Three-Dimensional Diffusion Tensor Microscopy of Fixed Mouse Hearts

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The relative utility of 3D, microscopic resolution assessments of fixed mouse myocardial structure via diffusion tensor imaging is demonstrated in this study. Isotropic 100-μm resolution fiber orientation mapping within 5.5° accuracy was achieved in 9.1 hr scan time. Preliminary characterization of the diffusion tensor primary eigenvector reveals a smooth and largely linear angular rotation across the left ventricular wall. Moreover, a higher level of structural hierarchy is evident from the organized secondary and tertiary eigenvector fields. These findings are consistent with the known myocardial fiber and laminar structures reported in the literature and suggest an essential role of diffusion tensor microscopy in developing quantitative atlases for studying the structure-function relationships of mouse hearts. Magn Reson Med 52:453–460, 2004. © 2004 Wiley-Liss, Inc.

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The fiber anatomy of the myocardium is known to have a profound impact on the electrical and mechanical properties of both normal and diseased hearts (1,2). Precise knowledge of the tissue fiber structure will contribute significantly in elucidating the complex structure–function relationships of the organ, especially in biomedical engineering studies based on the so-called “morphologically accurate” modeling. Advances in molecular biology have provided unprecedented opportunities to use versatile small animal (e.g., mouse) platforms (3–6) for studying genotypes and phenotypes of myocardial development, damage, or repair associated with specific pathologies. Due to the small size of the mouse heart (with overall volume typically less than 250 mm³), an efficient means to accurately and quantitatively characterize the tissue fiber structure at high or microscopic resolution would be of great benefit.

Most early studies of myocardial fiber structures were based on conventional histological techniques (7–11), which are not only destructive, but also labor-intensive. By probing the tissue microstructure via its influence on the diffusion of water, MR diffusion tensor imaging (DTI) (12) has been advanced as a promising nondestructive alternative to characterize the structure of ordered tissues such as the brain white matter (13–15), myocardium (16–22), other musculature (23), and cartilage (24). The underlying hypothesis in DTI is that the direction of fastest water diffusion coincides with the local tissue fiber orientation. Reports show evidence that validates a direct correlation between fiber orientations measured by DTI and conventional histology for freshly excised (16), perfused (17), and fixed myocardium (18).

In 3D space, the generalized diffusion tensor is a symmetric, second-order 3 × 3 matrix. A complete solution to the six independent parameters, plus an extra term for the diffusion-independent magnetization, requires that the DTI dataset consists of a minimum of seven images. The tradeoffs between scan time and the image signal-to-noise ratio (SNR), aggravated by the nature of diffusion sensitization (i.e., via signal attenuation) and the inadvertent T₂ weighting during the diffusion encoding gradient pulses, have made the SNR of DTI inherently low. Improvements in the acquisition methodology, such as optimized diffusion encoding gradient directional schemes (25) and parallel (26) and novel rapid imaging techniques (27), have been helpful, but practical applications of DTI continue to be hampered by relatively low spatial resolution because DTI is often compromised for faster scan time, improved SNR, or a combination of both.

In addition to the general SNR considerations of DTI, high-resolution DTI of the mouse heart is further complicated by the relatively low degree of diffusion anisotropy in the tissue as measured by, for example, the fractional anisotropy (FA) index (28). Compared to the reported brain white matter FA of 0.4–0.9 (13–15), myocardium has FA values in the range of 0.1–0.6 (17,20,21). (Larger FA values correspond to a greater degree of diffusion anisotropy.) It is known that image noise causes disproportionate larger diffusion tensor measurement errors of eigenvalues, anisotropy indices, and eigenvector direction estimates in tissues with low FA than those with high FA (28–30). Consequently, for the same desired degree of accuracy, DTI fiber orientation mapping in the myocardium would impose a more stringent SNR requirement than in the brain white matter.

