

# *MR imaging of ex-vivo mouse joints on a clinical 3T system with diagnostic SPION imaging using dUTE in an arthritis model*

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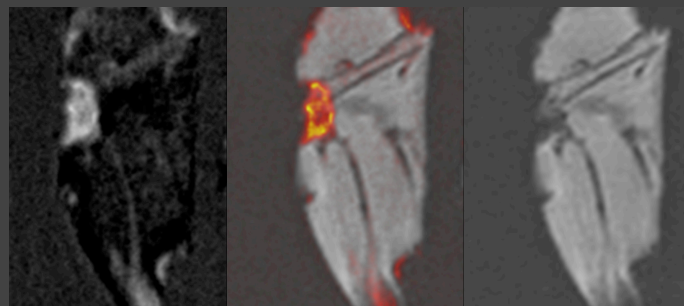
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**Mouse ex-vivo at 3T with SPION**

**# 3905, monitor 23,  
Wednesday 24 April, 11.00**

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- Normal and arthritic knee imaged using VIBE + SPION

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## Introduction

Super paramagnetic iron oxide particles (SPION) accumulate in macrophages in the synovium of arthritic knees<sup>1</sup>

We developed an MRI protocol for fast scanning of multiple mice knee specimens on a clinical 3T MR scanner

We present its performance for SPION detection in an antigen induced arthritis (AIA) model in mice

This protocol is based on a 3D difference Ultrashort Echo Time (dUTE) MR sequence that provides quantifiable positive contrast method for iron oxide detection in rodent AIA model in vivo<sup>2</sup>

1. Simon GH, von Vopelius-Feldt J, Fu Y, et al. Invest. Radiol. 2006;41(1):45-51.

2. Crowe LA, Tobalem F, Gramoun A, et al. Magn Reson Med 2012;68(5):1544-1552.

## Particles

All particles described in this work are amino-PVA-SPIONs provided by EPFL/Uni Fribourg with or without coupled fluorophore dye

The superparamagnetic iron oxide nanoparticles were manufactured by an aqueous co-precipitation method<sup>3</sup>

The colloidal particles were further coated with a mixture of poly(vinyl alcohol) (PVA) and vinyl alcohol/vinyl amine copolymer. Surface derivatization of polymer-coated particles with a dye in a magnetic bed reactor<sup>4</sup>

3. Chastellain M, Petri A, Hofmann H. J Colloid Interface Sci 2004;278(2):353-360.

4. Steitz B, Salaklang J, Finka A, O'Neil C, Hofmann H, Petri-Fink A. Bioconjug Chem 2007;18(5):1684-1690.

## Methods: Model

Arthritis was induced in mice using a standard AIA protocol in conformance with the institution ethical committee. SPIONS were injected at day 3 after AIA induction

At timepoints up to day 7 after SPION injection, mice were sacrificed to obtain multiple knee samples

- intra-articular (ia) injection of 6 $\mu$ g amino-PVA-SPION

- intra-vascular (iv) 1mg of the same amino-PVA-SPION

Both arthritic and control samples (right and left knees) were taken

Ex vivo mouse knees held tightly in sealed plastic tubes arranged in holder with joints aligned forward.

Scanning: Siemens 3T Tim Trio clinical scanner with the wrist coil  
3D difference Ultrashort Echo Time (dUTE)  
3D T1 gradient echo sequences, called 'VIBE' throughout

156 knees in total have been scanned with this method, with varying doses and timepoints. The results presented have n=3-6 for each route and dose of SPION injection as well as for each timepoint

