Application of Micro-Computed Tomography with Iodine Staining to Cardiac Imaging, Segmentation and Computational Model Development

Oleg V Aslanidi, Theodora Nikolaidou, Jichao Zhao, Bruce H Smaill, Stephen H Gilbert, Arun V Holden, Tristan Lowe, Philip J Withers, Robert S Stephenson, Jonathan C Jarvis, Jules C Hancox, Mark R Boyett and Henggui Zhang

Abstract— Micro-computed tomography (micro-CT) has been widely used to generate high-resolution 3D tissue images from small animals non-destructively, especially for mineralized skeletal tissues. However, its application to the analysis of soft cardiovascular tissues has been limited by poor inter-tissue contrast. Recent ex vivo studies have shown that contrast between muscular and connective tissue in micro-CT images can be enhanced by staining with iodine. In the present study, we apply this novel technique for imaging of cardiovascular structures in canine hearts. We optimize the method to obtain high resolution X-ray micro-CT images of the canine atria and its distinctive regions - including the Bachmann’s bundle, atrioventricular node, pulmonary arteries and veins - with clear inter-tissue contrast. The imaging results are used to reconstruct and segment the detailed 3D geometry of the atria. Structure tensor analysis shows that the arrangement of atrial fibers can also be characterized using the enhanced micro-CT images, as iodine preferentially accumulates within the muscular fibers rather than in connective tissues. This novel technique can be particularly useful in non-destructive imaging of 3D cardiac architectures from large animals and humans, due to the combination of relatively high speed (~1 hour per scan of the large canine heart) and high voxel resolution (36 µm) provided. In summary, contrast micro-CT facilitates fast and non-destructive imaging and segmenting of detailed 3D cardiovascular geometries, as well as measuring fiber orientation, which are crucial in constructing biophysically detailed computational cardiac models.

Index Terms—X-ray imaging and computed tomography, Heart, Animal models and imaging, Tissue modelling.

I. INTRODUCTION

Structural information, such as cardiovascular tissue geometry and fiber architecture, is important for understanding the associated function, both in health and disease [1, 2]. Thus, the distinctive conduction pathways formed by cardiac bundles determine the sequence of electrical activation of the healthy heart: the normal activation starts in the sinus node, spreads to the right atrium (RA) and through the muscular Bachmann’s bundle (BB) into the left atrium (LA), and then through the atrioventricular node (AVN) into the ventricles. Mapping 3D architecture of the myocardium is also important in relation to cardiac arrhythmias, and can guide clinical ablation treatments. For example, myocardial sleeves of the pulmonary veins (PVs) in the LA are recognized as primary sources of ectopic electrical activity during the most common cardiac arrhythmia, atrial fibrillation (AF), and ablation of the PVs is widely used to terminate AF [3, 4]. Connections between the atria via the BB allow fast synchronized atrial activation in healthy hearts [2], but can also be involved in atrial arrhythmogenesis [5]. Therefore, functional studies of atrial conduction and AF require detailed structural reconstruction of both atria, the BB and PV sleeves, as well as the AVN which protects the ventricles from fast atrial rates during AF and other arrhythmias [6].

Understanding of the electrophysiological mechanisms underlying cardiac arrhythmias often emerges from biophysically detailed computational models [7, 8] that use structural data as the source of 3D computational domains (geometry) and related sub-domains (tissue types). However, structural complexity can make it difficult to quantify fine 3D
details, such as distributions of tissue types and fiber orientation. Thus, even the most detailed 3D models of atrial activation have not included full descriptions of tissue architecture throughout the atrial chambers, although some have incorporated prescribed bundle anisotropy to account for the role of specialized conduction pathways [9, 10].

Although both experimental functional studies and computational modelling require accurate imaging of 3D structures, few widely applicable methods exist for non-destructive whole-volume imaging of soft tissues. The most established method for imaging soft tissues has been histological sectioning [7, 11, 12]. Magnetic resonance imaging (MRI) methods, such as diffusion tensor MRI, gained prominence in prominence for reconstructing the tissue geometry and fiber architecture non-destructively [13-16]. However, such techniques are relatively slow. An alternative non-destructive method of X-ray micro-computed tomography (micro-CT) has shorter acquisition times and is widely used for imaging diverse mineralized tissues [17, 18], but has been disregarded in soft tissue imaging due to the poor inter-tissue contrast.

