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Grayscale Inhomogeneity Correction Method for Multiple Mosaicked Electron Microscope Images

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ABSTRACT

Electron microscope image stitching is highly desired to acquire microscopic resolution images of large target scenes in neuroscience. However, the result of multiple Mosaicked electron microscope images may exist severe gray scale inhomogeneity due to the instability of the electron microscope system and registration errors, which degrade the visual effect of the mosaicked EM images and aggravate the difficulty of follow-up treatment, such as automatic object recognition. Consequently, the grayscale correction method for multiple mosaicked electron microscope images is indispensable in these areas. Different from most previous grayscale correction methods, this paper designs a grayscale correction process for multiple EM images which tackles the difficulty of the multiple images monochrome correction and achieves the consistency of grayscale in the overlap regions. We adjust overall grayscale of the mosaicked images with the location and grayscale information of manual selected seed images, and then fuse local overlap regions between adjacent images using Poisson image editing. Experimental result demonstrates the effectiveness of our proposed method.

Keywords: Image Stitching, Grayscale Correction, Image Processing, Electron Microscope Image.

1. INTRODUCTION

During the process of obtaining a series of Electron microscope (EM) images in some biomedical studies, electron microscope system can be affected by the change of the magnetic field environment, system parameter drift *et al,* which result in the gray scale inhomogeneity of the mosaicked EM images. In addition, there are some local discontinuity of the stitching seams in the overlapped area of two adjacent images. These gray level discontinuities depicted in Fig. 1(a), degrade the visual effect of the mosaicked EM images and aggravate the difficulty of further processing, such as object recognition [1]. Therefore, the grayscale correction method for multiple mosaicked images is an essential endeavor of EM image stitching research. To date, there are a few researches on grayscale correction. Brinkmann *et al.* [2] addressed grayscale inhomogeneity in magnetic resonance (MR) images quantitatively using simulated images with artificially constructed and empirically measured bias fields. Intensity inhomogeneity is a smooth intensity change inside originally homogeneous regions, Balafar *et al.* [3] proposed a filter-based inhomogeneity correction method to tackle it. In addition, Uyttendaele *et al.* [4] proposed a method for continuously adjusting exposure across multiple images by computing exposure corrections on a block-by block basis and smoothly interpolate the parameters using a spline to get spatially continuous exposure adjustment. While, these researches mostly focus on the MR images or the panorama, and they are unable to tackle the multiple mosaicked EM images which exist various severe gray scale inhomogeneity.

In order to provide a pleased data set for the subsequent EM image processing, in this paper, we design a grayscale correction process for multiple EM images, which exist severe gray scale inhomogeneity. We also introduce a novel method based on the seed images, which use the location information of the seed image to make a linear gain adjustment and the gray information of the seed image as a model to transform the grayscale, to alleviate the blocky grayscale difference and achieve the overall grayscale adjustment of multiple images. Then according to the boundaries characteristics of the EM images, Poisson Image Editing method [5] is used to hide the edges of each component images, achieving a smooth transition of image boundaries. The data used in this paper are collected by the Cryo-EM (200kV Tecnai F20 TEM), the magnification is 5000x. Fig. 1 (b) displays the workflow of grayscale correction for multiple mosaicked EM images.

