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Registration-Based Segmentation of Nerve Cells in Microscopy Images

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Abstract—**Nerve cell segmentation is important for better understanding of the connections between broken nerves. In this paper, we propose a registration-based segmentation to improve the segmentation from a series of microscopic image sequence. A global iterative closest point (ICP) registration is first employed to find the corresponding cells between neighboring frames. Then a local ICP registration is adopted to refine shape matching between corresponding cells. When registering the current frame to the previous frame, a missing cell in the current frame can obtain a dummy region with respect to the previous frame. The dummy region is then confirmed as a cell by checking the correspondence of the cell among the previous, current and next frames. Experimental results show that the proposed registration based algorithm recovers the cells that are missed in conventional segmentation.**

I. Introduction

NERVE cell segmentation is an important part of nerve reconstruction because it affects the accuracy of connection between two broken nerves. Many papers have discussed issues related to cell segmentation in recent years. Ko *et al.* [1] presented a cell nuclei segmentation method based on an adaptive attention window method which facilitates background removal and reduces processing time of segmentation. Kalviainen *et al.* [2] used the Laplacian of Gaussian filter with multiple scales as a blob detector, after which dynamic programming was used to segment cell contours. Yang *et al.* [3] presented an approach to the quantitative analysis of live cell images. Their method covered cell trajectories, cell cluster separation, mitotic cell detection and cell tracking. A modified watershed algorithm was developed by Tek *et al*. [4] to find initial cell positions, whereupon the circle Radon transform extracted cell centers. Jiang *et al.* [5] presented a white blood cell segmentation method based on scale-space filtering and watershed clustering. A two-step approach of cell segmentation for microscope images was proposed by Colantonio *et al.* [6]. They performed fuzzy clustering of color image in HSV color space, then exploited an artificial neural network to refine the nucleus contours. Although these prior segmentation methods mostly achieve good performance, the problem of non-uniform staining remains unsolved. In our previous work [7] achieved accuracy above 96%, but persistently lost a residual percentage of cells. We find that some cells got undetected because the method in [7] only considers a single frame. If the shape of a cell is too thin or long, or if the intensity of the cell is not uniform, then the cell detection may be misinterpreted as a false alarm. Fig. 1 shows examples of misdetection cells in our previous study. This proposed study reduces the number of missing cells by considering more than one frame, i.e. comparing inter-frame segmentation results by using registration-based methodology.

Registration assistance has been explored in various reported methods. Vladimir *et al.* [8] performed automatic registration based on mutual information for initial placement of the deformable models. Gerard *et al.* [9] performed landmark-based registration of left ventricle for the initial placement of deformable models. Frangi *et al.* [10] proposed a registration-based framework for propagation of corresponding landmarks from a 3-D atlas to 3-D shapes. Yezzi *et al*. [11] used parallel execution of both segmentation and registration, and demonstrated complementarities between segmentation and registration. We also take advantage of the registration-based segmentation to help the detection of missing cells.

The flowchart of registration-based segmentation is illustrated in Fig. 2 and presented as follows:

Initial:
Segment cell images and obtain the information of center and contour of each cell by previously proposed method [7].
Let t denote discrete time and set to 1.
Step 1: Let l and l+1 denote discrete time and set to t and t+1, respectively. Centers of cells in l and l+1 are put into S and M point sets, respectively.
Step 2: Register centers of cells in frame l to those in frame l+1 based on global ICP, and obtain paired cells.
Step 3: Refine shape matching of each paired cells between frame l and l+1 based on local ICP and construct their relationship.
Step 4: If $l = t$, the registered centers of cells in frame l are put into S, and centers of cells in frame t+2 are put to M, then $l = l + 1$ and go to step 2, else go to step 6.
Step 5: Check correspondence among cells on frames t, t +1 and t +2.
Step 6: Let $t = t + 1$, and go to step 1 until t is equal to the total number of frames – 2.