X-ray contrast enhancement agents are used routinely in clinical radiography, but only recently have been shown to allow quantitative characterization of soft tissues ex vivo. Thus, Metscher [19] has used micro-CT to visualize fine soft-tissue detail in embryos stained with iodine: the radio-opaque staining varied the tissue density and thereby resulted in the differential attenuation of X-rays. Micro-CT with iodine staining has also been used for imaging of cardiac geometries at various stages of embryogenesis in small animals [20]. Iodine staining of smooth muscular tissues has recently produced clear inter-tissue contrast in micro-CT images, as iodine preferentially accumulated in muscular fibers rather than in connective tissue [21]. Finally, Stephenson et al. [22] for the first time applied the contrast micro-CT to reconstruct cardiac structures, primarily the cardiac conduction system in small animal hearts.

We apply the contrast micro-CT for high resolution imaging of cardiovascular architectures in a large canine heart - primarily, to reconstruct structures involved in the electrical activation of the atria. Such a reconstruction can be used to create detailed 3D computational models of the atria and explore electrophysiological factors responsible for the development and maintenance of AF.

II. METHODS

A. Tissue preparation

The heart was removed after euthanasia of a healthy adult female boxer dog, ~8 years old, and body weight 36 kg. The dog was euthanized with pentobarbital sodium and its body was donated to Glasgow Veterinary School in accordance with the UK Veterinary Surgeons Act (1966). The body was immediately chilled to 4°C, and the heart was removed within 24 hours. Heart removal was with attached lung, to preserve PV anatomy. The heart was washed in saline and immersion fixed in 10% neutral buffered formaldehyde (NBF, Sigma-Aldrich). The heart was stored immersed in NBF until imaging. To study atrial anatomy and fiber orientation, dog atria were dissected and stained using a method described recently [22]. Staining was optimized in 8 sequential experiments with the same tissue sample and varying concentrations of iodine potassium iodide solution (5-10% I2KI) and also varying duration of sample incubation in the solution (4-7 days). Each time the tissue sample was stained and micro-CT imaged, after which the contrast agent was leached out by placing the tissue in NBF for at least a week. The leached sample was afterwards re-stained and re-scanned. Optimal contrast of micro-CT images was achieved after 7 days of incubation with 7.5% I2KI. After staining, the tissue was rinsed with NBF, excess solution drained and the sample was mounted in a plastic container onto the rotatory micro-CT scanner stage.

B. Micro-CT scanning

Samples were scanned using a Nikon Metris 225/320 KV housed in a customized bay system at the Henry Moseley X-ray imaging facility, University of Manchester. During the analysis the specimen was rotated through 360 degrees and the projections were recorded on a 2K x 2K Perkin Elmer 1621-16-bit amorphous silicon flat-panel detector with 200 pixel pitch. X-ray beam energy was adjusted to optimize resolution using a Mo-target, Cu-filter (thickness 0.5 mm) combination. The following settings were used for the analysis: scanning time 60 minutes, voltage 150 KV, current 125 µA, gain 16. As a result, 2001 projections per specimen were collected using a frame rate of 2000 ms and a voxel resolution of 36 µm.

Relation between the voxel resolution and the effective tissue resolution that can be used for computational purposes is discussed below (see Discussion).

C. Tissue segmentation

Post processing of the raw micro-CT data included its reconstruction using Nikon Metrolasis CT-Pro software (Metris XT 1.6) and visualization using Avizo 6.3.1 standard edition. Segmentation based on the iodine-enhanced inter-tissue contrast and subsequent volume rendering was used to reconstruct 3D atrial structures. Briefly, areas with distinctive fiber structure, such as atrial walls and myocardial bands of the BB and PV sleeves, had relatively high contrast in the acquired micro-CT images due to the preferential accumulation of iodine within the fibers. Such continuous high-contrast areas were tracked and segmented using the semi-automatic ‘Confidence Connected’ method in Avizo. In case when the semi-automatic tracking was not effective - for example, due to small tissue heterogeneity or sharp edges - manual image-by-image segmentation aimed at tracking over such heterogeneities was applied. The AVN was segmented manually through a series of images, as it stained differentially from the surrounding connective and myocardial tissues.

D. Structure tensor analysis

Grayscale intensity gradient information obtained from imaging can relate the structure of objects in an image to features of interest, e.g., the long axis of myocytes. The structure tensor method, representing gradient information for 3D imaging problems [12, 23], was implemented. The
features allowed us to (i) segment both atrial chambers and large blood vessels, (ii) segment smaller, but distinctive atrial structures - the BB, PVs and AVN - that play important roles in AF, and (iii) reconstruct fiber orientation in these structures.

