Abstract—Automatic computer-based methods are well suited for the image analysis of the different components in atherosclerotic plaques. Although several groups work on such analysis some of the methods used are oversimplified and require improvements when used within a computational framework for predicting meaningful stress and strain distributions in the heterogeneous arterial wall under various loading conditions. Based on high-resolution magnetic resonance imaging of excised atherosclerotic human arteries and a series of two-dimensional (2-D) contours we present a segmentation tool that permits a three-dimensional (3-D) reconstruction of the most important tissue components of atherosclerotic arteries. The underlying principle of the proposed approach is a model-based snake algorithm for identifying 2-D contours, which uses information about the plaque composition and geometric data of the tissue layers. Validation of the computer-generated tissue boundaries is performed with 100 MR images, which are compared with the results of a manual segmentation performed by four experts. Based on the Hausdorff distance and the average distance for computer-to-expert differences and the interexpert differences for the outer boundary of the adventitia, the adventitia-media, media-intima, intima-lumen and calcification boundaries are less than 1 pixel (0.234 mm). The percentage statistic shows similar results to the modified Williams index in terms of accuracy. Except for the identification of lipid-rich regions the proposed algorithm is automatic. The nonuniform rational B-spline-based computer-generated 3-D models of the individual tissue components provide a basis for clinical and computational analysis.

Index Terms—Active contour, atherosclerotic artery, segmentation, snake, 3-D reconstruction, vessel wall imaging.

I. INTRODUCTION

A CCURATE descriptions of arterial wall morphology would be helpful for quantitative diagnosis of atherosclerosis, treatment or surgical planning, monitoring disease progress or remission, and for comparing efficiencies of treatments. There are several image acquisition methods available for obtaining volumetric information about the arterial wall and plaque components; one example is intravascular ultrasound (IVUS), [1], [2], which provide high-resolution images but also artifacts and limited contrast. In addition, IVUS is an invasive method.

Noninvasive high-resolution magnetic resonance imaging (hrMRI) has considerable potential for characterizing atherosclerotic plaque components [3]–[6], and intravascular hrMRI provides improvement of the spatial resolution in in vivo measurements ([7] and [8]). In recent years, optical coherence tomography (OCT) has developed rapidly [9], [10]. Despite its limited penetration depth of 1–2 mm, OCT might also have potential for the characterization of smaller vessels such as coronary arteries.

To date, several studies on diseased arterial walls using computational mechanics have been based on oversimplified morphological (single-layer) models, which are inconsistent with histological evidence (for an overview of existing computational models, and a proposal for a new method of simulating, e.g., balloon angioplasty using a layer-specific three-dimensional (3-D) morphological model, hrMRI, and mechanical testing, see [11]). Appropriate morphological models that represent the 3-D boundary surfaces of histological and mechanically relevant components of the diseased wall are a prerequisite for the computation of meaningful stress and strain distributions in atherosclerotic arteries under various loading conditions. Assessment of the arterial wall structure is an issue of highest clinical priority. Unfortunately, existing standard imaging modalities, such as angiography, IVUS, or computer tomography provide little or no information on the structures and histological compositions of diseased walls and, hence, do not meet the requirements for mechanical investigations. Nonetheless important work has been carried out using IVUS imaging; see, e.g., the in vitro study [12] which presents a method based on RF IVUS data in which coronary plaque composition is estimated. One study [13] documents a 3-D active-surface system for border detection that facilitates the analysis of many images with minimal user interaction. In particular, an automatic 3-D detection of the luminal and

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and 500-μm slices, and was performed on a 0.234-mm-averaged image, which indicates a significant SNR improvement.

In this section, we describe the preparation of the specimens, the experimental setup, the process of image acquisition on a whole-body MR system and the acquisition of histological sections. Finally, we provide a brief description of the preprocessing technique for the MR images. This technique aims to remove (or correct) acquisition-based artifacts.

Fig. 1. Raw hrMRIs show the complex structure of atherosclerotic lesions: (a) PD-weighted image; (b) T2-weighted image; (c) T1-weighted image and (d) averaged image, which indicates a significant SNR improvement.

II. MATERIALS AND METHODS

In recent years, MR imaging has become more and more important. However, the image resolution achieved using standard clinical scanners is sometimes sparse when compared with the dimension of the underlying tissue component. For example, we are working with an in-plane resolution of 0.234 × 0.234-mm and 0.6-mm slice thickness. Considering the image resolution used and the wall thickness of an healthy iliac intima of about 0.1 mm and with reference also to the Shannon theorem, it must be concluded that it is not possible to image a nondiseased intima. To solve this problem, we propose to use a model-based active contour segmentation algorithm. By model-based we mean the use of an algorithm that includes statistical geometrical data of the underlying tissue for those regions where the image resolution is too low. For example, we restrict our active contour to segment a tube-like object with typical morphology and include statistical information on the wall thicknesses of the nondiseased intima, media and adventitia as “good guess” values for starting our algorithm. The diseased part of the intima and its components, i.e., calcification, lipid pool, fibrotic intima, can be imaged with a standard clinical scanner because they are much thicker than a nondiseased intima.
Tyrode solution (in mmol: NaCl 136.9, KCl 2.7, MgCl₂ 1.05, NaHCO₃ 11.9, NaH₂PO₄ 0.47, EGTA 2.0) until the MR scanner was available (approx. 4 to 8 hours after removal from the human body). The Tyrode solution is a salt solution, specifically a modified Locke-Ringer solution used in physiological experiments, tissue cultures and tissue preservation.

B. Experimental Setup

1) Markers: In order to align the MR image slices with their corresponding histological images, we have to mark the specimen. Hard markers (mechanically) cause problems in the cutting process used to obtain histological sections. They break out, or move and produce cutting artifacts in the histological section. For histological sections, colored markers are the best choice since they do not produce cutting artifacts; however, MR imaging requires different material properties and use of hard markers would be valuable. We propose the following method: surgical twine of approximately 0.1-mm diameter was imbued with a water-insoluble dye. We then placed the twine along the arterial wall, as illustrated in Fig. 2. The twine serves as a marker and is slightly visible in the MR images (e.g., at the bottom of the images in Fig. 1). After the MR experiments the twine was removed, and the residual dye marks the position of the twine, which can be seen clearly on the histological sections.

