SUPPLEMENTAL MATERIAL
Data S1.

Supplemental Methods

Ex-vivo MRI of human atria
Contrast-enhanced MRI (CE-MRI) was used to define surface geometry and micro-structure of atria as previously described.\(^1\) After the optical mapping experiment, the whole human atria were formalin fixed for 48-72 hours, then washed out with PBS and incubated at 4°C in 0.2% Gd-DTPA (dimeglumine gadopentetate Magnevist, Bayer Schering Pharma) for 7 days in order to perform a CE-MRI. The human atria were imaged at The Ohio State University using a 9.4 T Bruker BioSpin Spectrometer (Ettlingen, Germany) and a 72 mm volume coil. FLASH_3Dslab_bas protocol was used to obtain high-resolution images with the following parameters: echo time 3.4ms, repetition time 16.7ms, flip angle 30°, naver=8 and fat suppression on (BW = 1520 HZ). Volume images with ~180×180×360 µm\(^3\) resolution and dimensions of 107×61×85 mm\(^3\) were obtained within 5 hours.

A MatLab built-in Interpolate function (MathWorks, Inc.) was used to interpolate raw tissue MRI images along the Z-axis to obtain an isotropic resolution of ~180 µm\(^3\) for this 3D volume. 2D MRI images of the human atria with a resolution of ~180 µm\(^2\) were segmented using a carefully selected global threshold (134) to effectively eliminate most of the background noise. The whole procedure was processed and visualized in 3D using commercial software Amira (FEI Company). A manual interactive segmentation approach was employed on each 2D imaging slice by visualizing the image data in orthogonal views to help further segment atrial anatomical regions (Figure 1), such as pulmonary veins, superior vena cava, coronary sinus, and coronary arteries, and to remove tissue structures that were not directly related to atria (background noise, fat, ventricular tissue etc.). A minimal bounding box was employed on the original CE-MRI images to minimize space and memory usage. Finally a suite of image processing tools were employed to process the high-resolution images to smooth internal structures, extract tissue boundaries, and digitally construct a voxel-based 3D volume (Figure 1).\(^2\) The optical mapping and reconstructed 3D human atrial structure were reconciled using atrial anatomical landmarks.\(^3\)

3D atrial wall thickness estimation
A robust approach for 3D atrial wall thickness estimation across the two atrial chambers is to solve the Laplace equation with two boundary conditions specified at both epicardial and endocardial surfaces.\(^4\) As previously seen in our study\(^1\) and others\(^5\), simpler approaches, such as measuring normal projection or closest point from one surface to the other one, are error-prone.

We have extended this Laplace approach to the whole human atria imaged by CE-MRI. First of all, we needed to close atrial chambers manually by adding artificial barriers to the four pulmonary veins (PVs), mitral valve, tricuspid valve, superior vena cava and inferior vena cava. A region grow function was used to obtain the endocardial surfaces and then the epicardial surface, seeding from the corresponding cavity and background spaces, respectively (Figure S1). The non-surface tissue region and both atrial cavities were also extracted (Figure S2). Finally, 3D atrial wall variation across the two atrial chambers was estimated by solving the Laplace equation and then tracing the trajectories along the gradient field of the Laplace solutions connecting the two surfaces. Atrial cavity/tissue volume, right atrium (RA) vs left atrium (LA), was estimated as well (Table 1).

The Laplace equation for the given 3D human atrial volume is shown below:
\[
\frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2} + \frac{\partial^2 \phi}{\partial z^2} = 0, \quad (1)
\]

According to the Taylor expansion, a point \( \phi_i \) and its neighbouring points (\( \phi_{i-1} \) and \( \phi_{i+1} \)) were chosen along the x-axis, with a separation distance of \( h \) in one dimension, we obtained the following equations:

\[
\begin{align*}
\phi_{i-1} &= \phi_i - \frac{\partial \phi}{\partial x} \bigg|_i h + \frac{\partial^2 \phi}{\partial x^2} \bigg|_i \frac{h^2}{2!} - \frac{\partial^3 \phi}{\partial x^3} \bigg|_i \frac{h^3}{3!} + \frac{\partial^4 \phi}{\partial x^4} \bigg|_i \frac{h^4}{4!} - \ldots, \quad (2) \\
\phi_{i+1} &= \phi_i + \frac{\partial \phi}{\partial x} \bigg|_i h + \frac{\partial^2 \phi}{\partial x^2} \bigg|_i \frac{h^2}{2!} + \frac{\partial^3 \phi}{\partial x^3} \bigg|_i \frac{h^3}{3!} + \frac{\partial^4 \phi}{\partial x^4} \bigg|_i \frac{h^4}{4!} + \ldots, \quad (3)
\end{align*}
\]

