Off-Resonance Separation for Positive Contrast Imaging of Iron-Oxide Labeled Cells

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Introduction: Labeling of stem cells with super-paramagnetic iron oxide (SPIO) particles has shown great promise for visualization of these cells using MRI, and offers tremendous potential for tracking cells *in vivo* [1]. Traditional imaging using T2* weighted imaging rely on local disruption of magnetic field in areas with high concentrations of labeled cells. Hence, the labeled cells appear dark in a background of bright signal from areas with a more homogeneous magnetic field. Recently, Cunningham et al. introduced an elegant "positive contrast" method that uses spectrally selective RF pulses designed to excite off-resonance spins directly adjacent to iron labeled cells [2]. Stuber et al. have also reported a similar method [3]. In this way, regions that contain labeled cells appear bright, and background signal appears dark, increasing the conspicuity of the labeled cells through positive contrast. In this work, we describe a new positive contrast method that *encodes* off-resonance information into images acquired at different echo times, permitting the *separation* of off- and on-resonance spins.

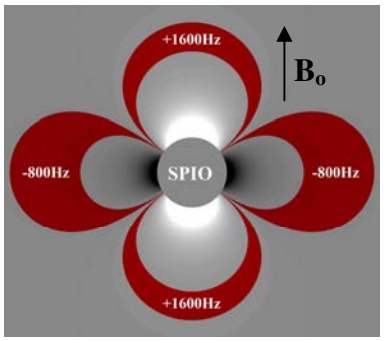

Figure 1: Schematic of the Bo field surrounding an iron oxide sphere. Polar lobes are positive while equatorial lobes are negative and half the magnitude of the polar lobes. Red areas contain spins near 1600Hz and -800Hz.

Theory: The signal from a voxel containing an on-resonance and one or more off-resonance population of spins is:

$$s(t_n) = \rho_o + \sum_{m=1}^{M} \rho_m e^{i2\pi \Delta f_m t_n} \quad (1)$$

where ρ_m and Δf_m are the signal contribution and off-resonance frequency of the m^{th} off-resonance population (m=1,…M), respectively. With a sufficient number of images (N≥M+1) acquired at different echo times, t_n, (n=1, …, N) the on- and off-resonance spin populations can be decomposed with a linear least-squares matrix inversion. Specifically, Eq. 1 can be written in matrix form: $\mathbf{S} = \mathbf{A}\boldsymbol{\rho}$ (2)

where $\mathbf{S} = [s(t_1)\ \ s(t_2)\ \ \ldots\ \ s(t_N)]$, $\boldsymbol{\rho} = [\rho_0\ \ \rho_1\ \ \rho_2\ \ \ldots\ \ \rho_M]^T$ and $\mathbf{A} = \begin{bmatrix} 1 & c_{11} & c_{21} & \ldots & c_{M1} \\ 1 & c_{12} & c_{22} & \ldots & c_{M2} \\ \ldots & \ldots & \ldots & \ldots & \ldots \\ 1 & c_{1N} & c_{2N} & \ldots & c_{MN} \end{bmatrix}$

with $c_{mn} = e^{i2\pi\Delta f_m t_n}$ for simplicity.

Estimates of the M+1 species are then made using the pseudo-inverse of Eq. 2,

$$\hat{\boldsymbol{\rho}} = (\mathbf{A}^H\mathbf{A})^{-1}\mathbf{A}^H\mathbf{S} \quad (3)$$

where the superscript H denotes Hermitian transpose.

As shown in Fig. 1 the magnetic field perturbation surrounding an iron oxide sphere has two polar lobes (θ = 0, 180° relative to the B_o field) which are symmetrical and positive, while the equatorial lobes (θ = 90°, 270°) have similar perturbations that are symmetrical, negative and half the magnitude of the polar field perturbations. For this reason, a spherical geometry lends well to a two off-resonance component system (M=2) with $\Delta f_2 = -\Delta f_1/2$. In the work by Cunningham *et al*, it was found empirically that frequency offsets of -800Hz, corresponding to the equatorial lobes of the field perturbation, gave optimal positive contrast. Thus, we chose M=2, and Δf_1=1600Hz and Δf_2 =-800Hz. Red areas in Fig 1. correspond to field perturbations of Δf_1 and $\Delta f_2 \pm$ 300Hz.

The frequency response of this model was calculated and plotted in Fig 2. The on-resonance signal (blue) and the sum of the two off-resonance components (abs(ρ_1)+abs(ρ_2)) (green) are plotted for a range of "test" frequencies, acquired using four echoes spaced by 300us. Note the wide passbands near -800Hz and 1600Hz.