A. Segmentation of blood vessels

The aorta (Ao) and pulmonary arteries (PAs) are large blood vessels that carry blood from the left and right ventricles, respectively. Smooth muscles forming the vascular walls (i) can be easily stained with iodine [21] and (ii) are anatomically distinctive from myocardial tissues of the atria [24]. This enabled semi-automatic reconstruction of the blood vessels from the contrast micro-CT images (Fig. 3). Fig. 3A shows the segmented volumetric 3D structures of Ao and PAs, and Fig. 3B illustrates the identification of these structures based on inter-tissue contrast in the images. Thus, micro-CT with iodine staining provides relatively fast and easy means for reconstructing geometries of blood vessels, which are in good agreement with more established but more time demanding

III. RESULTS

Fig. 1 shows the 3D tissue geometry of a canine heart reconstructed from micro-CT images. High resolution achieved with micro-CT allows for the high level of detail in the 3D geometry, such as fine structures of separate blood vessels (Fig. 1A) and pectinate muscles (PMs) in the right atrial appendage (RAA) of the heart (Fig. 1B). Note that micro-CT imaging of the heart at 36 µm voxel resolution took about ~1 hour, which is considerably faster than MRI techniques - for comparison, 300 µm reconstruction of the same heart on 3T medical MRI scanner took ~16 hours. Importantly, the 3D tissue geometry can be reconstructed from micro-CT either with or without iodine staining.

Atria of the heart seen in Fig. 1 were dissected by removing lung tissue and most of the ventricles (Fig. 2A) - however, parts of the ventricles were kept in order to preserve the AVN. The resultant atrial sample was stained with iodine and scanned to produce micro-CT images with both high voxel resolution (36 µm) and high inter-tissue contrast (Fig. 2B). The images and the knowledge of well-known anatomic
Segmentation of the RA included the superior vena cava (SVC) located superiorly (Fig. 4B) and a fine network-like structure of conductive PMs in the RAA (Fig. 4C). The segmented 3D atrial geometry (Fig. 4) was in good qualitative agreement with anatomical studies [2, 24, 25].

Thus, the contrast micro-CT enabled (i) reconstruction of

Fig. 3. Segmentation of the 3D geometry of blood vessels. A: Segmented geometry of Ao and PAs. Vascular regions are reconstructed and rendered as 3D volumetric digital masks. B: Identification of Ao and PAs (dotted lines) in the respective high contrast micro-CT images (orthogonal planes x-y and x-z). Similar to Fig. 2B, brighter colors in the images correspond to lower intensity/higher absorption of X-rays, which is due to higher accumulation of iodine in the respective tissues. Ao, aorta; PA, pulmonary arteries.

anatomical studies [2, 24]. Note that the PVs that carry blood from the lungs to the LA were segmented separately as part of the LA (see below). Although vascular walls of the PVs are similar to those in the arteries, their long sleeves branching from the LA are formed by myocardial tissue.

B. Segmentation of the atria

Fig. 4 illustrates the segmented 3D geometry of the canine atria. The RA, LA, BB and PVs were clearly seen in the micro-CT images (Fig. 4A) due to the accumulation of iodine within their myocardial fibers. This enabled semi-automatic segmentation of the atria into distinctive 3D tissue regions (Fig. 4B, C). First, the interatrial BB was segmented based on its anatomically-defined location and cable-like fibrous structure. Second, branching PVs were traced as vascular structures starting superiorly and then - as continuous myocardial sleeves - joining the LA. Finally, the LA was formally identified as the chamber adjacent to the already-segmented PV region, and the RA was identified as the second chamber separated from the LA by the BB (Fig. 4B).

Fig. 4. Segmentation of 3D geometry of the canine atria. A: Identification of the RA, BB, LA and PVs based on inter-tissue contrast in micro-CT images. As before, brighter colors correspond to lower intensity/higher absorption of X-rays, which is due to higher accumulation of iodine in the respective atrial tissues. B and C: Superior and posterior views of the segmented 3D atria. Segmented atrial regions are reconstructed and rendered as 3D volumetric digital masks. 3D atrial regions are shown using same colors as lines in A. Clear structure of PMs inside the RA is seen in C.
the 3D geometry of canine atria and (ii) their segmentation into major atrial structures (tissue types) with a high level of detail. High resolution and high contrast of the micro-CT images also allowed for the reconstruction of fiber orientations in muscular atrial bundles, such as the BB and PV sleeves (see below).

C. Fiber orientation in the BB

The BB is the largest atrial bundle that connects the atria and during normal sinus rhythm provides the primary pathway for the rapid interatrial spread of electrical activation. Despite its importance in the synchronized atrial activation and a role in AF arrhythmogenesis [5, 26], there are relatively few anatomic studies of the BB [2, 24, 27]. Detailed fiber orientation in the BB has been reconstructed only recently using time and labor demanding histological methods [12]. The established cable-like structure and clear arrangement of fibers make the BB an ideal object for reconstruction using the novel fast method of micro-CT with iodine staining.