Given these technical challenges, the goals of this study were to 1) investigate and demonstrate the relative utility of DTI in obtaining microscopic resolution measurements of myocardial fiber orientation, and 2) provide a preliminary description of the myocardial fiber structure in the mouse heart. Specifically, the utility of DTI is evaluated in terms of fiber orientation measurement accuracy for a given choice of imaging parameters (e.g., voxel size, TR, NEX), which in this study corresponds to 100 μm isotropic spatial resolution with scan time of ∼9 hr.
MATERIALS AND METHODS

Specimen Preparation

All procedures were carried out under protocols approved by the Institutional Animal Care and Use Committees of the University of North Carolina. Two 8-month-old male mice of 129/ola strain were used for these procedures. After the mice were euthanized the hearts from the animals were surgically isolated. Each isolated heart was fixed overnight in 4% paraformaldehyde at 4°C. In preparation for MR imaging, the intact heart was placed inside a plastic tube immersed with fomblin (Ausimont, NJ), a low dielectric, low signal perfluoropolyether for susceptibility matching.

DTI Acquisition

Imaging experiments were conducted on a 9.4 T MR instrument (Oxford Instruments, Oxford, UK) interfaced with a Signa console (General Electric NMR Instruments, Fremont, CA) with 17°C bore temperature. The sample tube was inserted in a custom-made 14-mm-diameter solenoid RF coil with long axis of the heart parallel to the coil axis. A standard diffusion-weighted spin-echo pulse sequence was used to acquire 3D volume images (12.8 × 12.8 × 12.8 mm FOV, 500 ms TR, 15.6 ms TE). Diffusion encoding was performed using a pair of half-sine gradient pulses (5.0 ms width, 7.5 ms separation, and 50 G/cm nominal gradient amplitude, which corresponds to a b-value of 1.13 × 10^4 s/mm^2). A reduced encoding DTI methodology (31) was employed, such that each dataset consisted of a fully encoded 128 × 128 × 128 (readout × phase × slice) matrix-size b0 (i.e., b = 0) and 12 reduced encoded (128 × 64 × 64) diffusion-weighted images sensitized in each of an optimized set of 12 directions (25). The acquisition time for each complete DTI dataset was ~9.1 hr.

Each reduced encoded diffusion-weighted image was reconstructed to 128 × 128 × 128 matrix size by a reduced-encoding imaging via generalized series reconstruction (RIGR) algorithm (32), using the b0 image as the constraining reference. The robustness of RIGR lies in that it offers maximal continuity between the constraining reference and reduced encoding data in the k-space, and that generalized series often allows a faster convergence of the reconstructed image than conventional Fourier series. For DTI, RIGR has been shown to provide significant improvements in data acquisition-time efficiency (i.e., fiber mapping accuracy for a constant total scan time) than zero-filling interpolation, direction data replacement (i.e., keyhole) reconstruction techniques, or proportionally reducing the number of full-encoding images in the dataset (31).

Diffusion tensors were estimated off-line on a pixel-by-pixel basis via nonlinear least-squares curve-fitting according to the signal intensity attenuation equation (12). Subsequently, the computed diffusion tensors were diagonalized, and the eigenvalues in descending order were labeled as the primary, secondary, and tertiary eigenvalues.

Characterization of Myocardial Structure

The eigenvectors corresponding to the primary, secondary, and tertiary diffusion tensor eigenvalues were taken to be the local myocardial fiber orientation (16), the sheet direction of the myocardial laminae, and the normal direction of the sheet structure (17), respectively. Although the structural directional information is fully embedded in the eigenvectors, for more intuitive visualization each eigenvector was converted to a pair of angles (two angles are needed to represent a given direction in 3D space) in a local cylindrical coordinate system (17,22). For each location on the left ventricle (LV), the coordinate system consists of three orthogonal reference planes (Fig. 1a): 1) transverse or short-axis plane, which corresponds to the imaging slice plane; 2) tangential or circumferential plane, which is parallel to the epicardial surface with the normal vector pointing in the radial direction passing through the myocardial centroid (for a perfectly cylindrical LV) in each transverse plane; and 3) radial plane, which is the plane spanned by the myocardial longitudinal and radial axes (9).

Directions of primary and tertiary eigenvectors were represented by the respective helix angles (7) and tangential residual angles (Fig. 1b), where the helix angle was the angle between the projection of the eigenvector on the tangential plane and the transverse plane, and the tangential residual angle was the angle between the eigenvector and the tangential plane. Rather than the previously defined transverse angle (17,18), which is the angle between the projection of the eigenvector on the transverse plane and the tangential plane, the tangential residual angle is used to avoid the amplified variability and error associated with projecting a vector onto a near-perpendicular plane.