## Methods: Protocol

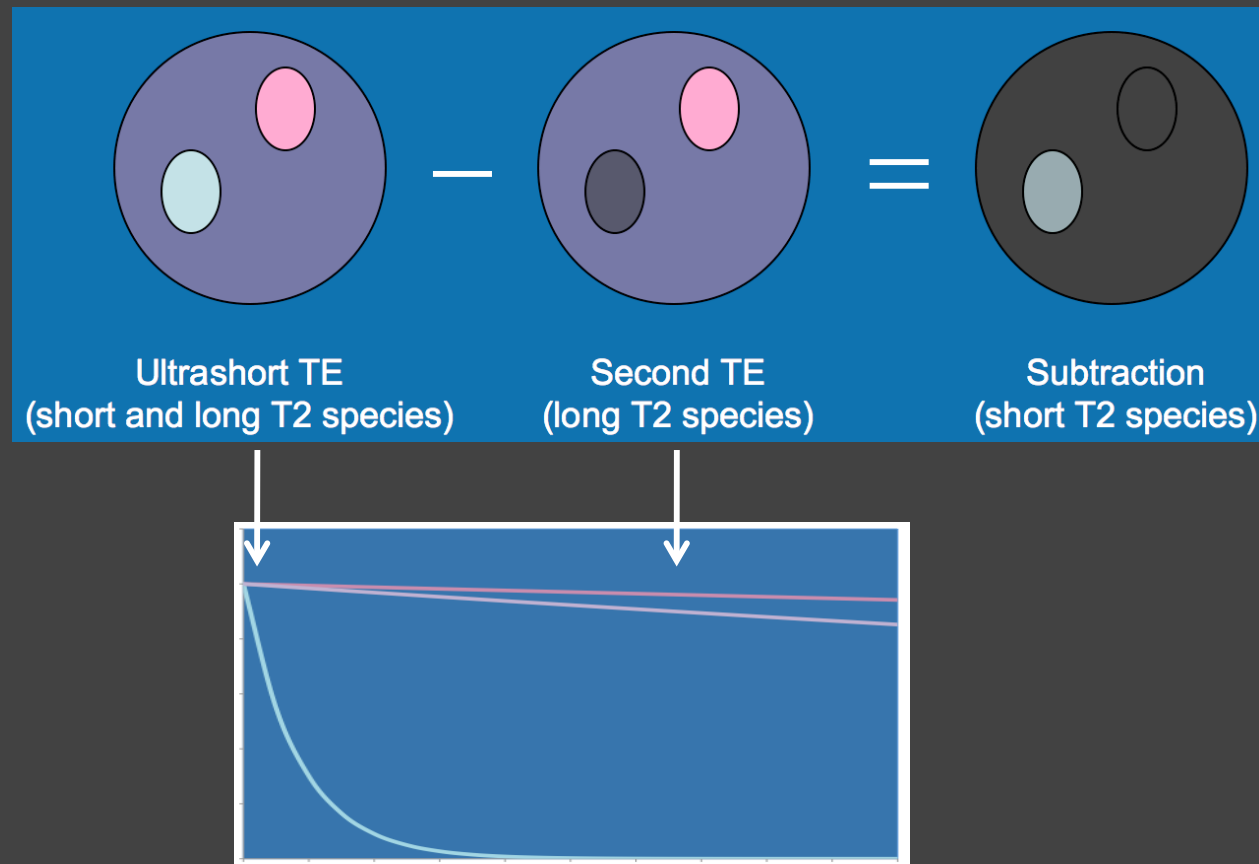
dUTE sequence parameters were: 3D isotropic matrix of 512, 90mm FOV (field of view), = 180 $\mu$ m isotropic resolution, 50000 radial projections, ultrashort TE (echo time) of 0.07ms, TE2 2.46ms, TR (repetition time) 9.6ms, flip angle 10°

VIBE parameters were: 3D isotropic resolution of 310 $\mu$ m, TR/TE 14.3/5.9ms and flip angle 12°

Whole examination for a single group: 20 minutes repeated for 7 signal averages with 42 knees at a time corresponding to an effective acquisition of <4 min for a single knee for both VIBE and dUTE

Score for presence of SPION to compare groups and MR sequences. Blind scoring was done on a 5-point scale on each knee, after random arrangement in the wrist coil set-up

## dUTE



dUTE gives high-resolution 3D anatomical images with suppressed background, reduced artifacts and high iron contrast, and allows automatic quantification of iron oxide

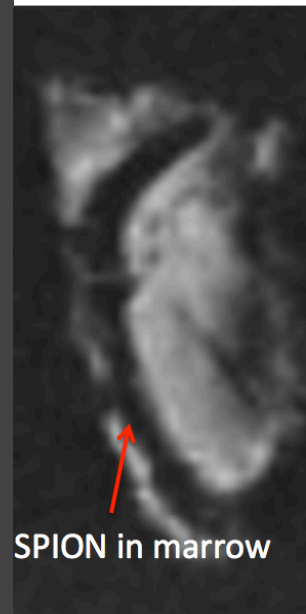
## Normal and arthritic knee imaged using VIBE + SPION

