Whole Brain Reconstruction from Multilayered Sections of a Mouse Model of Status Epilepticus

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Abstract—This research concerns confocal fluorescence microscopy imaging of the whole brain of C57BL/6 mice with single-cell resolution. These brains are too large for specimen holders in available 3D microscopes, so this research develops a set of volume reconstruction methods to reproduce a whole brain from multilayered, thin sections of the brain imaged using a confocal microscope. As the sections are in solution during imaging, their shapes warp differently, and their structures no longer align. The proposed two-stage reconstruction procedure consists of single-section correction and section-to-section alignment, towards producing a whole brain volume. In the first stage, the proposed method carefully unwarps the distorted shapes of each section. The second stage aligns prominent features between the layers of neighboring sections. This paper also newly considers how these stages influence each other in the broader context of whole brain volume reconstruction. Experimental results portraying each stage with real image data suggest that the proposed approach can produce consistent 3D volumes and largely correct the observed distortions.

Index Terms—3D mouse brain reconstruction, tissue flattening, epilepsy

I. INTRODUCTION

Microscopy is an important tool to investigate brain structures and activities [1]. However, imaging an entire mouse brain is usually impractical or inconvenient because of the limitations of the stain penetration depth and the size of microscopic chamber. Numerous brain reconstruction methods build whole 3D mouse brains with separately imaged specimen stacks [2]–[5]. Most existing brain reconstruction methods deal with frozen or fixed mouse brains before sectioning, and thus the stacks used for reconstruction are usually assumed to be flat [3]–[5]. In [3], [4], the structure jitter in reconstructed brains is corrected with the smoothness prior. Frozen 0.25-µm-thick brain slices are registered to form 3D brain volumes with reference to in vivo MRI scans at low resolution [5].

However, brain reconstruction with thin sections involves laborious brain specimen preparation [6]. Advanced tissue clearing techniques developed recently, such as CLARITY [7], SCALE [8] and PACT [9], together with confocal microscopy enable the quantitative analysis of immunohistochemical stains and reporter proteins in specimens with increased thickness. As a result, manual sectioning involved in specimen preparation is greatly reduced. On the contrary, tissue clearing techniques require immersing tissues in certain solutions to remove the lipids, and this procedure usually introduces sophisticated warping artifacts as shown in Fig. 1 (a). In [2], 50-µm-thick brain sections labeled with three stains are flattened and registered semi-automatically. Artifacts such as swelling are considered in [2], but the manual flattening scheme is not suitable for brain reconstruction on a larger scale.

In this study, brain cells in genetically engineered mice express red reporter protein during status epilepticus. After extracting and sectioning mouse brains, a tissue clearing technique, PACT [9], is applied before imaging each tissue specimen. As stated before, the warping artifact is a major distortion in the reconstructed brain with traditional reconstruction methods. We design a novel tissue flattening method that detects the tissue surface, fixes holes, and identifies the projection direction automatically. To fully automate the brain reconstruction process, both the flattening method and a coarse-to-fine registration pipeline are implemented with Matlab. In our implementation, the registration pipeline consists of rough alignment, affine registration [10] and non-rigid registration [11]. Fig. 1 (a) shows the reconstructed mouse brain without flattening. Although the major structure, such as
the hippocampus, is visible in Fig. 1 (a), the gaps between specimen stacks impair the visual quality of the reconstructed brain. After incorporating the tissue flattening method, the reconstructed brain in Fig. 1 (b) demonstrates less warping distortion and more consistent structure.

This paper is organized as follows. Section II introduces the registration pipeline and the tissue flattening method. The registration pipeline with a coarse-to-fine approach ensures a robust reconstruction performance, and the tissue flattening module improves the structure consistence in the reconstructed brain. Section III verifies the proposed registration pipeline and the tissue flattening technique with two reconstructed brains. Section IV summarizes the contributions and limitations of the proposed method, and discusses the future work.

II. IMPLEMENTATION
A. Registration Pipeline

The coarse-to-fine registration pipeline shown in Fig. 2 contains three steps: rough alignment, affine registration and non-rigid registration. Both flipped and non-flipped situations are considered in the first two steps. The first row shows the non-flipped registration results, and the second row shows the flipped registration results. Flip detection is based on the registration results of affine registration.

![Fig. 2: The coarse-to-fine registration pipeline contains three steps: rough alignment, affine registration and non-rigid registration. Both flipped and non-flipped situations are considered in the first two steps. The first row shows the non-flipped registration results, and the second row shows the flipped registration results. Flip detection is based on the registration results of affine registration.](image)

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Algorithm 1 Tissue Flattening

