Microscopic neural image registration based on the structure of mitochondria

Huiwen Cao¹, Hua Han¹,², Qiang Rao¹, Chi Xiao¹, Xi Chen¹
¹Institute of Automation, Chinese Academy of Sciences, Beijing, 100190, China
²The Center for Excellence in Brain Science and Intelligence Technology, CAS

ABSTRACT:
Microscopic image registration is a key component of the neural structure reconstruction with serial sections of neural tissue. The goal of microscopic neural image registration is to recover the 3D continuity and geometrical properties of specimen. During image registration, various distortions need to be corrected, including image rotation, translation, tissue deformation et.al, which come from the procedure of sample cutting, staining and imaging. Furthermore, there is only certain similarity between adjacent sections, and the degree of similarity depends on local structure of the tissue and the thickness of the sections. These factors make the microscopic neural image registration a challenging problem.

To tackle the difficulty of corresponding landmarks extraction, we introduce a novel image registration method for Scanning Electron Microscopy (SEM) images of serial neural tissue sections based on the structure of mitochondria. The ellipsoidal shape of mitochondria ensures that the same mitochondria has similar shape between adjacent sections, and its characteristic of broad distribution in the neural tissue guarantees that landmarks based on the mitochondria distributed widely in the image. The proposed image registration method contains three parts: landmarks extraction between adjacent sections, corresponding landmarks matching and image deformation based on the correspondences. We demonstrate the performance of our method with SEM images of drosophila brain.

Keywords: neural image registration, Scanning Electron Microscopy (SEM), mitochondria structure, landmarks

1. INTRODUCTION
With the development of electronic microscopy technology in recent year, the ability of image acquisition is no longer the limitation for the reconstruction of large scale neural circuit at synaptic level. In general, there are two different methods for volume electron microscopy imaging, which are block-face SEM imaging and serial sections SEM imaging. For block-face SEM imaging, tissue blocks are fixed in SEM vacuum chamber. The surface of block is imaged in SEM, and then removed using a diamond knife or a focused ion beam. This process is repeated with no manual intervention, and the acquired images are easy to be aligned. As the sections are lost when they are removed from tissue block, this method is destructive and not suitable for large scale neural circuit reconstruction. For serial sections imaging, the sections are cut in an ultra-microtome and collected onto conductive supporter, such as electron opaque support tape. The advantage of the method is that the sections can be saved and imaged many times. Moreover, for saving imaging time, the sections could be imaged in parallel, but more complicated alignment is needed to cope with section stretching and distortion.

In this paper, we introduce a novel image registration algorithm for SEM images of serial sections based on the structure of mitochondrion. As there are various distortions that are applied to the tissue during staining, sectioning and imaging, our purpose is to recover the 3D continuity and geometrical properties of specimen. The distortions include staining artifacts, mechanical deformation, missing sections and the fact that structures may appear dissimilar in consecutive sections [1]. As there is only certain similarity between adjacent sections, and the degree of similarity depends on local structure of the tissue and the thickness of the sections, traditional registration methods which use SIFT feature [2] could not reflect real landmarks. Besides, many similar structures may influence the matching result of corresponding SIFT landmarks. Therefore, in this paper we use mitochondrion information to identify corresponding landmarks across sections and achieve image registration.
We use mitochondria information as the landmarks of image registration for the reasons including the shape, the distribution and the size of mitochondria. In detail, the ellipsoidal shape of mitochondria ensures that the same mitochondria has similar shape between adjacent sections, and its characteristic of broad distribution in the neural tissue guarantees that landmarks based on the mitochondria distribute widely in the serial images. Generally speaking, the diameter of mitochondria is 0.5-1um and the length of mitochondria is 1-2um, thus a mitochondria can appear on more than 10 continuous sections when the thickness of sections are 50nm. These properties make sure that we can acquire the continuous landmarks in slice sequences. So we use centroid of mitochondria region as the corresponding landmark to align adjacent section images.

The proposed image registration method for serial sections of biological tissue could be divided into three parts, which are landmarks extraction between adjacent sections, corresponding landmarks matching and image deformation based on the correspondences. Figure 1 displays the flowchart of the proposed approach.

![Flowchart of the proposed approach](image)

**Fig 1.** The flowchart of the proposed approach.

2. METHOD

2.1 Landmarks extraction

Searching landmarks is not an easy task for serial sections of biological tissue, for the appearances of different sections are not same. There is only certain similarity between adjacent sections, and the degree of similarity depends on local structure of the biological tissue and the thickness of the sections. Thicker section means lower similarity, and it increases the difficulty of looking for the correspondences.

