Atrial fibrillation (AF) is one of the most common arrhythmias, but its mechanisms remain unclear because of the complex human atrial structure and pathology of this disease. High-resolution optical mapping and 3-dimensional (3D) structural imaging of the atria in ex vivo animal models have provided a wealth of information for better understanding of AF. However, these high-resolution techniques cannot currently be performed in patients to directly uncover the important role of atrial anatomic substrates in pathophysiological conduction. For the first time in the intact human heart, this study integrates functional data collected by high-resolution near infrared optical mapping with the 3D atrial structure of the same heart obtained by novel micro-computed tomographic (CT) imaging to investigate the structural basis for conduction during sinus rhythm, atrial pacing, and sustained atrial flutter (AFL) and AF.

An intact explanted human heart (unused donor, 63-year-old woman, chronic hypertension, heart weight 608 g) from the Lifeline of Ohio Organ Procurement Organization was obtained in the operating room at the time of cross-clamp and immediately preserved with cold cardioplegic solution (1–3°C) in accordance with The Ohio State University Institutional Review Board. Whole intact atria were dissected from ventricles and coronary-perfused with oxygenated Tyrode solution at constant pressure (55–60 mm Hg) and temperature (37°C). Subepicardial optical mapping of the whole coronary-perfused atria was conducted with near-infrared voltage-sensitive dye di-4-ANBDQSB to detect and map atrial activations during sinus rhythm, posterior left atrium pacing, and pacing-induced sustained AFL and AF (Figures 1–3). A high spatial (100×100 pixels, 1.16×1.16 mm$^2$) and temporal (1 frame/ms) resolution Ultima-L CMOS camera (SciMedia, Japan) was focused on both atria from the epicardial surface (Figure 1A). After functional mapping, the human atria was formalin-fixed for 48 hours, and then washed with PBS and incubated at 4°C in 25% Lugol iodine solution for 6 days. The whole atria (6×10×15 cm$^3$) were imaged by a micro-postion emission tomography-CT (Inveon, Siemens) scanner with a resolution of 49×49×49 μm$^3$ in 2 bed positions combined using the 30% overlap. Atrial structure was further smoothed and segmented according to their known anatomic features and digitally reconstructed in 3D (Figure 1B–1D; Figure I in the Data Supplement and Movies I and V in the Data Supplement). The structure tensor approach with 3D Eigen-analysis used the x-ray signal intensity of the original micro-CT images to characterize 3D myofiber field, adapted from histological reconstructions (Methods in the Data Supplement). The optical mapping and micro-CT images were reconciled using atrial anatomic landmarks and pin holes placed at the completion of optical mapping.

During sinus rhythm at baseline (59 beats per minute) and 10 nmol/L isoproterenol perfusion (115 beats per minute), integration of optical mapping and micro-CT imaging revealed that the electric wavefront originated from the sinoatrial node in the superior crista terminalis and propagated from the right to the left atrium (RA and LA) through interatrial muscular bundles such as Bachmann bundle and septal pathways (Figures 1E; Movie II in the Data Supplement). Posterior left atrium pacing (120 beats per minute; Figure 1F; Movie III in the Data Supplement) led to retrograde interatrial conduction from the LA to RA through the same structural pathways. Major atrial muscular bundles are highlighted in Figure 1D. Acceleration of posterior left atrium pacing led to conduction anisotropy, magnified in pectinate muscle bundles in the lateral RA, and eventually unidirectional block and reentrant AFL...
(267 beats per minute), whereas the LA was passively activated by the Bachmann bundle and septal pathways (Figure 2A; Movie IV in the Data Supplement). Micro-CT revealed that endocardial pectinate muscles and their intersection with the crista terminalis formed an anatomic track that anchored the AFL reentry (Figure 2B–2D). Burst atrial pacing also induced an episode of sustained AF (>75 minutes). Dominant frequency analysis indicates 2 discrete driver regions in the LA with higher frequency (7.6 Hz) and power (stability) than the RA (Figure 3A). Because of intermittent failure of 1:1 conduction, the atrial regions outside of the stationed reentrant AF drivers had slower activation frequencies (Figure 3B and 3D) and disorganized multi-wavebreak activity. Abrupt changes in myofiber orientation at intersections between the circumferential atrial roof/floor and the longitudinal posterior left atrium bundle provided substrates anchoring these drivers to the anatomic musculature (Figure 4B; Figure IB in the Data Supplement). Our study for the first time provides direct evidence that the atrial myofiber orientation determines the specific activation patterns in both RA and LA, during sinus rhythm, pacing, AFL and AF in intact human atria.