Whereas the primary and tertiary eigenvectors lie largely in the tangential plane, the secondary eigenvector (i.e., the sheet direction), is expected to point predominantly in the radial direction within the transverse plane (9,17,19,21). Therefore, for easier correlation to histological methods (as most histological studies of myocardial fiber orientations were performed on tangential plane (7,8), those of the sheet structure were conducted on the radial plane (9,10)), the secondary eigenvector was represented by the transmural and radial residual angles (Fig. 1c), where the transmural angle was the angle of inclination of the projection of the eigenvector on the radial plane from the transverse plane, and the radial residual angle was the deviation of the eigenvector from the radial plane.

Without loss of generality, unless otherwise indicated, quantification of the myocardial fiber structure was done on the mid-hemispherical plane (i.e., the widest short-axis plane) of the LV. After digitally removing the papillary muscle by visual inspection, myocardial fiber orientations were quantified at eight equally spaced sites spanning the entire arc of the LV free wall. At each site, the helix angle of the diffusion tensor primary eigenvector (see Fig. 1b) was measured as a function of transmural distance along the local radial axis emanating from the LV centroid. (The location where the fiber helix angle changes sign according to a second-order polynomial fit was taken to be the zero point of the transmural distance.) Likewise, myocardial fiber orientations were measured at eight sites in the LV septal wall. For all sites, the subepicardial fiber helix angle, range of transmural helix angular change, and the LV wall thickness were obtained. In addition, linear regression was used to determine the slope and the corresponding R^2 value of helix angle-transmural distance relationship to estimate the rate of trans-
mural helix angular rotation and the goodness-of-fit of the linear model. Each of the subepicardial fiber angle, range of transmural helix angular change, wall thickness, helix angle rotation rate, and $R^2$ goodness-of-fit were compared between the LV septal and free wall regions within each individual heart via Student's $t$-statistics, with $P < 0.05$ taken to be statistically significant.

In the absence of the histological “gold standard” fiber orientation angle, which is also subject to error, the DTI angular measurement accuracy was estimated by the root mean square (RMS) deviation of the fiber helix angles from each corresponding linear regression fit. Since it necessarily includes errors arising from using a linear model to characterize transmural fiber helix angular rotation, the RMS deviation represents the upper-bound of the DTI fiber orientation measurement error.

RESULTS

Figure 2 shows the myocardial structure within a mid-hemispherical rectangular region of interest pixel-by-pixel displayed as round cylinders pointing the directions of the primary eigenvector or flattened cylinders oriented along the primary and secondary eigenvectors. The classical counterclockwise rotation of myocardial fiber from epi-to-endocardium (7) is clearly evident. Fibers oriented largely in the longitudinal (i.e., apex-to-base) direction are found near the epicardium, endocardium, and in the papillary muscles, whereas in the mid-wall fibers run predominantly in the circumferential direction. Importantly, the conspicuous coherence of secondary eigenvector, as well as the primary eigenvector, suggests “sheet” or planar organization of the myocardial structure in addition to the fiber organization.

Figure 3 shows representative digitally segmented 3D angular maps displayed pairwise for the diffusion tensor eigenvectors, demonstrating the structural details obtainable for the mouse myocardium. At each tissue voxel, the helix angle and tangential residual angle of the primary eigenvector (Fig. 3a,b), tertiary eigenvector (3c,d), and the transmural angle and radial residual angle of the secondary eigenvector (3e,f) are displayed in different color according to the color scale. In Fig. 3a, the changing color clearly demonstrates the epi-to-endocardial counterclockwise (about $-60^\circ$ to $+90^\circ$) helix angular rotation for the primary eigenvectors in all transverse (i.e., short-axis) planes, while the helix angle of the tertiary eigenvector reveals a similar rotation of the sheet structure throughout the whole myocardium (Fig. 3c). The tangential residual angle of the primary eigenvector is relatively small throughout the LV, as seen in Fig. 3b. This is confirmed by