2) Vessel Preparation: After the markers were placed correctly, we prepared about 2 liters of Tyrode solution, which was mixed with 4 ml/liter contrast medium (Gadolinium with a concentration of 0.5 mmol/ml). The vessel was then put into a customized Perspex container and fixed at its ends with surgical suture in order to prevent movements of the vessel during scanning. The Perspex container was then filled with the prepared Tyrode solution. For MR measurements it is important that all air bubbles inside and outside the vessel are removed to avoid artifacts. For that reason we rinsed the vessel well using a surgical syringe. The Tyrode solution was then warmed up to 37°C and kept at this temperature by means of a heating spiral. The heating spiral was fixed inside the borders of the Perspex container, far away from the specimen. This was necessary to minimize flow artifacts. The temperature of the Tyrode solution was controlled by a heating controller. In that way the specimen was kept at 37 °C during the time of acquisition to provide conditions similar to those in vivo. The temperature was measured with Fluoroptic temperature sensors (fluoroptic system, Luxtron model 3000, Luxtron, Santa Clara, CA). The sensors were placed at three locations inside the Perspex container.

Since spatial resolution with an acceptable signal-to-noise ratio (SNR) is related to field strength and the signal acquisition (receiver coil system), we used a small circularly shaped surface coil with a diameter of 15 cm and provided a short coil to specimen distance of < 3 cm. The coil was placed directly under the Perspex.

C. Image Acquisition

The artery was scanned on a whole-body MR system at 1.5 T (Philips ACS-NT). Three-dimensional turbo spin echo (TSE) sequences were applied to achieve high spatial resolution and a sufficient SNR in an acceptable scan time of 10–15 min. By applying 3-D TSE sequences, we obtained a coronal proton density (PD)-weighted sequence (repetition time, 2000 ms; echo time, 17 ms; turbo-factor, 6; 256 × 256 matrix), a coronal T1-weighted sequence (repetition time, 600 ms; echo time, 15 ms; turbo-factor, 3; 256 × 256 matrix), and a coronal T2-weighted sequence (repetition time, 2000 ms; echo time, 70 ms; turbo-factor, 9; 256 × 256 matrix). The in-plane resolution for all sequences was 0.234 mm [field of view (FOV) 60 mm], and the slice thickness was 0.6 mm. The multispectral images are used for the segmentation of the different intimal plaque components. From the specimen, 32 MRI sections with a distance of 0.6 mm were obtained. Since the two outer wall boundaries of the adventitia and media provided nearly homogeneous signal, and since we observed that they contained no noticeable differences in the T1-, T2-, and PD-weighted images, we propose to fuse these three images in order to provide a better SNR, just for the first segmentation steps in which we separate the three wall boundaries. The influence of the inhomogeneous reception sensitivity was the same for each of the three scans (since the configuration coil-tissue specimen was not changed) and we, therefore, assumed the same intensity inhomogeneities for all three scans. Thus, we averaged the T1-, T2-, and PD-weighted images. This gave us a SNR improvement of approximately √3 (by assuming the same SNR for all three images). Fig. 1 shows the three raw images: PD-weighted, T2-weighted, T1-weighted, and the averaged image Fig. 1(d), in which we can observe a significant SNR improvement.

D. Histology

Due to the high signal contrasts, hrMRI can distinguish between different tissue components; however, in some case it cannot actually identify which component it is. Therefore, histological analysis is required, in particular for the identification of lipids.
After MR image acquisitions, the arterial segment of about 20-mm length was cut into two 10-mm segments; see Fig. 3. One section was used for histological analysis, and could be correlated with the corresponding hrMRIs, while the other section was used for mechanical tests. The mechanical testing part will not be discussed in the present work, but the interested reader is referred to [16], in which this aspect is documented extensively.

The 10-mm segment reserved for histomorphometric study was fixed in 70% alcohol, and then embedded in methyl methacrylate plastic (Sigma Chemical Company, St Louis, MO). The favorable properties of a plastic embedding include low viscosity for complete and rapid infiltration and the high hardness of the resulting polymer. These properties allow harder samples to be used without decalcification of the specimens. To match MR images, series of five histological 5-μm consecutive sections were taken every 0.6 mm.

In order to characterize and classify tissue components, three slides from each stack were stained with Ladewig’s trichrome (LT), elastica van Gieson (EvG), and Hematoxylin and Eosin (HE), or chromotrope anilin blue (CAB) trichrome. Typically, the LT-stained section is used to identify calcification, the EvG-stained section can be used to identify elastic fibers, the HE-stained section is appropriate for identifying the cell nuclei, while the CAB trichrome colors muscle cells red and fibers blue. The remaining two slides, which were not stained, were kept in stock in case additional material was required. Each section was then photographed by a photomicroscope with an average total linear magnification of 20 x. Digital images were acquired from the sections using a color charge coupled device digital camera (Nikon Coolpix 950 mounted on a Nikon Optiphot microscope) with a resolution of 1600 x 1200 and 8 bits per color. Pathologists inserted the borderlines of the adventitia, media, intima, lipid pool and the calcification into the digital images using a standard image processing software. Additionally, due to the relatively low-resolution images, the histological sections were examined under the microscope. This was often necessary for the determination of the lipid pool.

Fig. 4 presents three images of a representative stenotic human external iliac artery from our data-set (i.e., specimen I in [16]). Fig. 4(a) shows the macroscopic view, Fig. 4(b) the histological section (transmitted light microscopic photograph), and Fig. 4(c) the corresponding preprocessed PD-weighted hrMRI. The contrast between the macroscopic image of the section [Fig. 4(a)], and the corresponding histological and MR images can be observed. The borderlines in Fig. 4(a),(b) were drawn by a pathologist, while those in Fig. 4(c) were identified using the proposed segmentation scheme described in Section III-C.

