# Application of Positive Contrast SSFP Imaging to USPIO-labeled Macrophage Cells: Theory and In Vitro Experiment

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**Introduction:** MR imaging of macrophages (Mϕ) in atherosclerotic plaque has been demonstrated through labeling with intravenously delivered ultrasmall superparamagnetic iron oxide nanoparticles (USPIO) [1,2]. In this application, the most striking evidence of cellular labeling is encroachment of the magnetic susceptibility artifact into the vessel lumen. Therefore contrast is generated not only from the voxels containing the labeled cells but also, and perhaps more notably, from the field inhomogeneity extending beyond that region. Fast low-angle positive contrast SSFP (FLAPS) has been proposed as a method to generate positive signal enhancement from dipolar fields surrounding a magnetic perturber[3]. We hypothesized that a region of USPIO-labeled Mϕ in plaque could be treated as a magnetic perturber and thus imaged using FLAPS. We sought to investigate theoretically and experimentally the feasibility of imaging USPIO-labeled Mϕ with FLAPS at clinical field strength (1.5T) and resolution (0.8 x 0.8mm in-plane). The objectives were to first determine if groups of labeled cells could generate off-resonant conditions with the spatial extent required for detection with FLAPS, and second to evaluate the dependence of positive contrast on flip angle and concentration of labeled cells.

**Theory:** SSFP signal intensity (SI) was approximated using equations described in Haacke et. al. (1999)[4]. For typical TR=5ms and blood[5] T1/T2=5, contrast between off- and on-resonance was determined as: SI($f$) – SI($f$=0), where $f$ is frequency in Hz. The Full-Width at Half-Maximum (FWHM) of the lobes of the contrast curves defined the range of frequencies with potential to contribute to positive contrast (Fig. A, shaded regions). The frequencies corresponding to the FWHM were input to a model of a spherical perturber of radius r = 3 mm, and change in susceptibility[6,7] $\Delta\chi$ ~ 10 ppm. The purpose of the model was to simulate the spatial extent ($Area_{SIM}$, Fig. A, inset), and average contrast ($Contrast_{SIM}$) of the regions associated with the FWHM frequencies. Simulated Visibility ($VIS_{SIM}$) defined as: $Contrast_{SIM}$ x $(Area_{SIM})^{1/2}$ , was evaluated for several flip angles and volume fractions of iron.

**Methods:** Human monocyte-derived Mϕ cells (THP-1, ATCC) were labeled by incubation with 50µg Fe/mL USPIO (SH U 555C, Bayer Schering Pharma, AG) for 24hrs. USPIO internalization was verified by Prussian Blue staining, and intracellular iron content was evaluated by ICP-AES. Cells were washed, fixed and pelleted in 750µL test tubes (radius of cell pellet ~3mm). The volume fraction of labeled cells was varied (33%, 66%, and 100%), while maintaining a total of $12x10^6$ cells per tube. The samples were submerged in a water bath doped with $MnCl_2$ (T1/T2 = 5). Images were acquired on a 1.5T Sonata scanner (Siemens Medical Solutions) using a product head coil for signal reception. Scan parameters for SSFP imaging were: TR/TE=5.0/2.5ms, $\alpha$= 5°, 10°, 15°, 25°, 35°, number of averages = 12, acquisition time = 3.72min and voxel size=0.8 x 0.8 x 3mm$^3$. Images were evaluated using MATLAB. Pixels with contrast-to-noise ratio (CNR) greater than 10 were considered to contribute towards positive contrast. Overall positive contrast was quantified with the imaging metric Visibility ($VIS_{EXP}$): $VIS_{EXP} = CNR_{PC} \times (n_{PC})^{1/2}$, where $CNR_{PC}$ is the average CNR of all pixels contributing to positive contrast, and $n_{PC}$ is the number of pixels contributing to positive contrast. CNR was calculated as $CNR = (SI – SI_{BACKGROUND})/\sigma_{AIR}$.