1. $I^{max}(p) = \max_{i=1,\ldots,D} I_i(p)$ \hspace{1cm} $\triangleright$ MIP of the section
2. $M = I^{max} \setminus \emptyset$ \hspace{1cm} $\triangleright$ get the mask
3. $M_i = \text{erode}(M, \text{disk}(W_c))$ \hspace{1cm} $\triangleright$ interior mask
4. $M_b = \text{xor}(M, M_i)$ \hspace{1cm} $\triangleright$ boundary mask
5. $Z_1(p) = \min i, \text{ s.t. } I_i(p) > \frac{I^{max}(p)}{I^{max}_{c}}$ \hspace{1cm} $\triangleright$ top surface
6. $Z_2(p) = \max i, \text{ s.t. } I_i(p) > \frac{I^{max}(p)}{I^{max}_{c}}$ \hspace{1cm} $\triangleright$ bottom surface
7. $d = \text{median}(|Z_2 - Z_1|)$ \hspace{1cm} $\triangleright$ median thickness of the section
8. Within $M_i$, \hspace{1cm} $\triangleright$ fix the interior holes
   \begin{enumerate}
   \item if $|Z_2(p) - Z_1(p)| < d$ then
     \begin{enumerate}
     \item if $\|\nabla Z_1(p)\|_2^2 > \|\nabla Z_2(p)\|_2^2$ then
       \begin{enumerate}
       \item $Z_1(p) = Z_2(p) - d$
     \end{enumerate}
     \item else
       \begin{enumerate}
       \item $Z_2(p) = Z_1(p) + d$
     \end{enumerate}
   \end{enumerate}
   \end{enumerate}
9. Within $M_b$, \hspace{1cm} $\triangleright$ determine the projection direction
   \begin{enumerate}
   \item if $\|\nabla Z_1\|_2^2 \cdot M_b > \|\nabla Z_2\|_2^2 \cdot M_b$ then
     \begin{enumerate}
     \item project to $Z_2$
   \end{enumerate}
   \item else
     \begin{enumerate}
     \item project to $Z_1$
   \end{enumerate}
   \end{enumerate}

The key steps in tissue flattening are summarized in Alg. 1, and intermediate results are illustrated in Fig. 3. In Alg.

![Fig. 3: Intermediate results for flattening. The red dashed line in (a) indicates the position of other side views. Without hole fixing, the structure around ventricle is changed as shown in (e). With incorrect projection direction, the boundary contour is changed as shown in (f).](image)

Fig. 3: Intermediate results for flattening. The red dashed line in (a) indicates the position of other side views. Without hole fixing, the structure around ventricle is changed as shown in (e). With incorrect projection direction, the boundary contour is changed as shown in (f).
Two major artifacts exist in the reconstructed brain: the sawtooth-like structure inconsistence and black inter-layer gaps. To solve the first artifacts, we plan to utilize the structure and gaps. Reconstructed brains with MIP shows a following registration pipeline. Besides the obvious warping artifacts and gaps, reconstructed brains with MIP shows a cylinder-like contour because structures at different layers in a 3D section are mixed in the MIP. Whole brain reconstruction results in Section III demonstrate this phenomenon more clearly.

II. EXPERIMENTS

Two brains, Brain 1 with 17 sections and Brain 2 with 18 sections, are reconstructed to demonstrate the effect of tissue flattening and verify the robustness of the coarse-to-fine registration pipeline. The in-plane resolution of the brain stacks is 2.77μm, and the cross-plane resolution of the brain stacks is 10μm. Before reconstruction, each stack is tiled with several squared patches with the Zen2 software. Fig. 5 (a) and Fig. 6 (a) show the stacked raw tissue sections. The structures like hippocampus and outlines of the brains are disordered. Only with the registration pipeline implemented with [10] and [11], the reconstructed mouse brains are shown Fig. 5 (b) and Fig. 6 (b). The overall brain and hippocampus shapes are more prominent, and verify the robustness of the coarse-to-fine registration pipeline. However, the brains are in cylindrical shapes because the maximum intensity projections of two sections are used for registration. Fig. 5 (c)-(e) and Fig. 6 (c)-(e) are reconstructed brains with flattened sections after rough alignment, affine registration and non-rigid registration respectively. Rough alignment approximately matches two sections and provides a reasonable initialization for the affine registration. The difference between reconstructed brains after affine registration and non-rigid registration is minor. This is because the mouse brain sections are relatively thick (200μm), and thus in-plane stretching is not significant.

The main source of the structure inconsistence in the reconstructed brain is incorrect flip detection. Instead of causing the disparity at the joint interface, one incorrect flipping decision affects the registration and flip detection of the following stacks. A more robust flip detection is one topic of future work discussed in Section IV.

IV. CONCLUSIONS

Recent advances in tissue clearing and microscopy enable imaging brain tissues with certain thickness. Therefore the labor involved with specimen preparation is greatly reduced. However, the whole brain volume cannot be imaged at once because of the limitation of stain penetration depth. In this paper, we proposed a robust automated tissue flattening method that contains tissue surface detection, hole fixing and projecting direction identification. With the proposed flattening method, the reconstructed brain volumes are more compact and the 3D reconstruction is more natural.

Two major artifacts exist in the reconstructed brain: the sawtooth-like structure inconsistence and black inter-layer gaps. To solve the first artifacts, we plan to utilize the structure information within each stack, and improve the flip detection based on these structure changes. Reconstructing 3D tissue volumes with structure propagation in each section is discussed in [12]–[14]. However, these methods are based on vessel and
Data-driven methods are promising approaches to remove the black inter-layer gaps. Because the changes from layer to layer are smooth and consistent within each stack, the filling rules for the black regions between sections can be learned from the intra-section regions. More complete and quantitative evaluation on a larger set of brains is also planned for the future.

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REFERENCES