E. Preprocessing of the MR Images

Acquisition artifacts and image quality directly affect the segmentation process. Therefore, several preprocessing steps are required to remove these artifacts and to improve the quality of the image.

1) Intensity Nonuniformity Correction: A typical artifact is the intensity nonuniformity (INU), which refers to smooth, local changes in the image intensity induced by the data acquisition technique. This artifact is typically present in MRI data and depends on a combination of factors, including a) the shape and electromagnetic properties of the object being scanned; b) the spatial sensitivity of the radio frequency (RF) receiver coil; c) the gradient-driven eddy currents; d) the frequency response of the receiver; e) the spatial inhomogeneity of the excitation field. The influence of INU on the visual interpretation of images is significant. Since many segmentation methods use, at least partially, absolute image intensity values the INU leads to incorrect segmentations.

Correction of INU is typically based on a multiplicative model of the artifact. Ignoring random noise, the observed signal \( o(\mathbf{x}) \) can be related to the true (artifact-free) signal \( f(\mathbf{x}) \) according to (see [17, p. 17])

\[
 o(\mathbf{x}) = f(\mathbf{x})l(\mathbf{x}) 
\]

where \( f(\mathbf{x}) \) is the distortion factor (also called bias field) at spatial location \( \mathbf{x} \). Using (1), the true image signal \( f \) is obtained by multiplying the observed signal \( o \) by the reciprocal of the estimated field \( f \).
A number of methods for estimating the distortion factor $f$ have been proposed in the literature; see, e.g., [17]–[19]. The contribution [20] is based on a wavelet transform, while in [21] a minimization problem for the entropy is solved in order to estimate the bias field. A robust method, referred to as nonparametric nonuniform intensity normalization (N3), was proposed in [17]. This method is fully automatic, does not rely on an explicit segmentation of the image or on a priori information on tissue class statistics. Since this algorithm is easy to handle and gives satisfactory results for our specifications we use it for our analysis, although the N3 method is not actually the newest or best method. For example, the algorithm documented in [22] provides slightly better results than the N3 method. However, we observed that the results obtained from the N3 algorithm are good enough for our requirements and they have no significant influence on our segmentation algorithm. According to our tests the influence of the intensity uniformity does not play an important role since the considered objects are mainly small (about 10-mm diameter) and occupy less than 20% of the FoV. Moreover, the N3 code is freely available for noncommercial use.

2) Noise Filtering: In choosing the optimal parameters for the MR imaging, we have to compromise between (higher) spatial resolution and (lower) SNR. We are interested in a high spatial resolution with acceptable SNR. The image quality of the acquired image can be improved by enhancing the SNR with a noise filter. The disadvantage of the most basic filters (median, Gaussian, etc.) is that they also smooth edges. There is always a compromise between loss of information (by smoothing) and enhancing the image quality (reduction of noise). For this reason we need an anisotropic filter, and we apply the edge-flat-gray (EFG) filter for our analysis (see [23]). Many anisotropic filters lead to undesired staircasing in regions with gradual image variations. The EFG filter, however, treats such regions as consisting of flat scale gradients and, therefore, flattens such regions. These regions are often present in MR images; therefore, the EFG filter is well suited for segmentation preconditioning of these images. For a detailed algebraic treatment of the EFG filter see [23].

III. SEGMENTATION

For the segmentation of the arterial wall we use a deformable model or active contour-based model [24], also called “snake.” We briefly review the traditional snake model and then present our approach to generating 3-D models by stacking contours obtained from a series of two-dimensional (2-D)–derived contours. We describe the regularization process and define the different tissue components of the arterial wall. Finally, in Sections III-C3 and III-C4 we describe our segmentation method, comprising a fully automatic part and a manual part.

A. Snakes: Active Contour-Based Models

Automatic segmentation methods are often challenged by artifacts, noise and the limited spatial resolution characteristic of vascular MRI. It is, therefore, difficult to segment these types of images automatically. Many applications, however, require the segmentation of a set of image slices and a 3-D reconstruction of complex objects that comprise different tissue components. For such applications active contour-based models or snakes, first introduced in [24], are preferred. They have a wide range of application; see, e.g., [25]–[33].

In the last few years several formulations of active contours have been proposed, with their functionality enhanced by including, e.g., additional forces such as pressure forces [34], [35] and distance transform forces [36], which are used to drive the snake. In addition, there are studies that extend an initially 2-D representation to 3-D. In this respect, the application area has been extended. Active contours are used preferentially for ultrasound images and many other medical images. They are used for motion tracking, e.g., of the beating heart [37], the tongue [38]; for a survey see [39]. However, active contours are quite often associated with problems such as initialization and poor convergence to boundary concavities. The method, as documented in, e.g., [40], uses a new external force for active contours and largely solves both problems. It is called gradient vector flow (GVF) and differs fundamentally from traditional snake external forces. The paper [41] introduces the generalized gradient vector flow (GGVF) as an extension of the GVF formulation. It improves the convergence rate of active contours to long, thin boundary indentations while maintaining the features of GVF.

Here we present a model-based automatic segmentation method with active contours by using GGVF. The model-based formulation of the active contour allows us to use information on the composition and geometry of the object to be segmented. This model-based formulation is necessary since it is often difficult to find the boundaries between these layers due to the limited image resolution. The study [26] proposes a 3-D reconstruction mechanism that requires only 2-D contours. In accordance with this study we propose to use 2-D active contours. Our method has the following advantages: 1) it requires only minimum input, which can be obtained automatically; 2) it admits the support of the inflation mechanism, which allows us to overcome homogeneous regions and noise in the data; 3) any 3-D shape may be reconstructed; 4) it is computationally efficient.

