

QUANTIFICATION OF INHOMOGENEOUS IRON OXIDE UPTAKE IN A MODEL OF AIA IN RAT.

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Purpose

Iron oxide nanoparticles (SPION) as MRI contrast agents target macrophages in antigen-induced-arthritis (AIA) in rat. SPION are easily detectable on MRI as dark regions, but quantification in vivo remains extremely challenging. We developed and validated a method for SPION measurement based on positive contrast dUTE with a semi-automated segmentation and quantification software.

Context

Quantifying SPION uptake with conventional signal loss images is difficult due to early signal saturation. Heterogeneous nature of both the components of the natural progression of the disease and the biodistribution of contrast agents justified the development of a semi-automated segmentation method. This poster describes in vivo quantification of SPION uptake after intra venous injection in the clinically relevant antigen-induced arthritis (AIA) model in rat.

Following on from a previously published concentration phantom study with single region measurements of mean signal intensity after ia injection (3) where the monotonic signal increase was proportional to the iron concentration, we assess now by 3D semi-automated quantification an in vivo SPION signal and irregular uptake after iv injection.

We used the dUTE positive contrast method for image acquisition and home built software for automatic segmentation to give pixel intensity histograms to allow quantification of both size and intensity of the SPION biodistribution in the knee

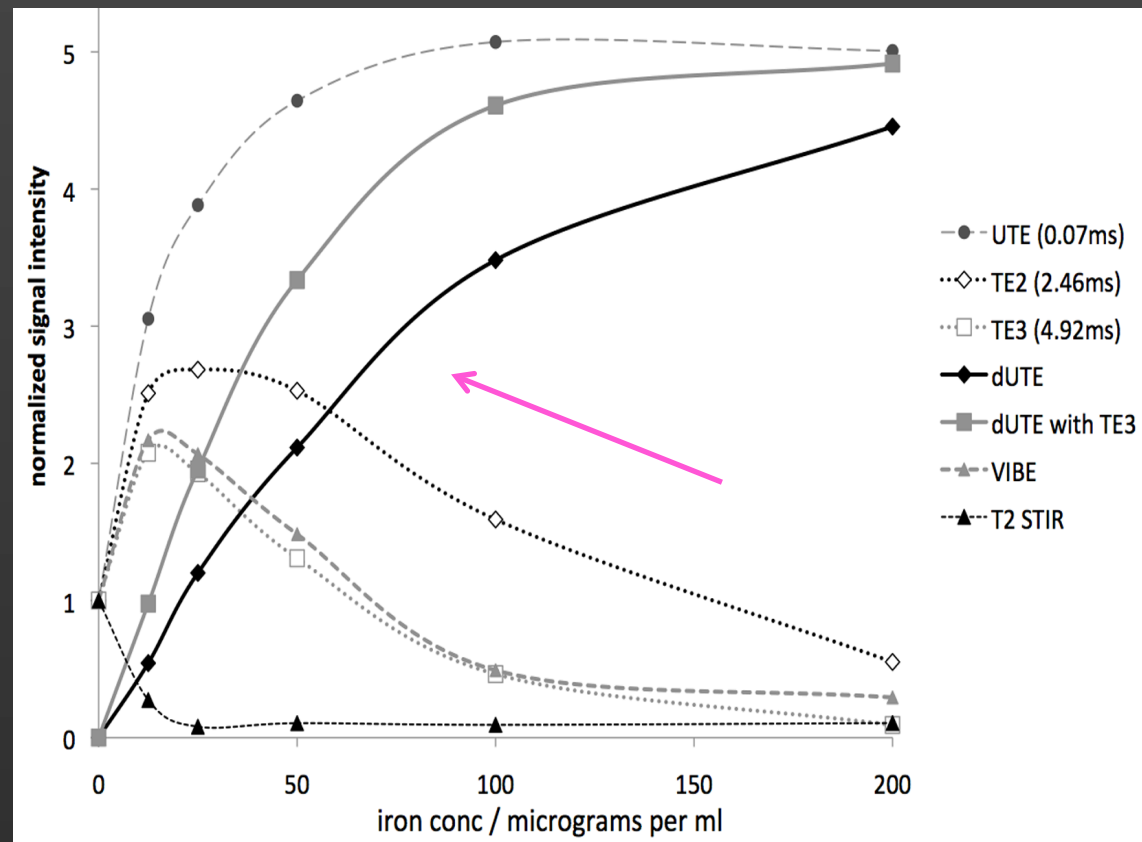


Figure: Signal vs conc of SPION in sample tubes comparing sensitivity and concentration ambiguity. The monotonic concentration dynamic of dUTE can be seen.

