

## On Possible Pitfalls in Working on SPIO Labelled Cells with 2D UTE Sequences

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### Introduction

In the past years many strategies for the in-vivo detection of cells have been proposed. Many of these approaches are based on superparamagnetic iron oxide (SPIO) nanoparticles to overcome the inherent low sensitivity of MRI to common T1 based contrast agents [1]. In addition new pulse sequences were introduced to gain positive contrast out of the native dark T2\* contrast of SPIO particles [2]. One promising method is the use of ultra short echo time sequences (UTE) [4,5] either to acquire the T1 signature of SPIO particles or to gain positive contrast through double echo with subtraction. Latter method obtains the first image from the free induction decay and the second some 100 $\mu$ s later. Within this study we would like to outline some crucial points concerning the use of 2D UTE sequences for this purpose. We show that especially in regions of high iron density many artefacts caused by the pulse sequence could yield to misinterpretations or wrong quantitative results. On the basis of an ectopic labelled cell population we discuss the artefacts caused by a common 2D UTE acquisition strategy with half-pulse excitation.

### Material and Methods

**Cell preparation:** Clinical scale EPC/MSK propagation was done in an animal serum free system. Cell labelling was performed with 3 $\mu$ g/ml protamine sulfate and 50 resp. 100 $\mu$ g/ml Fe Resovist (Schering, Germany) solution within 24 hours. Sub-cellular SPIO distribution was investigated and confirmed by transmission electron microscopy. 2 million cells (MSC/EPC: 20/80%) were injected into the left and right flank of athymic nude mice whereas the MSC were labelled with 50 and 100 $\mu$ g/ml Fe (left/right flank respectively).

Cellular iron quantification was measured through intracellular magnetization in-vitro. The used susceptometry technique was carried out as proposed by Bowen et al.[3] by fitting the magnetic field-profile caused by a cylindrical sample of the loaded cells. The iron uptake could be determined as 15.72  $\pm$  1.5 pg Fe/cell for 50 $\mu$ g/ml and 27.81 $\pm$  2.1 pg Fe/cell for 100 $\mu$ g/ml Fe respectively. Images were taken after 5 weeks after injection.

**Used Sequences:** The scans were performed on a 3T clinical scanner with an 8CH multipurpose coil array. A turbo-spin-echo sequence was performed as a reference image: 256x256 matrix, resolution 137x137x1500 $\mu$ m, TR/TE 2230/13ms, TF 9, NSA 5.

2D-UTE sequence with trajectory and phase correction. Image was taken in the ISO-center: 192x192 matrix, resolution 193x193x1150 $\mu$ m, BW 62.6 kHz /spoke, 400 half-echos over 2 $\pi$ , sampling on RO ramp-up, VERSE-half-pulse with 1140 $\mu$ s duration, TR/TE 50ms/75 $\mu$ s..1210 $\mu$ s. NSA 12.

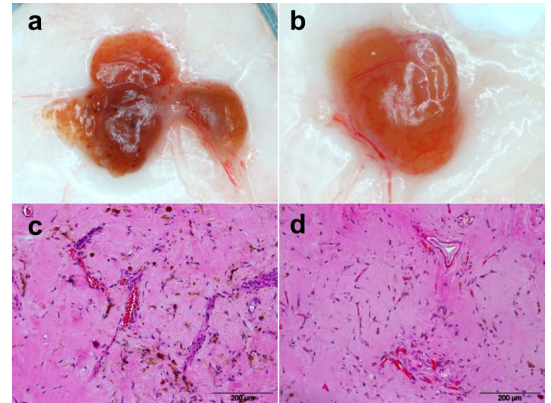
### Results and Discussion

Both labeled compartments can clearly be detected in the transversal TSE reference image (Fig. 2 a). Compared to this image pronounced blurring in the UTE results (Fig. 2 b,c). This smearing is caused by an improper combination of the two half-k space spokes with opposite gradient polarity. This problem is emphasized for in-vivo measurements influenced by chemical shift. In our case, the subcutaneous fat produces a displaced duplicate of the skin.

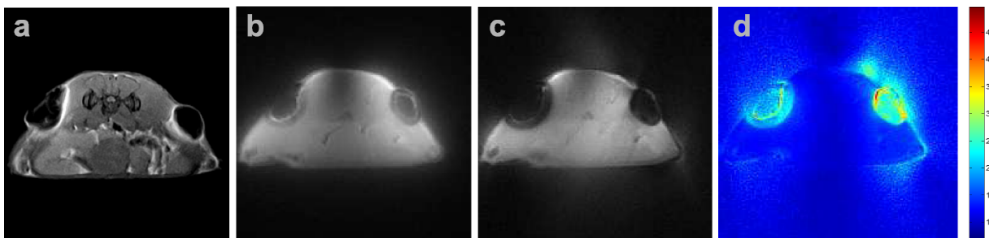
For the subtraction or the division of double echo images the in-phase and opposed-phase condition of fat and water have to be taken into account. Since the tissue without any changes in signal intensity is cancelled and differences are pronounced, fatty tissues will be attenuated if the 2<sup>nd</sup> echo is taken at the time of opposed-phase.

A more severe problem for detecting SPIO nanoparticles with 2D UTE sequences date from the used type of excitation pulses. In those experiments two VERSE-pulses which individually are not very slice selective and excite signal far from the intended slice location are used. But ideally, the out-of-slice magnetization from each excitation has opposite phase and cancels perfectly when the two excitations are combined [6]. Since line shapes of SPIO labeled components may vary widely in width, their phases after excitation vary also. This causes non cancellation of magnetization far away and therefore image-artefacts. Within our exemplary difference-image (Fig. 2d) misleadingly it seems that on the right hand side there are more SPIOs to detect. Additionally in Fig. 1b no compartmentalized area around the ectopic cell population can be seen although it would be expected judging from the differential image. This is obviously an artifact by the fatty tissue.

In summary it is important to note that for SPIO aided cell-tracking using a 2D UTE approach the artifacts addressed here have to be regarded particularly must not be ignored.



**Fig. 1:** Explanted ectopic cell population from the left (a) and right (b) flank. Microscopic view of the corresponding histologic specimen (c,d) show the cell-nuclei as blue and the SPIO particles as brown dots. The brownish color of the cell population is due to the SPIO nanoparticles. As can be clearly seen, the particles are homogeneously distributed over the cell implant.



**Fig. 2:** (a) Transversal TSE reference image. Left flank: 400 000 cells labelled with 28pg Fe/cell; Right flank: 400 000 cells labelled with 16 pg Fe/cell. (b) UTE FID image at 75 $\mu$ s k-space center. Around the cell pellet a doubling of the structure can be seen which is caused by B0 inhomogeneities and radial half pulse sampling. (c) image at 1210 $\mu$ s. Artefacts becoming worse, occurring phase errors cause

improper addition of the two VERSE excitation pulses and therefore signal nulling on edges. The resulting subtraction image (d) is overlaid by incorrect signal on regions totally free from SPIOs. On the SPIO dense region (left) the result is completely misleading since the signal decay in the surrounding region is as dominant as in the region of the particles yielding to equal contrast.

### Reference

[1] Bulte J., Kraitchman D., *NMR Biomed.* 2004;17:484-499. [2] Liu W., *NMR Biomed.* 2007;749-763. [3] Chris V. Bowen et al., *MRM* 2002; 48:52-61. [4] Coolen B.F. et al., *Proc. ISMRM* 2007, 1224. [5] Diwoký C. et al., *Proc. ISMRM* 2009, 804. [6] Josan S. et al., *MRM* 2009; 61:1083-1089.