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A fully automated trabecular bone structural analysis tool based on T2*-weighted magnetic resonance imaging

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Abstract

One major source affecting the precision of bone structure analysis in quantitative magnetic resonance imaging (qMRI) is inter- and intraoperator variability, inherent in delineating and tracing regions of interest along longitudinal studies. In this paper an automated analysis tool, featuring bone marrow segmentation, region of interest generation, and characterization of cancellous bone of articular joints is presented. In evaluation studies conducted at the knee joint the novel analysis tool significantly decreased the standard error of measurement and improved the sensitivity in detecting minor structural changes. It further eliminated the need of time-consuming user interaction, and thereby increasing reproducibility.

1. Introduction

The mechanical properties of the bone are determined by density, structure and composition. These features are altered in cases of bone pathologies such as osteoporosis (OP) leading to increased risk of fracture [1, 2]. In osteoarthritis (OA) several in vivo studies confirmed detectable disease-related changes in bone structure and thus providing evidence of an intrinsic connection between bone and articular cartilage [3, 4, 5]. A more thorough understanding of the bone-cartilage relationship involved in the OA process will improve early diagnosis based on the evaluation of the trabecular network within the cancellous bone. For such diseases magnetic resonance imaging (MRI) is the optimal modality of choice, it enables simultaneous examinations leading to a wealth of morphological and biochemical information within the affected site. In both OA and OP, detecting minor structural changes of the trabecular bone is of great clinical interest in order to evaluate and monitor disease progression and treatment response.

Current methods for assessing bone strength rely mainly on radiographic techniques. Bone mineral content and bone mineral density are measured using quantitative computer tomography (QCT) or dual X-ray absorptiometry (DXA) [6]. Trabecular bone structure can

be visualized through high-resolution computed tomography and micro-computerized tomography [7].

MRI as the method used in this study provides structure and density related information for the assessment of the cancellous bone. MRI has been reported to offer two different approaches, high-resolution MRI [8] and quantitative MRI, especially T2* relaxometry [9, 10, 11].

The potential of MRI as a diagnostic tool for the assessment of pathologies affecting the bone is today only partly utilized in daily clinical routine [7]. The process is a rather tedious and time consuming analysis of individual images and highly operator dependent. To further complicate matters, age- and sex-related alterations of the trabecular bone network are also site-specific and exhibit regional dependency [12, 13, 14]. For longitudinal studies, accurate tracing of identical regions of interest (ROIs) becomes a major determinant of sensitivity and reproducibility. There is a high probability that minor structural alterations triggered by a pathological bone remodelling process or treatment might remain undetected through ROI mismatch.

Several studies examined the reproducibility of quantitative in vivo MR measurements determining the effective transversal relaxation time T2*. At the sites of distal radius, distal tibia, and proximal femur short term mean relative precision errors of 2 to 11 % were reported [15]. Using manual ROI tracing long term precision errors varied up to 50 % [9]. Newitt et al. [16] studied the reproducibility, expressed as the coefficient of variation (CV), of specific apparent structural parameters derived from high-resolution MR images. Applying semi-automated analysis CVs ranging between 4 to 9 % were reported. The established radiographic methods for the assessment of BMD show in vivo precision errors between 1 to 4 %, only achievable by applying fully computer assisted image analysis [17].

One major source of imprecision in the assessment of the trabecular bone from high resolution MRI exists in an non-consistent alignment of the analysis volumes in longitudinal studies [18]. Automatic registration offers remedy for this failure and several authors have successfully shown significant improvement of the reproducibility applying specific registration approaches [19, 20]. Lazar et al. developed a software tool for bone quantification, capable of analysing multimodal data from QCT, DXA, high resolution MRI and magnetic resonance spectroscopy (MRS) [21]. Their tool features registration and automatic bone segmentation utilizing the fused image data. However, technical solutions capable to automatically perform quantitative analysis of the T2* relaxation time within standardized volumes of interest of synovial joints were to our best knowledge not published yet.

In this current paper we describe a novel analysis tool for the automated determination of the effective transversal relaxation time T2* obtained by a 3D fat-selective spoiled multi gradient echo MR sequence in specific regions of the distal femur. The developed tool features a novel trabecular bone segmentation algorithm, whereby a Level Set segmentation technique is driven by the inherent signal decay characteristic of bone marrow in the surrounding of a trabecular network.

The main purpose of this tool is to facilitate the entire imaging process and to increase the sensitivity for detecting minor structural changes of cancellous bone. Thus, an automatic analysis workflow was implemented to ensure identical ROI delineation between consecutive examinations. Hence, systematic errors introduced by the variations in manual ROI placement are entirely eliminated. Furthermore, during the process of ROI generation the spatial variation of the trabecular density is also taken into account. By subdividing a volume into regions of similar features, and thus decreasing the variance, minor changes are more likely to be retrieved.

The overall performance and reproducibility of the software was tested in a validation study including six healthy volunteers. In a reliability study the automatic analysis tool was compared to manual evaluation of cancellous bone structure.

2. Materials and Methods

Six healthy volunteers (five males and one female, ranging in age from 25 to 45 years, mean: 33 ± 8.5 years) gave written informed consent to participate in the study and underwent three consecutive MRI scans each at the right knee joint. All measurements were carried out on the same day with complete repositioning. Images were acquired on a clinical 3T system (Magnetom Trio, Siemens AG, Erlangen, Germany) using the manufacturer's 8-channel Tx/Rx knee coil in supine feet first position. Volumetric sagittal images were obtained using a 3D fat-selective spoiled multi gradient echo sequence (3D fs multi-GRE). The spectral excitation train was composed of five binominal pulses. The scanning parameters were set to: flip angle = 20° , bandwidth = 350 Hz/pixel, matrix size = 256×256 , slice thickness = 1 mm, and square FOV of 150 mm. Five different T2*-weightings were applied with an echo spacing of 5.22 ms starting with the first echo at an echo time TE = 5.57 ms and a repetition time TR = 35 ms. The number of slices varied between 80 to 112 according to individual knee size. Scan time was reduced using parallel imaging technique GRAPPA with an acceleration factor of 2. The knees were immobilized by inserting two wedge shaped bags between the knee and the coil casing on the left and the right site. The centre of the coil indicated by an external landmark served as a reference to ensure reproducible positioning between experiments.