B. Traditional Snake Model

Mathematically, a deformable contour is a curve of the form $x(s) = (x(s), y(s))$ in the image plane $(x, y) \in I^2$, where $s \in [0, 1]$ is a normalized arc length along the curve. The contour moves through the plane so as to minimize the energy functional

$$\Phi(x(s)) = \int_0^1 [E_{\text{int}}(x(s)) + E_{\text{ext}}(x(s))]ds$$

(2)

where $E_{\text{int}}$ and $E_{\text{ext}}$ are the internal and external energies of the snake, respectively. The internal energy defines an elastic property for the snake, and is defined by

$$E_{\text{int}} = \frac{1}{2}(\alpha|\kappa'(s)|^2 + \beta|\kappa''(s)|^2)$$

(3)

where $\alpha$ and $\beta$ are weighting parameters that determine to what degree the flexible contour can stretch or bend, respectively, at any point. Hence, the first term in (3) controls the energy due to stretching while the second term is associated with the bending energy. The variables $\kappa'(s)$ and $\kappa''(s)$ denote the
first and second derivatives with respect to \( s \). The external energy function \( E_{\text{ext}} \) is derived from the particular image so that it is driven by image features of interest, such as boundaries.

The final result is obtained after the minimization of the energy function \( G(\mathbf{x}(s)) \); see (2). This satisfies the Euler-Lagrange equations

\[
\frac{\partial}{\partial s} (\alpha \mathbf{x}'(s)) + \frac{\partial^2}{\partial s^2} (\beta \mathbf{x}'(s)) + \nabla E_{\text{ext}}(\mathbf{x}(s)) = 0
\]  

(4)

which may be written more concisely as

\[
\alpha \mathbf{x}''(s) - \beta \mathbf{x}'''(s) - \nabla E_{\text{ext}} = 0.
\]  

(5)

Equation (5) can be viewed as a force balance equation

\[
\mathbf{F}_{\text{int}} + \mathbf{F}_{\text{ext}} = 0
\]  

(6)

where the internal force is \( \mathbf{F}_{\text{int}} = \alpha \mathbf{x}''(s) - \beta \mathbf{x}'''(s) \) and the external force is \( \mathbf{F}_{\text{ext}} = -\nabla E_{\text{ext}} \).

To find a solution of (5), the snake is usually treated as a dynamical system by introducing \( \mathbf{x} \) as a function of time \( t \) and the parameter \( s \), i.e., \( \mathbf{x}(s, t) \). We then obtain

\[
\mathbf{x}_t(s, t) = \alpha \mathbf{x}''(s, t) - \beta \mathbf{x}'''(s, t) - \nabla E_{\text{ext}}
\]  

(7)

where \( \mathbf{x}_t(s, t) \) is the partial derivative of \( \mathbf{x}(s, t) \) with respect to \( t \). When the solution \( \mathbf{x}(s, t) \) stabilizes, \( \mathbf{x}_t(s, t) \) vanishes and we achieve a solution for (5).

The work [40] proposed to replace the external force \( \mathbf{F}_{\text{ext}} \) by a new static field \( \mathbf{F}_{\text{ext}} = \mathbf{v}(x, y) \), which is a GVF field. Consequently, for the dynamic snake equation, we obtain

\[
\mathbf{x}_t(s, t) = \alpha \mathbf{x}''(s, t) - \beta \mathbf{x}'''(s, t) + \mathbf{v}.
\]  

(8)

The more robust GGVF formulation, as proposed by [41], is based on a 2-D vector field, which is iteratively derived from the intensity gradient field of the original image, where the vector \( \mathbf{v}(x, y) \) at a specific image position \( (x, y) \) points in the direction of the nearest image edge.

C. Present 3-D Model Based on 2-D–Derived Contours

For a 3-D reconstruction of complex morphological tissue components a 3-D formulation of a snake, sometimes called a balloon, is required. The enhancement from a 2-D to a 3-D representation is straightforward but has some disadvantages, including increased computational costs. We propose to use here a semi–3-D model, which is based on a series of 2-D snakes constrained to move only in the 2-D plane of the associated image. The corresponding neighboring snake curves in the slices above and below are considered in order to produce a smooth 3-D surface. This is done by including a \( z \)-derivative directional energy term in the snake energy according to [13]. This formulation has the advantage that the constraint movement (in the 2-D plane) inhibits mis-movements in the axial direction, while it is suitable for any tube or cylindrically shaped objects. Any 3-D shape can then be represented as a stack of a series of 2-D contours. The use of a set of linked 2-D contours instead of a traditional 3-D surface representation leads to a number of simpler processes during the snake deformation and, consequently, to lower computational costs. Furthermore, the set of 2-D contours is easier to initialize, initialization being one of the main difficulties with use of snakes.

1) Regularization: A known problem of snakes is their regularization. The discrete points of a snake may concentrate in regions where the intensity gradient is high, while there may be only a few points in regions with a low intensity gradient. Therefore, regions with high point densities are preferred and the accurate boundary in regions with lower point densities may not be found. The regularization process redistributes the points in such a way that they are uniformly distributed along the boundary. In order to save computational costs we perform the regularization only after every tenth iteration step. By doing the regularization we eliminate the problem of oscillation points, which are points that move in opposite directions in successive iteration steps, i.e., they move forward and return back to the same location; they oscillate around a point.

