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Optical coherence tractography using intrinsic contrast

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Abstract

Organs such as the heart and brain possess intricate fiber structures that are best characterized with three-dimensional imaging. For instance, diffusion-based, magnetic resonance tractography (MRT) enables studies of connectivity and remodeling during development and disease macroscopically on the millimeter scale. Here we present complementary, high-resolution microscopic optical coherence imaging and analysis methods that, when used in conjunction with clearing techniques, can characterize fiber architecture in intact organs at tissue depths exceeding 1 mm. We anticipate that these techniques can be used to study fiber architecture *in situ* at microscopic scales not currently accessible to diffusion magnetic resonance (MR), and thus, to validate and complement macroscopic structural imaging techniques. Moreover, as these techniques use intrinsic signals and do not require tissue slicing and staining, they can be used for high-throughput, nondestructive evaluation of fiber architecture across large tissue volumes.

Both the heart and brain possess complex three-dimensional (3D) architectures that are essential for normal function. In the heart, the orientation of myofiber tracts plays a central role in the contraction of the ventricles. Seminal histological studies have shown that myofibers form a series of crossing spiral structures in the healthy myocardium [1]. Helix angles transition linearly from a right-handed helix in the subendocardium to a left-handed helix in the subepicardium. More recent studies using diffusion tensor magnetic resonance imaging (DT-MRI) show similar results on a macroscopic scale [2]. In the brain, changes in myelination and connectivity occur during both development and disease. Diffusion spectral imaging (DSI), a further advance, enables quantification of multiple fiber crossings within a voxel, which allows reconstruction of complex fiber pathways in neural tissue using tractography algorithms [3]. While informative, these MR techniques produce relatively macroscopic images that cannot resolve individual myofibers or axons.

Volumetric optical imaging techniques are nondestructive, eliminating artifacts induced by histological sectioning. Previous works used polarization-based linear birefringence [4] and optical projection tomography [5] to characterize the microstructure of cardiac tissue. In particular, optical coherence tomography (OCT) and optical coherence microscopy (OCM)

are imaging techniques capable of producing fast, high-resolution images using intrinsic scattering contrast, enabling imaging depths exceeding two-photon and confocal microscopy. OCT was previously used to quantify cardiac fiber orientation in a rabbit model [6], and polarization-sensitive OCT was used to determine fiber orientation in the brain [7]. OCM produces higher resolution images and can image single myelin fibers [8]. These characteristics make optical coherence imaging an ideal modality for bridging the gap between MRI and histology. The purpose of this study was to demonstrate optical clearing, imaging, image processing, and tractography protocols to quantify aspects of fiber architecture in the heart and brain.

All procedures were performed with local Institutional Animal Care and Use Committee approval. The C57BL/6 mice used in this study were between 12 and 14 weeks old. For heart imaging, animals were euthanized and each heart was removed, submerged in an optimal cutting temperature compound embedding medium, and frozen/thawed gradually. We empirically found that this procedure qualitatively improves OCT image contrast. The tissue was then placed in a 50% glycerol solution (Sigma Chemical, St. Louis, Missouri) for 24 h. A glass slide was placed on the anterolateral wall of the left ventricle and used to lightly compress the tissue, creating a flat perpendicular surface. Histology was then performed by sectioning 5 μm -thick slices taken parallel to the epicardial surface and staining with H&E. For brain imaging, animals were perfused transcardially with 4% PFA/PBS. Tissue was fixed in 4% PFA/PBS, cryoprotected, then optically cleared with (ScaleA2), as previously reported [9]. Care was taken to ensure that the brain cortical surface was perpendicular to the optic axis during imaging.