Acknowledgments
We sincerely thank the Lifeline of Ohio Organ Procurement Organization for providing the explanted heart and Benjamin Canan and Eric Schultz for help with tissue processing. We also thank the Wright Center of Innovation in Biomedical Imaging at The Ohio State University for imaging assistance.

Sources of Funding
This work was supported by the National Institutes of Health HL115580 (Dr Fedorov), by the International Mobility Fund from Royal Society of New Zealand (Drs Zhao and Fedorov), and by funding from Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University.

Disclosures
None.

References

Key Words: atrial fibrillation ■ atrial flutter ■ heart conduction system ■ microcomputed tomography ■ optical mapping, anatomic electrical activity

Figure 1. High-resolution optical and structural mapping of an explanted human heart during sinus rhythm (SR) and posterior left atrium (PLA) pacing. A, Coronary-perfused, explanted intact human atria; white box indicates optical mapping field of view from the epicardial surface. Novel micro-computed tomographic (CT) imaging of the optically mapped heart at a spatial resolution of 49×49×49 µm³ visualized from an posterior (B) and inferior (C) view. Significant anatomic regions across and within both atrial chambers are highlighted (D). In the activation map of SR at 115 beats per minute (E) and PLA pacing at 120 beats per minute (F), black arrows indicate fastest conduction along major muscular bundles (B, C, and D). BB indicates Bachmann bundle; BPM, beats per minute; CS, coronary sinus; CT, crista terminalis; Epi, epicardial; IAS, interatrial septum; IVC, inferior vena cava; LAA, left atrial appendage; LI, left inferior; LS, left superior; MV, mitral valve; PLA, posterior left atrium; PM, pectinate muscle; RAA, right atrial appendage; RI, right inferior; RS, right superior; SAN, sinoatrial node; and SVC, superior vena cava.
Figure 2. Atrial flutter: pectinate muscle (PM) structure sustains reentry in human lateral right atrium (LRA). A, Activation map of the human atria shows a re-entry circuit in the LRA (solid arrow) with passive left atrial activation through major bundles shown in Figure 1. B, PMs and crista terminalis (CT) in the LRA are highlighted and the location of re-entry is indicated from the epicardium of the reconstructed 3-dimensional (3D) atrial LRA; right, 3D myofiber orientation of the LRA in absolute inclination angle with the angle range from 0° to 90° is shown from the endocardium. Here, the inclination angle is defined as the angle between each individual fiber angle with its projection on the XY imaging plane; blue is 0°, green is 45°, and red is 90°. D, Left, Endocardial PMs in the LRA are highlighted; Right, 2D anatomic sections taken from the green and red planes are displayed to demonstrate tissue variation and major muscular bundles (CT and PMs) in the LRA. Epi indicates epicardial; IVC, inferior vena cava; LAA, left atrial appendage; LI, left inferior; LS, left superior; PLA, posterior left atrium; RA, right atrium; RAA, right atrial appendage; RI, right inferior; RS, right superior; and SVC, superior vena cava.
Figure 3. Sustained atrial fibrillation (AF). A, Dominant frequency analysis of the pacing-induced sustained AF displays estimated frequency across the human atria and indicates the highest frequency in the PLA. B, Power analysis at the highest dominant frequency (7.6 Hz) shows 2 discrete islands of stable activity; black arrows indicate the 2 competing stationary drivers. C, Locations of the reentrant AF drivers were marked by red arrows on the micro-computed tomographic (CT) reconstruction of atria. D, Optical action potentials (OAPs) from the left atrial roof, floor, and lateral right atrium denoted as 1 to 3, along with atrial ECG.  A.U. indicates arbitrary units; BB, Bachmann bundle; CS, coronary sinus; CT, crista terminalis; IVC, inferior vena cava; LA, left atrium; LAA, left atrial appendage; LI, left inferior; LS, left superior; PLA, posterior left atrium; PM, pectinate muscle; RAA, right atrial appendage; RI, right inferior; RS, right superior; and SVC, superior vena cava.