2) Arterial Wall Morphology and Definition of the Tissue Components Used: For the tissue components of an aged diseased human artery we use the definitions as proposed in [11] and illustrated in Fig. 5 (see, also, Fig. 4). In general, an arterial wall is composed of three layers: adventitia, media and intima I. The membrana elastica externa and the membrana elastica interna are thin layers that separate the adventitia/media and the media/intima, respectively. These two layers are very thin (about 10–30 \( \mu \text{m} \)), and are not considered in this work (they are too thin to be identified by MR imaging). Aged diseased arteries may also contain plaque components such as a calcification I-c and a lipid pool I-p. An aged nondiseased intima may be thickened (in comparison to a young intima) without any appearance of collagenous fibers. Such a nondiseased intima is referred to here as “not otherwise specified.” I-nos (the abbreviation “nos” is frequently used in histopathology) is the fibrous intima at the medial border is denoted as I-fm, while the fibrotic part at the luminal border (fibrous cap) is denoted as I-fc. The borders between these tissue components cannot be distinguished by MR imaging. We define them as borders between the parts that lie behind the plaque region I-lp and before the plaque region I-c, if
they are present; otherwise, the borders are defined as those lines that bisect the diseased intima. The border that separates the diseased wall from the nondiseased wall is defined as the position where the wall thickness increases to 150% of the smallest wall thickness. The media can then be segmented in a nondiseased (M-nos) and a fibrous (diseased) part (M-f). This separation is not possible with the MR contrast, so we consider significantly thinner regions of the media as M-f, which, in general, is located behind the calcification. For a summary of the abbreviations introduced see the caption of Fig. 5.

We define the following four wall boundaries: the outer boundary of the adventitia, the adventitia-media boundary (membrana elastica externa), the media-intima boundary (membrana elastica interna), and the intima-lumen boundary, labeled \( C_A, C_{AM}, C_{MI}, \) and \( C_{IL} \), respectively. The boundaries of the lipid pool and the calcification are labeled \( C_{LP} \) and \( C_C \), respectively. For the different wall layers we use the active contour-based model, for which different parameters for each layer have been chosen. In addition, the initialization of the snake is different. For a poor initialization the algorithm fails.

As far as numerics is concerned, we use a simple optimization strategy. For each iteration step, we move each discrete point of the snake a step toward the direction of the gradient of the total snake energy. The decision to terminate the iteration process is made by considering that gradient. For this reason we compute the total snake energy for each iteration step and smooth it using a Gaussian function. If the gradient of the smoothed energy function is lower than some initial threshold, the distance of the movement is divided by a factor of 1.2. This adaptive change in the movement distance makes it possible to start with a larger movement and, therefore, with a faster convergence of the snake. The iteration is stopped after the gradient of the smoothed energy function reaches a second threshold.

3) Model-Based Segmentation Scheme: Now we describe our segmentation method in detail, based on the concept of snakes. We use information on the composition (e.g., calcification) that is located in the intimal region and the media behind it is always thin and geometric data (thickness, smoothness of boundaries) of a diseased aged human artery to be segmented. This leads to a model-based design of our algorithm.

We start with the detection of the boundaries of the adventitia and the lumen from a preprocessed MR image, as shown, e.g., in Fig. 6(a). For the initialization of the snake outside the artery we place the snake so that it captures the region of interest (ROI) shown by the (outer) dashed circle in Fig. 6(b). The outer solid line in Fig. 6(b) indicates the final boundary of the adventitia, while the final boundary of the lumen is represented by the inner solid line. Next we initialize the snake to detect the lumen. In order to obtain an estimate of the lumen, we use a highly smoothed image of the section from which the approximate luminal region is found by an automatic intensity threshold. Finally, we initialize the snake with a circle near the center of the lumen, as shown by the (inner) dashed circle in Fig. 6(b) (the final boundary of the lumen is represented by a solid line). After these two steps the boundaries of the arterial wall are determined.

The next step is the detection of the calcification, which appears as a dark area in the T1-, T2-, and PD-weighted images, as shown in Fig. 1. Note that the contrast between the calcification and the surrounding tissue is sometimes weak for the T2 image, depending on the surrounding tissue. For example, the lipid pool in the T2- and PD-weighted images is also characterized by dark intensity values [see Fig. 4(c)]. Therefore, we use the T1-weighted image for the determination of the calcification, which offers the highest contrast between calcification and surrounding tissue.

For an initial estimate of calcified areas we use a threshold method. For each section we consider all image areas from which the vessel volume is computed and analyzed, and a threshold is estimated by maintaining several constraints. For example, the threshold must be located in a range of \([0, \ldots, 60]\), and the volume selected by thresholding must be less than 20% of the vessel volume. The calcified volume selected by the thresholding process is then treated by morphological operations, i.e., opening and closing. Labeling is used to filter out regions with only a few pixels and to close holes inside the calcified volume. Boundaries of the volume so obtained are then chosen as an initial estimate for a snake. The final boundaries of the calcification are found after a few iterations of the snake. During the iteration process we do not allow the boundary to move too far from its initial estimate and we do not allow the boundary to move too close to the boundary of the adventitia \( C_A \).

Determination of the boundaries \( C_{AM} \) and \( C_{MI} \) is not so straightforward as for the determination of the adventitia and the lumen boundaries. An adequate contrast between adventitia, media and intima is, in general, not given at each position of the wall, and, therefore, an accurate boundary estimate is not possible from the intensity gradient information alone. The reason for this low contrast may come from thin wall layers (e.g., for I-nos), and the subsequent partial volume effect, which causes averaging of MR tissue values with neighboring tissues along the borders. Therefore, we need a good initialization for these two boundaries. Hence, we use statistical information from aged human iliac arteries (wall thicknesses of the individual layers) for choosing a good initialization, as documented in [42]. In addition, we assume that the outermost layer has constant thickness within a single-cross-section, and the media can become very thin in wall sections that are diseased, particularly behind the calcification.

In the next step, we separate the wall into diseased and nondiseased sections. To do so we search the position of the smallest wall thickness and name it “nondiseased reference thickness.” Wall sections that are thicker than 150% of the nondiseased reference thickness and wall sections that contain
calcification are segmented as diseased [see Fig. 6(c)]. The initial positions of the boundaries $C_{AM}$ and $C_{MI}$ are then estimated for nondiseased regions so that the proportion of the wall thickness of adventitia, media, and intima corresponds to their statistical proportion, as summarized in Table I (see [42, Table 2]).

