Automatic Mouse Embryo Brain Ventricle Segmentation from 3D 40-MHz Ultrasound Data

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Abstract—Volumetric analysis of brain ventricles is important to the study of normal and abnormal development of the central nervous system of mouse embryos. High-frequency ultrasound (HFU) is frequently used to image embryos because HFU is real-time, non-invasive, and provides fine-resolution images. However, manual segmentation of ventricles from 3D HFU volumes remains challenging and time consuming. In this study, in utero and in vivo volumetric ultrasound data were acquired from pregnant mice using a 5-element, 40-MHz annular array. An automatic segmentation algorithm based on active shape model (ASM) was developed to segment the brain ventricles of the embryos; ASM allows us to efficiently “learn” from training data (i.e., manually segmented data). The algorithm was further enhanced by using detail-preserving reference shapes (also learned from training data) and region growing constrained by the reference shape. The hybrid algorithm was applied to three 12.5-day-old embryos. Results were qualitatively analyzed and compared with manual segmentation results in regions typically difficult to segment (e.g., thin brain ventricle connections). In addition, quantitative analysis using the Dice similarity coefficient (DSC) was used to compare the automatic segmentation results with manual segmentation. We obtained average DSC values of 0.848 ± 0.015 for the brain ventricles and our method produced morphologically accurate results. Therefore, our method could streamline current HFU longitudinal studies of brain development that require manual segmentation.

Index Terms—high-frequency ultrasound, segmentation, mouse embryo, brain.

I. INTRODUCTION

Due to the high degree of homology between the mouse and human genome, the mouse has been the premier animal model for studying mammalian development. Several imaging modalities are capable of providing 3D data sets from the embryonic mouse at sub 100-μm resolution. Micro-CT [1] and optical projection tomography [2] have been used, but these methods usually require specimen fixation, which makes them unsuitable for in vivo imaging. Due to its high tissue contrast, MRI has been used extensively to image the adult mouse in vivo and fixed embryos [3], but the imaging time is an extremely long 2.5 hours per embryo making it impractical for high-throughput translational studies. High-frequency ultrasound (HFU) imaging has become a standard tool for real-time, in vivo small-animal imaging [4] at fine spatial resolution (<100 μm). The applicability of this modality to developmental biology has been demonstrated in previous studies [5], [6]. The robustness of HFU to image embryos in utero over a wide range of developmental stages, and the sharp acoustic contrast between brain ventricles (BVs) and surrounding tissue, along with 3D acquisition times on the order of 2 minutes, makes HFU ideal for analyzing the morphology of the developing embryo, particularly the central nervous system (CNS).

In previous studies, our group developed an HFU imaging platform for 3D in vivo imaging of mouse embryos [5], [7]. This platform uses a 40-MHz 5-element annular array that permits 3D imaging of several mouse embryos from the same litter in a single imaging session. Following imaging, the BVs of the embryos were then semi-automatically segmented. This segmentation process is time consuming and requires a clear knowledge of mouse-embryo anatomy. Therefore, in this study, we propose a novel automatic-segmentation algorithm. The segmentation algorithm combines the advantages of active shape model (ASM) [8], [9] and a modified version of region growing [10] constrained by a reference shape extracted from training data. The automatic-segmentation algorithm was qualitatively and quantitatively compared to manual segmentation and other automatic segmentation algorithms.

II. METHODS

A. Custom HFU system

All the data were acquired using a custom HFU system entirely developed and designed at Riverside Research. In this study, an annular array was utilized to acquire in vivo, in utero, 3D HFU data sets from mouse embryos [5], [11]. The annular-array transducer had five, equal-area elements, with a 38-MHz center frequency, a 12-mm geometric focus and a 6-mm aperture [12]. To permit fast 3D imaging, the transducer was mounted to a high-speed linear actuator. To acquire HFU radio-frequency data, one element was pulsed using a chirp-coded excitation signal and the received radio-frequency (RF) echoes were simultaneously digitized on all five elements at 250 MS/s. This pulsing scheme was repeated for each of the five array elements, and a complete 2D image required five passes with a total scan time of 0.5 s. Beamforming was accomplished in post-processing using a synthetic-focusing algorithm [11]. Pulse compression was performed prior to beamforming using a mismatched filter [5]. To limit motion artifacts due to the breathing motion of the pregnant mouse,
respiratory gating was implemented using a pneumatic pillow [7].

B. In-vivo mouse embryo imaging

The mice used in these studies were imaged at New York University School of Medicine and maintained under protocols approved by their Institutional Animal Care and Use Committee. In this study, timed pregnant Swiss Webster mice (Taconic, Hudson, NY) were imaged at embryonic day (E)12.5 (E0.5 was defined as noon of the day after successful overnight mating). The pregnant mouse was anesthetized using isoflurane (4% in air for induction, 1-2% in air for maintenance) and placed on a heated custom stage to maintain the internal body temperature at 37°C. The annular array was lowered into a water bath positioned over the shaved abdomen. The self-contained water bath was made by cutting a 25-mm hole in a plastic Petri dish. After positioning, a full 3D data set of the embryo was acquired with 50-μm spacing between adjacent scan planes at a sampling rate of 250 MHz. Each B-scan was approximately 7-mm wide and 9-mm deep, and complete 3D data sets (100 slices) were acquired in 2.2 minutes.