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II. Cell Segmentation

We briefly review the nerve cell segmentation process that generates cell contours as the initial input to the proposed registration-based algorithm in this paper. Details of the segmentation method can be found in [7]. A multi-scale watershed algorithm is employed as the first step of cell segmentation. Based on the algorithm, we can detect cell nuclei in different scales. After watershed segmentation, we can obtain watershed regions and take them as the estimated positions of cell nuclei. Because these regions may be the interstices between cells, they need to be double-checked with all basic cell properties. In this cell nuclei identification process, we used some properties of cell to eliminate the falsely-detected cells. After cell nuclei were appropriately identified, we apply an active contour model based on fuzzy rules to refine the outer boundaries. The energy functions determined by using fuzzy rules provide more flexibility for cell contour deformation in the proposed active contour model.

III. Cell Registration

Based on prior knowledge of the center point of each cell and contour information from the previous study, the registration-based segmentation is adopted to improve the segmentation results. Its three steps are global ICP, local ICP and handling missing cells, and are described in the following sections.

A. Global ICP

A general statement of ICP is described as follows. Given two point sets, a source data $S=\{s_i\}_{i=1}^{N_s}$ and a target data $M=\{m_i\}_{i=1}^{N_m}$, then it is possible to find a transformation T that can register s to best align with M. The adopted formulation to find T is based on the least squares criterion as follows:

$$\min_{T,\, j(i)\in\{1,2,\ldots,N_m\}} \sum_{i=1}^{N_p} \| T(s_i) - m_{j(i)} \|_2^2 \qquad (1)$$

Let T of (1) denoted a rigid transformation. Eq. (1) is represented as follows:

$$\min_{R,t,\, j(i)\in\{1,2,\ldots,N_m\}} \sum_{i=1}^{N_s} \| (R\cdot s_i + t) - m_{j(i)} \|_2^2 \qquad (2)$$

$$s.t.\ R^T R = \mathrm{I}_m,\ \det(R)=1$$

where R is a rotation matrix and t is a translation vector.

An efficient method to handle rigid registration in (2) was suggested by Besl and Mckay [12]. They used two basic steps to register source data to the target data; the first step is to find the correspondence and the second is to calculate the rotation and translation. To find the correspondence $\{(j,\ C_k(j))\}_{j=1}^{N_s}$ between data sets S and M based on the $(k-1)^{th}$ rigid transformation (R_{k-1}, t_{k-1}).

$$C_k(j) = \underset{i(j)\ \in\ \{1,2,\ldots,N_m\}}{\arg\min} \| (R_{k-1}\cdot s_j + t_{k-1}) - m_{i(j)} \|_2^2 \qquad (3)$$

To calculate the k^{th} rotation and translation (R_k, t_k) based on the current correspondence $\{(j,\ C_k(j))\}_{j=1}^{N_s}$, we can use the following equation:

$$(R_k, t_k) = \underset{R^{\mathrm{T}}R=\mathrm{I}_m,\ \det(R)=1,\ t}{\arg\min} \sum_{j=1}^{N_s} \| R\cdot s_j + t - m_{c_k(j)} \|_2^2 \qquad (4)$$

In this procedure we use the centers of cells in two adjacent frames, e.g. the red points in Fig. 1, to execute global registration. The centers of the cells in frame *t* and *t*+1 are stored in the database *S* and *M*, respectively. After the global ICP, the matched centers of the cells in frame *t* and *t*+1 are called a paired cell. If the distance of centers between paired cells is less than 10 pixels, local ICP is performed to refine the shape contour of the paired cell. If the distance is larger than 10 pixels, the two cell centers are stored in the *S* and *M* dataset. Global ICP is again executed. The procedure is repeated until no further increase in the number of paired cells between frame *t* and frame *t*+1. Fig. 3 (a) and (b) show frame *t* and frame *t*+1 and the white contours of cells are the segmentation results obtained by our previously reported method [7]. Fig. 3(c) shows the pink contours which are the cells in frame *t* registered to those in frame *t*+1 after global ICP.