We used a 1310 nm spectral/Fourier domain OCT/OCM system built on a Nikon microscope platform to acquire image volumes [10]. Two unpolarized superluminescent diodes were combined with a 50/50 fiber coupler to create a light source with a bandwidth of 170 nm. The axial scanning speed was 47,000 axial scans per second achieved by a 1024 pixel InGaAs line scan camera (Goodrich-Sensors Unlimited, Princeton, New Jersey). The axial resolution was approximately 4.7 μm in air. OCT was performed with a 10 $\times$ objective (10 $\times$ Mitutoyo Plan Apo NIR Infinity-Corrected) and OCM was performed with a 40 $\times$ objective (Olympus LUMPLANFL/IR 40 $\times$ W). For OCT imaging, the transverse resolution was 3.6 μm FWHM. Volumes consisting of 768 frames with 1024 axial scans per frame were acquired over 1.9 mm $\times$ 2.5 mm. For OCM imaging, the transverse resolution was 0.9 μm FWHM. Volumes consisting of 512 frames with 512 axial scans per frame were acquired over 0.6 mm $\times$ 0.6 mm. Tractography [3] was performed on OCT images.

Our initial observations of myocardial tissue showed that the image features in *en face* OCT images were locally elongated along the fiber axis, as shown in Fig. 1(a). We confirmed this interpretation by imaging of myocytes using two-photon excited autofluorescence (red) and collagen using second-harmonic generation (green) as seen in Fig. 1(b) from the same specimen. Since a single voxel does not provide information about orientation, the intensity variations over a local region must be analyzed. This suggests that windowed analysis of the OCT image data could be used to quantify local fiber orientation. The choice of window size (0.36 mm) represents a trade-off between accuracy of angular estimation and spatial resolution. A two-dimensional (2D) discrete Fourier transform (DFT) was calculated after multiplication by a 0.36 mm symmetric Hann window that was scanned across the original image slice in step sizes of 0.09 mm. Based on the sum of the high-pass filtered 2D DFT slice magnitude squared at each angle, a local orientation distribution function (ODF) was constructed, as shown in Fig. 1(c):

$$\text{ODF}(\theta) = \int_{-\infty}^{\infty} |F_w(f_x, f_y)|^2 H(f_x, f_y) df, \quad (1)$$

where f_x is $-f \sin(\theta)$, f_y is $f \cos(\theta)$, F_w is the windowed Fourier transform, and H is a high-pass filter. The maximum of the ODF gives the local fiber orientation, and multiple maxima indicate multiple orientations within the windowed region. By definition, all angles fall between -90° and 90° . Similar analysis methods based on the Radon transform, Hough transform, or image auto-correlation also could be devised.

Angular data θ_n were characterized using the circular mean ($\bar{\theta}$) and variance (Var), defined using circular statistics and accounting for modulo 180° equivalence:

$$\bar{\theta} = \frac{1}{2} \text{Arg} \left(\frac{1}{N} \sum_{n=1}^N e^{i2\theta_n} \right) \quad \text{and} \quad (2)$$

$$Var = 1 - \sqrt{\left[\frac{1}{N} \sum_{n=1}^N \cos(2\theta_n) \right]^2 + \left[\frac{1}{N} \sum_{n=1}^N \sin(2\theta_n) \right]^2}. \quad (3)$$

Figure 1(d) shows a map of the local maxima of the ODFs obtained from the windowed analysis described above. The length of each red line has been scaled based on the ratio of the peak of the ODF to the variance of the ODF 90° away from the peak. While the distribution of ODF peak orientations can be analyzed alone, myofibers in the normal heart are known to form continuous tracts. These constraints can be incorporated by constructing tracts or streamlines from the ODFs. A streamline was therefore originated at each point and propagated along the direction of the ODF maximum. The direction was updated by multiplying the ODF by a circularly Gaussian window centered on the orientation from the previous iteration. Figure 1(e) shows the results of tractography, where tracts with a tortuosity (ratio of tract length to distance between endpoints) of greater than 1.3 or whose distance from the circular mean exceeded a threshold were removed. Figure 1(f) shows a histogram of tract angles compared to the histogram of ODF maxima angles, showing that tractography leads to a narrower spread and possibly better estimation of the fiber orientation.