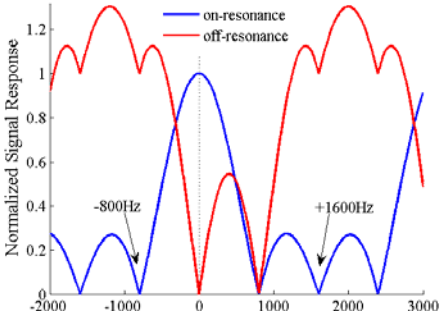

Figure 2: Frequency response of separation method for Δf_1=1600Hz and Δf_2 =-800Hz (M=2) and 4 echoes separated by 300μs.

Materials and Methods: Mouse skeletal myoblasts (C2C12; ATCC) were incubated with a mixture of SPIOs (100ug/ml; Feridex; Berlex Laboratories) and protamine sulfate (2ug/ml; American Pharmaceuticals Partner) in serum-free RPMI 1640 medium for 3hrs, followed by 24 hrs of incubation in supplemented DMEM medium (Gibco, 10% FBS, 1% P/S). Afterwards, cells were harvested by trypsinization, washed three times with PBS, and resuspended in PCR tubes at different cell concentrations (0.5 - 2.5x10^6 cells/350μl PBS). Tubes were embedded in 1% agar containing 0.5% copper sulfate.

Imaging was performed on a 1.5T GE TwinSpeed 11.0 MR scanner (Waukesha, WI) using a product extremity coil. Proton density weighted FSE images were obtained with a modified FSE sequence capable of shifting echo with respect to the spin echo, creating relative phase shifts from off-resonance effects. Echo shifts were 0.0, 0.3, 0.6, 0.9ms. TR/TE$_{eff}$= 2000/14.8ms, BW=±20kHz, 256 x 256 matrix size, FOV=10cm, slice=5mm, scan time=2:08min. Complex source images were processed with software written in Matlab (Mathworks, Natick, MA) that uses Eq. 3 to separate on- from off-resonance signal.

Results: Figure 3 demonstrates the separation of on- and off-resonance signal from SPIO labeled cells in axial images acquired through tubes containing 0.5x10^6 cells/350μl PBS. Although any presumed frequency shift can be used with this method, it was found empirically that Δf_1=1600Hz, and Δf_2 =-800Hz gave the best separation, in agreement with Cunningham's empirical finding that an excitation frequency of -800Hz gave optimal positive contrast. Recombination of the two images provides anatomical reference for the off-resonance signal. A small shift in the readout direction was necessary to correct for susceptibility induced shift.

Discussion: This work describes a new method for positive contrast imaging by *separating* signal from spins in regions of local off-resonance. This method encodes the off-resonance signal in complex images acquired at different echo times. Although this method requires a longer scan time to acquire multiple images, it has several important advantages over excitation methods. First, the reconstruction can retrospectively select different off-resonance frequencies, while excitation methods will fail if the wrong excitation frequency is chosen. Second, the separation approach provides an on-resonance image that acts as an anatomical reference as shown in Fig. 3 in a manner similar to hybrid imaging modalities such as PET-CT. Future work will address scan time reduction methods, characterization of the optimal echo spacing for SNR optimization, and sensitivity limits of this method.

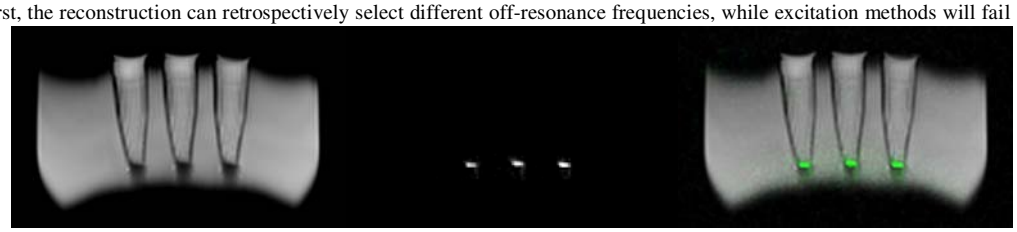

Figure 3: Separated A) on-resonance, and B) off-resonance images SPIO labeled myoblasts. Excellent separation of the signal from the labeled cells provides positive contrast with high conspicuity. C) Images can be recombined (off-resonance signal in green), providing anatomical reference for the off-resonance signal.

References:

1. Frank, et al. Radiology, 228: 480-487 (2003).
2. Cunningham et al, MRM 2005 53:999-1005
3. Stuber et al, ISMRM 2005, pg 2608