SPIO quantification using inversion recovery prepared bSSFP for targeted molecular imaging

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Introduction: Over the past two decades, pre-clinical MR molecular imaging studies using superparamagnetic iron oxide (SPIO) have significantly advanced the fields of cancer, immunology, regenerative medicine and drug development by providing non-invasive visualization of targeted cells and molecules. However, the vast majority of these studies are limited to showing the presence or absence of iron, rather than quantification of the amount of iron present. This lack of accurate quantification greatly limits these techniques in terms of investigating the comparative efficacy of therapeutic approaches, as well as gradations in immunological response. Most recently, balanced-SSFP (bSSFP) acquisitions have demonstrated exquisite sensitivity to SPIO labels [1], with superior off-resonance artifact resistance as compared to gradient echo acquisitions, suggesting their use for quantitative approaches. In fact, Schmitt et al. [2] showed that T₁, T₂ and proton density maps can be obtained from a single IR-prepped balanced SSFP (IR-bSSFP) acquisition.

In this work, we propose the use of IR-bSSFP for SPIO quantification by R₂ mapping. The two main challenges with quantifying SPIO over a practical dynamic range are the need to measure prohibitively short T₂ values, and the requirement that signal be suitably on-resonance for the Schmitt equations [2] to be valid. The difficulty with characterizing short T₂ values can be reduced with smaller flip angles, since this decreases the apparent recovery rate (R₁*) thus facilitating measurement, given that R₁* = R₀cos(ω/2) + R₂sin(ω/2) [2]. With a reduced flip angle, the signal becomes more R₁-weighted, thus decreasing R₁*. The difficulty with B₀ inhomogeneity is notable in pre-clinical animal imaging due to significant susceptibility to off-resonance banding artifacts, thus necessitating the acquisition of multiple acquisitions with different RF phase cycling (or frequencies). This problem was addressed through novel post-processing of RF phase-cycled acquisitions, after which IR-bSSFP quantification was achieved in vitro by exploiting the linear relationship between relaxation rate and iron concentration.

Methods: Phantoms were prepared using 20 nm SPIO nanoparticles (Ocean Nanotech, Springdale, AR) over a wide range of iron concentrations (0 to 200 µg/mL in 25 µg/mL increments). The nanoparticles were placed in 5 mm diameter NMR tubes after suspension in 4% gelatin doped with 8.4 µm/mO/L of Mn to better emulate physiological R₁/R₂ values. An array of tubes having different SPIO concentrations were supported in a 50 mm diameter, Mn-doped water-filled cylinder for imaging. All scans were performed on a 3T magnet equipped with a 21 cm ID gradient coil (Magnex Scientific, Oxford, UK) interfaced with a Varian DD Console (Varian Inc., Palo Alto, Ca) with an in-house built 52 mm diameter quad RF coil. Two-dimensional bSSFP was applied as previously described [3], with IR-prepped trains having half flip angle RF catalyzation (single 10 mm slice, 100 echoes, TI/TR/TE=10/5.25/2s, flip angle 20° or 35°, 50mmx50mm FOV, 128x128 matrix, 128 PE segments, 4 RF phase-cycled acquisitions).

Bloch simulations were performed in MATLAB (MathWorks, Natick, MA) to evaluate the B₀ inhomogeneity tolerance of R₁ and R₂ estimates in the range applicable for SPIO applications (Fig. 1). Voxel-wise fits of the IR curves for each RF phase-cycled acquisition (i.e., each frequency) were performed in MATLAB to obtain R₁ and R₂ values using the functions described in [2] (Fig. 2). To select the on-resonance frequency index, traditional MIP processing is inappropriate because low flip angles produce an off-resonance peak signal. Instead, the most on-resonance IR curve of the four phase-cycled acquisitions was chosen on a voxel-wise basis using a two stage process. First, the acquisition with the largest residuals was rejected due to oscillatory, non-monoeponential behaviour characteristic of stop-band signal, resulting in an unreliable fit [4]. Second, of the remaining three acquisitions, the smallest R₂ value was deemed on-resonance, in accordance with the Bloch simulation results.

Results and Discussion: Bloch simulations (Fig. 1) illustrated that four phase-cycled acquisitions were required, since these produced R₂ quantification errors limited to 11% (peak off-resonance of π/4), whereas two phase-cycled acquisitions produced unacceptably large R₂ deviations (50% for π/2 peak off-resonance). IR curves for a representative voxel (Fig. 2) revealed the oscillatory behaviour of off-resonant stop-band signal, and the significant sensitivity to off-resonance from the remaining three curves. Fig. 3 shows a cross-sectional bSSFP image of the phantom (A), along with maps of the R₂ values (B) resulting from the selected frequency indices (C). Very little contrast is evident between tubes with SPIO (A), since traditional bSSFP techniques have good detection, but poor quantification of SPIO concentration across practical dynamic ranges. However, very homogeneous R₂ maps are achievable with our method (B) despite a significant variation in off-resonance within the phantom (C). Fig. 3D shows the capacity of IR-bSSFP to produce linear R₁ and R₂ calibration curves across a substantial dynamic range of SPIO concentrations, with little variance resulting from off-resonance (small error bars showing standard deviation across voxels within tubes). Quantification was achieved up to concentrations of 75 µgFe/mL with 35° flip angles, and 100 µgFe/mL with 20° flip angles. This demonstrates the potential for dynamic range extension through use of lower flip angles.

Conclusion: We have demonstrated the ability of the IR-bSSFP pulse sequence to quantify SPIO using MRI by employing small flip angles and by addressing issues posed by B₀ inhomogeneity. This technique has potential to significantly advance the use of MRI for pre-clinical studies targeting molecules involved in drugs and disease.