4) Manual Part: The scheme for segmenting the tissue boundaries and corresponding tissue components, as described in Section III-C3, works fully automatically.

The contrast of the lipid pool $I_{lp}$ in the MR image is, however, not high enough to separate lipid regions from the fibrous intima (i.e., I-fc and I-fm, see Fig. 4(c)) in a fully automatic way. Hence, this separation is performed manually and the corresponding histological sections have to be considered. The reason for relatively continuous intensity gradients between the lipid pool and the surrounding tissue in the MR image is clear from looking at the histological section. There are not two different materials visible with a clear boundary, the transition is smooth. In addition, note that the density of cholesterol crystals in certain regions may vary from a very low concentration to a very high concentration (advanced stage of atherosclerosis are frequently accompanied with large lipid regions). Since a tissue with only a few cholesterol crystals cannot be identified from the MR image, a separation of sparse lipid regions is not straightforward; this is similar to difficulties that occur with segmentation of lipid regions during histological analysis. Hence, in the present analysis we consider only large lipid regions, which are characterized as yellow areas in the associated HE-stained images. This characterization is performed by experts using an image segmentation tool. In order to simplify the comparison of MR images and histological images, the histological images were registered by a simple rigid registration algorithm (translation and rotation), which is based on mutual information.

It turns out that about 90% of the specimens did not show large lipid regions, so that the (interactive) manual segmentation, as described, could be skipped.

### IV. Validation

In the present work, we are particularly interested in a comparison of the 3-D geometries of the different tissue components. To validate the quality of the boundaries obtained from our automatic segmentation algorithm we would have to compare the automatic segmentation results with the “true” segmentation (real boundaries), which we do not know. All we can do is to compare the (algorithmic) computer results with those segmented tissue components obtained by experts manually; we (usually) call this “ground truth.”

#### A. Manual Segmentation by Four Independent Experts

Comparing the results of the segmentations from only one expert with the computer segmentations would, in general, probably not be sufficient, because we do not know how accurate the segmentation of this single expert is. Hence, we consider four experts who are familiar with MR images of atherosclerotic plaque components. For the validation we select a set of 100 MR image slices (including the three contrast images for each slice), which came from 15 arterial specimens. The four experts were then asked to manually segment each image into four tissues A, M, I, and I-c by drawing five boundaries $C_A$, $C_{AM}$, $C_{MI}$, $C_{IL}$, and $C_C$. Note that the calcification boundary $C_C$ can be absent or can be present more than once in an image (if there are several regions of calcification in a specimen). Attention has to be paid to the segmented calcification regions ($C_C$ boundaries) because one expert may segment a calcified region as one closed boundary while another may segment the same region with two nearly connected boundaries. For the purpose of comparison we have then used the convex hull of the two boundaries.

The experts’ manual segmentation was performed using the medical imaging software Osiris [43]. Each boundary was drawn with the polygon ROI tool, which allows a boundary to be drawn using a polygon (between 50 to 100 points were used for each polygon). Each of the polygon ROI was then assigned an annotation, which corresponds to the segmented tissue name. The segmented images were stored in DICOM format. Finally, our validation software reads the DICOM files, with the segmented ROI polygons and their corresponding tissue names. To simplify the comparison between the boundaries drawn by the different experts we use the curve averaging method, as proposed in [44]. Using an iterative technique it averages the multiple experts’ boundaries to generate a new averaged curve, which is then used as our “ground truth” (gold-standard boundaries) for the evaluation. Now we have a clear relation between the segmented boundaries obtained from our automatic segmentation tool and the experts’ segmented boundaries.

#### B. Evaluation Methodology – Statistical Analysis

For the actual evaluation we use a methodology for boundary-based segmentation of images similar to that in [44]. In order to compare two given boundaries, we choose two (very different) distance measures: 1) the Hausdorff distance [44], [45], which is the maximum of the set of shortest distances between corresponding points of two shapes (it is a local measure); 2) the average distance [44], which measures the mean of the distance between every pair of corresponding points in the two boundaries of interest (it is a more global measure).

As a first step in the comparison of computer-generated boundaries and boundaries drawn by experts, we check if all tissue components are present in both segmentations, and if they are classified in the same way. In the next step we determine the geometric differences between the computer-generated boundaries and the experts’ segmented boundaries (in the form of the computed averaged curves), and use the two distance measures discussed. They were computed for each boundary type.

### Table I

Wall Thickness of Human Aged Non-Diseased Iliac Arteries (Adopted From [42, Table 2])

<table>
<thead>
<tr>
<th></th>
<th>Mean thickness (mm)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.43</td>
<td>0.08</td>
</tr>
<tr>
<td>M</td>
<td>0.73</td>
<td>0.15</td>
</tr>
<tr>
<td>I</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Total</td>
<td>1.3</td>
<td>0.16</td>
</tr>
</tbody>
</table>
For our statistical evaluation we consider any of the segmented boundaries separately in order to show the accuracy of our algorithm boundary-wise. Given this set of data we perform two statistical tests; the first one is based on the modified Williams index (WI), which is a generalization of the WI [46], and the second is based on the percentage statistic (PS); both were introduced in [44]. The WI computes the ratio between the average computer-to-expert variability and the average interexpert variability, while the PS computes the percentage of cases for which the computer-generated boundaries lie within the interexpert range. Since we have four experts the expected percentage of PS is 80%. In [44], it is claimed that the two statistical tests have very different behaviors when tested against the set of data. Note, however, that in a recent paper [47] it is shown that the comparative results discussed in [44] are not conclusive, mainly due to the simplicity of PS compared with WI. Finally, the 95% confidence interval (CI) for the estimate of the WI and of the PS is computed using the nonparametric technique (jackknife) in [48].