C. Manual segmentation

3D stacks of B-mode images were imported into Amira visualization software (v5.2, Mercury Computer Systems, San Diego, CA) for volumetric visualization and semi-automatic segmentation. Before segmentation, a 3D Gaussian filter and a contrast-limited adaptive histogram equalization filter were applied to reduce noise and to optimize contrast between cerebral BVs and the surrounding tissue. The 3D volume was then transformed to a standard sagittal orientation. BV masks were created semi-automatically by choosing voxels within the region of interest and applying a 3D threshold-detection algorithm with an empirically-determined window of image gray levels. The masks were then inspected and manually corrected to reflect correct morphology. The BVs were then isolated by applying a logical arithmetic “AND” between the masks and the 3D volume. (The embryonic head was also manually segmented by drawing region of interests around the head tissue.) To accomplish these tasks, it took an expert user about 90 minutes per embryo.

D. Automatic segmentation

The BVs are fluid-filled and are less echogenic than the other parts of the brain. Therefore, a possible way to segment BVs is by using region-growing methods with seeds which have grayscale intensities lower than a threshold \( T \). Region growing works iteratively, starting with all seed pixels as the current region. In each iteration, we compute the mean \( \eta \) and standard derivation \( \sigma \) of pixel gray levels inside the current region, and we then check each neighboring pixel of the current region, to see whether it has a gray level that is within the range of \( \eta - \alpha \sigma \) to \( \eta + \alpha \sigma \). The challenge in applying region growing for BV segmentation lies in how to choose the parameter \( \alpha \). If \( \alpha \) is too high, pixels that are in the space between the head and the uterus which are also dark may be falsely connected with the BVs (which we call leakage). If \( \alpha \) is too low, then some thin connections between the BVs may be missed.

An alternative approach is to make use of a prior shape model learned from training data (i.e., manually-segmented data sets) through the ASM method [8], [9].

With ASM, corresponding feature points on the surfaces of the training shapes are identified, and the mean shape and the principal modes of shape variations are learned: the eigenvectors of the covariance matrix of the training shape vectors (each containing the feature positions a training data set). Furthermore, the distribution of the variation pattern of each feature point along the surface normal is also learned. The mean shape, principal modes, and distributions of the variation patterns of all feature points together form the point distribution model (PDM) of the shape prior. Given a test data set, each feature point is moved so that the variation pattern along its normal surface matches with the learned pattern as best as possible under the constraint that the resulting shape vector can be represented as the mean shape plus a weighted sum of the principal modes. Because there are variations among the manually segmented BV shapes in different training data sets, it is difficult to obtain sufficient feature points that are common among all training shapes and yet preserve all important shape details. For example, some thin connections in Fig. 1(b) are not present in Fig. 1(a), hence points along those connections cannot be used as feature points. We can only choose “strong” feature points for which we can reliably establish correspondence among all training shapes. These strong feature points will result in a mean shape shown in Fig. 1(c).

![Fig. 1](image_url)
In addition to the PDM built from a relatively coarse set of feature points, we also generate a reference shape based on the union of points on the registered training shapes (Fig. 2). This reference shape keeps all the thin connections, but may also contain extra points (Fig. 1(d)). As outlined in Fig. 2, we first use the PDM to generate an initial segmentation map, which may miss some shape details. Then, we deform the reference shape so that it is aligned with the initial segmentation map to produce an intermediate segmentation map. This result is likely to keep all the shape details, but the shape boundary may not fit closely with the underlying image, because the reference shape was an aggregation of all training shapes after proper registration. Therefore, in the final step, we apply a modified region-growing method that allows growing and shrinking so that the resulting shape boundary fits with the actual ventricle boundary more accurately.

III. RESULTS

In this study, we applied our algorithm to nine 3D HFU data sets of E12.5 mouse embryos. Six data sets were used to train the algorithm and the remaining three were used for testing. (All data sets include the manual segmentation result segmented by a medical expert.)

Three segmentation algorithms were implemented: region-growing, ASM using the trained PDM, and the proposed method. For the region-growing algorithm, parameters $T$ and $\alpha$ were chosen so that the average DSC value among all training data sets was maximized. In order to prevent significant leakage, we also applied a distance threshold, so that the grown pixels were not more than a certain distance away from the nearest seed pixel. This threshold was also optimized using the training data. We used these parameters to segment the testing data sets. The computation time for region growing was about 10 seconds per 3D data set using MATLAB on PC with a 2.6-GHz i7 processor.

