

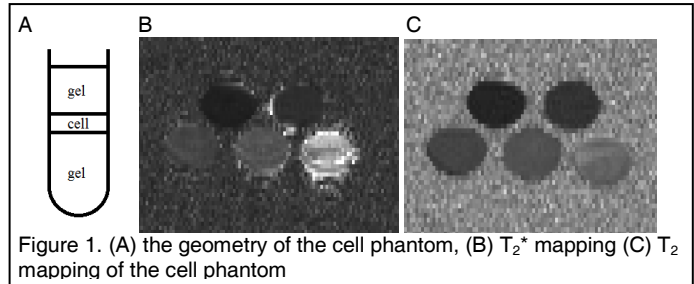
# Quantification of Cells Labeled by Superparamagnetic Iron Oxide Particles Using $R_1$ , $R_2$ , and $R_2^*$ mappings: A Comparative Study Using Cell Phantoms

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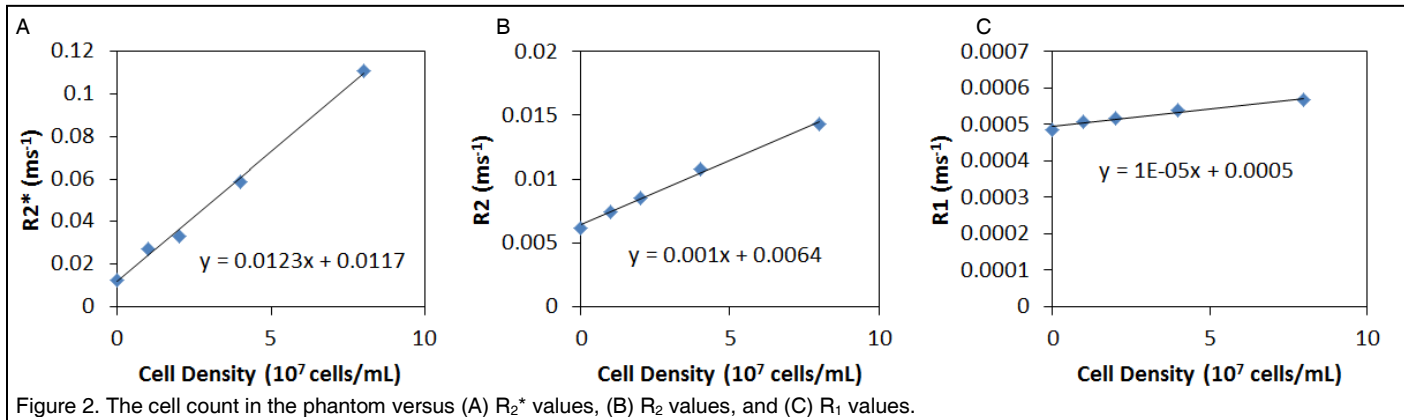
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**Introduction:** Ultra-small superparamagnetic iron oxide (USPIO) and micrometer-sized superparamagnetic iron oxide (MPIO) particles have been used to label macrophages and to detect the tissue inflammation based on  $T_2^*$ -weighted [1][2][3]. Quantification of the cell density using iron-oxide particles, however, is complicated by the blooming effect of the iron-oxide particles accumulated in the cells, which renders MR signals different from that of free particles in homogeneous distribution [4][5]. In this study, we created a cell phantom that simulates the in vivo condition, where the different density of labeled cells were mixed with raw matrix cells to form a mixture of cell samples that mimic in vivo conditions. We acquired  $R_1$ ,  $R_2$ , and  $R_2^*$  maps to investigate whether we can quantify the amount of label cells by these relaxation time mappings.

**Methods:** Cell preparation Inbred male BN rats were euthanized and the splenocytes were isolated by repeated division and perfusion of the spleen in a Petri dish. Macrophages were isolated by decanting the culture medium and removing non-adherent cells. The adherent macrophages were allowed to grow for 10 days and labeled with USPIO particles (Molday ION, BioPAL, Worcester, MA) (2 mg Fe/mL) in 5 mL of cell culture medium for 24 hr. The averaged iron concentration into the labeled cells was 1.25 pg Fe/cell, measured by colorimetric assay. The labeled macrophages were divided into two five different cell counts before mixing with raw cells (cells not labeled with USPIO) to create 5 phantoms with labeled cell density of  $1.0 \times 10^7$ ,  $2.0 \times 10^7$ ,  $4.0 \times 10^7$ , and  $8.0 \times 10^7$  cells per mL, respectively. The number of cells was measured by trypan blue. The cell phantoms were then embedded in 2% agarose gel, as shown by Fig. 1(A).



**MRI scan** The phantom was scan on a 7-T Bruker scanner. The  $T_2^*$  map of the phantoms was acquired by a multiple gradient echo (MGE) sequence. A total of 20 effective echo times (2, 4, 6,..., and 40 ms) were used to acquire the images. The repetition time was 2 sec. The  $T_2^*$  map was obtained by applying a log-linear regression to the MR signals. The  $T_2$  map of the phantoms was acquired by a RARE  $T_2$  sequence, and a total of 64 effective echo times (6, 12, 18,..., and 384 ms) were acquired. The repetition time was 2 sec, and number of average=2. The  $T_2$  map was obtained by applying a log-linear regression to the MR signals. The  $T_1$  map of the phantoms was acquired by a RARE sequence with variable repetition time TR (RAREVTR) sequence. A total of 8 repetition times (100, 253, 433, 654, 937, 1334 ms, 2 sec and 5 sec) were acquired. The effective TE was 11.4 ms, and a RARE factor of 4 was used. The  $T_1$  map was obtained by applying a log-linear regression to the MR signals. The spatial resolution for the MGE, RARET2, RAREVTR scan was 0.2 mm x 0.4 mm. Slice thickness = 0.6 mm.



**Results and Discussion:**  $R_1$ ,  $R_2$ , and  $R_2^*$  values shows a good linear correlation with the labeled cell density (the correlation coefficients are 0.980, 0.997, and 0.998, respectively), suggesting that any of them can be used to quantify density of the labeled cells in the cell phantom. The sensitivity of  $R_1$ ,  $R_2$ , and  $R_2^*$  mappings, however, varies a lot.  $R_2^*$  values show the highest sensitivity (coefficient=0.01), which is approximately 10 times more sensitive than that of the  $R_2$  values (coefficient=0.001), and 1,000 times sensitive than that of the  $R_1$  values. This result suggests that the  $R_2^*$  imaging is the most sensitive measurement to quantify the cell density at  $10^7$ - $10^9$  cells per mL. As the density reaches  $10^9$  cells per mL,  $T_2$  value is a better index to quantify cell density, since the projected  $T_2^*$  value will be smaller than 1 ms, a value that may not be accurately measured. Furthermore, as the cell density reaches  $10^{10}$  cells per mL,  $T_1$  values can provide better quantification. The optimal setting will depend on the labeling efficiency and/or the type of labeling particles used

**References:** <sup>1</sup>Wu, YL et al. PNAS 2006, 103, 1852-1857. <sup>2</sup>Ye, Q et al. Circulation 2008, 118, 149-156. <sup>3</sup>Wu, YL et al. JACC Cardiovasc Imaging 2009, 2, 731-741. <sup>4</sup>Mowat, P.2007. NMR Biomed 20, 21-27. <sup>5</sup>Rad, A.M.2007. J Magn Reson Imaging 26, 366-374. This work is supported by NIH grant P41EB-001977