### VIBE

control  
No SPION



control +  
iv SPION



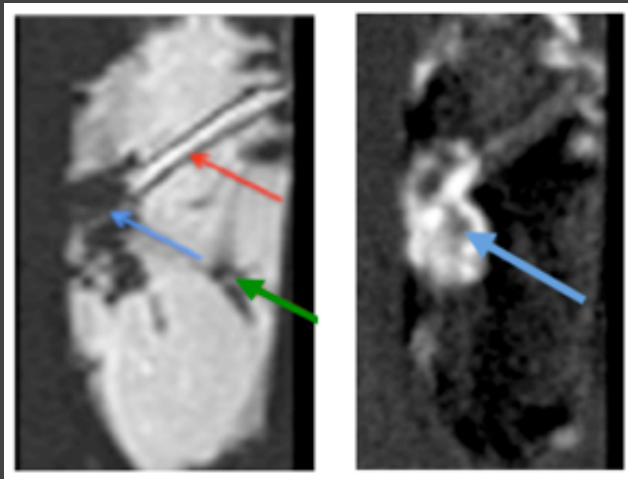
arthritis +  
iv SPION



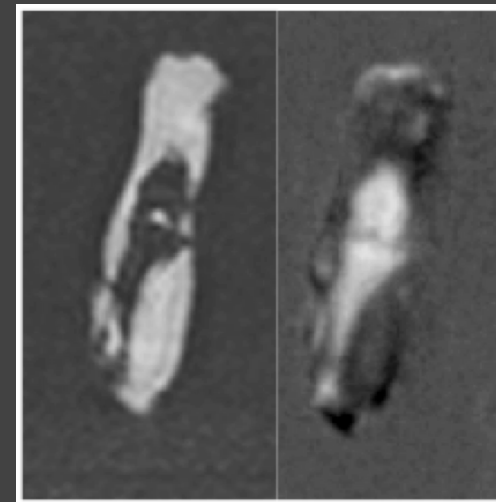
*Sagittal slice of mice knee specimens imaged by a VIBE sequence in control (left), after an iv iron oxide (SPION) injection in normal (middle) and arthritic (right) mouse knee. Accumulation of SPION in the synovium is only seen in arthritic knee whereas bone marrow uptake is present in both normal and arthritic knee*



## Normal and arthritic knee imaged using dUTE + SPION



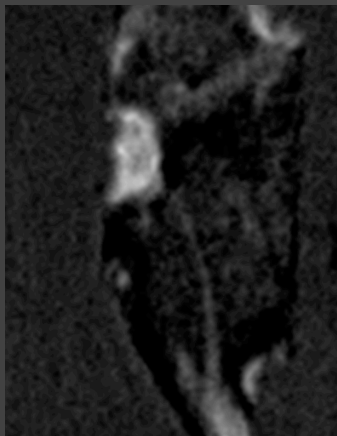
*False positive signal drop on VIBE image (green arrow) not seen in the dUTE image. SPION induced signal drop on VIBE (blue arrow) but signal enhancement on the dUTE sequence, Bone did not show SPION uptake (red arrow) after ia injection in AIA knee*



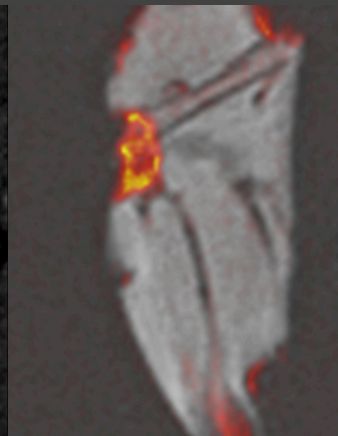
*Bone marrow SPION uptake after high dose iv injection in a control knee on VIBE and dUTE*

In the figure we see that false positive signal drop on VIBE image is not seen in the dUTE image. Due to reduced “false positive” from artifacts dUTE outperformed VIBE for the quantification of SPION uptake. We have SPION induced signal drop on VIBE but signal enhancement on the dUTE sequence. In addition, bone did not show SPION uptake (red arrow) after ia injection in AIA knee