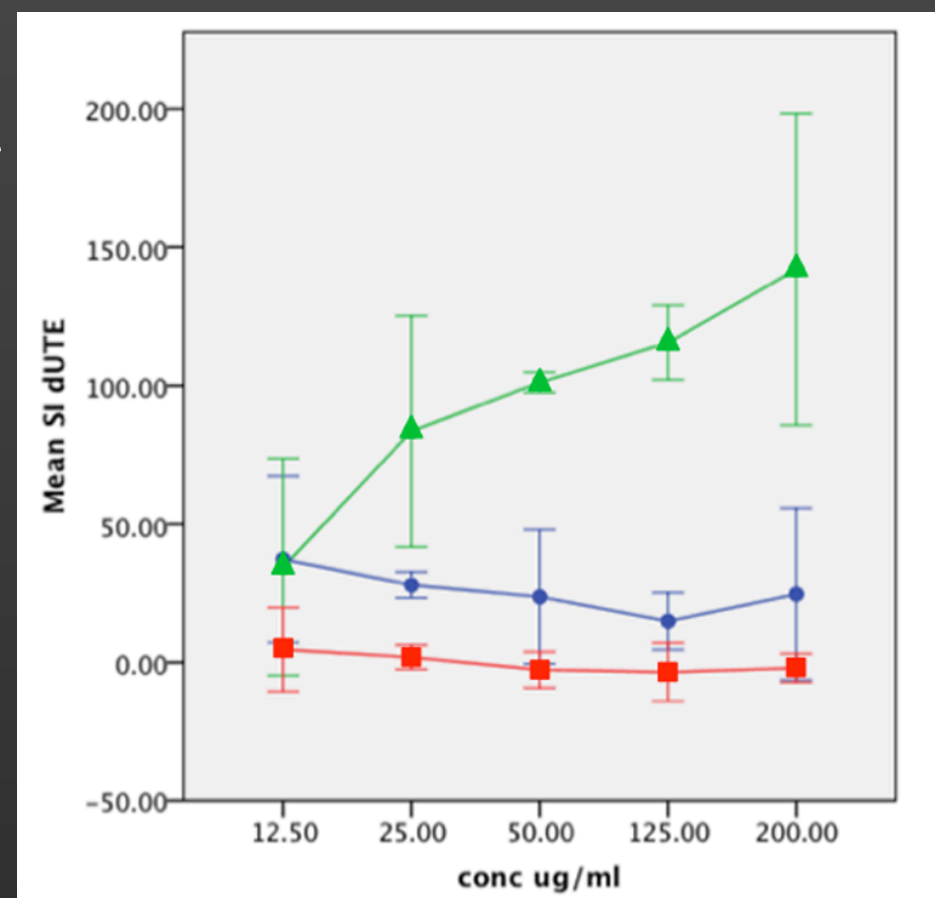


Figure: In-vivo results showing **synovial iron signal** (after 50uL iv injection) emphasizing both the enhanced sensitivity to concentration from the slope and the contrast due to the separation of the **iron signal** from **muscle and bone**. Error bars 95%.