The proposed image registration method uses the centroids of mitochondria regions as the landmarks. To obtain the region of mitochondria, there are three steps: mitochondria detection, mitochondria segmentation and segmentation result filtering.

A. Mitochondria detection

Mitochondria detection is the first step of mitochondria segmentation, and it decides the position and scale of the segmentation area. In this part, Faster R-CNN (Region with Convolutional Neural Network Features) is adopted to detect the mitochondria in each microscopic neural image.

![Detection results of mitochondria in adjacent SEM images](image)

**Fig 2.** Detection results of mitochondria in adjacent SEM images

The core task of Faster R-CNN [5] network is to design and train Region Proposal Network (RPN) that shares full-image convolutional features with the detection network. As a consequence, four basic steps of target detection: region proposal, feature extraction, object proposals classification and bounding-box regression are unified to a deep-learning-based
object detection system. Since the Faster R-CNN network can be fully completed in the GPU, it is 10 times faster than Fast R-CNN [4], and the learned RPN also improves region proposal quality and the overall object detection accuracy. So Faster R-CNN offers a guarantee both in detection accuracy and calculating speed.

Consider the memory of GPU, we cut the original image into small images to train faster R-CNN network. To be specific, we divide the original image (size of 4k*4k) into 16 images (size of 1k*1k) and utilize the plug-in of training Image Labeler to label mitochondria, then flip these images and obtain nearly 8000 training samples. We exploit VGG16 [6] network to detect mitochondria in small image (size of 1k*1k) and use effective stitching method to obtain the detection result of original image (size of 4k*4k). Figure 2 shows the promising detection results.

B. Mitochondria segmentation
After mitochondria detection, for each mitochondria we get its position and scale. Then, region growing algorithm is used to segment the region of mitochondria with these information.

Region growing method [7] is a 2D segmentation algorithm which is based on minimizing an energy function incorporating a weighted Total Variation regularization and a data term taking into account initial classification information from user. The initial classification is the seed point which is defined by the user-provided scribble annotation. In our experiment, we calculate the seed points by the center of detected external rectangle of mitochondria, and then compute the 2D segmentation of the mitochondria by globally minimizing the following variation segmentation energy:

$$\arg\min_{\mu_i} \int (g_i |\nabla \mu_i| + f_i \mu_i) dx$$

Where $i$ refers to the index of the mitochondria, $x \in \Omega_i$ and $\Omega_i$ is the 2D image domain which is equal to the mitochondria external rectangle. The function $\mu_i$ encodes the 2D segmentation result for each mitochondria, where $\mu_i \geq 0.5$ is foreground and $\mu_i \leq 0.5$ is background. The function $f_i$ is defined according to the seed point which is calculated by the detected mitochondria external rectangle center. With globally minimizing the variation segmentation energy [8], we can obtain the segmentations of all the detected mitochondria. Thus, we get the mitochondria segmentation region. Figure 3 shows the promising segmentation results.

![Fig 3. Segmentation results of mitochondria in adjacent SEM images](image)

C. Filtering segmentation result with Random Forest algorithm
There may exist some segmentation errors with region growing method, especially when the mitochondria is near membrane structures, which results in false location of landmarks. So Random Forest (RF) algorithm is adopted to filter out those errors.

Random Forest algorithm [9] is a combination of tree predictors. Each tree depends on the values of a random vector sampled independently, and all trees have the same distribution in the forest. The final result is voted by each tree. The shape features of segmentation region are used as the random vector in RF to filter out wrong segmentation results.
2.2 Corresponding landmarks matching

The goal of this part is to assign correspondences between point sets. We have obtained the landmarks which are the centroids of mitochondria regions and we consider the landmarks in each image as a point set. In this part, Coherent Point Drift (CPD) algorithm is adopted to assign correspondences between point sets.

For two point set registration, CPD [10] considers the first point set as the data points generated by Gaussian Mixture Model (GMM) and the second point set as GMM centroids. And then it fits the GMM centroid to the data by maximizing likelihood. The correspondence probability between two points is defined as the posterior probability of the GMM centroid given the data point. The corresponding landmark is selected with the maximum posterior probability.