Figure 4. Atrial fibrillation (AF) reentrant driver locations correlate with myofiber structure in the posterior left atrium (PLA). A, Anatomic structure of the PLA is displayed from posterior/epicardial (left) and anterior/endocardial (right) views, respectively. B, Three-dimensional (3D) myofiber orientation of the PLA in absolute inclination angles with the angle ranging from 0° to 90° is shown from the posterior/epicardial (left) and anterior/endocardial (right) surface; blue is 0°, green is 45°, and red is 90°. The 2 AF driver locations are indicated on the 3D reconstructed atrial anatomy by red arrows (A) and black arrows (B), where a longitudinal PLA bundle intersects the circumferential atrial roof and floor. Epi indicates epicardial; LI, left inferior; LS, left superior; PLA, posterior left atrium; RI, right inferior; and RS, right superior.
Integration of High-Resolution Optical Mapping and 3-Dimensional Micro-Computed Tomographic Imaging to Resolve the Structural Basis of Atrial Conduction in the Human Heart

Jichao Zhao, Brian J. Hansen, Thomas A. Csepe, Praise Lim, Yufeng Wang, Michelle Williams, Peter J. Mohler, Paul M.L. Janssen, Raul Weiss, John D. Hummel and Vadim V. Fedorov

_Circ Arrhythm Electrophysiol_. 2015;8:1514-1517
doi: 10.1161/CIRCEP.115.003064

_Circulation: Arrhythmia and Electrophysiology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/8/6/1514

Data Supplement (unedited) at:
http://circep.ahajournals.org/content/suppl/2015/12/16/CIRCEP.115.003064.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Arrhythmia and Electrophysiology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Arrhythmia and Electrophysiology_ is online at:
http://circep.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Supplemental Methods

Atrial Segmentation and 3D Reconstruction

The 3D atrial structure obtained using the novel micro-CT approach was further processed using a suite of image processing tools and a variety of software (ImageJ, Matlab (MathWorks), and Amira (FEI)). Due to the high quality of micro-CT images, a simple threshold filtering removed most of the background and noise. Additional unwanted ventricular tissue and attenuated noise were manually segmented out using Amira. The structure was then further interpolated to smooth internal structures and epicardial/endocardial surfaces. Additional operations were employed to remove isolated small tissue/noise from the main atrial tissue. Two different ways to visualize 3D atrial structure, surface view (Figure 1B) and volume rendering (Figure 1D and Supplemental Figure IA) were employed in this study.

For better analyzing key regional atria structure, e.g., pectinate muscles (PMs), crista terminalis (CT) and Bachmann’s bundle (BB), we further segmented the human atria manually according to their known anatomical features using Amira. The resultant 3D segmented atria were digitally reconstructed in 3D and used to better understand the structure-function relationship (Figures 1-4 and Supplemental Movies I and IV).