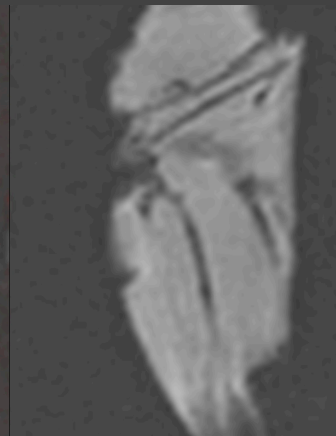
## Control knee imaged using dUTE + SPION



dUTE

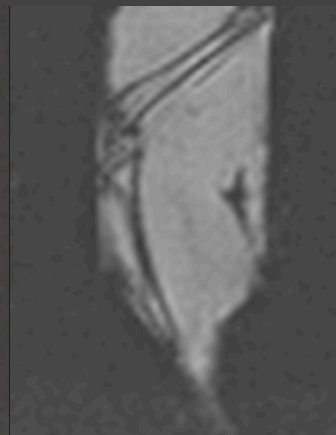
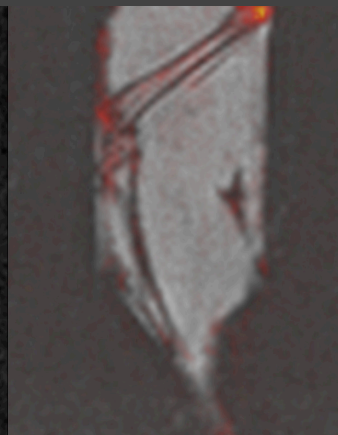
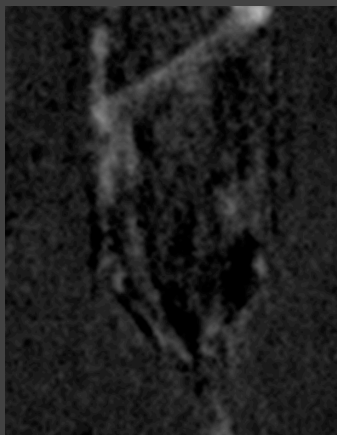


fusion



VIBE

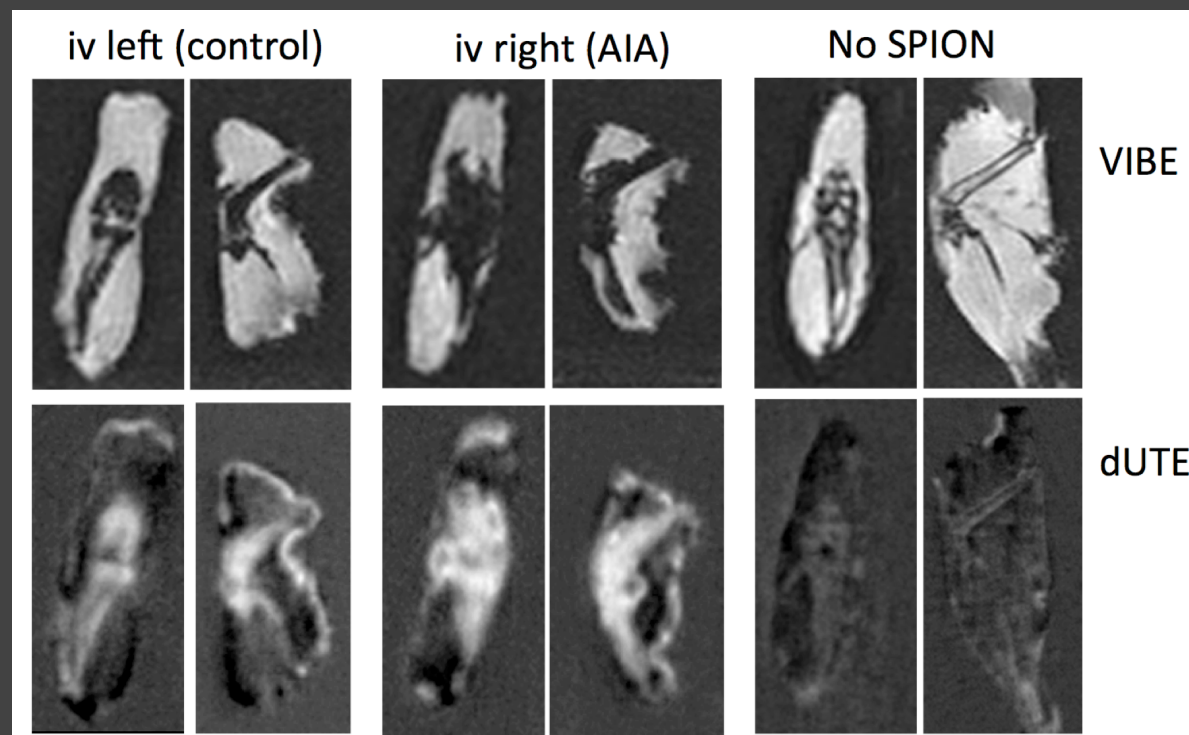
After ia SPION



No SPION

*Image Fusion of dUTE  
SPION on VIBE anatomy  
in a control knee after ia  
injection, and without  
SPION*