Adding Equations (2) and (3) and removing third and higher order terms, we could rearrange it into the following so-called central difference method\(^6\):

\[
\frac{\partial^2 \phi}{\partial x^2} \bigg|_i \frac{h^2}{2} = \frac{\phi_{i+1} - 2\phi_i + \phi_{i-1}}{h^2}, \quad (4)
\]

Extending this into three dimensions, we now had expressions of each of the terms in the 3D Laplace equation. Substituting into Equation (1):

\[
\frac{\phi_{i+1} - 2\phi_i + \phi_{i-1}}{h^2} + \frac{\phi_{j+1} - 2\phi_j + \phi_{j-1}}{h^2} + \frac{\phi_{k+1} - 2\phi_k + \phi_{k-1}}{h^2} = 0, \quad (5)
\]

Rearranging it into

\[
\phi_{i,j,k} = \left( \frac{\phi_{i+1,j,k} + \phi_{i-1,j,k} + \phi_{i,j+1,k} + \phi_{i,j-1,k} + \phi_{i,j,k+1} + \phi_{i,j,k-1}}{6} \right), \quad (6)
\]

With the above central difference formula, we could solve the Laplace solution with a second-order accuracy iteratively. For each iteration, the maximum relative change for each pixel was calculated in order to decide whether the numerical solution had converged or not:

\[
\varepsilon(i, j, k) = \left| \frac{\phi^{\text{Iteration}-1}(i,j,k) - \phi^{\text{Iteration}}(i,j,k)}{\phi^{\text{Iteration}-1}(i,j,k)} \right|, \quad (7)
\]

In our implementation, the Laplace solver was terminated when numerical solutions were solved with a maximum relative error of 0.02\%. Additionally, a successive over relaxation was used to significantly decrease computation time and the most common value for the relaxation factor \( (\omega = 1.4) \)\(^7\) was adopted

\[
\phi(i, j, k)^{\text{(New)}} = (1 - \omega) \phi(i, j, k)^{(0\,ld)} + \omega \phi(i, j, k), \quad (8)
\]

Where \( \phi(i, j, k) \) is given by Equation (6).

To solve the Laplace equation (1) or (6) over the 3D atrial volume, constant boundary conditions have to be specified at the epicardial and endocardial surfaces.\(^4\) In our case, we used 100 and 300 respectively in our 3D numerical solver. Due to the fact that human atria are composed of dual chambers, it was necessary to divide the atrial tissue into RA and LA, and then solve the Laplace equation separately on each atrial chamber.

After obtaining the Laplace solution field, the gradient map was calculated for each of the solutions using MatLab’s built-in gradient function and then normalised by magnitude at each
point. The 3D wall thickness at any location in atrial volume was estimated by using the following procedures: Starting from any point and its gradient from one surface, a line travelling along the local gradient will keep propagating using a new point and its’ gradient within the 3D volume, till it reaches the other surface of interest in the Laplace solutions. The distance travelled from the original point on the surface along its traveling path was recorded as the atrial wall thickness. This procedure was then repeated for every point on the surface and therefore, to complete the calculation of the atrial wall thickness map. Finally, a MATLAB built-in interpolation function, interp3, was used to smooth the wall thickness map and fill in any unassigned values within the 3D atrial volume.

Other important information we can extract from the Laplace solutions was the transmurality of atrial volume. Given that the values of the Laplace solutions varied from 100 (fixed at the epicardium) to 300 (fixed at the endocardium), we chose the regions with values from 175 to 225 as transmural/mid-wall tissue, values smaller than 175 as the subepicardial regions and values larger than 225 as the subendocardial regions. In this way, we can separate the 3D atrial wall into three different partitions and study the difference in atrial structure in these partitions throughout our study.

