# Expanding Applications of Cellular MRI by Combining Fluorine-19 and Iron-Oxide Techniques

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## Introduction

The ability to visualize cells and cell migration non-invasively with imaging is a powerful tool for basic biomedical research and medicine. For cellular MRI, cells labeled with iron-oxide nanoparticles can be detected with high sensitivity because of the effect on the nuclear relaxation properties of the surrounding water. Conversely, cells labeled with perfluorocarbon (PFC) can be uniquely detected in low SNR images with $^{19}$F MRI. Combining these two techniques has the potential to expand and improve cellular MRI applications. For example, if PFC labeled cells can be imaged and quantified in the presence of iron-oxide labeled cells, then two cell populations can be simultaneously tracked. In addition, a change in PFC $T_2$ and $T_2$* due to the presence of iron-oxide nanoparticles may be used to identify cell-cell proximity or co-labeled cells.

## Methods

A series of labeled cell phantoms were prepared to investigate the effect of iron-oxide nanoparticles on $^{19}$F NMR relaxation and imaging properties of PFC-labeled cells. Three types of cell phantoms were prepared: PFC-labeled cells, mixed populations of PFC- and iron-oxide-labeled cells, and PFC/iron-oxide co-labeled cells (i.e. two different labels within one cell). Fetal Skin-derived Dendritic Cells (FSDCs) were labeled in culture with PFC (CS-1000, Celsense, Pittsburgh PA). Iron-oxide-labeled cells were prepared by co-culture with different concentrations of iron-oxide particles obtained from Bangs Laboratories (Fishers IN), BioPal (Worchester MA), or ITRI-IOP (1). Co-labeled cells were prepared by either consecutive or simultaneous co-incubation with two reagents. $6 \times 10^6$ cells were pelleted for each sample. Mixed cell populations were prepared by mixing $3 \times 10^6$ PFC labeled cells with $3 \times 10^6$ iron-oxide labeled cells. Samples were imaged at 7 Tesla (Bruker Biospec) using FLASH, RARE, and Ultra-short TE (UTE) pulse sequences, for $T_2$*-weighted, $T_2$-weighted, and density-weighted images, respectively. NMR spectroscopy was also used to investigate relaxation properties and $^{19}$F NMR linewidths in cell pellets and cells diluted 5x in agarose.

## Results and Discussion

In cell phantoms, the presence of iron-oxide particles did not significantly affect the $^{19}$F PFC $T_1$. For mixed populations of cells, small-to-modest non-homogeneous line broadening and down-field shift were observed depending on iron-oxide concentration and cell-cell proximity. For co-labeled cells, line broadening was more severe (Figure 1), especially for cell pellets (not shown). In co-labeled cells, less than 1 pg Fe/cell (ITRI-IOPC) was needed to reduce the $^{19}$F $T_2$ by a factor of 10. $^{19}$F images of cell pellets demonstrate that the $^{19}$F relaxation properties can be used to identify different cell populations. A $^{19}$F UTE image can be used to show all $^{19}$F-labeled cell populations (Figure 2A), a $T_2$-weighted RARE can be used to filter out co-labeled cell populations (Figure 2B), and a $^{19}$F gradient-echo can be used to filter out signal from mixed and co-labeled cell populations (Figure 2C). Studies using rodent models are underway to demonstrate *in vivo* application of this technique.

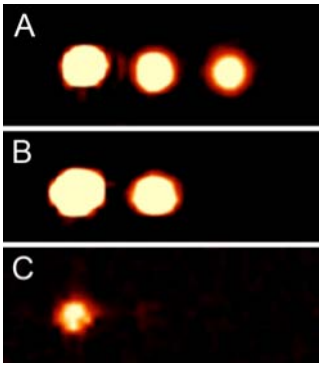


Figure 2. $^{19}$F UTE (A), RARE (B) and FLASH (C) images of cell pellets containing PFC-labeled-FSDCs (left), a mixed population of PFC- and ITRI-IOP-labeled FSDCs with 0.45 pg Fe/cell (center), and co-labeled FSDCs with 0.56 pg Fe/cell (right).

## Conclusions

Combining $^{19}$F and iron-oxide cellular MRI techniques has the potential to expand and improve cellular MRI applications. Two cell populations could be tracked with $T_2$*-weighted $^1$H imaging for iron-oxide cell populations, and $^{19}$F RARE imaging for $^{19}$F-labeled cells. In addition, systemic iron-oxide labeling of macrophages may be used to quench the $^{19}$F signal taken up from dead PFC-labeled cell transplants, a major limitation to current MRI cell tracking studies.

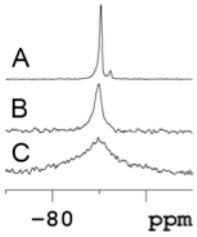


Figure 1. $^{19}$F NMR Spectra of cells in agarose: (A) PFC-labeled cells; (B) mixed population and (C) co-labeled cells. B and C contain 0.45 pg Fe/cell for ITRI-IOP labeled cells.

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[1]Chen CL, Zhang H, Ye Q, Hsieh WY, Hitchens TK, Shen HH, Liu L, Wu YJ, Foley LM, Wang SJ, Ho C. Mol Imaging Biol. 5, 825-39 (2011).