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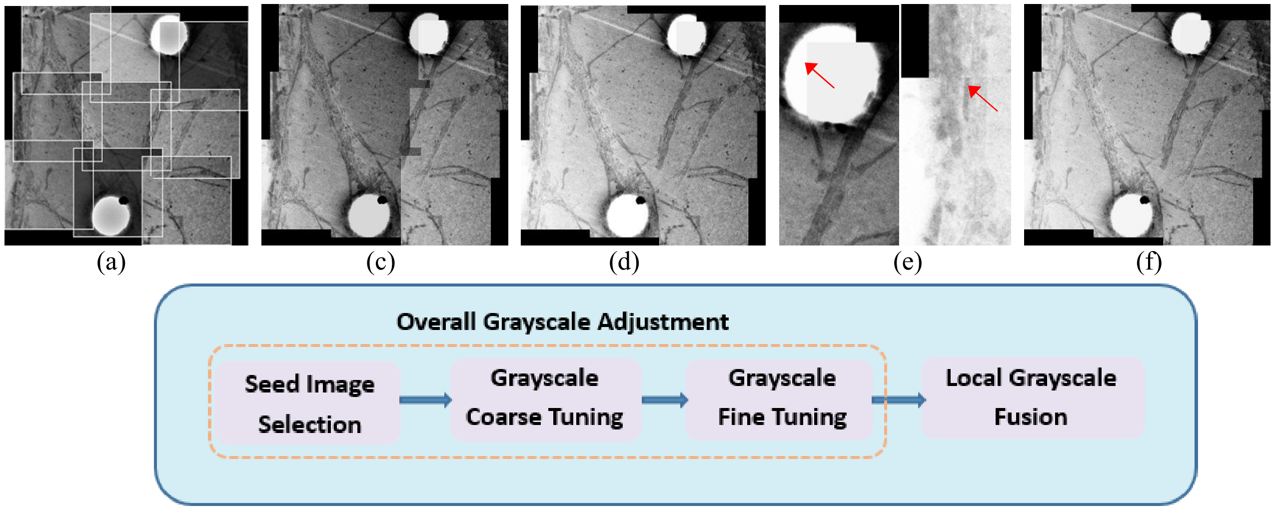
The rest of the paper is organized as follows: the detailed method is described in Section 2; the preliminary results of the study are presented in Section 3; the followed Section 4 concludes the whole paper.



(b)

Fig.1. (a) Multiple mosaicked EM images with severe gray scale inhomogeneity. (b)The whole workflow of grayscale correction for multiple mosaicked EM images. (c) Mosaicked EM image by coarse tuning. (d) Mosaicked EM image by fine tuning. (e) Partial mosaicked EM image by overall grayscale adjustment with red arrows points to the local discontinuity of the stitching seams. (f) Mosaicked EM image after grayscale correction

2. PROPOSED METHOD

In this section, we provide algorithmic details about our grayscale correction process. The proposed algorithm is divided into two parts, which are overall grayscale adjustment and local grayscale fusion. The whole workflow of grayscale correction for multiple mosaicked EM images is shown in Fig. 1(b).

2.1 Overall grayscale adjustment

The overall grayscale adjustment for multiple images need global optimization. Each image is adjacent with the four images (the up, down, left and right image), once an image is adjusted improperly, multiple images which are adjacent to the adjusted one will degenerate correspondingly. Therefore, in order to avoid the accumulation and propagation of errors, we proposed a grayscale correction method based on the seed images. This part is divided into three steps. To begin with, a seed image selection operation decides the direction and goal of the adjustment. After that, we use the information of seed images to do the linear adjustment. Finally, the nonlinear transformation is used to alleviate the blocky grayscale difference further and implement overall gray scale adjustment.

2.1.1 Seed image selection

Seed image selection is the first step of overall grayscale adjustment. In this part, the seed images are marked by artificial selection. We choose gray balanced image as seed image, which gray level distribution is even. This is determined empirically through trial and error.

2.1.2 Grayscale coarse tuning

After seed images have been selected, we get the starting position and template of the grayscale adjustment. In this section, gain compensation method is solved for the overall gain between images. We use this method with the seed images position information as a constraint.

Gain compensation method defines the sum of gain intensity errors for all images as an error function over all overlapping pixels [6].

$$e=\frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n}\sum_{u_i\in R(i,j)}(g_iI_i(u_i)-g_jI_j(u_j))^2 \tag{1}$$

Where g is the gain parameter, and R (i, j) is the overlapped region between images i and j. To simplify the computation, the images $I_i(u_i)$ are replaced by the mean of the overlapping region $\overline{I_{ij}}$ in (2). And to be practical, the gain should not be equal to zero, so we change the function by adding the constraint items σ_N And σ_g . They represent the deviate degree of the gain parameter and brightness. hence the function becomes as (3).

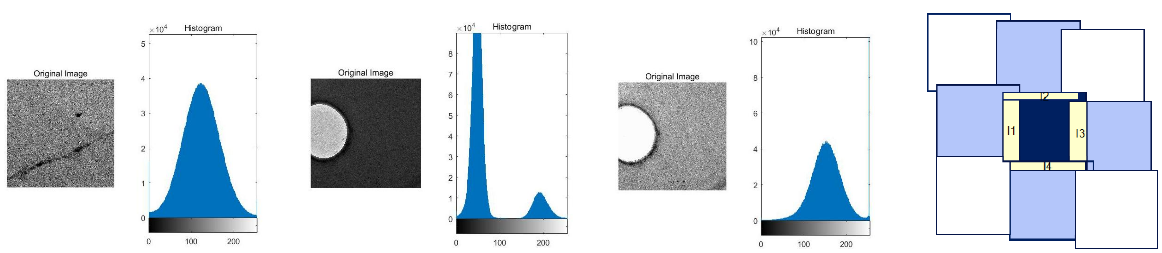
$$\overline{I_{ij}}=\frac{\sum_{u_i\in R(i,j)}I_i(u_i)}{\sum_{u_i\in R(i,j)}1} \tag{2}$$