Such a reconstruction is shown in Fig. 5. The arrangement of fibers along the BB is illustrated by a histological tissue slice (Fig. 5A) - a similar pattern can be seen in micro-CT images due to iodine staining of the BB fibers (Fig. 4A). The BB segmented from these images (Fig. 5B) was used as a 3D digital mask for applying the structure tensor method for fiber tracking (Methods). The resultant cable-like arrangement of fibers along the BB can be clearly seen in Fig. 5C, which is in good qualitative agreement with the histological data from sheep [12] and human anatomical studies [2, 24]. Although direct quantitative comparisons between data from various species are not straightforward, the contrast micro-CT reconstruction of this prominent bundle in dog (Fig. 5C) generally agrees with the histological [12] and anatomical [24] observations: (i) the vast majority of fibers are strictly aligned along the BB with the inclination angle of <10°; (ii) a small fraction of fibers have higher inclination angles of ~20-30°. This provides a validation for the micro-CT imaging of fiber orientation in the canine atria.

D. Fiber orientation in the PVs

The myocardial sleeves of the PVs are recognized as the primary source of ectopic electrical activity during AF [3, 4], which may be due to complex arrangement of fibers in this distinctive region of the LA [28, 29]. The myocardial sleeves of the PVs were clearly seen in micro-CT images (Fig. 4A), as iodine staining of their fibers resulted in high contrast with the surrounding connective tissues. The segmented 3D structure of the PV region is seen in Fig. 4B: four branching PVs are located at the superior side of the LA.

Fig. 6 shows the novel micro-CT reconstruction of fiber orientation in the PV region. Similar to the BB, the segmented

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Fig. 5. Reconstruction of fiber orientation in the BB. A: High resolution histological section (8 μm) of the sheep atria [12]. Clear arrangement of fibers along the BB can be seen. B: Segmented geometry of the BB (see also Fig. 4) used as a 3D digital mask for reconstructing fiber orientation. C: Reconstructed fiber orientation in the BB. Fibers are colored according to their inclination angle (“rainbow” palette). Fibers are aligned in the direction along the BB, which is in good agreement with histological studies.

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Fig. 6. Reconstruction of fiber orientation in the PVs. A and B: Segmented geometry of the PV region (see also Fig. 4) used as a 3D digital mask for reconstructing fibers. C and D: Reconstructed fiber orientation in the PVs. Fibers are colored according to their inclination angle. Fibers are mostly aligned along the PV sleeves, but their arrangement becomes more complex - with multiple changing directions - towards the LA (dark arrow), which agrees with other studies [12, 25, 28]. A characteristic pattern of fibers [25] can be seen in the inter-pulmonary area, where circumferentially aligned strands meet with predominantly longitudinal strands (white arrows). Posterior (A, C) and anterior (B, D) views are shown.
PV region (Figs. 6A, B) was used as a digital 3D mask for applying the structure tensor method for fiber tracking. The resultant fiber orientation was in agreement with available (however, limited) knowledge of this region [12, 25]. Fibers were aligned along the myocardial sleeves of the PVs, but the orientation became complex - with fibers arranged in multiple directions and changes in the arrangement - as the PV sleeves extended towards the LA (Figs. 6C, D). Such a complex arrangement of fibers at the PV-LA junctions is difficult to characterize [25]. However, characteristic changes in the orientation of fiber strands can be seen in the inter-pulmonary areas, where obliquely or circumferentially aligned strands meet with predominantly longitudinally aligned strands [25]. Qualitatively similar patterns are observed in the micro-CT reconstruction (see Fig. 6C, with arrows showing the strand directions), as well as in histological [12] and electro-anatomical [28] studies. Along with the recent histological reconstruction by Zhao et al. [12], this study provides unique high resolution information on the fiber orientation in the PVs.

E. Atrioventricular node

The AVN plays a key role in coordinating electrical conduction from atria to the ventricles. Studies of the AVN have been limited due to its small size, complex anatomy and inaccessible location in depth of the atrioventricular septum [6, 30]. Micro-CT with iodine staining provides high resolution and high contrast images, and allows for non-destructive in-depth tissue studies. This novel method combined with the knowledge of cardiac anatomy is perfectly suited for fast tracing of the AVN and reconstruction of its 3D geometry.