Figure 2(a) and 2(b) shows H&E stained histology and streamlines overlaid on OCT images in the epicardium, midwall, and endocardium of the left ventricular lateral wall, while Fig. 2(c) shows a histogram of the tract orientations. The change in fiber angle with depth was highly linear, as shown in Fig. 2(d). Figure 2(e) shows similar changes in orientation across six hearts, from the epicardial to endocardial surfaces.

Similarly, OCM and OCT visualized white matter architecture in intact brains. Figure 3(a) shows OCM imaging of myelinated axons in the intact mouse neocortex from the cortical surface to a depth of more than 1.5 mm, including the corpus callosum (CC). Myelination increases with cortical depth as expected. As shown in Fig. 3(b) and 3(c), imaging and tractography of the intact corpus callosum is possible, where tracts show a predominantly mediolateral orientation. For comparison, Fig. 3(d) shows crossing fibers at the optic chiasm, where an exemplary ODF [Fig. 3(e)] and the tract histogram [Fig. 3(f)] show two peaks corresponding to the different orientations.

In conclusion, we demonstrated methods for imaging and quantitative analysis of fibrous architecture in deep tissues. We anticipate that clearing and volumetric imaging techniques have significant advantages over histology and staining where complex fiber architecture needs to be preserved and studied, and could be useful in studying damage, recovery, and remodeling after myocardial infarction or white matter injury. Tractography techniques

impose constraints of a continuous fiber-like structure over large spatial scales, which can be incorporated to aid data analysis.

These techniques only require estimation of the local orientation distribution and do not require tracing the same fiber over long distances, or even the capability to resolve individual fibers. Limitations of the methods presented here include reduced image contrast at large depths, preparation time required for clearing, and the fact that orientation determination and tractography can only be performed in the *en face* plane. Alternative imaging geometries, processing, and analysis techniques may enable 3D tractography. Optimal fixing and clearing protocols, to be investigated in the future, will represent a trade-off between improved penetration depth (from reduced scattering) and reduced contrast (from reduced backscattering). Finally, similar concepts could be applied to other imaging modalities including two-photon, CARS, or third-harmonic generation.

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Fig. 1.

Myofiber direction can be quantified from OCT images. (a) Representative OCT image of the left ventricle of a mouse is shown. (b) Image of the same specimen from a two-photon microscope, showing myocytes (red) and collagen (green). (c) Example orientation distribution function (ODF) from the OCT image shows a maximum at -42.4° (red line). Orientations of (d) ODF peaks and (e) streamlines, show myofiber orientation over the region of interest. (f) Representative tract histogram shows consistent fiber direction. From the ODF peak distribution (blue), the mean direction was -48.0° with a circular variance of 0.13. With tractography (red), the mean direction was -37.1° and had a lower circular variance (0.02). Scale bars are 0.1 mm.

Fig. 2.

Myofiber orientation changes with depth. (a) Representative H&E stained histology; (b) OCT images with tracts overlaid showing myofiber direction of the lateral left ventricular wall exhibiting a clear transition in orientation with depth. Directions are shown for a single heart as histograms at selected depths. (c) Directions are shown for a single heart as a continuous function of depth. (d) Fiber orientations from six hearts show an approximately linear transition with respect to depth from -60° in the epicardial layer to 60° in the endocardial layer. (e) Images are shown in the same orientation as Fig. 1. Scale bars are 0.1 mm.

Fig. 3.

(Color online) Representative imaging and tractography of two intact mouse brains. (a) Sagittal section of cortical myelination (maximum intensity projection over $25\ \mu\text{m}$), showing the corpus callosum (CC). (b) Imaging of the corpus callosum at roughly 1.5 mm depth; (c) both tracts (circular variance of 0.02) and ODF maxima (circular variance of 0.22) show a predominantly horizontal (mediolateral) orientation of 90° . (d) Example of crossing fibers in the optic chiasm. (e) ODF showing two local peaks (dotted and solid red lines) corresponding to the crossing fibers. (f) Histogram of tract orientations shows two predominant orientation populations, representing contralaterally projecting fibers. Scale bars are 0.1 mm.