'MultiPaw': High throughput MR imaging of ex-vivo AIA mouse joints with injected SPIONs on a clinical 3T system.

Lindsey A Crowe[1], Eline A Vermeij[2], Marije I Koenders[2], Fons A J van de Loo[2], Frank Tobalem[1], Thomas Goget[1], Azza Gramoun[1], Wim B Van den Berg[2], and Jean-Paul Vallée[1]

[1]*Faculty of Medicine/Department of Radiology, University of Geneva, Geneva, Switzerland,* [2]*Rheumatology Research and Advanced Therapeutics, Radboud University Nijmegen Medical Centre, Nijmengen, Netherlands*

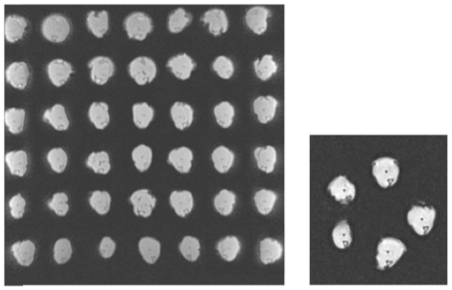
Introduction: The experimental protocol was developed in the context of joint image of an antigen induced arthritis (AIA) model in mouse for detection of disease components and iron oxide contrast agents (SPIONs). Visualization of oedema, bone erosion and SPIONs has been reported with the sequences used in this comparison study.

Methods: All animal handling procedures were in conformance with the institution ethical committee. Scanning was carried out on a Siemens 3T Tim Trio clinical scanner. Samples consisted of ex vivo mouse knees arranged with joints aligned forward. The coils compared in this study are those that are suitable for use in rodent imaging on the clinical scanner, either small parts of the anatomy or 'whole body': '4cm loop' designed for small structures near the surface (e.g. joints of fingers, toes or wrist and skin), that takes 5 of the sample tubes used to hold the knee joints in this study; 'wrist', 8-element coil with 8 integrated preamplifiers; an 8-channel phase array coil, dimensions 9 x 9 x 5cm that will take 42 samples. These were held tightly in small, sealed, sample tubes and placed in a wooden holder with a regular arrangement of appropriately sized indentations. The number of averages required was calculated theoretically from the relative SNR observed in a phantom study, based on a simple $\sqrt{2}$ proportionality. With the wrist coil, 7 signal averages were acquired. With 42 samples in the wrist coil this represents a gain in time over 7 groups of 5 in the loop coil.

A standard localization sequence, multi-slice FLASH (fast low angle shot), was used to check sample positioning, followed by the two sequences of interest. UTE sequence parameters (for iron oxide particle quantification and joint/bone structure) were 3D isotropic matrix of 512 and 90mm FOV (field of view), giving a resolution of 180µm in all three directions, 50000 radial projections, ultrashort TE (echo time) of 0.07ms, TR (repetition time) 9.6ms and flip angle 10°. A 3D T1 gradient echo sequence, called VIBE throughout, (for iron oxide signal loss imaging and joint structure) used a 3D isotropic resolution of 310µm, TR/TE 14.3/5.9ms and flip angle 12°. The whole examination for a single group (of 5 or 42 depending on the coil used) took around 20 minutes. Signal intensity was measure and SNR (signal divided by the standard deviation of the noise) calculated for 10 regions per coil group each 3 mm^2.

Demonstration of the potential application in screening for contrast agent in multiple samples involved mouse knees with intra-venous (100µg) or intra-articular (6µg) injections of SPION sacrificed at time points up to 7 days. Right knees had AIA and the left knees acted as internal controls. The SPION observed after intra-articular and intra-venous injection was scored on a scale of 0-5 in an AIA model with the left knee as an internal control in both VIBE signal loss and positive contrast dUTE images.

Results: The sample arrangement is illustrated in figure 1. In order to increase throughput, the number of samples in relation to the maximum 5 in the loop coil must be greater than the number of signal averages needed for the same SNR. The calculated number of averages for the same signal with the wrist coil is between 3.4 and 6.2 depending on the region taken, and therefore the optimum next integer value of 7 (for the maximum signal level) was chosen. A regular arrangement of 42 samples was easily attained in the wrist coil giving an excellent scan time efficiency with 7 averages, comparing an identical scan time of only 35 samples in the loop coil. The expected SNR values were realized in the actual samples. Further advantages of the wrist coil include a larger area for easy regular arrangement of samples for ease of image reading (figure 1) and homogeneous signal intensity over the samples. Even in such small samples the signal drop-off away from the loop coil is significant. The comparison of signal-to-noise (SNR) homogeneity can be seen in figure 2. With radial acquisition, as in the UTE sequence, image quality, as well as SNR can be improved by replacing signal averages with more radial projections. The diagnostic ability of these acquisitions for the detection of injected SPIONs, even on a clinical scanner in mouse knee samples, is illustrated (figure 3). Intra-articular injected iron is clearly seen and 'false positives' from other regions of VIBE hypointense signals that make scores less consistent are avoided by the use of the dUTE positive iron contrast method (figures 4 and 5).