Measured scans were transferred to a personal computer for offline data processing using the developed analysis tool. The novel image processing system was implemented in our laboratory using MATLAB (The MathWorks, Inc.).

2.1 Image processing

The automatic region of interest generation requires accurate extraction of the femoral epiphysis, which was achieved through Level Set segmentation. The process was initialized by bone marrow priors using threshold operations. The following subsections explain the entire image processing steps in detail which are summarized in Figure 1A-1D.

2.1.1 Background classification—The intensity histogram of a 2D section from the volumetric fat-selective images of the knee joint can be considered as a composition of three different compartments. One is formed by voxels with low signal intensity (background and

mainly water). Bone marrow and other fatty tissues (fat pad, subcutaneous fat) are distributed over moderate to high signal intensity range and compose the remaining two classes. Within three specific slices of the image data at the first TE an expectation maximization (EM) algorithm assigned every voxel to one of these three possible groups, yielding three different classes in each of the slices [22]. The applied EM algorithm assumed that the image pixels follow a Gaussian mixture model, the possible classes were predefined. The pseudo code of the EM algorithm is shown in Figure 2. Subsequently, for each of the slices a Background set was identified as the class exhibiting the lowest mean signal intensity. The maximum gray value of the obtained three Background sets was then utilized as the appropriate threshold to exclude non-fat containing voxels.

2.1.2 Bone marrow classification—Generally, the MR signal is predominantly affected by longitudinal and transversal relaxation phenomena. During the gradient echo formation process the magnetization is continuously influenced by the effective transversal relaxation, characterized by the time constant $T2^*$ of the specific tissue. Cancellous bone containing trabeculae exhibits short $T2^*$ caused by the trabecular network disturbing the homogeneity of the magnetic field. The impact of the dephasing effect of such local magnetic field inhomogeneities can be controlled through an appropriate TE setting. Hence, a long TE results in a pronounced $T2^*$ weighting, respectively, in a significant MR signal loss.

As a consequence of the inherent $T2^*$ difference between the two tissues to separate, the extraction of bone marrow from remaining fatty tissues was conducted by utilizing its faster signal decay. This $T2^*$ related effect can be enhanced when deriving two volumetric ratio data sets from the measured data at $TE_{short} = 5.57$ ms and $TE_{long} = 16.01$ ms:

$$ratio1 = \frac{image(TE_{short})}{image(TE_{long})}, \quad ratio2 = \frac{image(TE_{long})}{image(TE_{short})} \quad (1)$$

In ratio1 the values of voxels containing bone marrow surrounding trabeculae exhibited higher values than the ones containing the remaining fatty tissues. In ratio2 these voxels behaved in opposite manner, consequently marrow having lower ratio values. Additional spatial smoothing was applied on the ratio1 data set to account for the regional varying trabecular bone density, resulting in homogeneous ratio values within the marrow. At this point, dividing ratio2 by the smoothed version of ratio1 generated a new volumetric ratio3 data, whose slices revealed a bimodal distribution. The lower mode contained mainly bone marrow, while the higher mode was composed of other fatty tissues. As a result the ratio computations enhanced the two-compartmental appearance of the tissues to separate. Further, it entirely eliminated the impact of the spatial varying receiver characteristic.

The formation of a bimodal distribution by ratio computations enabled a reliable marrow classification. Prior classification, ratio3 data had to be normalized using linear scaling to cover the data range [0, 1]. Slice wise binarization of the volumetric ratio3 data was accomplished using Otsu's algorithm [23]. Consequently, a size filter was applied to the marrow mask to preserve the three largest connected regions and to remove misclassified voxels from overlapping parts of the histogram. A morphological erosion process finalized

the processing block yielding the desired bone marrow templates for the initialization of the contour refinement.

2.1.3 Contour refinement—Imperfect selective excitation and similar relaxation behaviour of the tissues to be separated can lead to segmentation artifacts when performing thresholding. Adjacent fat tissue in connection with partially excited cartilage might get wrongly classified as marrow, thus corrupting the ROI generation.

Therefore, a Level Set formalism with stopping force controlled by the image gradient as proposed by Malladi et al. [24] was performed to account for technical imperfections and normal tissue variation. In practice, using the original formalism, the zero level contour may eventually pass through object boundaries, therefore the steering force had to be appropriately adapted for the bone refinement task [25]. We therefore propose to relate the impact of the stop function k_I to a certain data range of the ratio3 data. Deriving the mean value C of the ratio3 data within the initially segmented bones, the lower and upper bounds of such an admissible region were defined as $C \pm$ window width w . Hence, voxels with ratio3 values which lie outside the obtained range will force the front to evolve with decreased speed. The proposed modifications of k_I were implemented as following:

$$k_I(\vec{x}) = \begin{cases} 0 & \text{for } \vec{x} \in \text{Background class} \\ \frac{1}{1+\Gamma(\vec{x})} & \text{for } \vec{x} \notin \text{Background class} \end{cases} \quad (2)$$

Consequently, voxels being a member of the Background class force the evolution speed of the zero Level Set to halt. In the non-member situation k_I was solely determined via $\Gamma(\vec{x})$. $\Gamma(\vec{x})$ was obtained according to:

$$\Gamma(\vec{x}) = \begin{cases} 0 & \text{for } |\gamma(\vec{x}) - C| < w \\ \rho|\gamma(\vec{x}) - C| & \text{for } |\gamma(\vec{x}) - C| \geq w \end{cases} \quad (3)$$