$$e=\frac{1}{2}\sum_{i=1}^{n}\sum_{j=1}^{n}N_{ij}\left(\frac{\left(g_i\overline{I_{ij}}-g_j\overline{I_{ji}}\right)^2}{{\sigma_N}^2}+\frac{(1-g_i)^2}{{\sigma_g}^2}\right) \tag{3}$$

Where Nij |R (i, j)| equals the number of pixels in image i that overlap in image j. In this paper, the value of σ_N and σ_g are affected by the seed images, if the image I_i is marked as a seed image, σ_g of this image should be much smaller to keep the gain close to 1. Several values were used to achieve the best correction results. The error function is a quadratic objective function in the gain parameters g which can be solved in closed form by setting the derivative to 0. Therefore, this method is guaranteed to yield a globally optimal solution with respect to the constraints provided by seed image. The gray scale correction result by parameter σ_N 5and σ_g 0.001/0.6 is depicted in Fig. 1(c).

2.1.3 Grayscale fine tuning

Gain compensation method is linear gain, it can have a partial improvement for the mosaic results of multiple images, but can't achieve the overall grayscale consistency. Histogram normalization algorithm is suitable for the mapping of nonlinear grayscale matrix and can play an image enhancement role [7]. For the sake of visual interpretation, we plot the gray level histogram of two images in Fig. 2(a) (b), and the result of histogram normalization mapping from (b) to (a), in Fig. 2(c), from which we can see that after mapping, the gray level histogram and visual sense of the second image is similar to the first one. To our knowledge, there are few studies using this method for the multiple mosaicked EM images. So, we proposed an improved histogram normalization algorithm using the information of seed images for the multiple mosaicked EM images to do the further fine-tuning.



(a) (b) (c) (d)

Fig.2. (a)The model image and its gray level histogram. (b)The inconsistent gray image and its gray level histogram. (c)The image after histogram normalization mapping. (d) Mosaicked model for the histogram normalization mapping

According to the position of seed images, we choose the start point of gray scale fine-tuning. Next, the histogram normalization operation is used for the overlapped region, namely mapping from the adjacent images to the seed images. Then, centered on the seed images and spread outside, the images which have been corrected become the model image to do the next round mapping, until all the images are iterated. In order to demonstrate the operation method, we show the mosaicked model in Fig. 2(d). The dark blue image is the seed image; the yellow part is the overlapped region of the two adjacent images. First do the gray mapping of the overlapped region from the light blue images to the dark one, then, map from the white to the light. The result of this section is depicted in Fig. 1(d).

2.2 Local grayscale fusion

After the adjustment of the previous section, which achieves gray consistency roughly, some image edges are still visible due to slight distortions, intensity decreases towards the edge of the image and so on, depicted in Fig. 1(e). In this section, we aim to eliminate the local gray discontinuity of the stitching seams and implement the whole grayscale correction.

Poisson image editing [5] is a popular algorithm to deal with the visible image edge. It is a kind of image fusion algorithm by mixing the gradient domain of the source and destination image, keeping the texture information of the source image while fusing with the destination image without visible boundary. In this paper, this algorithm is adopted to fuse the overlapping region of the adjacent images by minimizing the difference between the gradient fields of the two adjacent images, and replacing the boundary value of the overlapping region with the closest pixels' value to keep the boundary of the overlapping region continuous. In order to demonstrate the operation method, we show the mosaicked model in Fig. 3(a). The purple and the orange region are the boundary values needed to be replaced. The result of local grayscale fusion for multiple Mosaicked EM image is depicted in Fig. 1(f). By the way, due to the gradient domain operation of Poisson image editing algorithm which avoid the problem of blending image dimming or blurring, the result of eliminating the visible edges is more natural and realizing the smooth transition. The comparison between this method and the traditional multi-resolution image blending method [8] indicates the advantages of the former in Fig. 3(b).

