shown to be an effective paramagnetic *in vivo* MRI reporter (1). The stark positive-contrast images produced by this method highlighted arbitrary distributions of the paramagnetic ferritin. The contrast-to-noise ratio (CNR) in these images was found to significantly exceed the CNR of conventional magnitude gradient-echo images by a factor of 4-25. Using whole-brain MRI, we visualized the ferritin distribution using 3D renderings. Importantly, this method requires no special pulse sequences or extra scan time and works on previously acquired data.

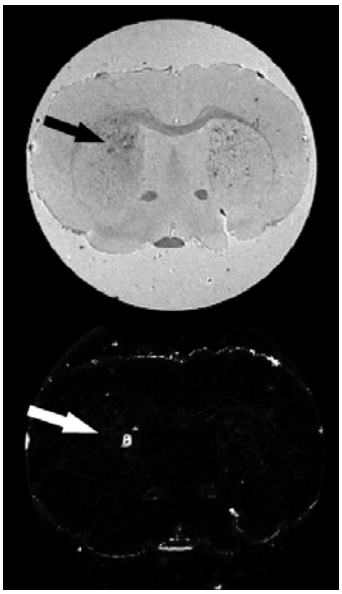

FIG 1. Magnitude image (top) and phase slope magnitude (PSM) image (bottom) for the same 2D slice of a mouse brain injected with an AdV-TfR-1 vector into the striatum. The arrows mark the location of the primary ferritin expression. LacZ-containing AdV was injected into the contralateral side as a control. In this particular axial volume slice, the ferritin deposit does not generate robust contrast for unambiguous detection in the magnitude image. However, the PSM image exhibits very substantial positive contrast.

Theory An MRI dataset is generally complex-valued, and typically only a magnitude image is displayed while phase angle information, which is related to the magnetic field distribution, is discarded. To extract magnetic field information from a phase map, a 'phase unwrapping' algorithm is applied that eliminates all $-\pi$ / $+\pi$ boundaries in the raw phase image (2). Next, to remove the low frequency phase ramp created by macroscopic and background magnetic field gradients, we apply a high-pass filter to the image; the result is a phase angle offset image. To further accentuate the magnetic field perturbations in the phase angle offset image, we calculate the phase gradient map via the relationship:

$$\vec{\phi}_{Slope} = \sum_{i=1}^{n} \frac{d\phi}{dx_i} \hat{x}_i \qquad [1]$$

where ϕ is phase angle offset and n indicates the number of image dimensions. Eq. [1] results in a vector field consisting of two components, the phase slope magnitude (PSM) and the phase slope direction (PSD). The simplest image one can generate from this vector field uses only the PSM values. To eliminate phase contributions from random noise, we use statistical methods (3) to automatically threshold the final images to generate more refined maps of the local magnetic field disturbances.

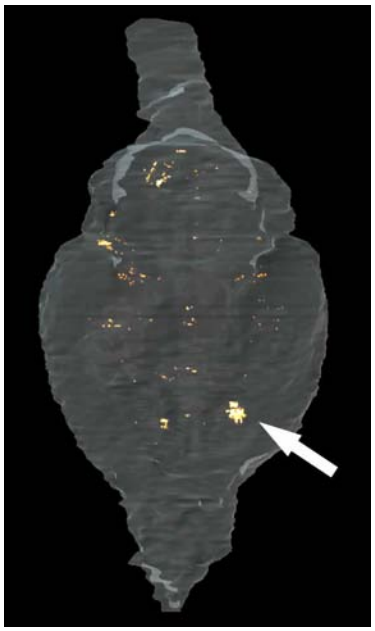

FIG 2. 3D visualization of the mouse brain shown in Figure 1. This ventral view magnitude image is rendered in translucent gray and overlaid with the PSM image. The arrow marks the location of ferritin expression; and the contralateral side (control) shows minimal contrast. The ferritin expressions shows pronounced contrast compared to other structures in the brain.

Methods To test the effectiveness of this method for highlighting paramagnetic agent deposits, we applied it to 3D MRI data acquired in an *ex vivo* C57BL/6J mouse brain with a transgene-induced localized ferritin deposit. The AdV-TfR-1 virus was inoculated unilaterally into the striatum, and a control virus carrying a LacZ gene was inoculated on the contralateral side. The AdV-TfR-1 construct has been shown to upregulate native ferritin in transduced cells, and is made superparamagnetic by sequestering endogenous iron from the animal (4). Five days post-inoculation the mouse was perfused and the brain excised and fixed in 4% paraformaldehyde. The brain was embedded in 1% agarose and imaged on an 11.7 T/89-mm vertical bore micro-imaging system. A gradient echo sequence was used for data acquisition with parameters TR/TE=6.4/50 ms, NEX=16, and 50 μm isotropic voxels. The resulting MR phase data were unwrapped using an open-source unwrapping algorithm (2). The data were then high-pass filtered to remove the phase angle contribution from applied magnetic field gradients. The 'phase slope vector field' was calculated using Eq. [1] for each image voxel. The magnitude component of this vector field was mapped to grayscale to form PSM images. The CNR of the PSM image and the conventional magnitude image were computed for comparison.

Results and Discussion Figure 1 displays a coronal PSM image and the corresponding magnitude image of the mouse brain. In the magnitude image, the ferritin deposit does not significantly alter the image contrast, while the PSM image displays dramatic contrast in the same deposit. The PSM image shows a unilateral ferritin distribution as expected, and no observed contrast from the contralateral control inoculation. Histology for reporters in the same brain corroborates MRI results (data not shown). Figure 2 shows a PSM whole-brain 3D visualization. The rendering shows that the ferritin produced in the brain has a magnetic perturbation effect that is more robust than any other source present. The calculated CNR values in a region of interest encompassing the ferritin deposit exhibits approximately a 4-to-25-fold increase in the CNR for the PSM image compared to the magnitude image.

Overall, the phase gradient method generates dramatic positive-contrast images that significantly highlight the contrast of arbitrary distributions of T_2 and T_2* paramagnetic contrast agents. The positive contrast images can be overlaid onto conventional magnitude images to selectively identify deposits of the labeled cells in an anatomical context. No additional scans are required, thus allowing for retrospective data analysis. Furthermore, the method requires no special acquisition parameters or pulse sequences, and it is applicable to a wide range of contrast agents.

References 1. Genove G *et al.,* Nat Med 2005;11:450-454.
2. Chen CW *et al.,* J Opt Soc Am A 2001;18:338-351.
3. Gudbjartsson H *et al.*, Magn Reson Med 1995; 34:910-914
4. Robison C, Ahrens ET, *et al.*, In Preparation