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Towards Automated Quantification of Atrial Fibrosis in Images from Catheterized Fiber-Optics Confocal Microscopy Using Convolutional Neural Networks

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Abstract

Clinical approaches for quantification of atrial fibrosis are currently based on digital image processing of magnetic resonance images. Here, we introduce and evaluate a comprehensive framework based on convolutional neural networks for quantifying atrial fibrosis from images acquired with catheterized fiber-optics confocal microscopy (FCM). FCM images in three regions of the atria were acquired in the beating heart in situ in an established transgenic animal model of atrial fibrosis. Fibrosis in the imaged regions was histologically assessed in excised tissue. FCM images and their corresponding histologically-assessed fibrosis levels were used for training of a convolutional neural network. We evaluated the utility and performance of the convolutional neural networks by varying parameters including image dimension and training batch size. In general, we observed that the root-mean square error (RMSE) of the predicted fibrosis was decreased with increasing image dimension. We achieved a RMSE of 2.6% and a Pearson correlation coefficient of 0.953 when applying a network trained on images with a dimension of 400×400 pixels and a batch size of 128 to our test image set. The findings indicate feasibility of our approach for fibrosis quantification from images acquired with catheterized FCM using convolutional neural networks. We suggest that the developed framework will facilitate translation of catheterized FCM into a clinical approach that complements current approaches for quantification of atrial fibrosis.

Keywords

Atrial Fibrosis; Fiber-Optics Confocal Microscopy; Machine Learning

1 Introduction

Atrial fibrosis, defined as excessive formation of connective tissue in the atria, is associated with a variety of conditions including heart failure, ischemia, hypertension, and mitral valve

disease [1]. Atrial fibrosis is also associated with an increased risk of atrial fibrillation. Recent studies showed that many atrial fibrillation patients exhibit atrial fibrosis, which is thought to be the major contributor to arrhythmogenic tissue remodeling in these patients [2, 3]. Also, atrial fibrosis is an important predictor of outcome in patients undergoing catheter ablation for the treatment of atrial fibrillation [4].

Several approaches for atrial fibrosis identification and quantification at the microscopic and macroscopic scale have been developed. Recently developed macroscopic approaches are based on magnetic resonance imaging (MRI) and include T1 mapping and late gadolinium enhancement MRI [5]. Further approaches for identification of fibrotic remodeling and scarring apply endocardial electrical measurements. In particular, fractionated electrograms and reduced voltage amplitudes are thought to identify fibrosis [6, 7]. Microscopic approaches for fibrosis quantification apply stains to assess collagen content in sections of excised tissue. For both, macroscopic and microscopic approaches, image processing is required to quantify fibrosis from the image data.

Here, we present a methodology for evaluating microscopy-based fibrosis quantification in the beating heart in situ. Our studies are based on a transgenic goat model of fibrosis, which was found to exhibit a significant increase in the atrial collagen area fraction versus control [8]. We imaged the atria in this model applying previously introduced catheterized fiber-optics confocal microscopy (FCM) of the beating heart in-situ [9]. We histologically assessed fibrosis based on excised atrial tissues from these imaged regions. The histologically derived fibrosis was indexed to their corresponding FCM images, which were subsequently applied for training of convolutional neural networks. We explored this machine learning approach by varying parameters of the networks and training. We evaluated the trained networks in quantifying fibrosis from a test set of FCM images and assessed its utility using the root mean square error (RMSE) of predicted fibrosis levels.

2 Methods

2.1 Animal Model

All experimental procedures in this study conformed to the National Institutes of Health Guide for the Care, and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Utah (IACUC protocol #17-08008). We used a transgenic goat model of atrial fibrosis produced by cardiac specific overexpression of human transforming growth factor- β 1 [8]. To further increase atrial fibrosis based on an approach previously applied in canine [10], we implanted a pacemaker in one animal, and paced the right ventricle at 240 bpm for 3 weeks prior to FCM imaging.

2.2 Catheterized Fiber-Optics Confocal Microscopy

Transgenic goats ($n=2$) weighing 40 to 60 kg were anesthetized with Propofol and mechanically ventilated. We performed cardiac catheterization in these anesthetized animals using a percutaneous femoral approach. Under intracardiac echocardiographic and fluoroscopic guidance, a transseptal puncture was performed and a bi-directional steerable guiding sheath (14F Destino; Oscor Inc, Palm Harbor, FL, USA) was placed into the left