**Results:** Theoretically, as flip angle decreased from 35° to 5°, maximum contrast increased, but spatial extent decreased (Fig. A). $VIS_{SIM}$, a metric combining contrast and spatial extent, peaked at a flip angle of about 15°. Similar results were obtained experimentally; positive contrast ($VIS_{EXP}$) was maximal at a flip angle of 15° for all concentrations of labeled cells (Fig. B). The spatial extent of positive contrast for cell concentrations of 100% and 66% was greater than 15mm$^2$ (~20 pixels); however for 33% the spatial extent was less than 1mm$^2$ (~1 pixel) (Fig. C). Iron content per labeled cell was 1.24±0.02 pg Fe/cell (mean ± standard deviation).

**Discussion:** Theoretical and experimental results indicate that it is possible for groups of USPIO-labeled Mϕ to generate the off-resonant conditions required for detection with FLAPS at clinical field strength and resolution. Detection was achieved at a labeled cell concentration of 66% or greater for a perturber of radius r = 3mm with $\alpha$ = 15° and background T1/T2 = 5. These results may guide application of the FLAPS technique for plaque imaging or other applications of iron-oxide-labeled cells.

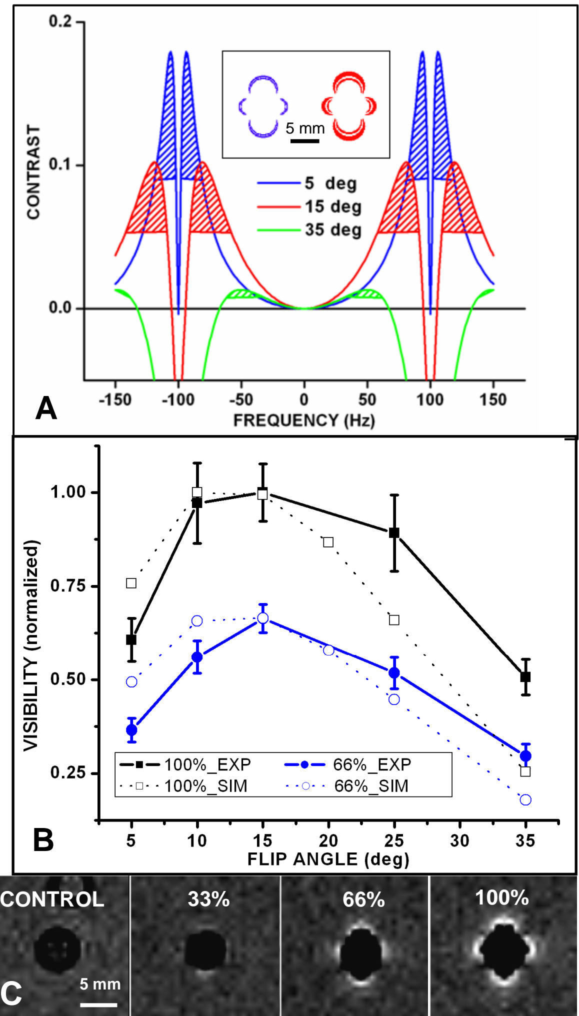


**Figure**. **A)** Theoretical contrast curves for $\alpha$ = 5° (blue), 15° (red), and 35° (green). Shaded regions indicate the FWHM range for positive contrast at each flip angle. Inset: Spatial extent of each FWHM band surrounding a spherical magnetic perturber. **B)** Normalized visibility, both experimental (solid lines) and theoretical (dashed lines) for flip angles 5°-35°. **C)** Representative cross-sectional images of cell pellets with 0%, 33%, 66%, and 100% labeled cells for $\alpha$ = 15°.

**References:** [1] Ruehm SG et al, Circ 200; 103:415; [2] Hyafil F et al, ATVB 2006; 26:176; [3] Dharmakumar R et al, Phys. Med. Biol. 2006, p. 4201; [4] Haacke EM et al, Magnetic Resonance Imaging 1999, p. 350, 474; [5] Stanisz GJ et al, MRM 2005; 54:507; [6] Weisskoff RM et al, MRM 1992; 24:375. [7] Bowen CV et al, MRM 2002; 48:52.