

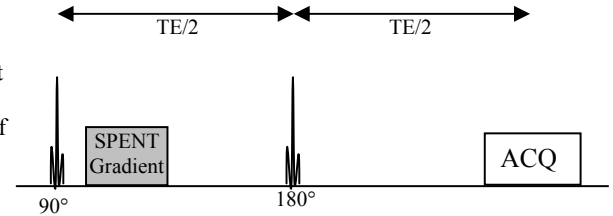
# Detection of human mononuclear cells labelled with micron-sized iron oxide particles using the Sub-pixel Enhancement of Nonuniform Tissue (SPENT) sequence

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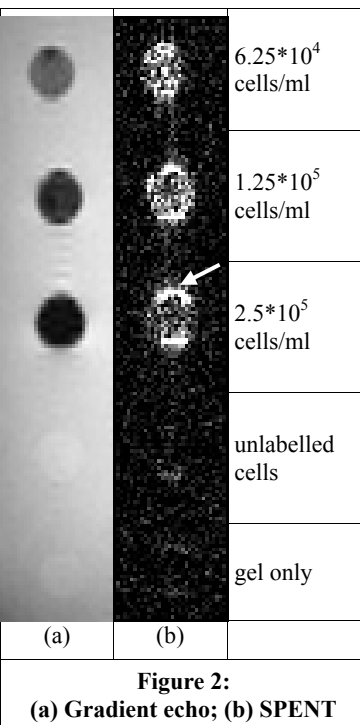
**Introduction:** There has been increasing interest in the use of micron-sized iron oxide particles (MPIO) for cell tracking [1]. T2\*-weighted acquisitions are traditionally used for the spatial localisation of MPIO labelled cells, resulting in large hypointense regions in the images. Endogenous tissue contrast, such as lungs or bone marrow, may also produce similar hypointensities which is problematic for unambiguous spatial localisation of MPIO labelled. Recently, positive contrast techniques have been shown to produce hyperintensities from superparamagnetic contrast agents [2], which would traditionally generate hypointensities. In this study, we have assess a novel technique know as Subpixel Enhancement of Nonuniform Tissue (SPENT), which has recently been used for quantification of Bone Mass Density [3], as a positive contrast spin echo technique for the detection of MPIO labelled human mononuclear cells. Spatial localisation of SPIO using a gradient echo SPENT sequence has recently been reported [4]. Spin-echo acquisition would help mitigate signal loss that is seen in gradient echo acquisitions.

**Pulse sequence and theory:** A simplified schematic of the SPENT sequence is presented in figure 1. The SPENT sequence consists of a conventional spin echo sequence with an additional gradient between the excitation and refocusing pulses that applies  $2\pi$  phase dispersion across a voxel. The amplitude of the SPENT gradient applied is  $G_s = 2\pi/\gamma t_s r$ , where  $t_s$  is the duration of the SPENT gradient,  $r$  is the length of the pixel. In magnetically homogeneous regions, the SPENT gradient causes the net signal in a voxel to be zero. Therefore the phases of the transverse magnetisation of spins across the length of the voxel in which the gradient is applied is uniformly distributed over 1 cycle ( $2\pi$  radians); thus the phase of spins at a particular position is anti-parallel to the phase of spins half a voxel length away. In the case where there are magnetic inhomogeneities within a voxel, the phase of spins across the voxel are no longer uniformly distributed because the inhomogeneities would perturb the net phase of the spins. Thus, signal should be visible in regions of sub-pixel magnetic inhomogeneities.



**Figure 1 Schematic of SPENT sequence implemented**

**Methods: MRI acquisition:** The sequence was implemented on a Varian Inc 9.4T imaging system equipped with a Rapid Biomedical GmbH 72mm diameter r.f. coil. The following parameters were used for a SPENT acquisition: SPENT gradient was applied in the read direction for 1ms at 75.2 mT/m; TE = 17ms; TR = 3s, 256x128 matrix, 80x40mm FoV (read and phase directions respectively), 1 slice, 1mm slice thickness, receiver bandwidth of 52kHz. A gradient echo scan of the same slice was acquired with 20° excitation pulse, TR=200ms, TE=5ms, 128x128 matrix, and other parameters identical to those of the SPENT acquisition. **Sample preparation:** Human mononuclear cells (MNCs) were labelled with 120µg per ml media of 1.5 µm diameter BioMag particles (Bangs Laboratories Inc) for 24 hours. They were then trypsinized, washed twice with PBS, magnetically separated and re-suspended in PBS. The cell suspension was diluted with equal volumes of PBS and 2% agarose solution to produce solutions containing  $2.5 \times 10^5$ ,  $1.25 \times 10^5$  and  $6.25 \times 10^4$  cells/ml. The agarose solutions were then placed into 5mm diameter wells formed in a 2% agarose gel phantom. Once set, the phantom was filled completely with 2% agarose to reduce susceptibility artefacts.



**Results and Discussion:** Figure 2 shows gradient echo and SPENT images of the gel phantom with MPIO labelled cells (only part of the total field of view shown). The gradient echo scan clearly shows hypointensities in the gel wells that contain MPIO labelled cells. The hypo-intense regions appear to be quite homogeneous in intensity. Conversely, the SPENT images clearly show hyperintensities in the gel wells that contain MPIO labelled cells. In regions that are magnetically homogeneous (e.g. surrounding gel), no contrast is seen. Hyperintensities appear more prevalent in regions near the boundary between gel containing MPIO labelled cells and the surrounding gel (for example, arrow in figure 2(b)). In particular, contrast is seen where the boundary has a component normal to the direction of the applied SPENT gradient (top-bottom of page in figure 2); the SPENT gradient could be applied in different directions to obtain an isotropic measurement. The method is spin echo based hence large signal drop out problems are mitigated. For both MPIO labelled cells and boundary regions, sub-pixel susceptibility gradients perturb the field, causing incomplete phase cancellation within a voxel and thus signal is seen; future work will try to use multiple SPENT acquisitions to separate these effects. Equivalent scans of a phantom that only consists of MPIO in gel (i.e. not inside the cells) show no contrast except at boundary regions (data not shown): the distribution of the MPIO is more evenly spaced over an imaging voxel, making those regions more magnetically homogeneous and thus not generating any SPENT contrast at this resolution; different resolution images could be used to probe different SPIO distributions and concentrations by causing net signal of a different length scale.

**Conclusions:** The spin-echo SPENT sequence clearly demonstrates positive contrast in regions that contain subpixel inhomogeneities in the magnetic field. Hyperintensities in SPENT images match up well with hypointensities in the gradient echo images. These results demonstrate that SPENT is a viable positive contrast technique that has directional information. Potential advantages over traditional positive contrast techniques are that the sequence may provide both directional and quantitative information on iron concentration and distribution. The additional information that SPENT provides may lend itself to the assessment of susceptibility effects of top-down microfabricated superparamagnetic particles [5].

**References:** 1. Shapiro, E.M. et al. MRM, 2005. 53: p. 329 ff. 2. Cunningham, C.H., et al. MRM 2005. 53: p. 999 ff. 3. Yiannakas M.C. et al, J Bone Miner Res 2009;24:324 ff. 4. Carmichael, D.W., Proc 13th ISMRM;2005 p2613. 5. Zabow, G., et al., Nature, 2008. 453(7198): p. 1058 ff.