Observations: Images

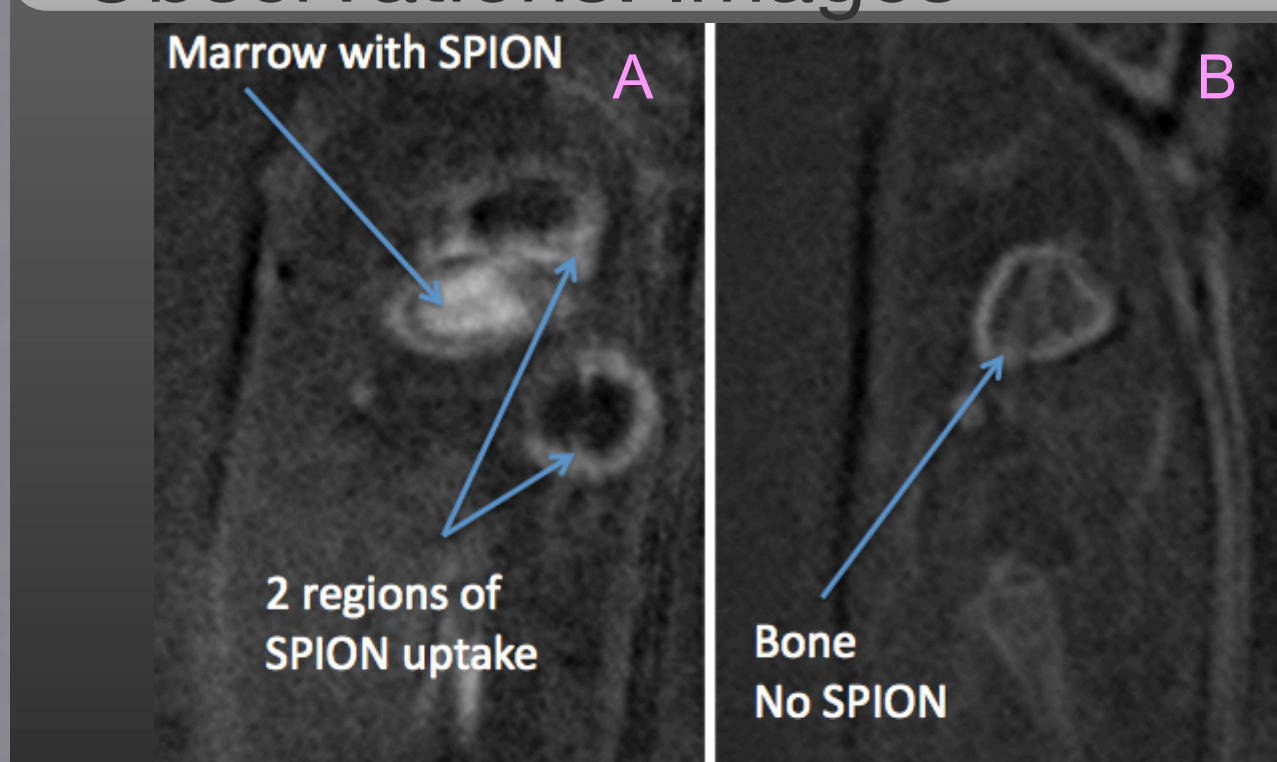