atrium. Afterward, we performed cardiac mapping (EnSite Precision-, Abbott St. Jude Medical, Green Oaks, Illinois, USA) to generate 3D models of the atrial chambers. Following mapping, fluorescein sodium (AK-Fluor 10%, Akorn Inc., Lake Forest, IL) was intravenously injected at a dose of 7.7 mg/kg to label the extracellular space. The FCM imaging microprobe (UltraMiniO, CellVizio, Mauna Kea Technologies, Paris, France) was steered and positioned via the steerable sheath on the endocardial surfaces within atrial chambers. We acquired images at a field of view (xy) of 245 μm at a rate of 12 images/s. The lateral and axial resolution as well as depth of field of the image system was 1.4, 9, and 66 μm , respectively. The distal tip of our imaging microprobe was customized with electrodes to track its 3D position via cardiac mapping. Image sequences, tip positions, and 3D anatomical models were annotated, indexed and stored on a file server for subsequent analyses. Following the imaging study, the animals were sacrificed and their hearts excised and formalin fixed.

2.3 Histological Assessment of Fibrosis

Full-thickness biopsies from the imaged left atrial anterior and right atrial free wall were paraffin embedded, transmurally sectioned, and Masson's trichrome stained. The slide sections were imaged with a digital slide scanner (Axio Scan.Z1, Zeiss, Jena, Germany) equipped with a 40 $\times$ lens at a lateral resolution of 0.89 μm (Fig. 1A).

Quantitative fibrosis assessment was performed on these acquired images using the image processing software Fiji[11]. In short, we selected a rectangular region of interest through the full myocardial thickness excluding endo- and epicardium, as well as greater vessels (Fig. 1A). We applied color deconvolution for Masson's trichrome stains to separate out the contribution of the blue and red dyes based on their RGB absorption within the selected region (Figs. 1B and C). The deconvolved blue and red images corresponding to their respective dyes were subsequently histogram-derived thresholded (Figs. 1D and E). The thresholded red component image was subtracted from the blue component image. The resulting binary image displayed only the blue component pixels that correspond to collagen (Fig. 1F). A selection was created of all dark pixels in the original blue component image and applied to the collagen-only image. The area fraction of the collagen component within the selected blue component was used as our measure of fibrosis.

2.4 Fibrosis Quantification Using Convolutional Neural Networks

We configured a convolutional neural network (Table 1) for quantification of fibrosis in FCM images from 3 atrial regions using histological assessment as ground truth. In short, we inspected all FCM image sequences and selected images free of artifacts. We avoided inclusion of replicates. Through this process we were able to stratify these images into three groups based on imaged region and their histologically-derived fibrosis level. The 3 imaged regions were of the right atrial free wall in both the paced and non-paced transgenic goat as well as the left atrial anterior wall in the paced transgenic goat. Eight FCM images from each of the three atrial regions were selected and subsequently rotated 35 times in 10° increments to expand the image pool (n=864). The original images from each of the 3 atrial regions and their corresponding 35 rotated variants were randomly split into a training and test set. A split of 75% and 25% for the training (n=648) and test (n=216) sets, respectively

was determined to adequately reduce variance and overfitting in the training process as well as eliminating overlap of images and their variants across the training and test groups. Initially, we trained the network on full resolution images, but also investigated image dimensions of 50, 100, and 200 pixels. We compared a stochastic gradient descent and the Adam optimizer for training of the network. Training was terminated after a maximal number of epochs of 1000 or when the training RMSE did not decrease for 500 iterations. Batches of training data were used in each epoch and shuffled after each epoch. We varied batch sizes between 64, 128, and 256. Each combination of batch size and image dimension was applied 10 times for training of a network.

3 Results

Example Masson's trichome images for the 3 different regions with low, intermediate and high fibrosis in the atria of transgenic animals are presented in Fig. 2A, D and G, respectively. Corresponding images of the detected fibrosis are shown in Fig. 2B, E and H. The measured degree of fibrosis is presented in table 2. We annotated images from catheterized FCM from these regions with the measured degree of fibrosis. Example collagen-only images from atrial regions with low, intermediate and high fibrosis are presented in Fig. 2C, F, and I, respectively.

Statistical information on RMSE for training using the stochastic gradient descent optimizer and with varying batch sizes and image dimensions is summarized in Fig. 3A. In general, decreasing image dimension or batch size increased training RMSE. The number of iterations for each training process ranged between 350 and 2200 (Fig. 3B). We calculated RMSE for application of trained neural networks to the test image set (Fig. 3C). In general, test RMSE decreased with increasing image dimension. The stochastic gradient descent optimizer outperformed the Adam optimizer when applied to both the training and test image sets. The smallest training and test RMSE achieved with the Adam optimizer was 0.80 and 7.9%, respectively. The stochastic gradient descent optimizer achieved a training and test RMSE of 0.50 and 2.6%, respectively, for a network trained on images with a dimension of 400×400 pixels and a batch size of 128. The relationship between batch size and test RMSE was weak.

