UTE Imaging for Single Cell Detection with Positive Contrast

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Introduction

Employing gradient recalled echo (GRE) techniques to image cells labelled with super paramagnetic iron oxides (SPIO) is known to be an efficient method for the detection of small cell quantities in-vivo [1]. Dephasing within a voxel, and therefore negative contrast of the pixel, depends on the local magnetic dose within this voxel [2] and the global field-distortions caused by SPIO compartments in close proximity of the imaging volume. This is the reason why contrast changes with different imaging volumes, for example larger slice thickness, in a non-linear way. For the quantification of cells, the use of small imaging volumes has proved to be essential [3]. On the other hand, isotropic imaging volumes in the region of 50µm are highly time consuming and not applicable in a clinical setup.

Within this study, we propose the use of a T1 weighted 2D ultra short echo time (UTE) sequence [4] to image single labelled cells at a fraction of the acquisition time needed for isotropic 3D-GRE images. Single cell detection is presented based on a new in-vitro model using endothelial progenitor cells (EPC) [5] which build cell networks as an in-vitro correlate of the vasculogenesis process. We will show that the UTE approach is more sensitive to single cells than the 2D-GRE with the same slice thickness.

Theory

Since SPIO nanoparticles offer longitudinal relaxivities in the range of r1=20 $mmol^{-1}s^{-1}$ they can be used as T1 contrast agents too. If one would like to acquire T1 weighted images of fast dephasing components, one has to sample the free induction decay. Therefore UTE sequences have to be employed where sampling starts within the first microseconds after excitation. Within this time period, SPIO labelled cells reach positive contrast compared to their environment (**Fig. 1**).

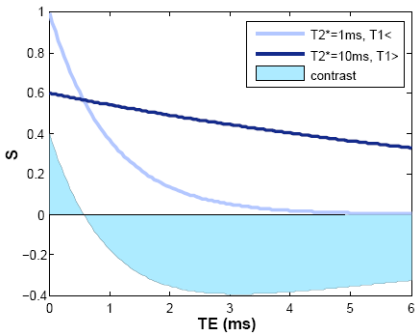

Fig. 1: Contrast between SPIO containing voxels (light blue) and regions without a contrast agent (dark blue). For very short echo times the contrast of SPIO labelled cells get positive dependent on the T1 relaxation.

Materials and Methods

Cell preparation: Clinical scale EPC propagation was done in a novel animal-serum free system. Cell labelling was performed without the aid of transfection agents within 24h in 280 µg/ml Fe Resovist (Schering AG, Germany) solution to prevent cell death or dysfunction. Single cell SPIO uptake was visualized using Prussian Blue staining and quantified by flow cytometry. Sub-cellular SPIO distribution was investigated by transmission electron microscopy. Cell cultures were seeded in 24 well plates containing extracellular matrix solution for the formation of endothelial networks. Resulting networks were washed and the wells were filled with Gd-DTPA (0.5mmol/l) doped agarose-gel in order to reduce T1 of the cell environment.

Pulse sequences: The scans were performed on a 7T (Bruker BioSpin, Germany) system with a maximum gradient performance of 1500mT/m using a 25mm birdcage coil. After 2nd order shimming, 3D FLASH images were acquired with 384x384 matrix, resolution 57x57x78 µm, TR/TE 200/3.2 ms, FA 30°, NSA 4, Taq 2:14h and 2D FLASH images as follows: 512x512 matrix, resolution 47x47x800 µm, TR/TE 200/3.9 ms, FA 30°, NSA 4, Taq 6'50" and a 2D UTE protocol with an reconstruction matrix of 384x384, acquired with 804 projections, resolution 57x57x800 µm, TR/TE 50ms/360µs, FA 15°, NSA 4, Taq 2'40".

Results and Discussion

The image with isotropic resolution (**Fig. 2b**) showed every single detail of the cell network presented in the microscopic photograph (**Fig. 2a**). Comparing the isotropic GRE image and the 2D image with enlarged 800µm slice (**Fig. 2c**), the latter looks somehow cloudy. This is caused by global field inhomogeneities within the imaging volume which tend to obscure contrasts. The deficit in details is also clearly documented within the network structure in the 2D image. Nevertheless, it is important to point out that the acquisition time was reduced from 2 hours to 7 minutes for the 2D image.

Acquiring the UTE image (**Fig. 2d**) required only 2:40 min for the needed T1 weighting and high in-plane resolution. Despite the fact that the UTE image was taken with the same slice thickness, much more detail of the cell structure is dissolved. The visible blurring occurs because of dephasing within the 1.28 ms sampling period which penalizes high spatial frequencies. Using half pulse excitation with the ability of echo times smaller than 100 µs would administer this problem. Future work will enhance this contrast with differential UTE [6] or additional T2* weighting acquired with variable rate UTE [7].

Finally we would like to point out that for complex structures such as bone marrow, the positive contrast of UTE would be highly desirable and the reduced sensitivity to shimming procedures compared to other positive contrast techniques based on susceptibility induced frequency shifts might enable the detection of labelled cells in those tissues in the first place.

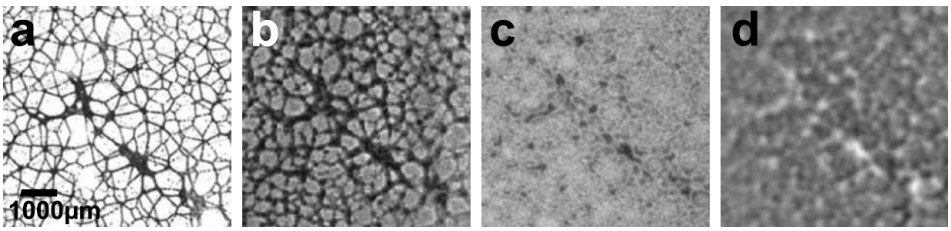

Fig. 2: Microscopic (a), isotropic 3D GRE (b), 800µm 2D GRE (c) and 800µm 2D UTE (d) image of the SPIO labelled cell network.

Acknowledgement

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Reference

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