As the variation between adjacent sections is not significant, the transformation between the point sets is considered to be rigid. Therefore we choose rigid transform method to achieve the point set registration. For rigid point set registration, in [10] it is shown that the transformation of the GMM centroid locations is defined as $T(y_m; R, t, s) = sRy_m + t$, where $R_{DxD}$ is a rotation matrix, $t_{Dx1}$ is a translation vector, and $s$ is a scaling parameter. We reparameterize the GMM centroid locations by rigid transformation parameter and estimate them by maximizing the likelihood. We use Expectation Maximization algorithm to find the solution and achieve the point set registration.

After CPD, the correspondences are assigned between the point sets. There would be a small number of mistakes in the correspondences, so Random Sample and Consensus (RANSAC) algorithm [11] is adopted to remove wrong corresponding point pairs. Figure 4 shows the corresponding landmarks matching results.

![Corresponding landmarks in adjacent SEM images](image)

2.3 Image deformation

In this part, with the positions of those correspondences in section 2.2, Moving-Least-Square (MLS) [12] method is used to warp each section image. The deformation result produced by Moving-Least-Square method is globally smooth, and as a result of using rigid transformations, rigidity and scale are maintained locally so that biological specimens could retain their relative shapes as much as possible, which is one of the reasons that MLS is widely used in registration of biological specimens.

Let $p$ be a set of landmarks in deforming image, and $q$ their correspondence in reference image. As described in [13], given a point $v$ in deforming image, the new position of the point $v$ in reference image is calculated by minimizing under-mentioned formula,

$$\sum_{i} \omega_i |l_v(p_i - q_i)|^2$$

Which $\omega_i$ is defined by $\omega_i = \frac{1}{|p_i - v|^2}$, and $l_v$ is a rigid transformation to be calculated. Considering algorithm implement, instead of applying the deformation function to every pixel in the image, we approximate the deforming image with a grid and apply the deformation function to each vertex in the grid. The grid width has a great influence on the amount of
computation. Small grid width means that more vertexes are involved, which could increase registration accuracy but also computation burden.

3. RESULTS

We implement the described algorithm on two adjacent sections from Drosophila brain. The thickness of sections is 70 nm imaged with SEM in Institute of Automation, CAS, where the pixel size is set to 5 nm and dwell time is 2us.

For landmark extraction, the mitochondria detection results are displayed in figure 2. Table 1 presents the training result of three different deep learning methods. Compared to selective search method, RPN extracts 300 proposals which is more accurate. With 8000 training samples, RPN+VGG16 method achieves the highest mean Average Precision (mAP) which is 85.2%. As VGG16 is more complex, the detection rate is slower relatively.

<table>
<thead>
<tr>
<th>Method</th>
<th>proposals</th>
<th>Data</th>
<th>mAP(%)</th>
<th>rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>2k</td>
<td>8000 samples</td>
<td>78.1</td>
<td>0.7 fps</td>
</tr>
<tr>
<td>RPN + ZF, shared</td>
<td>300</td>
<td>8000 samples</td>
<td>83.4</td>
<td>16 fps</td>
</tr>
<tr>
<td>RPN + VGG16, shared</td>
<td>300</td>
<td>8000 samples</td>
<td>85.2</td>
<td>5 fps</td>
</tr>
</tbody>
</table>

Table 1. Detection AP (%) and Timing on a TITAN X GPU

Fig 5. Two adjacent SEM images, (a) template image, (b) the original image

Fig 6. Registration result of proposed method, (a) template image, (b) the deformed image
Figure 3 is the segmentation result of mitochondria, regions in red indicate mitochondria segmentation result. The corresponding landmarks are shown in figure 4 and the numbers around markers indicate the correspondences in adjacent SEM images. Figure 5, 6 display registration results of two SEM images using the proposed method.

4. CONCLUSION

In this paper, we present a novel image registration method for SEM images of serial neural tissue sections based on the structure of mitochondria. Because of various distortions generated during section preparation and the dissimilarity between adjacent sections, it is difficult to extract real corresponding landmarks with SIFT feature. Unlike traditional methods, our proposed method chooses the mitochondria centroid as landmarks and it is robust to morphological distortions. Besides, broad distribution characteristic of mitochondria in the neural tissue guarantees that landmarks based on the mitochondria are distributed widely in the image.

Finally, in the future we would segment mitochondria directly using Deep Convolution Neural Network (DCNN). DCNN has achieved great success in image classification area, it may improve accuracy of mitochondria segmentation.

5. ACKNOWLEDGMENT

This paper is supported by Strategic Priority Research Program of the CAS (No. XDB02060001), National Science Foundation of China (NO. 61673381, NO. 61201050) and Special Program of Beijing Municipal Science & Technology Commission (No.Z161100000216146).

REFERENCE