The predicted degree of fibrosis for each region using the network with the smallest training RMSE is listed in Table 2. The Pearson correlation coefficient between predicted and measured fibrosis was high (0.953).

4 Discussion and Conclusion

In this study, we introduced and evaluated a framework for quantification of atrial fibrosis in the beating heart in situ based on microscopic imaging and convolutional neural networks. We explored our approach in a transgenic animal model of atrial fibrosis and using histological analyses from excised atrial tissues as ground truth. An important finding was that reduction of image resolution reduced the accuracy of fibrosis quantification. The overall performance of the neural network for fibrosis prediction was promising. The

predicted fibrosis values in our test image set was close to the ground truth values based on our RMSE and cross correlation analysis.

Our results suggest that catheterized FCM may translate into a clinical approach complementing current approaches for quantification of atrial fibrosis. Currently, assessment of fibrosis burden is based on MRI or endocardial voltage mapping. MRI and voltage mapping lack the spatial resolution to accurately and precisely localize atrial fibrosis. In addition, these macroscopic techniques have pigeonholed fibrosis into only two categories: patchy or diffuse. Microscopic approaches such as FCM may expand these definitions and etiologies of fibrosis based on insights that only becomes apparent at this scale. These opportunities motivate a need for a high-resolution imaging technology to accurately and precisely localize atrial fibrosis and assess fibrosis burden. The introduced framework takes steps towards translating catheterized FCM for clinical assessment of atrial fibrosis at a meaningful resolution.

We note several limitations of our study. We selected only images without notable motion artifacts for training and testing of the network. Future refinements to our framework could automatically detect such artifacts using the neural network and remove detected images from subsequent analysis. In addition, we focused our analyses on a small number of atrial regions and animals which limited the diversity in the images acquired. However, even with this small dataset there appeared to be learnable features that produced reliable quantification of fibrosis using our framework. Randomization of the images into their respective training and test sets was performed once. A more comprehensive evaluation of our framework would be to perform several iterations of the randomization process and how this parameter affects the overall performance of the neural network. We also evaluated only two of many numerical optimizers available for training. The two optimizers were limited in that they produced largely varying outputs due to the stochastic nature of the learning process. Alternative approaches such as particle swarm optimization could be more efficient.

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Fig. 1. Image processing for fibrosis assessment. (A) Representative Masson's trichrome cross-section of the right atrial free wall. (B-F) Processed region of interest outlined in green. Color deconvolved region separated into (B) blue, (C) red components and after (D,E) histogram-derived thresholding. (F) Visualization of fibrosis from subtracting (E) from (D). Scale bar in (B) applies to (C-F).

Fig. 2.

Representative images of atrial tissue with (A-C) low, (D-F) intermediate, and (G-I) high fibrosis. Zoom-in of Masson's trichrome stained regions depicting fibrosis (blue pixels) at (A) low, (D) intermediate, and (G) high levels. (B,E,H) Visualization of fibrosis (bright pixels) in the Masson trichrome zoom-ins post-process. Representative images from FCM image sequences acquired from the (C) low, (F) intermediate, and (I) high fibrosis regions *in-vivo*. Scale bar in (A) applies to (B,D,E,G,H). Scale bar in (C) applies to (F,I).

Fig 3. Statistical analysis of convolutional neural network trained with varying batch sizes and image dimensions. (A) RMSE and (B) number of iterations upon termination for training. (C) RMSE after applying the trained neural network to the test image set.

Table 1.

Convolutional Neural Network Configuration.

Layer	Layer Type	Parameters and Range
1	Input	Dimensions (pixels): [50 50], [100 100], ..., [400 400]
2, 6, ..., 18	Convolution	Filter size: 2; Number of filters: 2, 4, ..., 32
3, 7, ..., 19	Normalization	
4, 8, ..., 20	Rectified linear unit	
5, 9, ..., 17	Maxima pooling	Pool size: 2; Stride: 2
21	Fully connected	
22	Regression	

Table 2.

Comparison of fibrosis measured in Masson's trichrome and predicted from FCM images and the convolutional neural network.

FCM Image Set	Transgenic Model	Tissue Region	Measured Fibrosis (%)	Predicted Fibrosis (% , mean $\pm$ stdev)
1	Paced	Left atrial anterior wall	16.5	16.7 $\pm$ 2.3
2	Non-Paced	Right atrial free wall	21.8	23.0 $\pm$ 3.8
3	Paced	Right atrial free wall	39.7	37.7 $\pm$ 1.8