Where C is defined as the mean value of the ratio3 data within the initially segmented bone, w stands for the window width in percentage of C , and $\gamma(\vec{x})$ is the ratio3 value in the voxel at position $(\vec{x})$ [26]. The impact of Γ is controlled by the weighting factor ρ . Following the approach of Sethian [27], the Level Set equation [24] including the modified stop term (2) can be written as:

$$\frac{\partial \phi}{\partial t} = \varepsilon k_I(\vec{x}) |\nabla \phi| \operatorname{div} \left(\frac{\nabla \phi}{|\nabla \phi|} \right) + F_A k_I(\vec{x}) |\nabla \phi| \quad (4)$$

where the weighting factor ε controls the remainder force. Initializing the segmentation through the obtained bone priors, the advection force F_A permits surface expansion in the direction normal to the contour. The curvature driven remainder force, first term on the right hand side of equation (4), acts as a counterpart of the advection flow, resulting in a contour regularization effect. Consequently contour outflow is hindered by the penalizing behaviour of the remainder term on high local concave curvature. The steering term k_I prevents the segmentation of pixels with ratio3 values lying outside the admissible range. Practically, this 3D Level Set algorithm was implemented using a sparse field technique [28, 29], a constant

advection term F_A [24], and upwind schemes to estimate $F_A |\nabla \phi|$ in 3D [30, 31]. Equation (4) was evolved using the forward Euler method, an explicit time integration scheme, whereby the time step size was constrained through the Courant-Friedrichs-Lewy criteria [32]. The convergence of the contour evolution was ensured by limiting the maximal number of iterations.

2.1.4 Generation of ROIs—In the distal femur trabecular density, orientation of the trabeculae, and their mechanical property are spatially varying [12, 33]. To increase the sensitivity for detecting minor changes of the trabecular architecture, the femoral head was divided into medial and lateral condyle. The condyles were subdivided in regions of interest (ROIs) with similar bone density and geometry.

Initially the segmented bone underwent rigid rotation. The inferior and posterior outermost surfaces of both condyles were aligned along an orthogonal coordinate system set up by a transversal and a coronal plane. Subsequently the first grouping was performed by bisectioning the aligned bone into medial and lateral condyle. Figure 1D shows an example of an aligned segmented bone together with its corresponding coordinate system. The resulting medial and lateral compartments were orthogonally projected onto a 2D sagittal plane, wherein the specific ROIs were derived. Starting with the construction of four triquetrously shaped areas, an ellipse was approximated to the contour of the projected condyle. The epiphyseal region was subdivided into four sections using the centre of the ellipse as the common vertex. A further division of each section into three subregions was performed by three times intersecting with a resized ellipse, scaling factor one quarter each. The final ROIs were obtained by slice-wise intersecting the resulting 12 regions of each medial and lateral projection through the corresponding bisectioned bone.

2.1.5 T2* Quantitative Magnetic Resonance Imaging—In each slice the mean signal intensities of the autonomously generated ROIs at the five different echo times were used to generate a single T2* value by means of a nonlinear least-squares-approximation to a three parameter fit function:

$$S = A + B \exp^{-TE/T2^*} \quad (5)$$

The introduction of the constant A to the standard monoexponential model has been successfully applied in order to account for remaining deviations of the gradient echo relaxation decay from a monoexponential decay for longer echo times. The fitting model is equivalent to a bi-exponential function with one term having a decay constant greater than the maximum TE (26.45 ms). A high value of the ratio A/B corresponds to a decay that deviates significantly from a single exponential [34, 35].

The T2* quantities reflecting the trabecular status in each of the 12 ROIs at lateral and medial site were finally obtained by averaging the T2* times of all appropriate slices corresponding to the current ROI.

2.1.6 Statistical Analysis

Short term variability: The automatic data analysis was applied to all six study subjects. On the same day each subject was scanned three times and was instructed to leave the scanner table after each measurement. For each subject the precision errors of the T2* quantification in each ROI were expressed as the coefficient of variation. The technique's short term precision error and the CV were computed as proposed by Glüer et al. [36].

Intraoperator reliability: The manual data analysis within ROI #11, a trabecular bone region similar to lateral/medial femoral condyle (LCF/MFC) as defined by Lindsey et al. [37] was carried out by three operators. The reliability coefficients ρ_{intra} , their 95 % one-sided lower-limit confidence interval and estimates of the standard error of measurement (SEM) were computed. Furthermore an assessment of the minimum difference was performed that needs to be exceeded to be fairly certain that a real change has occurred. Statistical processing was conducted using MATLAB (The MathWorks, Inc.); ANOVA calculations were carried out as described by Bortz [38]. The intraoperator assessment for fixed operator effects was computed similar to Eliasziw et al. [39].

3. Results

Image processing

A visual reconstruction of the bone marrow segmentation is shown in Figure 3. Using steering parameters $\epsilon = 0.0005$, $p = 100$, a window width of $w = 0.8$, and scaling factor of the bone template of 0.75 the proposed segmentation led to smooth bone surfaces without major artefacts. The adapted stopping term k_I performed flawlessly in contour refinement of the femoral condyles.

In Figure 4 the generated standardized ROIs are superimposed over sagittal images of the lateral and medial site of the bone. The triquetrously shaped areas are shown in different colours varying in intensity within each subsection.

MR relaxometry

The mean relaxation times T2* and the standard deviation computed in the autonomous generated ROIs of three subjects at base line are given in Table 1. Comparing the mean T2* within the four groups composed of ROI #1, 5, 9, #2, 6, 10, etc. prominent variations towards the articular surface are evident. The shortest T2* values were found in the outermost regions where the most densely trabeculae are reported [9, 40, 41].

Short term variability

Table 2 presents the reproducibility data of the subject's structural measure T2* obtained using the fully automated software tool. The Table lists the minimum and maximum values of the individual CVs and the precision error CV_{SD} within the autonomously generated ROIs. The individual CVs range between 0.6 to 8.9 % and 0.4 to 8.6 % at medial and lateral site, respectively. The CV_{SD} of the T2* measurement for each ROI varied at the medial site from 2.4 to 4.0 %, and from 1.8 to 4.9 % at the lateral site.

Intraoperator reliability

The results of the assessment of the intraoperator reliability are summarized in Table 3 and 4. The mean $T2^*$ values represent the average values within ROI #11 across the repetitive analysis of each subject. Comparing the mean $T2^*$ values between the operators and the automatic processing, variations caused by different delineations of ROI #11 are observable.