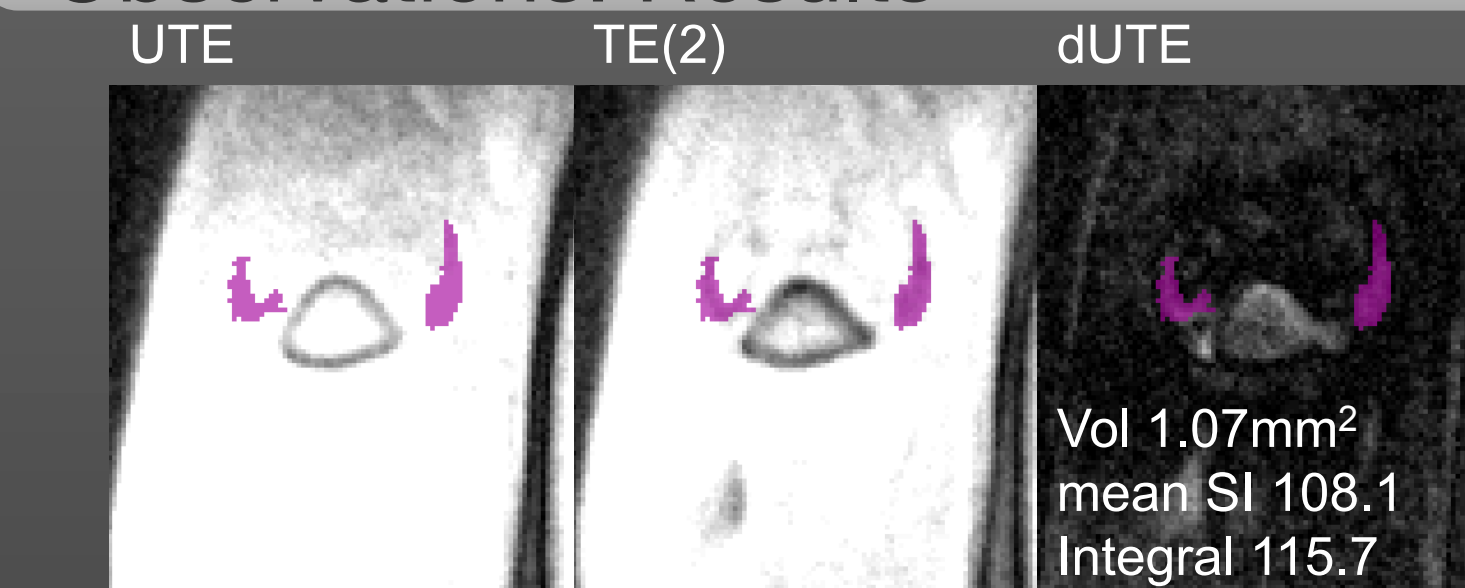
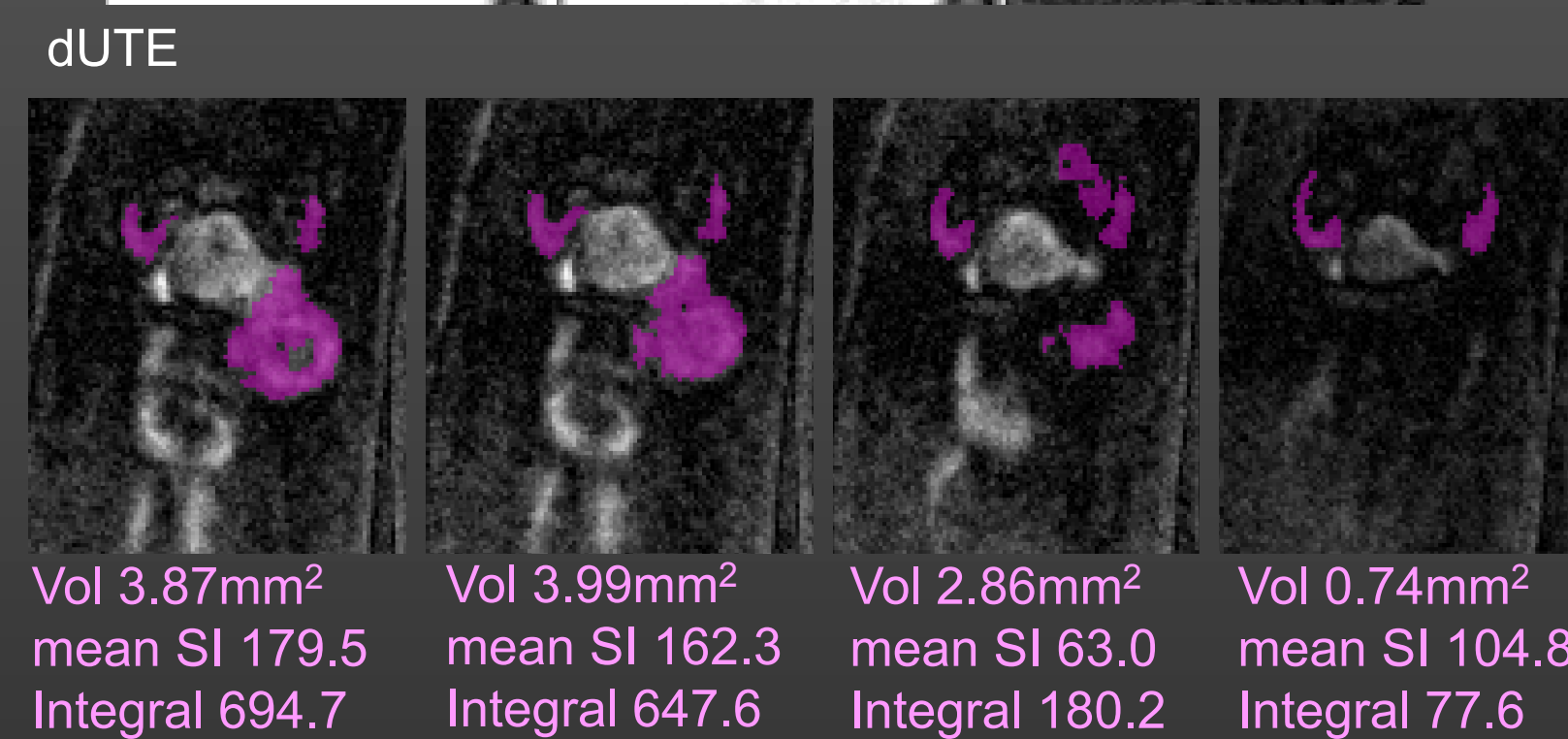