## SPIONs VIBE cf dUTE



*Coronal and sagittal slices of mice knee specimens imaged by a VIBE sequence (upper) and dUTE (lower) after an iv iron oxide (SPION) injection in control and AIA mice knees. Accumulation of SPION in the synovium is only seen in arthritic knee whereas bone marrow uptake is present in both normal and arthritic knee. The third case shows an AIA knee with no SPION*

## AIA in mice with SPION: VIBE cf dUTE

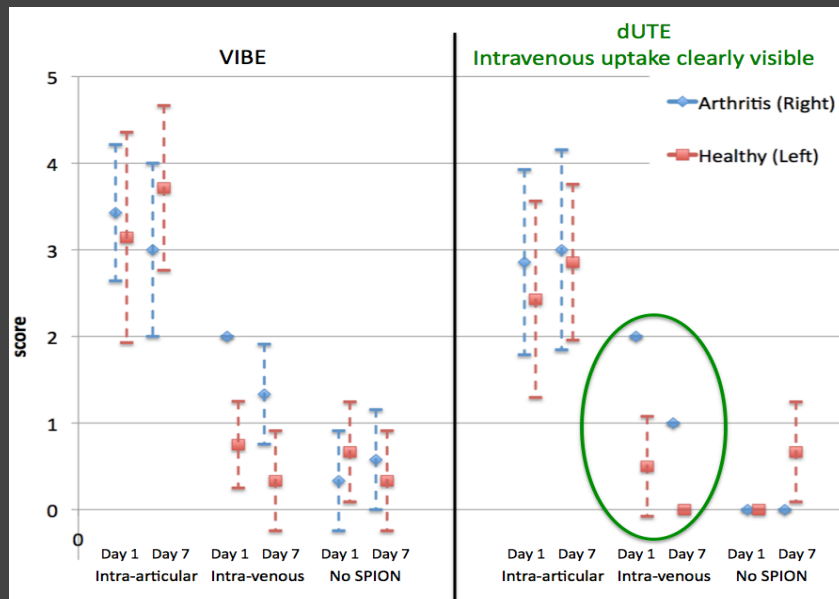
The spatial resolution achieved by our protocol was high enough to identify relevant anatomical landmarks of the mice knee

The presence of SPION was easily detected by a strong signal loss in the bone marrow after iv injection

Accumulation of iron in the synovium of arthritic knee was visible on both the VIBE and dUTE sequence

However, dUTE sequence was more efficient than VIBE sequence when grading the amount of SPION

## Scores



*Comparison of SPION accumulation scoring (n=3-6 per group) at day 1 and 7 using both VIBE (left) and dUTE (right) sequences*

*Due to reduced “false positive” from artifacts dUTE outperformed VIBE for the quantification of SPION uptake*

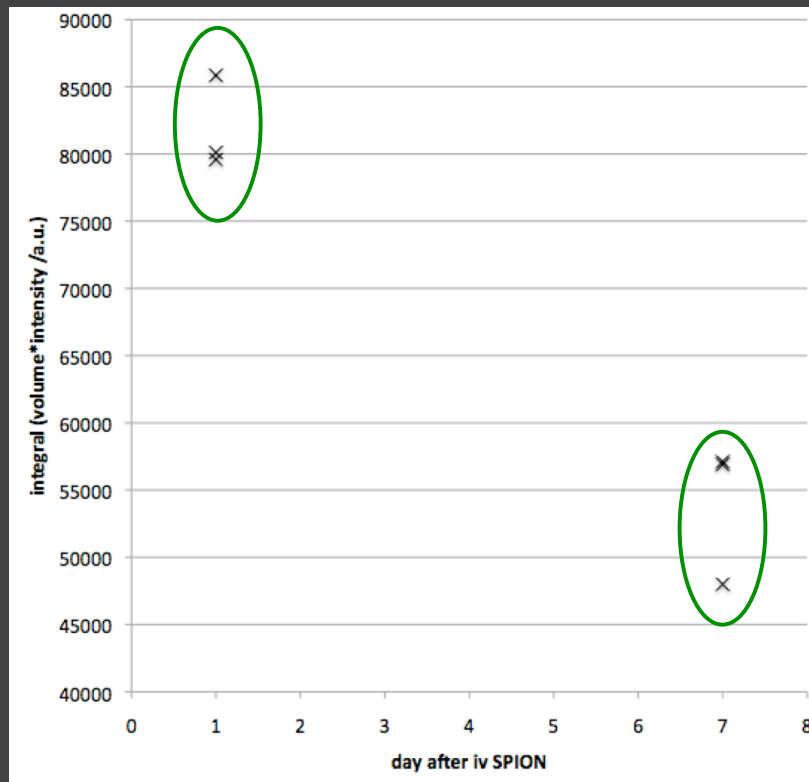
*After iv injection of SPION, only dUTE was able to demonstrate a statistically significant uptake at day 1 and following decrease at day 7*