At lateral site the standard error of measurement (SEM) varied for the different operators between 0.23 to 0.34 ms. The automatic analysis resulted in an improved SEM of 0.15 ms, a decrease of 35 %. At the medial compartment SEM was found to range between 0.30 to 0.35 ms for the manual evaluations whereby the automatic system reduced the SEM by 27 % to 0.22 ms. The developed automatic analysis detected even the smallest significant changes of the $T2^*$ quantity in ROI #11 of 0.43 ms and 0.61 ms, at lateral and medial compartment, respectively. In contrast the best repeatable operator A at lateral site achieved 0.64 ms and operator B at medial site 0.83 ms, corresponding to a loss of sensitivity by 33 % and 27 % respectively.

4. Discussion

In this study, a customized image processing on MR images of articular joints has been developed. In a validation study including six healthy volunteers good reproducibility has been proven and the superiority over manual analysis was clearly demonstrated.

MR imaging

The applied spectral excitation scheme performed well for the majority of the experiments. In two volunteers insular regions of the distal femoral cartilage were partially excited, resulting in a signal level of the cartilage similar to the adjacent Hoffa's fat pad and the bone marrow.

Background classification

The threshold was obtained via EM algorithm and accurately removed background and water containing voxels. Three sagittal slices at different positions were utilized within the EM assignment. This operational procedure was confirmed to be sufficient by the thresholding outcome. The selected slices corresponded to the centre slice and two slices displaced by centre slice ± 50 % of the centre slice's position.

In case of partially excited cartilage water containing pixels were not removed. However the $T2^*$ pronounced steering of the contour refinement prevented outflow at these sites.

Bone marrow classification

The outcome of the separation of the bone marrow from remaining fatty tissue depended on the two sampling points TE_{short} and TE_{long} . Before choosing these echo times two facts had to be considered. A signal difference, hence $T2^*$ contrast between the two tissues, must be already developed at TE_{short} . Further, at TE_{long} this contrast should ideally be evolved to its maximum. In this study the first sampling point was chosen as the shortest possible TE, restricted by the MRI scanning parameters. The second point was experimentally found as

the TE_{long} yielding accurate extraction results. TE_{long} was derived from data of one subject and consequently used throughout the entire analysis. This set up might be applicable for a wide range of trabecular morphology, pending further patient studies.

Contour refinement

Along the femoral metaphysis, regions belonging to the medullar cavity were not enclosed by the contour evolution. These regions contain mainly marrow fat with a sparse trabecular network. Thus, their ratio3 values corresponded to the remaining fatty tissue. None of these regions impaired the structural analysis, since all ROIs were placed within the femoral epiphysis.

Alternative approaches for bone segmentation were proposed by [42, 43, 44, 45]. Fripp et al. [42] were using active shape models (ASM), which were initialized using affine registration to an atlas. In the current work ASM was avoided since its performance is strongly dependent on the quality of the atlas and the used shape constraints. The texture feature driven classifier [43] makes use of MR phase images, resulting in bone templates with coarse surface of similar quality as obtained by the preceding threshold scheme. Accurate bone segmentation can be obtained when applying a semi-automatic multi spectral approach as proposed by Tamez-Pena et al. [44]. Huang et al. [45] implemented a fast automatic two-step segmentation workflow. The position of the bones were defined through masks, obtained by a distance-weighted directional gradient method. Within these masks bones were segmented using the Chan-Vese model. A sufficiently high image contrast between bone and cartilage is necessary but difficult to achieve, if cartilage is partially excited in fat selective GRE images.

Generation of ROIs

For the analysed bone the standardized ROI generation scheme was mainly influenced by (a) the known spatially varying trabecular arrangement and (b) its distance dependency [40, 41]. Merging cancellous bone of similar composition is believed to increase sensitivity in detecting minor structural bone changes. To our knowledge, the proposed ROI generation process introduced in this paper is the first attempt to improve the probability in detecting minor alterations by decreasing the variance of the trabeculae's morphology within the sample.

The alignment of the segmented bone enables a reliable separation into lateral and medial compartments. Additionally it improves comparison of corresponding regions between both compartments.

In case a displacement of the subject between consecutive measurements cannot be avoided, a registration of the scans at different points in time is recommended. In the current study the use of the compact knee coil enabled a repeatable positioning of the subject.

MR T2* relaxometry

It must be noted that in MR Osteodensitometry, alterations in bone mass and morphological changes of cancellous bone are affecting T2* [46, 14]. As can be seen in Table 1, the

observed behaviour of increasing mean $T2^*$ with increasing distance from the joint line can be explained by the structural dependence on the distance from the articular surface. Along the first 5 to 10 mm, Takechi [41] and Patel et al. [40] observed variations in density, connectivity and thickness of the trabeculae, using radiographic modalities. To summarize, in this zone the trabecular density decreased with increasing distance, which is in accordance with our finding of the spatial varying $T2^*$.

A more accurate merging of slices of similar regions into ROIs can only be achieved once precise data on the interior spatial arrangement of the cancellous bone is available. This would then allow to further decrease the standard deviation of the $T2^*$ values of the ROIs and to improve sensitivity of the structural changes of cancellous bone.

Short term precision

The susceptibility effect and source of the magnetic field disturbances both depend on the orientation of the main trabecular direction to the static magnetic field [47, 48]. Hence, variations of the individual CVs are believed to be caused by the alteration in repositioning. In repetitive studies performed on the appendicular skeleton these variations can be minimized using conventional extremity coils. Such coils can be locked to the base tray and minimize patient re-positioning.

Intraoperator reliability

The assessment of the intraoperator reliability was performed in ROI #11, since newly cancellous bone analysis were carried out in similar regions within the condyles using MRI [4, 37]. The evaluation clearly favoured the automated analysis as shown in Table 3 to 4. Besides eliminating systematic errors rising from manual ROI tracing, a significant decrease of the SEM was demonstrated. Furthermore the sensitivity for detecting $T2^*$ changes improved by 33 % and 27 %.

The current validation study has a few limitations. Firstly, the sample size of six subjects is limiting the statistical power of the resulting reliability estimates. Secondly, only healthy volunteers were examined. Since the estimates rely on the variability of the measurements in the study sample, they can only be interpreted within other populations that have similar attributes to the study's population [39].