Figure: Coronal slice from 3D dUTE of arthritic knee (A) showing **SPION uptake** after iv injections of 7mg given 5 days before imaging in AIA rat showing two different parts of the synovium with iron uptake in the medial side of the knee and in the bone marrow. The right hand image (B) shows only the cortical bone as hyperintense signal (less than SPION intensity) in a control knee without SPION injection.

Observations: Results



The images of UTE, TE(2) and dUTE show a snapshot of the software with simultaneous segmentation of both echos and the subtraction image. The automatic method was successful in all the cases and took around 20 minutes per knee: about 3 times faster than manual segmentation.



Two repeated measurements using the automatic method on the same subject gave a whole 3D integral difference of less than 10% for all slices. Variation of up to 200% was seen between animals with different disease severity (measured over a group n=9). Our reproducibility for repeated measurements was well below these differences we detected with mild, moderate and severe disease.

Figure: This illustration shows a complete semi-automatic segmentation of SPION in different locations, showing an example with the two individual echoes and the difference image used for segmentation of positive contrast. Volume, mean signal intensity and integral are given below each different slice in the lower row.

References and Acknowledgements

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Methods

All particles described in this work were amino-PVA-SPIONs provided by EPFL, Lausanne, and University of Fribourg (7).

Animal model: Female Lewis rats (Janvier, France, weighing 150-175g, age 2 months) were used in this study. Ethical committee approval was obtained for the protocol and animals were kept in the institutions animal facility with free access to food and water. Rats (n=23) with antigen-induced arthritis in the right knee were given intravenous injection of 7mg iron oxide on day 5 after AIA induction. Intra-venous (iv) injections gave a low, unknown and irregular uptake in the synovium with a complex shape that requires 3D quantification.

Magnetic Resonance Imaging: Scanning used a Siemens Magnetom Trio 3T clinical scanner and the manufacturers 4cm loop coil. The protocol included 3D T1 gradient echo (VIBE) with parameters:

TR/TE 14.3/5.9ms, flip angle 12°, fat suppression, isotropic resolution 0.31mm, and FOV 100mm. Quantifiable iron oxide particle detection by dUTE MRI (4) consisted of simultaneous acquisition and subtraction of two echo times leading to positive contrast from short T2* species and reduced signal elsewhere. Parameters were: 3D isotropic resolution of 0.18mm, an 80mm FOV, 50000 radial projections, UTE/TE2 0.07ms/2.46ms (for in-phase fat/water image), TR 9.6 ms and flip angle 10°.

Image analysis: The new analysis software allowed segmentation of the two simultaneously acquired UTE and TE2 images and the dUTE positive contrast iron oxide image. Important features of the software included: semi-automatic segmentation (thresholding: a single pixel click filled a region using intensity threshold and a radius constraint), quantification of volume and signal intensity for both echoes and the difference image with the segmentation and export of signal intensity measurements for further analyses in statistical software. The semi-automatic threshold method selected regions within a defined radius of similar intensity around a user-defined point and this was repeated for the regions of SPION in 3D. A histogram of pixel intensities as well as mean value and total volume were reported. Manual segmentation (n=16) was used as a gold standard for the validation of the analysis software.