Extracting and analyzing fiber orientations

How to characterize 3D myofiber orientations in atria is an important and challenging task. It is known that the diffusion tensor MRI (DT-MRI) cannot resolve the fiber structure of the atria due to the extremely thin walls. 3D structure tensor analysis was first proposed and employed on the sheep atria to quantitatively depict 3D myofiber orientations\(^1\)\(^-\)\(^2\). The structure tensor approach utilized the color intensity variation of the original images and 3D Eigen-analysis to reconstruct 3D fiber orientations, and has achieved great success for histological images\(^1\)\(^-\)\(^2\).

To describe 3D atrial fiber orientation quantitatively, the inclination angle \(\alpha\) is defined as the angle between this vector and its projection onto the XY imaging plane (as the reference plane), and the value of the inclination angle \(\alpha\) is between -90° and 90°. Under most circumstances we may only concern atrial fiber pattern generally, i.e., circumferential or longitudinal, therefore, the absolute value of the inclination angle \(\alpha\), denoted by \(|\alpha|\), is used to indicate fiber trends. The absolute inclination angle \(|\alpha| = 0°\) corresponds to lateral orientation and \(|\alpha| = 90°\) corresponds to...
SUPPLEMENTAL MATERIAL

longitudinal direction, as we used for Figures 2 and 4. We employed the relative inclination angle to demonstrate that myofiber orientation of the PV sleeves is circumferential in Supplemental Figure 1B.

In this study, we consider the resolution of 49×49×49 µm$^3$ to be of appropriate spatial accuracy for 3D fiber field computing since the dimension of a typical atrial cell is about ~80×30 µm$^2$.

Supplemental References

Movie Legends

Movie I. The whole intact human atria imaged by a micro PET-CT (Inveon, Siemens) scanner with a resolution of 49×49×49 µm$^3$ were further segmented according to their known anatomical features and digitally visualized in 3D. The highlighted regions include the Bachmann’s bundle (BB) and its extension into both right and left atria, coronary sinus (CS), crista terminalis (CT), left superior/left inferior/right superior/right inferior pulmonary vein (PV), pectinate muscles (PM).

Movie II. Top panel: electrical activations of the atria (from red to blue) during sinus rhythm with a white arrow showing conduction along the BB. Bottom panel: optical action potentials (OAPs) from selected regions in the left atria (blue) and sinoatrial node (SAN, green), along with Atrial ECG. BB: Bachmann bundle; LAA: left atrial appendage; RAA: right atrial appendage. This movie is represented in the activation map from Figure 1E.

Movie III. Top panel: electrical activation pattern of the atria during posterior left atrium (PLA) pacing. Bottom panel: optical action potentials (OAPs) from selected regions in the left atria (blue) and sinoatrial node (SAN, green), along with Atrial ECG. This movie is represented in the activation map from Figure 1F.
**SUPPLEMENTAL MATERIAL**

**Movie IV.** Top panel: electrical activation pattern of the atria during sustained flutter in the right atrium. Bottom panel: two optical action potentials (OAPs) from selected regions in the right atria (blue and green), along with Atrial ECG. This movie is represented in the activation map from **Figure 2A**.

**Movie V.** The whole intact human atria imaged by a micro PET-CT scanner with a resolution of 49×49×49 µm³ were digitally visualized in 3D.

**Supplemental Figure I:**

**A** Major Atrial Bundles Segmented from Micro-CT

**B** 2D Sectioning Perpendicular to PV Sleeve Openings

**Supplemental Figure I.** **A.** Further segmented major atrial bundles of the 3D human atria acquired by the micro-CT scanner with a resolution of 49×49×49 µm³. **B.** 2D sectioning perpendicular to the pulmonary vein (PV) sleeve openings (across the Z-axis) is displayed here to demonstrate the circumferential myofiber orientation of the PV sleeves. Here the enhanced inclination angles with the angle range from -90° to 90° are used to highlight the smooth transition around the PV openings. BB: Bachmann’s bundle; PM: pectinate muscles; CT: crista terminalis; LS/LI/RS: left superior/left inferior/right superior pulmonary vein.