The AVN was segmented by tracing its margins through serial images. Its location near the thick fibrous skeleton that connects the central fibrous body with the aortic, mitral and tricuspid valves was clearly seen in the micro-CT images. Image contrast allowed us to define 3D geometry of the AVN in situ, without distorting its relation to the surrounding fine structures (Fig. 7). Primarily, the AVN was identified at the crest of the ventricular septum and traced posteriorly and anteriorly along the septum based on the inter-tissue contrast (Figs. 7A, B). The reconstructed 3D geometry was further subdivided into the posterior nodal extension (PNE), the compact node (CN) and AV bundle (AVB) (Fig. 7C). Note that the subdivision was based on the knowledge from previous studies of the AVN anatomy and function [30, 31]. Note also that a histological validation for the micro-CT segmentation for the atrioventricular conduction axis in a small rat heart has been performed recently [22] (see Discussion). Thus, the contrast micro-CT enabled the reconstruction of the 3D structure of such a small (~13.0x4.0x0.5 mm³) and complex anatomical object as the canine AVN.

IV. DISCUSSION

We have demonstrated a variety of applications of X-ray micro-CT with iodine staining to imaging of cardiovascular tissues. The high resolution (voxel size of 36 µm) and high contrast imaging was used to (i) reconstruct 3D geometry of the canine atria (Fig. 1), (ii) segment the atria into major myocardial tissue types - RA, LA, BB and PV (Fig. 4), (iii) segment large vascular structures - Ao and PAs (Fig. 3), (iv) segment fine structure of the AVN (Fig. 7), and (v) reconstruct fiber orientation of distinctive atrial bundles - the BB (Fig. 5) and sleeves of the PV (Fig. 6). Such segmented 3D geometries and fiber orientations are crucial in constructing biophysically detailed computational models and applying them to study mechanisms of arrhythmias. Thus, the detailed 3D architecture of the atria reconstructed from micro-CT can be used to create computational models for exploring atrial conduction and AF arrhythmogenesis. Below we discuss potential applications of our methods and results, as well as their limitations.

A. Micro-CT with iodine staining

X-ray micro-CT is a high resolution version of medical CT scanning, which has been increasingly used in non-clinical research over the last decade. A sample is placed in the path of an X-ray beam to generate a projection image on an X-ray-sensitive detector. After the sample is rotated and imaged at a large number of angles, the sequence of projection images is used to reconstruct the X-ray attenuation at each point within the scanned 3D volume. Thus, micro-CT imaging represents the sample as a 3D matrix of intensity values, equivalent to a stack of aligned 2D digital images.

Advantages of micro-CT include 1) non-destructive approach to tissue imaging, 2) relatively short image acquisition times, 3) high resolution (up to ~1 µm) of the images and 4) high contrast between tissues with differential attenuation of X-rays. Therefore, the method has been widely

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Fig. 7. Reconstruction of the AVN from the contrast micro-CT images. A: Identification of the AVN margins (dotted line) in the contrast-enhanced images. B: Segmented 3D structure of the AVN. As in Figs. 3-6, the segmented region is reconstructed and rendered as a 3D volumetric digital mask. C: Anatomy of the AVN. The PNE is identified at the base of the tricuspid valve and continues anteriorly as the CN and AVB. D: Relationship of the segmented AVN to surrounding tissues seen in micro-CT images. Ao, aorta; AS and VS, atrial and ventricular septum; PNE, posterior nodal extension; CN, compact node; AVB, atrioventricular bundle; RV, right ventricle; RA and LA, right and left atria.
used for high resolution imaging of diverse mineralized tissues \textit{ex vivo} [17, 18]. Limitations of micro-CT arise from the facts that 1) high energies of the X-ray beam are dangerous to living organisms, which makes \textit{in vivo} studies nearly impossible, and 2) tissues with similar X-ray absorption may have poor inter-tissue contrast. Therefore, micro-CT has until recently been disregarded in soft tissue imaging. However, recent studies have shown that X-ray absorption in various soft tissues can be greatly enhanced by using contrast agents such as iodine. Micro-CT with iodine contrast enhancement has been used to image details of \textit{ex vivo} embryonic [19, 20] and smooth muscle [21] tissues. Recently the contrast micro-CT has been applied to cardiac tissues [22]. In such studies, resolutions of \(~1\) µm can be achieved in a small field of view [18, 32].

Mechanisms for the iodine tissue staining and contrast enhancement can be explained by its diffusion through tissue layers and binding to glycogen within muscle cells [21, 33]. The resultant accumulation of iodine in the muscular fibers leads to a relative increase of X-ray absorption in the fibers compared to connective tissues. Our results demonstrate that the combination of micro-CT with iodine staining can be used for the direct high resolution, high contrast imaging of cardiovascular tissues, including details of fiber architecture.