Image parameter setting

Currently the operator task consists solely in adjusting the image processing parameters at the base line scan. The smoothing kernel size should be matched to the in-plane resolution. The window width w has to be adapted to the expected trabecular density. The number of maximal iterations should be set according to the size of the bone templates, and can be experimentally determined from the segmentation of the baseline scan. Furthermore, the parameters p and e controlling the contour evolution have to be set accordingly.

5. Conclusion

In the current work we are presenting an image processing tool for the structural analysis of trabecular bone applicable to quantitative MRI of articular joints. The software features automatic bone marrow segmentation and automated determination of the effective transversal relaxation time $T2^*$ in specific ROIs of cancellous bone. For the first time bone structural analysis was carried out fully automated in predefined ROIs, whereby the regions were generated in a standardized manner and subdivided with respect to the interior cancellous bone architecture.

A validation study including six healthy volunteers assessed reproducibility values similar to user operated analysis systems [4, 34, 15]. In the intraoperator reliability study the automatic analysis demonstrated a significant improvement of the SEM and an increase of the sensitivity compared to manual operators. For small tissue changes within an clinical important time frame this is a decisive aspect.

The presented approach of segmentation is based on the general concept of performing a preclassification through tissue selective imaging and utilizing a priori knowledge supporting the exact classification. Applying MRI to study the trabecular microstructure, this concept was implemented using fat-selective excitation. Further, the a priori known different inherent MR signal decay was emphasized by ratio computations improving the segmentation performance. In an initial study the proposed workflow was additionally applied to a fat-selective 3D MRI dataset of the hip joint of a volunteer. The resulting automatically segmented bone marrow is given in Figure 5. From visual inspection of the result in Figure 5 it can be seen, that although the applied TE settings were not optimal chosen the proposed method can be successfully applied and is robust. However, this initial experiment reflects the potential of the general applicability of the proposed scheme for classifying cancellous bones within synovial joints. The existing limitation is more or less a restriction to MRI as this is the only modality that delivers different contrasts weightings without using contrast agents.

Based on these promising results, future work will aim on applying the tool to analyse the cancellous architecture of the femoral head at the site of the hip joint. For this application the generation of the anatomy dependent ROI's must be adapted. The application of DIXON [49, 50] fat-water separation technique instead of the fat-selective excitation is also a possible future development. Thus, the segmented bones, obtained from the fat images, could aid the characterization of the cartilage within the water data. Such fully automatic processing would facilitate the entire analysis and promote the routine monitoring of the whole articular joint using MRI in degenerative joint diseases.

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References

1. Briggs AM, Greig AM, Wark JD, Fazzalari NL, Bennell KL. A review of anatomical and mechanical factors affecting vertebral body integrity. *Int J Med Sci.* 2004; 1:170–80. [PubMed: 15912196]
2. Rho JY, Kuhn-Spearing L, Zioupos P. Mechanical properties and the hierarchical structure of bone. *Med Eng Phys.* 1998; 20:92–02. [PubMed: 9679227]
3. Beuf O, Ghosh S, Newitt DC, Link TM, Steinbach L, Ries M, et al. Magnetic resonance imaging of normal and osteoarthritic trabecular bone structure in the human knee. *Arthritis Rheum.* 2002; 46:385–93. [PubMed: 11840441]
4. Bolbos RI, Zuo J, Banerjee S, Link TM, Ma CB, Li X, et al. Relationship between trabecular bone structure and articular cartilage morphology and relaxation times in early OA of the knee joint using parallel MRI at 3T. *Osteoarthritis Cartilage.* 2008; 16:1150–9. [PubMed: 18387828]
5. Messent EA, Ward RJ, Tonkin CJ, Buckland-Wright C. Brief report: Differences in trabecular structure between knees with and without osteoarthritis quantified by macro and standard radiography, respectively. *Osteoarthritis Cartilage.* 2006; 14:1302–5. [PubMed: 16990028]
6. Engelke K, Glüer CC. Quality and performance measures in bone densitometry: part 1: errors and diagnosis. *Osteoporos Int.* 2006; 17:1283–92. [PubMed: 16821003]
7. Bauer JS, Link TM. Advances in osteoporosis imaging. *Eur J Radiol.* 2009; 71:440–9. [PubMed: 19651482]
8. Wehrli F. Structural and Functional Assessment of Trabecular and Cortical Bone by Micro Magnetic Resonance Imaging. *J Magn Reson Imaging.* 2007; 25:390–409. [PubMed: 17260403]
9. Majumdar S, Genant H. In vivo relationship between marrow T2* and trabecular bone density determined with a chemical shift-selective asymmetric spin-echo sequence. *J Magn Reson Imaging.* 1992; 2:209–19. [PubMed: 1562773]
10. Wehrli F, Ford J, Haddad J. Osteoporosis: Clinical Assessment with Quantitative MR Imaging in Diagnosis. *Radiology.* 1995; 196:631–41. [PubMed: 7644622]
11. Wehrli F, Song HK, Saha PK, Wright AC. Quantitative MRI for the assessment of bone structure and function. *NMR Biomed.* 2006; 19:731–64. [PubMed: 17075953]
12. Burgers TA, Mason J, Niebur G, Ploeg HL. Compressive properties of trabecular bone in the distal femur. *J Biomech.* 2008; 41:1077–85. [PubMed: 18206893]
13. Lochmüller EM, Matsuura M, Bauer J, Hitzl W, Link TM, Müller R, et al. Site-specific deterioration of trabecular bone architecture in men and women with advancing age. *J Bone Miner Res.* 2008; 23:1964–73. [PubMed: 18665791]
14. Link TM, Majumdar S, Augat P, Lin JC, Newitt D, Lane NE, et al. Proximal femur: Assessment for osteoporosis with T2* decay characteristics at MR imaging. *Radiology.* 1998; 209:531–6. [PubMed: 9807585]
15. Grampp S, Majumdar S, Jergas M, Lang P, Gies A, Genant HK. MRI of bone marrow in the distal radius: in vivo precision of effective transverse relaxation times. *Eur Radiol.* 1995; 5:43–8. [PubMed: 11539927]
16. Newitt DC, van Rietbergen B, Majumdar S. Processing and analysis of in vivo high-resolution MR images of trabecular bone for longitudinal studies: reproducibility of structural measures and micro-finite element analysis derived mechanical properties. *Osteoporos Int.* 2002; 13:278–87. [PubMed: 12030542]
17. Genant HK, Engelke H, Fuerst T, Glüer CC, Grampp S, Harris ST, Jergas M, et al. Noninvasive assessment of bone mineral and structure: state of the art. *J Bone Miner Res.* 1996; 11:707–30. [PubMed: 8725168]
18. Gomberg BR, Wehrli FW, Vasilic B, Weening RH, Saha PK, Song HK, et al. Reproducibility and error sources of μ -MRI-based trabecular bone structural parameters of the distal radius and tibia. *Bone.* 2004; 35:266–76. [PubMed: 15207767]
19. Blumenfeld J, Carballido-Gamio J, Krug R, Blezek DJ, Hancu I, Majumdar S. Automatic Prospective Registration of High-Resolution Trabecular Bone Images of the Tibia. *Ann Biomed Eng.* 2007; 35:1924–1931. [PubMed: 17705036]