FIG. 1. Schematics of the cylindrical coordinate system used to characterize and visualize myocardial structure. On any location on the LV, a set of three orthogonal planes, transverse, tangential, and radial planes (a), serves as local basis of reference. The diffusion tensor primary and tertiary eigenvectors (b) are then represented by the helix angle and the tangential residual angles. In contrast, the secondary eigenvector (c) is characterized by the transmural angle and the radial residual angle.
the mean primary eigenvector tangential residual angles of 2.5 ± 16.2° (mean ± SD, n = 5760) on the mid-hemispherical plane for the two hearts examined. Figure 3d shows the tangential residual angles of the tertiary eigenvector (with an average of −9.6 ± 28.1°), which is somewhat larger and distributed in a more complex pattern than those of the primary eigenvector. Histogram analysis reveals that the majority (63%) of tertiary eigenvector tangential residual angles on the mid-hemispherical plane are within the range of −30° to 30°. Although directional information of secondary eigenvector can be inferred from the primary and tertiary eigenvectors, interpretation and analysis of the myocardial structure can be done more easily by direct visualization (Fig. 3e,f), which reveals generally small and slowly varying transmural angle and radial residual angle throughout the LV.

Figure 4a shows a short-axis confocal microscopy section (obtained subsequent to DTI for comparison) of a mouse heart near the mid-hemispherical plane. The varying myocyte cross-section size is indicative of the transmural rotation of the myofibers, although quantification of the orientation angles cannot be directly obtained. In contrast, the DTI fiber helix angles measured for the same heart are plotted in Fig. 4b.

The descriptive statistics of the myocardial fiber structure are summarized in Table 1. There appear to be trends of steeper (more longitudinally oriented) subepicardial fiber orientation, larger transmural helix angular range, faster rate of the angular rotation, and better linear regression fit (i.e., higher $R^2$) in the LV septal wall than the free wall. Student’s t-tests revealed that the differences in the transmural helix angular range and rate of rotation were statistically significant for both hearts, whereas the significance of the subepicardial helix angle difference was mixed. No statistically significant differences in the LV wall thickness and linear regression $R^2$ were observed in either heart. It is worth noting that not only were the mean linear regression $R^2$ values in all LV regions equal or above 0.96, but also the individual $R^2$ values were all higher than 0.91 among the 32 radial transmural directions characterized (data not shown). This implies that the transmural rotation of the fiber helix angle can be well characterized by using a linear model. From the average linear regression RMS deviation of the helix angles, the accuracy of the current fiber orientation mapping experiment was estimated to be 5.5 ± 2.0° (mean ± SD, n = 32).

The eigenvalues of the diffusion tensor provide orientation-independent measures of water diffusivity along three diffusion principal axes. These eigenvalues are found to be relatively uniform throughout the whole myocardium in both hearts. The average values of the diffusion tensor eigenvalues, in descending order, are $\lambda_1 = 0.75 ± 0.13$, $\lambda_2 = 0.60 ± 0.13$, and $\lambda_3 = 0.51 ± 0.13 \times 10^{-3}$ mm$^2$/s (mean ± SD, n = 360,957). The corresponding mean FA value (28), a rotationally invariant index of diffusion anisotropy, is 0.27 ± 0.06.

**DISCUSSION**

**High-Resolution 3D DTI of Mouse Heart**

To our knowledge, this is the first report of 3D diffusion tensor microscopy of the mouse heart and 3D DTI of hearts
of any species. In contrast to multislice acquisition, 3D scans in general offer improved spatial resolution in the slice-selection direction, which can facilitate the visualization or accurate quantification of myocardial structures at arbitrary plane orientation. The spatial resolution (100 μm in each spatial dimension or 10⁻³ mm³ voxel

FIG. 3. Myocardial structures of a digitally truncated 3D LV volume represented in the local cylindrical coordinate system and shown in false-color: (a) helix angle of the primary eigenvector; (b) tangential residual angle of the primary eigenvector; (c) helix angle of the tertiary eigenvector; (d) tangential residual angle of the tertiary eigenvector; (e) transmural angle of the secondary eigenvector; and (f) radial residual angle of the secondary eigenvector.