\textbf{B. Comparison of micro-CT with histology}

The most established method for reconstructing soft tissues is dissection/sectioning combined with staining of the resultant histological sections and followed by microscopic imaging. This method has been used for reconstructing 3D geometries (and consequently biophysically detailed models) for various parts of the heart. These include several models for the ventricles [7, 34, 35], atria [12, 36], sinus node [11] and AVN [30]. Such studies are considered the reference standard for the cardiac model development due to high resolution (achieved by fine tissue sectioning and microscopy) and high contrast (achieved by staining) of histological images. However, the method has several limitations: 1) tissue sectioning is destructive, such that each sample can be used only once, 2) resolution within and between histological sections is limited by two different factors (resolutions of the microscope and the sectioning method used, respectively), 3) sectioning can result in damage and deformation of the sample such that meaningful data are lost, 4) variations in staining between serial tissue sections may give rise to artificial heterogeneities, 5) reconstruction of the 3D geometry from a stack of 2D histological sections/images requires their alignment, which can also lead to artifacts; 6) as most stages of tissue sectioning, staining, imaging and reconstruction are done manually, the method is extremely time and labor demanding.

The contrast micro-CT technique described in this study has several potential advantages over histological methods for studying cardiovascular architectures. Unlike sectioning, the method is non-destructive and iodine staining is easily reversible [22]. This means the same sample can be repeatedly scanned to optimize the imaging of different features, which was used in the present study with the canine atria. The non-destructive nature of the method is an important consideration when dealing with rare tissues (e.g., human) or unusual pathologies, which can help improve the understanding of cardiovascular disease. The micro-CT method is much faster: scanning the canine atria took \(~1\) hour, compared to several months required for histological studies [11, 12, 30]. Finally, it can produce high resolution 3D images that are intrinsically isotropic and free from tissue deformation artifacts.

\textbf{C. Comparison of micro-CT with MRI}

In clinical cardiac imaging MRI is the reference standard modality for assessing cardiac anatomy and evaluating myocardial contractile function [37]. Cardiac CT has mainly been used for the exclusion of coronary artery disease. The main disadvantage of CT for clinical cardiac imaging is the exposure of the patient to ionizing radiation. Experimental cardiac imaging has largely been developed from clinical approaches and therefore MRI is also the dominant imaging modality [38]. However, exposure to ionizing radiation is not important during \textit{ex vivo} studies of cardiac microstructure, and micro-CT may have an advantage over MRI in resolution, speed and tissue contrast. These factors are discussed below.

Associated with the unique manner in which MRI generates an image (from radio-frequency radiation induced in water molecules) are physical factors which limit the use of MRI for high resolution imaging. The main limitation is the extent of movement of the water molecules within the tissue, known as self-diffusion: over a typical MRI pulse of ~100 ms water molecules can diffuse over the distance of \(~8\) µm, hence limiting imaging resolution to \(>8\) µm [39]. In comparison, micro-CT resolution is limited primarily by scanner design, and resolutions of \(~1\) µm can be achieved [18].

Another limitation of MRI is related to the signal-to-noise ratio (SNR): increasing the resolution leads to a proportional increase of the SNR for a given scan time. SNR can be decreased (and hence, achievable resolution increased) by increasing imaging time. As a result, long MRI imaging sequences are required to obtain the highest quality images. For example, \textit{ex vivo} high resolution MRI (\(25 \times 25 \times 37\) µm) of whole fixed rat hearts has required 20 signal measurements over 72 hours and averaging the result [16]. This may be practical for stable fixed samples, but prevents the acquisition of high resolution MRI images from fresh tissue. Therefore, micro-CT may have more potential for the high resolution imaging of non-fixed cardiac tissues.

Conventional MRI images provide poor myocardial contrast and poor resolution of fibers. This can be enhanced by using perfused contrast agents. Targeted contrast agents have been developed for MRI that allow labeling of tissue features of interest, e.g., collagen in fibrotic tissue [40]. However, contrast MRI has not been used in studies of cardiac architectures.

Diffusion tensor MRI (DT-MRI) has been widely applied for measuring the fiber and laminar orientation from hearts \textit{ex vivo} [13-15]. Accuracy of these measurements has also been validated against histology [13, 41]. DT-MRI provides global cardiac architecture maps that are useful in studies of the
myocardial organization and can be applied as computational domains in 3D cardiac modelling [8, 11, 42]. However, the maximum resolution of DT-MRI reported in the ex vivo myocardium is ~100 μm, with acquisition times of ~10 hours for volumetric scans of a small mouse heart [15]. DT-MRI is limited to this resolution as the SNR of the imaging is inherently low [15] and the tensor estimation is highly noise-sensitive. As a result, DT-MRI images with low SNR result in poorly assigned fiber orientations [43].