20. Magland JF, Jones CE, Leonard MB, Wehrli FW. Retrospective 3D registration of trabecular bone MR images for longitudinal studies. *J Magn Reson Imaging*. 2009; 29:118–126. [PubMed: 19097098]
21. Lazar, VR.; Liney, GP.; Manton, DJ.; Gibbs, P.; Lowry, M.; Gregson, CL.; Rittweger, J., et al. Software tools for MR and pQCT bone quantification; Proceedings of the ISMRM-ESMRMB Joint Annual Meeting; 2010; Stockholm. Proc. Nr. 785
22. Dempster AP, Laird NM, Rubin DB. Maximum likelihood from incomplete data via the EM algorithm. *J R Stat Soc Series B Stat Methodol*. 1977; 39:1–38.
23. Otsu N. A threshold selection method from gray-level histograms. *IEEE Trans Syst Man Cybern*. 1979; 9:62–6.
24. Malladi R, Sethian JA, Vemuri BC. Shape modeling with front propagation: A level set approach. *IEEE Trans Pattern Anal Mach Intell*. 1995; 17:158–75.
25. Yezzi A, Kichenassamy S, Kumar A, Olver P, Tannenbaum A. A geometric snake model for segmentation of medical imagery. *IEEE Trans Med Imag*. 1997; 16:199–209.
26. Rink, K.; Törsel, AM.; Tönnies, K. Segmentation of the vascular tree in CT data using implicit active contours. In: Handels, H.; Ehrhardt, J.; Horsch, A.; Meinzer, H.; Tolxdorff, T., editors. *Bildverarbeitung für die Medizin*. Springer; Berlin Heidelberg: 2006. p. 136–40.
27. Sethian, J. Level set methods and fast marching methods: Evolving interfaces in computational geometry, fluid mechanics, computer vision, and materials science. University Press; Cambridge: 2003.
28. Lankton, S. Sparse field active contours. <http://www.shawnlankton.com/2009/04/sfm-and-active-contours/>
29. Whitaker RT. A level-set approach to 3D reconstruction from range data. *Int J Comput Vis*. 1998; 29:203–31.
30. Sethian J, Strain J. Crystal growth and dendritic solidification. *J Comput Phys*. 1992; 98:231–53.
31. Suri JS, Liu K, Singh S, Laxminarayan SN, Zeng X, Reden L. Shape recovery algorithms using level sets in 2-D/3-D medical imagery: A state-of-the-art review. *IEEE Trans Inf Technol Biomed*. 2002; 6:8–28. [PubMed: 11936600]
32. Courant R, Friedrichs K, Lewy H. On the partial difference equations of mathematical physics. *IBM J*. 1967; 11:215–35.
33. Hildebrand T, Laib A, Müller R, Dequeker J, Rügsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: Microstructural data from spine, femur, iliac crest, and calcaneus. *J Bone Miner Res*. 1999; 14:1167–74. [PubMed: 10404017]
34. Fransson A, Grampp S, Imhof H. Effects of trabecular bone on marrow relaxation in the tibia. *Magn Reson Imaging*. 1999; 17:69–82. [PubMed: 9888400]
35. Newitt DC, Majumdar S, Jergas MD, Genant HK. Decay characteristics of bone marrow in the presence of a trabecular bone network: in vitro and in vivo studies showing a departure from monoexponential behavior. *Magn Reson Med*. 1996; 35:921–27. [PubMed: 8744022]
36. Glüer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK. Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. *Osteoporos Int*. 1995; 5:262–70. [PubMed: 7492865]
37. Lindsey CT, Narasimhan A, Adolfo JM, Jin H, Steinbach LS, Link T, et al. Magnetic resonance evaluation of the interrelationship between articular cartilage and trabecular bone of the osteoarthritic knee. *Osteoarthr Cartil*. 2004; 12:86–96. [PubMed: 14723868]
38. Bortz, J. Statistik für Human- und Sozialwissenschaftler. 6th ed. Springer; Berlin, Heidelberg: 2005.
39. Eliasziw M, Young SL, Woodbury MG, Fryday-Field K. Statistical methodology for the concurrent assessment of interrater and intrarater reliability: using goniometric measurements as an example. *Phys Ther*. 1994; 74:777–88. [PubMed: 8047565]
40. Patel V, Issever AS, Burghardt A, Laib A, Ries M, Majumdar S. MicroCT evaluation of normal and osteoarthritic bone structure in human knee specimens. *J Orthop Res*. 2003; 21:6–13. [PubMed: 12507574]
41. Takechi H. Trabecular architecture of the knee joint. *Acta Orthop Scand*. 1977; 48:673–81. [PubMed: 607757]