**3D fibrosis distribution estimation**

Fibrosis has higher intensity compared with non-fibrotic tissue when imaged using the CE-MRI approach. Fibrosis percentage was measured from 2D CE-MRI sections by applying a fibrosis enhancement mask. The mask was based on signal intensity threshold differences of connective and muscular tissue, and was validated with 2D histology sections. After the CE-MRI scan, key regions of the same heart were stained by Masson’s trichrome with 0.5×0.5 μm² resolution. 3D CE-MRI structures were registered with the corresponding high-resolution histological data using anatomical landmarks, allowing for comparison of similar slice sections in MRI imaging and histology staining (Figure 3C). Then, a series of 2D re-sliced CE-MRI images were analyzed to obtain an optimal global threshold value to identify fibrosis throughout the human atria by comparing corresponding histological sections.

To characterize fibrosis in histological data, non-fibrosis pixels must be removed, namely areas dominated by nuclei, cytoplasm (red/purple) and background (white). First, white pixels were filtered out by removing pixels with luminance greater than 128 (uint8 RGB) using a weighted formula \((0.2126*\text{red} + 0.7152*\text{green} + 0.0722*\text{blue})\). Red channel was then filtered out by removing all pixels greater or equal to 100. Next, pixels with insignificant amounts of blue were removed (< 100 in the blue channel). Finally, a median filter was applied to the remaining pixels to capture areas dense in connective tissue, i.e. fibrosis. We also calculated the total tissue area of each histological slice. Then the fibrosis percentage in each 2D section was estimated.

The matched CE-MRI sections were used to find the intensity threshold required to obtain the same fibrosis percentage found from the histological slices for the regions separately. A least square fitting was employed to minimize the difference between the calculated fibrosis percentage in CE-MRI and the expected fibrosis percentage in histological sections. Therefore, a global threshold value (184) for CE-MRI (Figure 3) was found.

To evaluate the fibrosis distribution in atrial volume, we color-coded and visualized fibrosis transmurally using a rainbow colour spectrum: blue for endocardium and red for epicardium (Figure 3). Furthermore, we measured fibrosis density by counting the percentage of fibrotic voxels out of the total volume of a sphere with a radius of 5 pixels.
Figure S1. A-D: 3D epi and endo surfaces are shown from posterior and anterior views, respectively. A and B: the LA and RA epi (red and blue, respectively); C and D: the LA and RA endo (gold and cyan) and the interatrial septum region (yellow and green). LA/RA – left/right atrium, BB – Bachmann's bundle, CS – coronary sinus, Endo – endocardium, Epi – epicardium, LS/LI/RS/RI PV – left superior/left inferior/right superior/right inferior pulmonary vein, IAS – inter-atrial septum, IVC/SVC – inferior/superior vena cava, LAA/RAA – left/right atrial appendage, PLA – posterior left atrium.
Figure S2. The atrial wall (light blue) of both atrial chambers (LA (red) and RA (green)) were manually closed, and Epi (dark blue) and Endo (teal) surfaces were selected before the Laplace equation was solved for 3D atrial wall thickness. Here a single 2D atrial mask (inferior section of the atria) was displayed, and different regions were highlighted in different colors. LA/RA – left/right atrium, BB – Bachmann’s bundle, CS – coronary sinus, Endo – endocardium, Epi – epicardium, LAA – left atrial appendage.
Figure S3: Current clinical ablation strategies failed to terminate atrial fibrillation (AF) in the computer model. A. The AF driver at the inferior PLA was replicated in the computer model, and the anatomy-based pulmonary vein isolation (PVI) was employed but had no impact on AF cycle length or maintenance. B. A driver regional ablation approach that targeted the area of rotation with an area ~3.1 cm² slowed AF. LS/LI/RS/RI PV – left superior/left inferior/right superior/right inferior pulmonary vein, IVC/SVC – inferior/superior vena cava, LAA – left atrial appendage, PLA – posterior left atrium.
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