D. Effective atrial tissue resolution

3D atrial tissue architectures were reconstructed in this study from the contrast micro-CT data at the voxel resolution of 36 μm. However, the latter value is not the "true" tissue resolution that can be incorporated into computational models as the space step of numerical integration. There is no straightforward procedure for measuring the "true" tissue resolution from micro-CT imaging data. This is because the reconstructed resolution may depend upon several factors: (i) The focal spot size on the target sample. In our experiments this was 3 μm for the voltage of 150 KV, however the size generally varies from experiment to experiment depending on the used voltage value. (ii) The sample to source and sample to detector distances. (iii) The signal to noise ratio. A clear signal is obtained by empirically selecting the correct voltage and current for the sample, such that a ~30% transmission of X-rays through the sample is obtained. The signal is afterwards maximized, which was done in our experiments through ~50000 counts on the X-ray detector. The reported voxel size of 36 μm was calculated during the reconstruction stage by the Nikon Metrolasis CT-Pro software, which takes into account factors (i)-(ii), but not (iii). As an empirical rule, for the Nikon Metris custom bay system and the signal-to-noise ratio optimization procedure used the "true" resolution is about (or less than) twice the voxel size. Hence, the effective resolution of the reconstructed atrial tissue is approximately 70 μm. This value can be used in computational modelling of the 3D atria. Note that the integration space step of 70 μm is more than sufficient for computational purposes. For example, Zhao et al. [12] histologically reconstructed the 3D sheep atrial model with the voxel resolution of 50 μm; in simulations performed with the model, no quantitative difference between results obtained with the space steps of 50 and 100 μm was observed.

E. Computational models of the atria

Computational models with high degree of biophysical detail have been developed for major parts of the heart. Primarily, anatomically detailed 3D geometries have been developed for the ventricles utilizing histological [7, 34, 35] and later MRI [13, 14] techniques, and used as computational domains in cardiac function modelling [8, 42].

Models of the atria have been based on the histologically reconstructed Visible Female human geometry [9, 10, 36]. Reconstructions based on volumetric MRI [44] and CT [45] have also been used to obtain generic surface geometries of the atria. While these models included various details of atrial anatomy, their segmentation into electrophysiologically and anatomically distinctive tissue sub-domains was either absent [36, 44] or based on phenomenological estimations of the sub-domain locations [9, 45]. Moreover, even the most detailed 3D models have not included accurate descriptions of fiber architecture in the atria. Only few models have incorporated prescribed local bundle anisotropy to account for the role of specialized atrial conduction pathways, such as the BB [9, 10].

The model of the human atria by Aslanidi et al. [10] overcame many limitations of earlier models by considering detailed 3D geometry, as well as accurate electrophysiological heterogeneity and local anisotropy (which was partly based on DT-MRI data [11]). The latter two features of the model were particularly important in simulations of AF arrhythmogenesis. Primarily, the simulations showed that tissue heterogeneity caused the break-down of the normal activation wavefronts at rapid pacing rates, which initiated a pair of re-entrant scroll waves - and tissue anisotropy resulted in a further break-down of the scrolls into multiple meandering wavelets characteristic of AF. This provided insights into the 3D dynamics of AF in depth of the atria, which is beyond the current technical capabilities of experimental or clinical set-ups.

However, even the most detailed 3D atrial model [10] has not accounted for electrophysiological heterogeneity and anisotropy of the PV sleeves, which are crucial in the genesis of AF [3, 28, 29]. The contrast micro-CT method used in this study provides (i) the novel segmentation of the PV subdomain and (ii) reconstruction of detailed fiber orientation in the PV sleeves. Moreover, the method reconstructs the entire segmented 3D atria with the effective resolution of ~70 μm, which is a great improvement over the resolution of 330 μm provided by the widely used Visible Female dataset. A new family of heterogeneous electrophysiological models for the canine RA, LA, BB and PV cells has been developed recently [46]. Currently these models are being incorporated into a new 3D computational model that integrates the atrial cell electrophysiology with the tissue geometry and fiber orientations reconstructed from the contrast micro-CT data.

F. Limitations

Although time and labor intensive histological experiments were beyond the scope of this study, a histological validation will ultimately be required in order to evaluate the accuracy of reconstruction of structural features (such as fiber orientations in the BB and PVs) reconstructed using micro-CT.