42. Fripp J, Crozier S, Warfield SK, Ourselin S. Automatic segmentation of the bone and extraction of the bone-cartilage interface from magnetic resonance images of the knee. *Phys Med Biol.* 2007; 52:1617–31. [PubMed: 17327652]
43. Bourgeat P, Fripp J, Stanwell P, Ramadan S, Ourselin S. MR image segmentation of the knee bone using phase information. *Med Image Anal.* 2007; 11:325–35. [PubMed: 17482502]
44. Tamez-Pena JG, Barbu-McInnis M, Totterman S. Knee cartilage extraction and bone-cartilage interface analysis from 3D MRI data sets. *Proc SPIE Medical Imaging.* 2004; 5370:1774–84.
45. Huang F, Chen X, Ye D, Hertel S. A distance weighted directional gradient method for fully automatic bone segmentation of knee MRI. *Proc Intl Soc Mag Reson Med.* 2008; 16:3631.
46. Grampp S, Majumdar S, Jergas M, Newitt D, Lang P, Genant HK. Distal radius: In vivo assessment with quantitative MR imaging, peripheral quantitative CT, and dual X-ray absorptiometry. *Radiology.* 1996; 198:213–18. [PubMed: 8539382]
47. Selby K, Majumdar S, Newitt DC, Genant HK. Investigation of MR decay rates in microphantom models of trabecular bone. *J Magn Reson Imaging.* 1996; 6:549–59. [PubMed: 8724422]
48. Yablonskiy D, Haacke E. Theory of NMR signal behavior in magnetically inhomogeneous tissues: The static dephasing regime. *Magn Reson Med.* 1994; 32:749–63. [PubMed: 7869897]
49. Dixon WT. Simple proton spectroscopic imaging. *Radiology.* 1984; 153:189–94. [PubMed: 6089263]
50. Reeder S, Pineda AR, Wen Z, Shimakawa A, Yu H, Brittain JH, et al. Iterative “Dixon” water-fat separation with echo asymmetry and least squares estimation (IDEAL): application with fast spin-echo imaging. *Magn Reson Med.* 2005; 54:636–44. [PubMed: 16092103]

Figure 1.

(A) Flow chart of the Background classification step, where the threshold is obtained through EM-classification of the voxels within three specific slices, followed by utilizing the signal intensities of the resulting EM-classes. (B) Bone marrow classification is carried out in two single steps. Ratio computation yields the volumetric ratio3 data with its feature of a bimodal pixel value distribution within each slice. Hence bone marrow segmentation was obtained applying slice wise thresholding. (C) Contour refinement is carried through an adapted 3D Level Set algorithm, where the steering force is derived from an admissible value range of the ratio3 data. (D) Three involved processing steps of the standardized ROI

generation within the lateral and medial femoral condyles. 12 ROIs at both compartments were constructed utilizing the elliptical shape of the condyles after projection onto a 2D sagittal plane.

Initialization step	
user defined L partitions	
assume Gaussian mixture model (GMM)	
within partition l, pixels have a probability distribution represented by $p_l(\mathbf{x} \theta_l)$	
initialize estimates of θ_l	
Expectation Maximization step	
Repeat	
	step 1 (E-step): obtain an estimate of the labels based on the current parameter estimates
	step 2 (M-step): update the parameter estimates based on the current labeling
Until $ \text{current-previous} < \epsilon$	
Classification step	
for each pixel	
choose that label which maximizes $p_l(\mathbf{x} \theta_l)$	

Figure 2.

Pseudo code of the EM algorithm classifying the pixels into L defined partitions. θ_l represents the parameters of the data in segment l, mean and variance of the grey levels.

Figure 3.
Smoothed 3D reconstructions of the bones, obtained after automated bone marrow extraction and contour refinement.

Figure 4.
Representative labelling of the 12 different regions of interest for the lateral and medial compartment used throughout the study.

Figure 5.

First results (b) obtained applying the proposed bone marrow classification workflow on 3D MRI data of the hip joint (a). Scan parameters were set as follows: $TE_{\text{short}} = 4.84$ ms and $TE_{\text{long}} = 15$ ms, $TR = 30$ ms, flip angle of 20° and $0.7 \times 0.7 \times 2$ mm³ resolution.

Table 1

Comparison of the T2* relaxation times (mean $\pm$ standard deviation) within 12 ROIs of three subjects at lateral and medial site of the base line scan, T2* in ms.

ROI	subject 1		subject 2		subject 3	
	lateral	medial	lateral	medial	lateral	medial
1	11.96 $\pm$ 2.15	11.20 $\pm$ 2.79	13.21 $\pm$ 1.22	12.34 $\pm$ 2.04	13.69 $\pm$ 1.83	14.73 $\pm$ 1.85
2	10.23 $\pm$ 2.58	9.77 $\pm$ 4.39	11.46 $\pm$ 2.03	10.02 $\pm$ 3.11	10.58 $\pm$ 2.01	11.98 $\pm$ 2.39
3	9.23 $\pm$ 2.02	10.10 $\pm$ 3.27	9.98 $\pm$ 2.52	9.76 $\pm$ 2.45	9.54 $\pm$ 2.94	10.96 $\pm$ 2.95
4	10.43 $\pm$ 1.92	11.18 $\pm$ 2.66	13.26 $\pm$ 2.42	11.01 $\pm$ 2.43	10.97 $\pm$ 2.70	11.54 $\pm$ 2.16
5	8.24 $\pm$ 1.91	11.28 $\pm$ 3.27	9.09 $\pm$ 2.33	10.15 $\pm$ 3.96	9.01 $\pm$ 3.06	11.14 $\pm$ 3.32
6	8.22 $\pm$ 2.49	10.24 $\pm$ 4.32	8.48 $\pm$ 2.82	8.92 $\pm$ 3.27	7.71 $\pm$ 2.70	9.07 $\pm$ 3.67
7	10.20 $\pm$ 1.46	10.94 $\pm$ 2.66	9.47 $\pm$ 2.01	9.84 $\pm$ 3.15	9.29 $\pm$ 2.31	8.70 $\pm$ 1.99
8	11.26 $\pm$ 2.72	12.10 $\pm$ 2.22	11.62 $\pm$ 2.46	11.29 $\pm$ 2.29	11.70 $\pm$ 2.30	11.24 $\pm$ 1.80
9	7.19 $\pm$ 1.85	8.55 $\pm$ 1.81	8.22 $\pm$ 2.67	9.40 $\pm$ 4.01	6.30 $\pm$ 1.84	10.46 $\pm$ 3.11
10	8.43 $\pm$ 3.07	7.99 $\pm$ 2.75	7.95 $\pm$ 2.31	8.68 $\pm$ 2.90	6.33 $\pm$ 1.57	8.18 $\pm$ 3.20
11	8.36 $\pm$ 1.05	8.15 $\pm$ 2.42	7.95 $\pm$ 1.71	8.3 $\pm$ 4 3.27	7.20 $\pm$ 1.47	7.75 $\pm$ 3.15
12	10.57 $\pm$ 1.43	9.76 $\pm$ 0.80	10.55 $\pm$ 1.50	9.19 $\pm$ 2.12	8.77 $\pm$ 1.28	8.65 $\pm$ 2.44