FIG. 4. Short-axis confocal microscopy section of a mouse heart LV near mid-hemispherical plane. (The dark half circle is a registration marker used for compositing the piecewise acquired images.) The transmural fiber helix angles of the same heart obtained from DTI are plotted below as functions of absolute (i.e., not normalized to the wall thickness) transmural distance. Data from the LV septal wall and free wall regions are shown in red and blue, respectively. For both the confocal microscopy image and helix angle plot, the epicardium is shown to the left.
volume) allowed the myocardial structure to be quantitatively measured at more than 170,000 locations throughout the mouse heart, which is about 10 times better than the highest number reported for histological studies (performed on rabbit (11) and canine (8) hearts that are larger). A previous light microscopy histological study of the mouse heart (33) reported a discrete “sandwich” layering of the myocardium, with myofibers in the epicardium, mid-wall, and endocardium oriented predominantly in the oblique longitudinal, circumferential, and longitudinal directions, respectively. While the observation is true, though only qualitatively, Figs. 3 and 4 of the present study reveal the additional structural detail (e.g., on the order of 20 measurement locations across the myocardial wall including papillary muscles) and the quantitative nature of the measurement obtainable via diffusion tensor microscopy. From the linear regression RMS deviation of the helix angles, the accuracy of the current fiber orientation angle measurement is estimated to be 5.5°. Combined, these findings demonstrate the practical utility of diffusion tensor microscopy as a reliable, quantitative alternative to conventional histology for assessing tissue microstructure.

The FA value of 0.27 ± 0.06 measured in the current study is consistent with those previously observed for perfused rabbit (17), fixed goat (20), and in vivo human (21) myocardial tissues. Moreover, the mean diffusion tensor eigenvalues (in descending order) of 0.75 ± 0.13, 0.60 ± 0.13, and 0.51 ± 0.13 × 10⁻³ mm²/s are in good agreement with the values reported in the literature for the fixed rat (22) myocardium. The similarities of these diffusion parameters suggest not only that there exists minor interspecies variation, but also that the fixation process has minimal impact on the exhibited anisotropy of water diffusion of the myocardium. The latter was consistent with observations reported for the mouse brain (34). Due to the relatively small difference between the secondary and tertiary eigenvalues, it is possible that the difference reflects merely the effects of sorting of an otherwise axisymmetric (i.e., λ₂ = λ₃) system. Since image noise would be the predominant cause, this sorting artifact would manifest itself in the degree of randomness in the corresponding eigenvector fields (30). Consequently, the conspicuous organization of the corresponding directional structures (i.e., eigenvectors) shown in Figs. 2 and 3 indicates that the contribution of sorting artifact in the observed DTI results is likely small.

### Myocardial Structure of Mouse Heart

Despite its significant implication for tissue functions, due in part to the labor-intensive demands of conventional histology, few studies have been performed to examine and model the 3D myocardial structure. In contrast to the previously reported discrete “sandwich” layering (33) of the myocardium, the results in Fig. 4 and Table 1 clearly show that the fiber orientations in the mouse heart also undergo the rather smooth, counterclockwise rotation observed in other species (8,11,20–22). Moreover, at least for myofibers on the LV mid-hemispherical plane, it is evident that the fiber orientation rotation can be adequately characterized by a linear (i.e., constant rate) model, and that the rate of rotation in the septal wall is faster than that in the free wall. Both of these observations are consistent with those reported for the goat heart (20), which are indicative of some interspecies similarity of the myocardial structure. Given the significantly different transmural helix angular span but insignificant difference in the transmural wall thickness, the faster rotation rate would remain true in the septal wall even if the rotation rate were expressed in terms of percentage transmural distance. Although mechanical or electrophysiological implications of these structural observations are yet to be clearly understood, detailed and reliable measurements of the myocardial structure made feasible by diffusion tensor microscopy are expected to be instrumental for the development of “morphologically accurate” functional models of the heart.

In addition to the fiber structure, it has been proposed that the myocardium exhibits a laminar organization formed by 3–4 cells-thick myocyte “sheets” separated by extracellular collagen network (9,10). Evidence for this higher hierarchy of myocardial structure has been reported in previous myocardial DTI studies in larger animals (17,19,21), albeit direct histological validation is yet to be obtained. The myocardial laminar structure has been described as a twisted sheet orienting predominantly in planes spanned by the myocardial fiber and the transmural radial direction, which corresponds to the local planes formed by the diffusion tensor primary and secondary eigenvectors, with the tertiary eigenvector pointing in the sheet normal direction. Results shown in Figs. 2 and 3 indicate that this higher level of myocardial structural organization may also exist in the mouse heart, with the myocardial sheets (secondary eigenvector) orienting in the