V. RESULTS

A. Tissue Boundary Detection and 3-D Reconstruction

For the representation of the segmentation and the computer-detected tissue boundaries we use nonuniform rational B-splines (NURBS) [49], which allow analytical descriptions of the individual boundaries. The use of NURBS provides smooth surfaces and a suitable basis for mesh adaptation procedures within the finite element method. NURBS allows mesh refinement with respect to the (original) reference geometry, which is advantageous [11]. In addition, the use of NURBS allows easy generation of surfaces that come from a set of 2-D boundaries. A 3-D reconstruction of a representative atherosclerotic human iliac artery and its individual tissue components in the form of smooth surfaces are shown in Fig. 7. Table II shows the Hausdorff and the average distances by comparing the computer-generated boundaries $C_A, C_{AM}, C_{MI}, C_{IL}$, and $C_C$ with the boundaries drawn by the four experts for the individual tissues averaged over the set of 100 images. In addition, Table II summarizes the WI and PS estimates, and their 95% confidence intervals for the five boundaries. Thereby the values for the mean computer-to-expert differences (CED) and the mean expert-to-expert differences (EED) are provided in pixels and (related) millimeters ± standard deviation (SD).

B. Performance Tests of the Algorithm

As can be seen from Table II the computer-to-expert differences are nearly in the same range as the interexpert differences. These differences are less than 1 pixel (0.234 mm) for the outer boundary of the adventitia $C_A$, the adventitia-media boundary $C_{AM}$, the media-intima boundary $C_{MI}$, the intima-lumen boundary $C_{IL}$, and also for the calcification boundary $C_C$ when the average distance is considered. This is a good result for an automatic segmentation algorithm, and means that the disparity between the computer is not much higher than the disparity between the experts themselves.

The WI for each of the detected boundaries $C_A, C_{AM}, C_{MI}$ is close to 1, which means that the boundaries generated by our proposed automatic segmentation tool differ from the manually
The image intensity value of a voxel obtained, which includes two components by using the associated automatically generated boundaries that marked the image regions. In order to minimize the influence of partial volume voxels, we eroded those regions. The image intensity is represented by 8 bits. We normalized the intensities by the average intensity of the Tyrode solution, which is reversed. Given the inhomogeneous and the calciﬁcation boundary is not deﬁned in MR images. Therefore, an exact detection of such boundaries is difﬁcult. Due to the partial volume effect, the color display of Fig. 8 shows the feature space as intensity 3-D distributions of the different tissue components and the lumen using three contrasts. The 3-D plot is also projected to the 2-D planes for illustration purposes. The intensity distributions of the tissue components A, I-nos, I-fc, I-fm indicate a relatively large standard deviation. Although we eroded those tissue regions associated with the partial volume voxels, the partial volume effect still plays a considerable role due to the asymmetric voxel dimension.

C. MR Signal Characteristics

We computed the signal characteristics of the different tissue components by using the associated automatically generated boundaries that marked the image regions. In order to minimize the influence of partial volume voxels, we eroded those regions. The image intensity is represented by 8 bits. We normalized the intensities by the average intensity of the Tyrode solution, which was 177±15 for all three contrasts, and computed the statistical distributions.

Table III shows the MR signal intensities for the different tissue components and contrasts, and computed the statistical distributions.

<table>
<thead>
<tr>
<th>Boundary</th>
<th>Distance</th>
<th>CED</th>
<th>EED</th>
<th>W1</th>
<th>95% CI</th>
<th>PS (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₐ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hausdorff</td>
<td>0.931 ± 0.122</td>
<td>0.218 ± 0.029</td>
<td>0.913 ± 0.122</td>
<td>0.218 ± 0.029</td>
<td>0.98</td>
<td>(0.87, 1.01)</td>
<td>75.4</td>
</tr>
<tr>
<td>Average</td>
<td>0.492 ± 0.051</td>
<td>0.115 ± 0.012</td>
<td>0.482 ± 0.051</td>
<td>0.113 ± 0.012</td>
<td>0.97</td>
<td>(0.88, 1.02)</td>
<td>74.4</td>
</tr>
<tr>
<td>CₐM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hausdorff</td>
<td>0.965 ± 0.183</td>
<td>0.226 ± 0.043</td>
<td>0.952 ± 0.132</td>
<td>0.223 ± 0.031</td>
<td>1.02</td>
<td>(0.88, 1.05)</td>
<td>76.3</td>
</tr>
<tr>
<td>Average</td>
<td>0.513 ± 0.099</td>
<td>0.120 ± 0.023</td>
<td>0.856 ± 0.312</td>
<td>0.201 ± 0.073</td>
<td>1.01</td>
<td>(0.89, 1.04)</td>
<td>76.1</td>
</tr>
<tr>
<td>CₐL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hausdorff</td>
<td>0.930 ± 0.152</td>
<td>0.218 ± 0.036</td>
<td>0.893 ± 0.139</td>
<td>0.209 ± 0.033</td>
<td>0.97</td>
<td>(0.88, 1.01)</td>
<td>73.1</td>
</tr>
<tr>
<td>Average</td>
<td>0.573 ± 0.080</td>
<td>0.134 ± 0.019</td>
<td>0.855 ± 0.302</td>
<td>0.200 ± 0.071</td>
<td>0.98</td>
<td>(0.87, 1.02)</td>
<td>74.2</td>
</tr>
<tr>
<td>CₐC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hausdorff</td>
<td>1.103 ± 0.542</td>
<td>0.259 ± 0.127</td>
<td>1.091 ± 0.471</td>
<td>0.256 ± 0.110</td>
<td>0.67</td>
<td>(0.46, 0.72)</td>
<td>53.7</td>
</tr>
<tr>
<td>Average</td>
<td>0.856 ± 0.287</td>
<td>0.201 ± 0.067</td>
<td>0.849 ± 0.292</td>
<td>0.199 ± 0.068</td>
<td>0.66</td>
<td>(0.46, 0.73)</td>
<td>52.1</td>
</tr>
</tbody>
</table>