Table 2

Reproducibility of the effective transversal relaxation T2*: Individual min-max CV of T2* measurements in the corresponding ROI and CV_{SD} across the subjects are reported separately for the lateral and medial site.

subject	lateral				medial			
	min (%)	ROI	max (%)	ROI	min (%)	ROI	max (%)	ROI
1	1.01	6	6.79	10	0.69	4	4.20	2
2	1.12	5	6.48	10	0.75	1	5.46	9
3	1.10	8	8.59	9	0.73	3	3.93	5
4	0.58	7	6.42	9	0.55	8	6.00	3
5	0.59	10	3.45	6	1.44	2	8.88	5
6	0.35	4	4.84	2	1.14	3	3.85	5
CV _{SD}	1.79	11	4.87	2	2.38	7	4.04	5

Table 3

Assessment of the intraoperator reliability, lateral: Mean T2* of the manual and automatic analysis within ROI #11 of three repetitive scans are presented. Furthermore, the reproducibility expressed as CV and CV_{SD}, the standard error of measurement (SEM), the reliability coefficient ρ_{intra} , the 95% one-sided lower-limit confidence interval (conf. int.), and the minimum T2* difference (min. diff.) are reported. The F-value supports the rejection of H_0 for an intrater reliability hypothesis testing $H_0: \rho \leq \rho_0$ versus $H_1: \rho > \rho_0$, with $\rho_{\text{intra}} = 0.9$.

	operator A		operator B		operator C		automatic	
	T2* (ms)	CV(%)	T2* (ms)	CV (%)	T2* (ms)	CV(%)	T2* (ms)	CV(%)
subject1	9.57 ± 0.29	3.01	9.17 ± 0.35	3.77	9.17 ± 0.09	0.96	8.39 ± 0.13	1.59
subject2	8.19 ± 0.41	4.95	8.68 ± 0.32	3.68	7.51 ± 0.44	5.90	7.85 ± 0.14	1.83
subject3	8.49 ± 0.13	1.53	8.72 ± 0.20	2.32	7.60 ± 0.09	1.22	7.35 ± 0.18	2.41
subject4	8.29 ± 0.04	0.49	8.31 ± 0.09	1.09	7.64 ± 0.20	2.58	7.50 ± 0.16	2.10
subject5	8.57 ± 0.06	0.72	8.28 ± 0.04	0.48	8.33 ± 0.07	0.82	8.50 ± 0.18	2.10
subject6	13.11±0.22	1.65	13.26±0.38	2.83	13.03±0.67	5.15	12.07± 0.13	1.08
	F		F		F		F	
SEM	0.23 ms		0.26 ms		0.34 ms		0.15 ms	
CV _{SD}	2.45 %		2.79 %		3.86 %		1.79 %	
ρ_{intra}	0.99	7.40**	0.93	5.67**	0.97	3.32*	0.99	16.35**
conf. int	(0.96,1)		(0.94,1)		(0.91,1)		(0.98,1)	
min diff.	0.64 ms		0.73 ms		0.95 ms		0.43 ms	

Table 4

Assessment of the intraoperator reliability, medial: Mean T2* of the manual and automatic analysis within ROI #11 of three repetitive scans are presented. Furthermore, the reproducibility expressed as CV and CV_{SD}, the standard error of measurement (SEM), the reliability coefficient ρ_{intra} , the 95% one-sided lower-limit confidence interval (conf. int.), and the minimum T2* difference (min. diff.) are reported. The F-value supports the rejection of H_0 for an intrater reliability hypothesis testing $H_0: \rho \leq \rho_0$ versus $H_1: \rho > \rho_0$, with $\rho_{\text{intra}} = 0.8$.

	operator A		operator B		operator C		automatic	
	T2* (ms)	CV(%)	T2* (ms)	CV(%)	T2* (ms)	CV(%)	T2* (ms)	CV(%)
subject1	8.61 ± 0.48	5.55	7.50 ± 0.30	4.04	7.68 ± 0.33	4.23	8.15 ± 0.12	1.41
subject2	10.53±0.23	2.16	9.87± 0.19	1.92	9.36 ± 0.13	1.39	8.31 ± 0.26	3.17
subject3	8.04 ± 0.34	4.27	7.26 ± 0.42	5.77	7.10 ± 0.40	5.62	7.80 ± 0.14	1.80
subject4	8.73 ± 0.52	5.94	8.19 ± 0.29	3.53	7.43 ± 0.40	5.34	7.71 ± 0.37	4.77
subject5	9.80 ± 0.17	1.71	9.53 ± 0.31	3.28	8.93 ± 0.22	2.46	8.47 ± 0.17	2.03
subject6	12.41±0.22	1.81	12.01±0.23	1.91	11.36±0.33	2.91	11.21± 0.15	1.36
		F		F		F		F
SEM	0.35 ms		0.30 ms		0.32 ms		0.22 ms	
CV _{SD}	3.64 %		3.30 %		3.65 %		2.56 %	
ρ_{intra}	0.95	4.44*	0.87	6.16**	0.96	5.55**	0.98	11.38**
conf. int	(0.85,1)		(0.89,1)		(0.88,1)		(0.94,1)	
min diff.	0.98 ms		0.83 ms		0.87 ms		0.61 ms	