### Table 1

<table>
<thead>
<tr>
<th>Specimen/region</th>
<th>Angle at subepicardium (degrees)</th>
<th>Range of rotation (degrees)</th>
<th>Wall thickness (mm)</th>
<th>Slope (degrees/mm)</th>
<th>R²</th>
</tr>
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<tbody>
<tr>
<td>Heart #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>−45.7 ± 18.7</td>
<td>128.5 ± 15.4</td>
<td>1.62 ± 0.24</td>
<td>79.9 ± 13.0</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>Septal</td>
<td>−64.7 ± 13.6</td>
<td>150.9 ± 17.0</td>
<td>1.72 ± 0.10</td>
<td>93.5 ± 7.2</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>P</td>
<td>0.036*</td>
<td>0.016*</td>
<td>0.31</td>
<td>0.021*</td>
<td>0.089</td>
</tr>
<tr>
<td>Heart #2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>−55.6 ± 16.6</td>
<td>138.4 ± 16.7</td>
<td>1.59 ± 0.24</td>
<td>81.0 ± 11.6</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>Septal</td>
<td>−71.5 ± 9.5</td>
<td>155.9 ± 10.5</td>
<td>1.70 ± 0.13</td>
<td>93.8 ± 6.5</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>P</td>
<td>0.053</td>
<td>0.036*</td>
<td>0.28</td>
<td>0.033*</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Each entry represents the average of n = 8 measurements, reported as the mean ± SD. Student’s t-test P values are reported below each region pair, with statistically significant (P < 0.05) comparisons denoted by asterisks.
radial direction with some level of variation. Since the eigenvectors are linked via their mutual orthogonality, the sheet structures may have in part contributed to not only generally larger tangential residual angles of the tertiary diffusion tensor eigenvector, but also a higher degree of complexity of the diffusion tensor tertiary eigenvector field (Fig. 3a,b) compared to the myofiber structures (Fig. 3a,b). Quantitative modeling of the myocardial sheet structures was not done, as it is beyond the scope of the current study.

The representation and description of the myocardial fiber structure (Figs. 3, 4, Table 1) may be limited in two aspects. First, the local cylindrical coordinate system employed is accurate only when the shape of the LV is perfectly cylindrical. The natural longitudinal curvature of the epicardium and noncircular shape of the LV cross-section would cause the local tangential plane to deviate from being parallel to the epicardial surface, and thus induce errors in the measured fiber helix angle and tangential residual angle (20,22). Second, since the location of the endocardium was defined by digitally segmenting out the papillary muscle via visual inspection, there may be some subjective variability in the measurement of transmural helix angular span, rate of rotation, and transmural wall thickness. Steps were taken in the current study to address these issues. For example, the myocardial fiber structures were analyzed only on the mid-hemispherical plane of the LV where the longitudinal curvature of the epicardium is minimum. The relatively low tangential residual angle of the diffusion tensor primary eigenvector provides empirical evidence that departure of the LV epicardium is minimum. The relatively low tangential residual angle of the diffusion tensor primary eigenvector provides empirical evidence that departure of the LV cross-section from a circular shape is small. Moreover, a separate linear regression fit of the transmural fiber helix angles over only the mid-wall “compact” region of the myocardium (data not shown) yielded only marginal improvements in the $R^2$ value. Consequently, these potential limitations are not only unlikely to undermine the general conclusions reached, but also point out the necessity of more sophisticated structural models based on precise knowledge of the myocardial structure, such as that made available by the current study.

CONCLUSION

Isotropic 100 ¬m-resolution 3D diffusion tensor microscopy with a scan time of ~9 hr and estimated fiber orientation measurement accuracy of 5.5° has been demonstrated in fixed mouse hearts. Preliminary analyses of diffusion tensor primary eigenvector field reveal a largely linear rotation of the fiber orientation across the LV wall and a faster rate of rotation in the LV septal wall than in the free wall. The observed organizations of the diffusion tensor primary and secondary eigenvectors are qualitatively consistent with the known myocardial fiber and sheet structures reported for larger animal hearts. These findings underscore the utility of diffusion tensor imaging and microscopy as a practical alternative to conventional histology for assessing myocardial structures, and pave the way for the development of 3D structural atlases and “morphologically accurate” functional models of mouse hearts.

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