Observations: Quantification

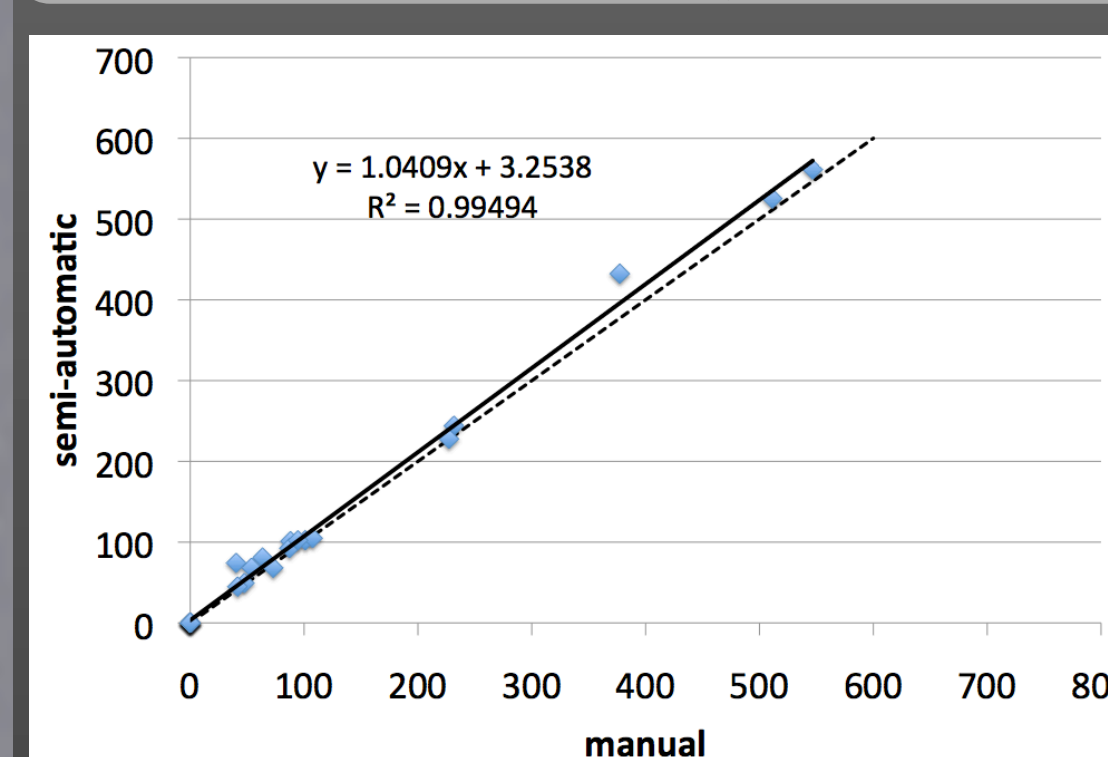


Figure: Correlation between manual and semi-automatic segmentation in slices of inhomogeneous regions of uptake (n=16). Both axes show pixel intensity*number of pixels. Dotted line: x=y, solid line: fit of data showing excellent agreement (p<0.0001).