The graph shows the blinded reader scores for both AIA knees and control (no arthritis) knees

For clarity, the results presented on the figures are from day 1 and day 7 with groups also assessed statistically including 4 hour and 4 days. A Bonferroni ANOVA was carried out over all the cases (20 groups in all) with a significance set at 0.05

After ia injection, both arthritic and control knees demonstrated an important SPION signal not evolving over time (no significant difference between knees and timepoints). VIBE showed non-significance comparing iv and no SPION, including comparison of control and AIA knees. For dUTE the arthritic knee after iv showed significant difference from the control, from ia SPION and no SPION

## Time evolution of the SPION uptake in AIA knee: Quantification

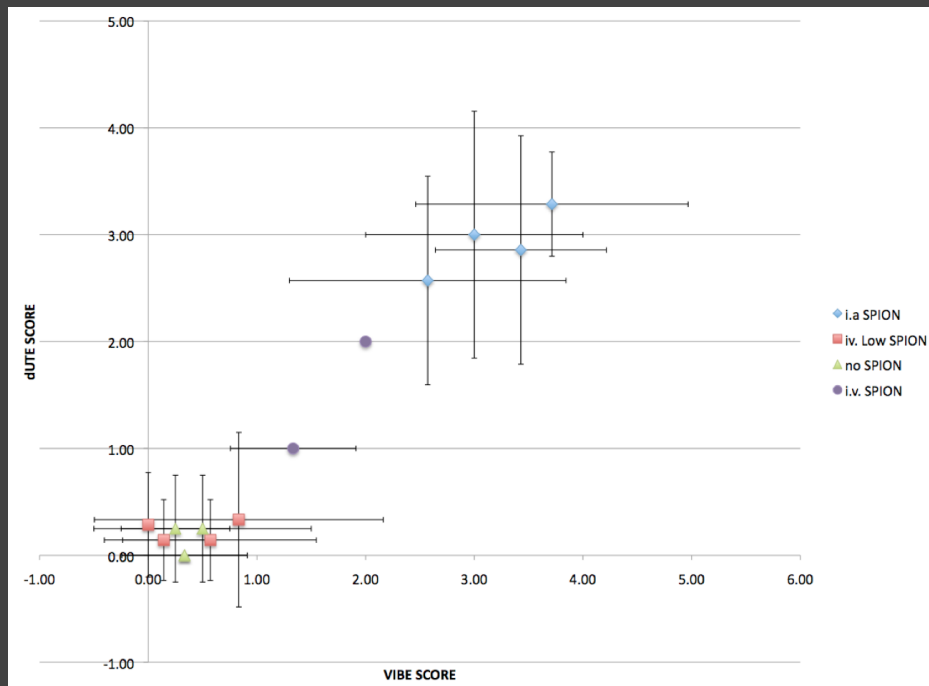


*Quantification of  
SPION signal after iv  
injection on dUTE  
positive contrast  
images 1mg dose, 3  
animals per group)*