a) b)

Figure 1 *Arrangement of samples in (a) wrist and (b) loop coil*

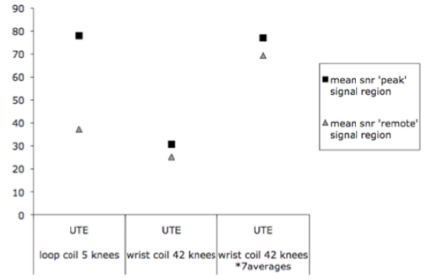


Figure 2 *Signal homogeneity problems with the loops coil and maximization of SNR over the whole sample in the wrist coil also showing the effect of averaging.*

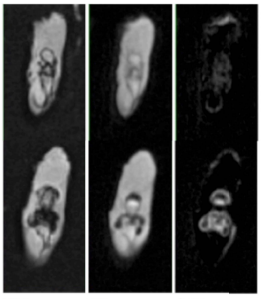


Figure 3 *After injection in synovial cavity, top: no iron, bottom: with iron*

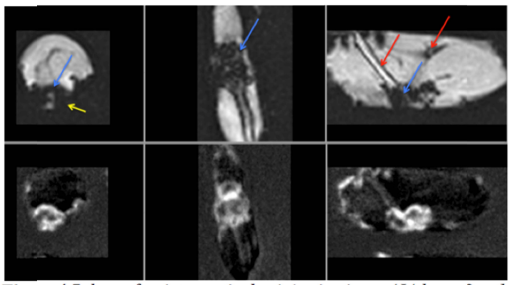


Figure 4 *7 days after intra-articular injection in an AIA knee. 3 orthogonal reconstructions of VIBE (top) and dUTE (bottom) showing difficulties in distinguishing SPION signal (blue arrows) from other regions (red arrows) and from the sample edge (yellow arrow) on the signal loss image. Iron uptake boundaries are clear on the background suppressed positive contrast dUTE image.*

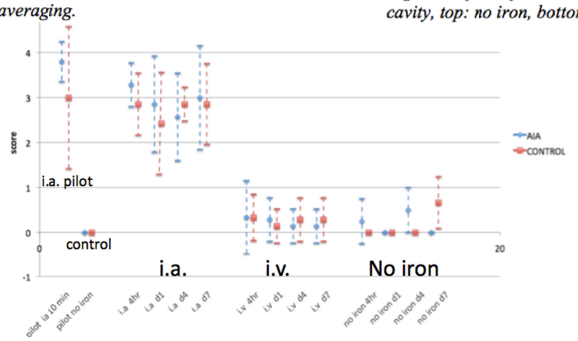


Figure 5 *dUTE scores (0-5) of Iron signal detection after injection in mouse knee for AIA and control at times up to 1 week after intra-articular and intra-venous injections*

Discussion and Conclusions: **Protocol:** The loop coil gives same maximum SNR as wrist coil with 7 averages and has the advantage of having more easy alignment of samples, giving easier segmentation of images. The wrist coil has homogeneous (maximum) signal over the whole sample region. Particularly with ex-vivo samples, one long scan in replacement of several individual shorter scans is not a disadvantage. Long duration is not a problem as it would be with anesthesia etc. in vivo, and a single scan means consistent scanner tuning and protocol for the whole sample set. **Application:** Ex-vivo imaging of the SPION injected mouse knees shows that: •IV dose is too low to be detected due to potential losses to the liver, •Artifactual signal loss on VIBE gives overestimate of iron score demonstrating the advantage of the dUTE positive contrast method, •Only intra-articular iron is bright on dUTE removing any background signal, •Iron remains in the joint over the timescale (7 days) with no significant signal change in both diseased and control knees. Future work will include development of a histological protocol to allow visualization of iron location for correlation. We conclude that the 'MultiPaw' imaging protocol is appropriate for optimized small sample scanning at high-resolution in a clinical MRI system and that dUTE has diagnostic potential for the detection of SPIONs in such a model.