TABLE III

MR SIGNAL INTENSITY RELATIVE TO THE AVERAGE INTENSITY OF THE TYRODE SOLUTION FOR DIFFERENT TISSUE COMPONENTS AND CONTRASTS (IN %). ABBREVIATIONS FOR TISSUE COMPONENTS ARE ACCORDING TO FIG. 4

<table>
<thead>
<tr>
<th>PD</th>
<th>T2</th>
<th>T1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>54 ± 14</td>
<td>36 ± 18</td>
</tr>
<tr>
<td>M-nos</td>
<td>63 ± 11</td>
<td>39 ± 12</td>
</tr>
<tr>
<td>I-nos</td>
<td>61 ± 14</td>
<td>53 ± 17</td>
</tr>
<tr>
<td>I-fc</td>
<td>67 ± 16</td>
<td>55 ± 20</td>
</tr>
<tr>
<td>I-fm</td>
<td>45 ± 12</td>
<td>26 ± 15</td>
</tr>
<tr>
<td>I-c</td>
<td>15 ± 11</td>
<td>10 ± 9</td>
</tr>
<tr>
<td>I-lp</td>
<td>52 ± 10</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>M-f</td>
<td>61 ± 11</td>
<td>37 ± 10</td>
</tr>
</tbody>
</table>
of the voxel volume contribution. For example, the mean thickness of the nondiseased intima of iliac arteries is 0.17 mm (see Table I), where the size of 1 pixel is 0.234 mm. In our study this seems not to be a severe limitation because the nondiseased intima of iliac arteries is not of such clinical importance. For several other vessels with smaller dimensions, such as coronaries, however, the 1.5 T-based resolution of the MR imaging is too poor for performing an accurate segmentation. Scanners with higher field strengths and better customized coils may then be preferred. The assumption that the outermost layer has constant thickness might be true for nondiseased parts of arterial sections. However, the uniformity of the adventitial thickness for the diseased part within a single cross-section has yet to be established. Currently our algorithm works only for ex vivo specimens. The algorithm is limited to well-prepared vessels from which loose connective tissue can be removed.

VI. CONCLUSION AND DISCUSSION

hrMRIs have the ability to display the geometrical structure of atherosclerotic lesions. The present novel results, which come from a snake-based method for identifying contours in high resolution MR images of excised atherosclerotic iliac arteries, demonstrate the feasibility of producing highly resolved 3-D multicomponent models. The proposed semi-automated methodology uses a series of 2-D contours including the outer adventitia border and the boundaries of the adventitia-media, media-intima, intima-lumen, lipid pool and calcification. Except for the identification of lipid-rich regions the proposed algorithm is automatic. The segmentation of the lipid pool was performed manually, and both MR and histological images were used for this segmentation process. The performance of the automated contour mapping was tested by comparing boundaries that were generated automatically by computer with boundaries drawn by four experts. The computer-to-expert differences and the interexpert differences for all relevant boundaries of the tissue components were less than 1 pixel for the average distance measure. For the Hausdorff distance these differences were also less than 1 pixel with the exception of the lipid pool boundary, which yielded a mean pixel difference of 1.1. Segmented tissue components of diseased arteries provide a sound basis for subsequent biomechanical modeling and computational (finite element) stress-strain analysis.

The most severe problem of the proposed snake approach seems to be the reliable identification and segmentation of lipid pools and the surrounding fibrotic tissues, for which histological images are required. Atherosclerosis is a slow-growing disease, which means that we have smooth changes between the different pathological stages of this disease. For example, tissue may contain low or high quantities of lipids and may vary between a tissue with a few cholesterol crystals, lipid pool and calcified tissue, which have completely different mechanical behaviors. Therefore, the MR signal is also mixed and it makes it difficult to distinguish between these different materials on the basis of hrMRIs alone. Although it is sometimes also not that straightforward to draw clear boundaries between these materials on the associated histological images, histological analyses are helpful in this respect and were used additionally in the present study. This problem may be overcome by employing high-field magnets, customized receiver coils or in vivo intravascular MR imaging [6]–[8]. Alternatively, we propose to assign each pixel with a lipid concentration and then to identify regions of different lipid concentrations by the application of area detection algorithms.

3-D morphological modeling of arterial walls will play an essential role in cardiovascular research and medicine in the near future. Morphological modeling will provide the basis for the fusion of clinical imaging/diagnostics and computational biomechanics and may lead to better prevention of injuries, treatments of diseases, surgical planning and intervention, analysis of the progression of diseases, and to optimization of biomedical engineering designs for tissue engineering or, e.g., vessel implants such as stents. The proposed semi-automated approach, which uses 2-D active-surface border detection and produces 3-D morphological models, provides a faster, less error prone and less tedious alternative to complete manual tracing for the assessment of artery anatomy in vitro. The proposed method could be applied to other arterial beds of similar dimensions; for the smaller coronary arteries, however, the imaging quality needs to be improved by using higher field strengths and better coils.
Basically our segmentation algorithm can be applied to imaging methods such as IVUS and OCT; however, major adaptations would be required. Our proposed segmentation algorithm could also be applied to in vivo situations. However, a few adaptations, such as changes in the initialization and reference intensity values, are required. For example, the study [52] documents in vivo MR evaluations of atherosclerotic plaque components of human thoracic aortas; in particular, a comparison with transthoracic echocardiography is discussed. In an in vivo situation the registration of images acquired with different contrast weightings remains a challenge due to the motion that occurs, the small vessel size, and the complexity of the plaque components. Hence, assessment of the in vivo effectiveness awaits a solution of the registration problem (see the review [53]). Nevertheless, there is hope that in vivo MRI of atherosclerotic human arteries and related 3-D morphological modeling in combination with subsequent patient-specific biomechanical analyses will be used in medical treatment decisions and to improve procedures such as balloon angioplasty, which often fail due to restenosis.

ACKNOWLEDGMENT

The authors wish to thank the anonymous reviewers for their constructive criticisms and suggestions.

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