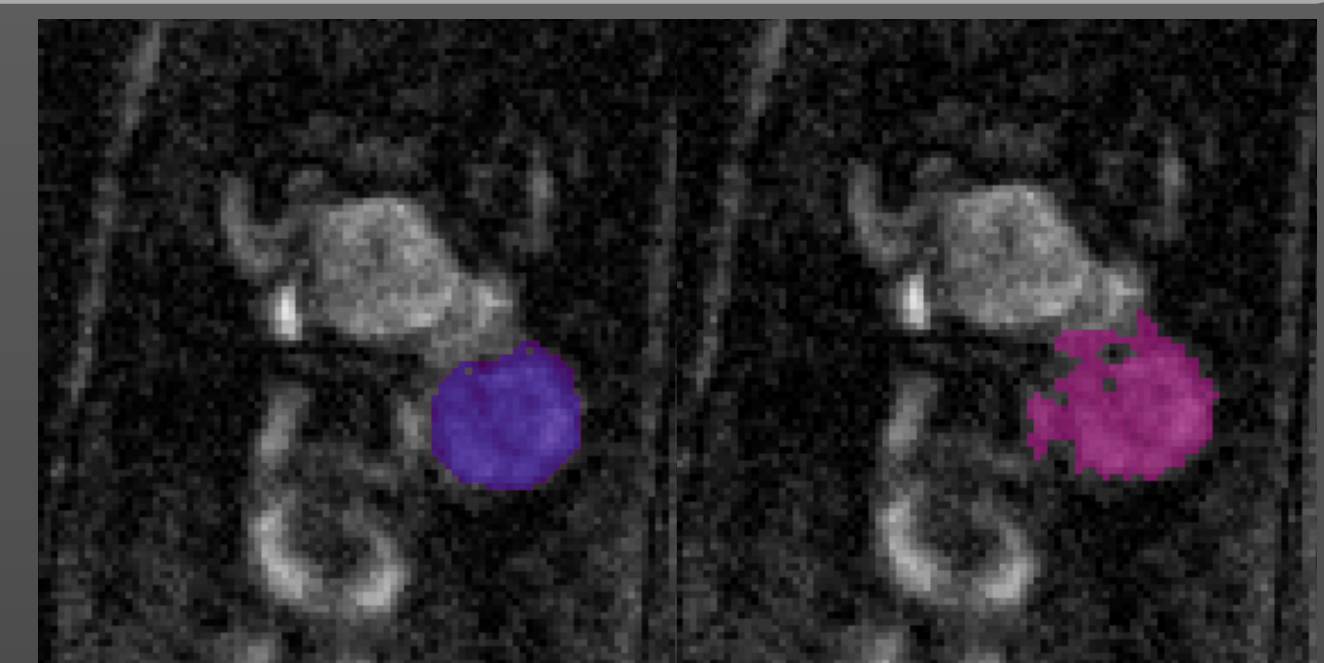


Figure: Manual (left) and semi-automatic (right) segmentation in inhomogeneous regions of uptake. This illustration shows visual difference for a single region of SPION behind the knee.

Manual segmentation
Volume = 2.15mm²
mean signal (sd) = 175.3 (50.8)
Integral = 377.6,
Semi-automatic segmentation
Volume = 2.45mm²
mean signal (sd) = 178.3 (43.8)
Integral = 432.4.

Correlation was good between measurements of manual and semi-automatic segmentation in heterogeneous regions of uptake (n=16) and there was no difference between manual and automatic segmentation for a single region of SPION uptake. Automatic segmentation avoided pixels within the region that were not of the required intensity to indicate SPION, especially when the SPION formed a contour around the synovium. The shape of the uptake could be more clearly represented on the automatic segmentation (unless done by manually selecting individual pixels – a method that would be prohibitively time consuming).

Discussion

SPION biodistribution in the AIA knee is a complex process with heterogeneous accumulation of iron all over the synovium. As a result, the density of SPION varied in the pixels. Such variation is generally not assessable by traditional GRE T2* MR sequences used to evaluate the SPION distribution due to the saturation induced by the signal drop resulting from even small amounts of iron inducing heterogeneous local B0.

The **dUTE sequence offered the advantage of positive, concentration dependent, signal** - useful in the case of heterogeneous iron distribution such as after intra-articular injection of SPION. Therefore, it became possible with dUTE to quantify both the number and the intensity of the pixels where the SPION were distributed. The total 'iron quantification' integral gave a more complete assessment of the irregular SPION uptake than just their distribution volume or mean intensity.

A second advantage of the dUTE sequence was provided by the segmentation options related to the positive signal. By removing artifactual hypointense regions in the image and **improving delineation of the iron-enhanced synovium from the cortical bone**, semi-automated segmentation of iron became possible.

The efficiency of the semi-automated segmentation was well illustrated by the validation against manual segmentation.

Conclusions

We demonstrated 3D quantification of irregular SPION uptake with robust, easier and faster assessment using semi-automated segmentation and dUTE, as applied to intravenous SPION uptake in arthritic rat knee.