(a) (b)

Fig.3. (a) The mosaicked model image of using Poisson image editing. (b) Original Mosaicked EM image (up) and the result contrast of multiresolution image blending (left) and Poisson image editing (right).

3. EXPERIMENTAL RESULT

In order to demonstrate the effectiveness of the proposed grayscale correction method, we test our method on mosaicked EM images, which consist of 35 images with severe gray scale inhomogeneity. The position of seed images is indicated by red rectangles in Fig. 4(a). The mosaicked EM images after grayscale coarse tuning, grayscale fine tuning and local grayscale fusion are illustrated in Fig. 4(b), (c) and (d) respectively.

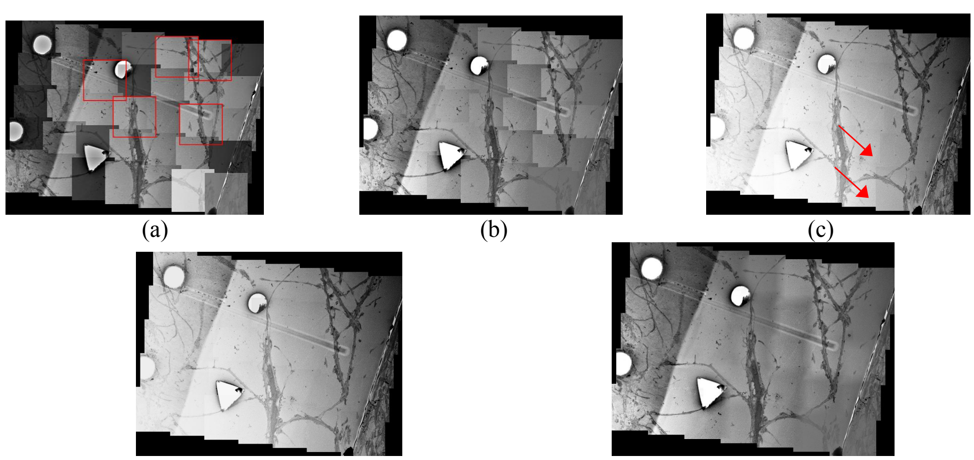

(a) (b) (c) (d) (e)

Figure 4. (a) Multiple mosaicked EM images with severe gray scale inhomogeneity. (b) the mosaicked EM images after coarse tuning. (c) the mosaicked EM image after fine tuning. (d) the mosaicked EM image after local grayscale fusion. (e) correction result using [6].

We also compare the proposed method with the correction method in [6] which uses gain compensation and multi-band blending. As shown in Fig. 4(e), it is clear that the correction result of the method in [6] still exists blocky visual effect, while our method achieves the consistency of the overall grayscale of the mosaicked images, and eliminates the local discontinuity of the stitching seams. The average of the summation of the absolute difference (SAD) value of the overlapped image regions before and after the gray correction are shown in Table 1, which illustrate the effectiveness of our method objectively.

Table 1. The average of SAD value of the overlapped regions between original images and the images after the grayscale correction.

SAD	Column1		Column 2		Column 3		Column 4		Column 5		Column 6		Column 7	
	Original	After	Original	After	Original	After	Original	After	Original	After	Original	After	Original	After
Row 1	84.5	58.2	142.2	102.9	120.5	85.8	138.0	90.9	170.1	113.6	131.9	106.3	102.9	68.6
Row 2	116.9	69.7	190.0	107.8	171.8	93.3	235.5	123.1	244.3	153.7	175.3	145.2	127.8	104.9
Row 3	122.9	56.7	208.8	79.4	189.7	82.1	188.9	125.8	210.1	146.1	193.9	144.0	143.1	101.5
Row 4	131.8	49.4	194.1	68.7	233.1	81.1	205.1	116.6	184.1	140.3	223.2	146.1	200.4	100.3
Row 5	91.9	26.5	149.0	32.7	220.1	42.2	200.9	77.5	193.9	105.8	246.8	110.7	137.7	68.5

4. CONCLUSION

In this paper, we designed a complete grayscale correction process for multiple EM images, which improve the visual effect of the mosaicked EM images with severe gray scale inhomogeneity. The grayscale correction process can be divided into several steps, which includes overall grayscale adjustment with the location and grayscale information of the manual selected seed images, and local overlap region fusion using Poisson image editing. The experiment shows that our proposed method can obtain satisfactory effect.

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