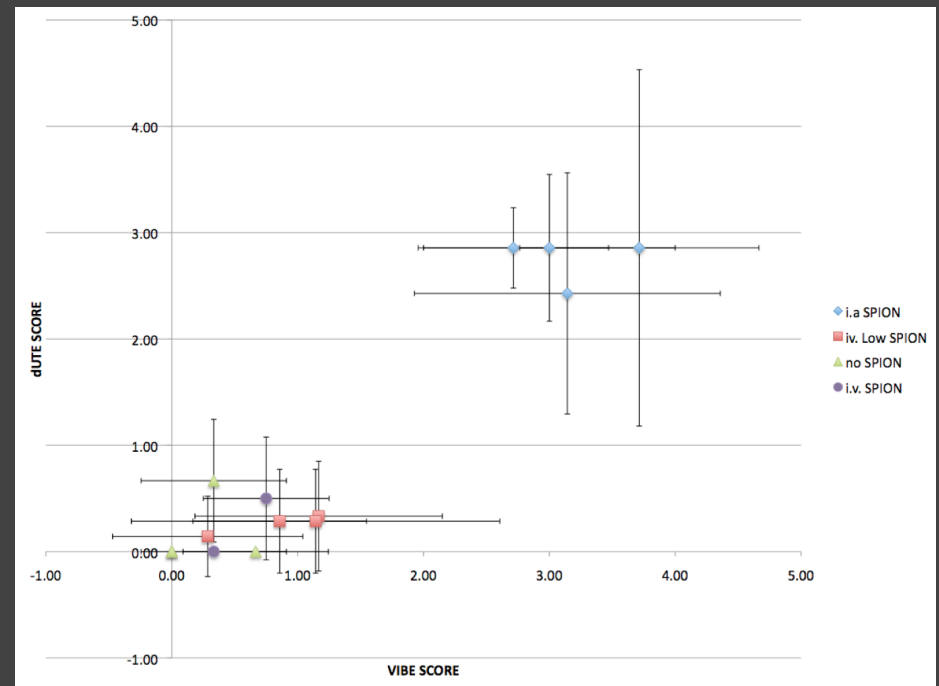
This graph demonstrates that dUTE is able to detect a time decrease of the SPION uptake in the synovium of AIA. This could reflect the migration of the macrophage as the inflammation is resolving or a destruction of the SPION and further recycling of the iron secondary to apoptosis of the macrophages

# Improved scoring with dUTE by comparison to VIBE

## AIA knee



## control knee



Scatter plot show separation of UTE scores, but not VIBE. Point for each condition and timepoint. For the iv SPION scores there is separation of error bars on the vertical axis (dUTE), but not the horizontal axis (VIBE)

dUTE shows reduced error bars and clear separation of iv SPION score from other conditions

## Discussion

After iv injection of SPION, only dUTE was able to demonstrate a statistically significant uptake at day 1 and following decrease at day 7

On day 1 after iv SPION injection, there was a significant change of signal intensity in the synovium of arthritic knee that was not present in control knee. This SPION signal decreased at day 7 but was still detectable in arthritic knees indicating that the SPION quantification could be efficient to monitor effect of treatment in this arthritic model in mice

dUTE but not VIBE was able to demonstrate after iv iron injection an uptake of SPION in arthritic knee that decreased after 1 week



## Discussion

The diagnostic ability of dUTE acquisitions on a clinical scanner in mouse knee samples is illustrated

In agreement with previous publications, dUTE was the most efficient MR sequence to quantify small variation of SPION accumulation in the knee

Applying this protocol on 156 mice knee specimens demonstrated that the signal loss after ia injection of iron was not different between control and arthritic knee and that it did not evolve over a 7 day period. Signal saturation related to a too high dose of injected ia SPION may explain this paradoxical absence of signal clearance

## Conclusions

Therefore, we demonstrated a robust and easily implementable protocol on a clinical MR system for fast assessment SPION uptake in knee specimens of arthritic mice with a strong potential for drug studies

Multipaw increases scan efficiency and signal homogeneity

False positives on VIBE images give overestimate of iron score

Only intra-articular iron is bright on dUTE, with all other tissue signals suppressed

Iron remains in place over the timescale (7 days) and can be quantified or scores in the different groups

## Acknowledgements

- HUG Radiology, CIBM



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- Thank you
- Questions?



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## References and contact

1. Simon GH, von Vopelius-Feldt J, Fu Y, et al. Invest. Radiol. 2006;41(1):45-51.
2. Crowe LA, Tobalem F, Gramoun A, et al. Magn Reson Med 2012;68(5):1544-1552.
3. Chastellain M, Petri A, Hofmann H. J Colloid Interface Sci. 2004;278(2):353-360.
4. Steitz B, Salaklang J, Finka A, et al. Bioconjug Chem 2007;18(5):1684-1690.

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