Currently, we can only qualitatively compare the micro-CT reconstruction of canine atria with histological results from sheep atria [12]. Quantitative comparisons between various experimental studies of the atrial fibers are extremely difficult considering the varying atrial shape, size, wall thickness and complex fiber pattern. Thus, Zhao et al. [47] have recently compared fibers in human and sheep atria and illustrated qualitative similarities of the structure of large atrial bundles in these two species. But the same study has also suggested that quantitative comparisons between atrial micro-architectures from two different species were virtually impossible as the
fiber information belonged to different tissue geometries. Hence, qualitative similarities between the contrast micro-CT reconstruction of the PVs (Fig. 6) and the respective histological [12], anatomical [25] and electro-anatomical data [28] may be the only validation currently available for this region. Note that although direct quantitative comparisons between data from various species are not straightforward, fiber inclination angles in the prominent BB reconstructed from the contrast micro-CT (Fig. 5) were in good agreement with histological [12] and anatomical [24] observations.

For the AVN segmented from the contrast micro-CT images (Fig. 7), a direct histological validation was once again beyond the scope of the present study. However, a histological validation for the contrast micro-CT segmentation of the atrioventricular conduction axis in a rat heart has been done [22]. Direct comparisons of the micro-CT and histological images of the same AVN tissue have shown that structures ~100 μm can be distinguished, and only individual cardiac myocytes (<20 μm) are unclear. The canine AVN segmented in the present study is ~13000 × 4000 × 500 μm and hence sufficiently large to be reconstructed from micro-CT images.

Note that even high resolution histology may not always provide clear information about atrial fiber directions. For the sheep atrial free wall, Zhao et al. [12, 47] have histologically reconstructed fiber orientation in several segments with the resolution of 50 μm, and concluded that the fiber micro-architecture in the wall is highly complex, with no clear fiber direction in many regions. This is consistent with anatomical observations [25]. Our preliminary reconstruction of fiber orientation in a segment of the canine atrial free wall (not shown) also reveals a complex fiber pattern. Efforts of quantifying atrial micro-architectures in different species and using various techniques (and hence, providing validated data for computational modelling) are ongoing.

V. CONCLUSION

Understanding the spatio-temporal electrical dynamics during normal sinus rhythm and atrial arrhythmias (such as AF) requires full in-depth access to the atria. This is extremely difficult to implement in an experimental or clinical set-up. Computational models of the 3D atria, which are based on multiple imaging modalities, can provide biophysical validated means for dissecting and explaining electrical processes underlying the normal and arrhythmic atrial dynamics.

Recent developments in semi-automated volumetric histology [12, 35] and MRI/DT-MRI [13-16] have enabled faster and higher resolution imaging of cardiovascular tissues, but the problem of efficient reconstruction of computational domains for biophysical modelling is still not fully resolved. Micro-CT with iodine staining can have an advantage over other methods in non-destructive imaging of 3D cardiac architectures from large animals and humans, due to the unique combination of high speed (~1 hour/scan for a large canine heart) and high voxel resolution (36 μm) provided. However, further validation of this novel method may be needed. As none of the existing techniques has yet delivered the golden standard for cardiovascular imaging, a combination of multiple mutually-validating modalities may provide the required solution. A combination of DT-MRI, used for non-destructive reconstruction of fiber orientation, with subsequent histological sectioning, to obtain fine details of the tissue structure, has been applied to create a small-scale 3D model of the sinus node [11]. Combining contrast micro-CT, for fast non-destructive imaging of 3D tissue geometries and fiber orientation, with semi-automated volumetric histology and microscopy [12], for further validation of the fiber orientation and tissue characteristics, may provide means for effective high-throughput generation of large-scale computational models of cardiac chambers and the entire heart.

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REFERENCES

In human and sheep atrial models, microscopic imaging of the heart has provided insights into the conduction system and potential sources of arrhythmia. In a study by Gilbert et al. (2008), visualization and quantification of whole heart laminar structure using high-spatial resolution contrast-enhanced MRI was performed, highlighting the potential of this technique in understanding cardiac anatomy.

F. Neues and M. Eppe (2009) used X-ray microcomputed tomography for the study of biomineralized endo- and exoskeletons of animals, providing a novel approach to understanding the structure and function of these complex systems.

In a swine model, conduction patterns were studied using microCT for comparative morphology, showing the potential of this modality in dissecting the pharmacological effects on propagation and arrhythmogenesis. This was reported by Metscher et al. (2008).

Moreover, the application of MRI in clinical cardiology has been explored, particularly in assessing the atrial septum and Bachmann's bundle. However, this requires further validation in human subjects.

The article by Sands et al. (2004) discussed the use of MRI in the diagnosis of cardiac arrhythmia, highlighting the potential of this imaging modality in clinical practice.

In conclusion, the combination of imaging modalities, such as MRI and microCT, offers a comprehensive approach to understanding the cardiac conduction system and arrhythmia in both mammalian hearts and small animals. Further research is needed to fully validate the use of these techniques in human subjects.