For the ASM and the proposed method, training of the PDM took about one hour using the six training data sets. Segmentation times were about 20 and 50 seconds using ASM and the proposed method, respectively.

Representative results obtained from a E12.5 embryo are shown in Fig. 3. For these illustrative cross sections, the improved performance of our proposed algorithm was partially visible in the regions of weak boundary and implicit detail. For the cross-section shown in the first row, the upper left boundary of the ventricle was weak, and using the classic region growing (Fig. 3b) leads to leakage outside of the boundary. Our method alleviates the above problem: the intermediate segmentation map (Fig. 3d) automatically restricted growth in the third step of our algorithm, yielding a final segmentation that was free from leakage (Fig. 3e). For the cross-section shown in the second row, there was a thin connection between two ventricles in the right side, which was missed even in the manual segmentation (Fig. 3f). Region growing (Fig. 3b) and ASM (Fig. 3c) results do not include this thin connection. This connection was maintained by the proposed method (Fig. 3e) because this structural feature was preserved in the reference shape. To further illustrate the performance of our method, Fig. 4 shows 3D renderings of the segmented BVs using region growing, our method, and manual segmentation. Fig. 4a clearly shows the segmentation leakage when region growing is used alone. Our method (Fig. 4b) shows a thin connection between the BVs which is not seen in the manual segmentation image (Fig. 4c), but is morphologically correct.

Table I compares the volume and DSC values for three E12.5 embryos using different segmentation methods. For embryo 1, the proposed method yielded better results in terms of DSC value than region growing and ASM for embryo 1. For embryos 2 and 3, DSC values obtained using our method were less than those obtained using region growing, because our third step result was strongly dependent on the result of ASM. When the error of the segmentation result from ASM is significant, traditional region growing may perform better. However, even for embryos 2 and 3, visual inspection of the segmentation results demonstrated that our method was able to provide details (e.g., thin connection between BVs) not always present even in the manual segmentation.

IV. CONCLUSIONS AND PERSPECTIVES

The segmentation results obtained are encouraging and reveal that with sufficient training data the proposed approach, which combines benefits from ASM and region growing, has strong potential for automatic segmentations of the BVs in 3D HFU data sets from mouse embryos. The method is computationally efficient (i.e., 3D segmentation requires less than 1 minute). Therefore, the proposed segmentation methods could potentially streamline HFU longitudinal studies of brain development that require manual segmentation. The performance of the proposed method is limited by the ASM method, because it uses the ASM result as a starting point. The ASM method does not perform well, at present, because we have limited training data (6 data sets) and the manual segmentations of these training data are not always consistent (e.g., some were missing the thinly connected ventricles). With more training data that have consistent manual segmentation results, the ASM method, and consequently the proposed method, should perform better. Nevertheless, the need for training data at each development stage can limit applicability and we are currently developing novel segmentation methods.

![Flowchart of the proposed segmentation algorithm.](chart.png)
Fig. 3: Illustrative segmentation results for E12.5 embryo 1. In each row, (a) is the raw image; (b) is the segmentation result of region growing; (c) is the segmentation result of ASM, which is also the initial segmentation of the proposed method, (d) is the intermediate segmentation of the proposed method, which is obtained by registering the reference shape to (c); (e) is the final segmentation result of the proposed method; (f) is the manual segmentation result.

TABLE I: Quantitative comparison of automatic BV segmentation results by region growing, ASM, and our method

<table>
<thead>
<tr>
<th></th>
<th>Manual Volume (mm³)</th>
<th>Active shape model Volume (mm³)</th>
<th>DSC</th>
<th>Region growing Volume (mm³)</th>
<th>DSC</th>
<th>Our method Volume (mm³)</th>
<th>DSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E12.5 embryo 1</td>
<td>2.11</td>
<td>1.91</td>
<td>0.714</td>
<td>3.15</td>
<td>0.800</td>
<td>2.62</td>
<td>0.842</td>
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<tr>
<td>E12.5 embryo 2</td>
<td>2.81</td>
<td>2.16</td>
<td>0.773</td>
<td>2.70</td>
<td>0.948</td>
<td>2.60</td>
<td>0.865</td>
</tr>
<tr>
<td>E12.5 embryo 3</td>
<td>2.65</td>
<td>1.77</td>
<td>0.712</td>
<td>2.25</td>
<td>0.890</td>
<td>2.06</td>
<td>0.838</td>
</tr>
<tr>
<td>Average</td>
<td>2.52±0.37</td>
<td>1.95±0.20</td>
<td>0.73±0.03</td>
<td>2.70±0.45</td>
<td>0.879±0.075</td>
<td>2.42±0.32</td>
<td>0.848±0.015</td>
</tr>
</tbody>
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Fig. 4: 3D rendering of the segmented brain ventricles of E12.5 embryo 1 obtained using region growing (a), our method (b), and manual segmentation (c).

which do not need training data, but require minimal user interaction for initialization.

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REFERENCES