B. Local ICP

It can be seen that the pink contours sometimes do not match the cell boundary in frame *t*+1. If the displacement between the centers of paired cells in consecutive frames is less than ten pixels, we execute local ICP for the paired cells for better shape matching. The global and local ICPs are necessary steps which register the centers of cells in frame *t* to frame *t*+1, and then from frame *t+1* to frame *t*+2. The global ICP use the centers of all cells to register the two adjacent frames. And the local ICP selects twenty points from the two contours of the paired cells with equal angular separation for registration. An example can be seen in Fig. 3(d), the pink and white colors show sample points around cell contours. After local ICP, the resulting contours in green color show better matching in Fig. 3 (e).

C. Handling Missing Cells

After global and local ICP, we obtain the registration parameters between neighboring frames. Missing cells can be recovered during the course of registration based segmentation. Fig.4 shows three sequential frames of two parallel nerve cells, cell #1 and #2. Cell #2 is detected in frames 1 and 3 but is missed in the frame 2. By properly employing the relationship of neighboring frames, the inter-frame information can be used for detecting this type of missing cell like cell #2. The strategy of handling missing cells includes creating a dummy cell and validating the dummy cell. In the dummy cell creation procedure, if a cell in frame *t* can't find a corresponding cell in frame *t*+1 then

create a dummy cell in frame t+1 based on the registration parameters of the global ICP from frame t to frame t+1. The dummy cells in frame t+1 will participate in the global ICP process which registering cells from frame t+1 to frame t+2. Thus, based on the registration parameters from frame 1 to frame 2, we create a dummy cell #2 in frame 2.

After finishing the registration from frame t to t+1 and frame t+1 to t+2, the relationship of a cell among these frames is built. In confirming the existence of the dummy cell procedure, the cell in frame t+2 registers backward to frame t+1 and then t. If it also finds the same corresponding cell in the previous forward registration from frames t to t+1 and t+2, the existence of this cell is validated. Cell #2 in frame 2 is thus confirmed if cell #2 was found in both the forward and backward registration processes and is discarded otherwise.

IV. Experiments

The goal of the proposed system is to segment the un-segmented cells via registration-based segmentation. To verify the accuracy of the proposed method, we apply our algorithm to a micrographic sequence which consists of 8 frames. We measured recovery rate to evaluate the cell registration–based segmentation algorithm. The recovery rate is the ratio of the number of recovered cells to the number of missing cells. In addition, cells less than ten pixels and cells that are too vague to be identified by experts were ignored in the performance evaluation. The recovery rates of 6 intermediate frames (excluding the first and last of the 8 frames) are 81%, 67%, 75%, 58%, 67% and 81%, respectively. Fig. 5 shows frame 7 of the segmented images by the proposed method whose recovery rate is 81%.

V. Results and Discussion

This paper presents an automatic segmentation method to improve the nerve cells segmentation results of our prior study by using the registration based technique. It addresses a class of thin and long cells, e.g. nerve cells, which are usually falsely or miss detected due to the poor image quality. The proposed method begins with ICP registration, and then detects the missing cells based on the continuity among neighboring frames. Experimental results show that the proposed method significantly improves the accuracy of our cell segmentation results. The proposed segmentation method can also be applied to the other segmentation problems with large number of contiguous objects in a sequence of images.

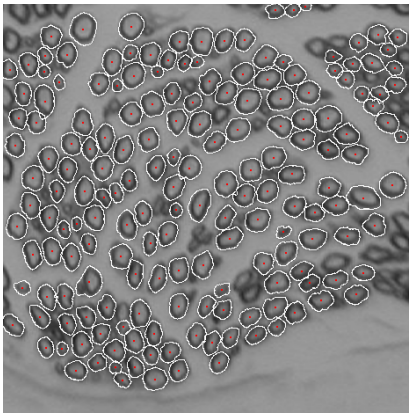

Fig. 1. A frame shows the center (red) and contour (white) information of the cells.

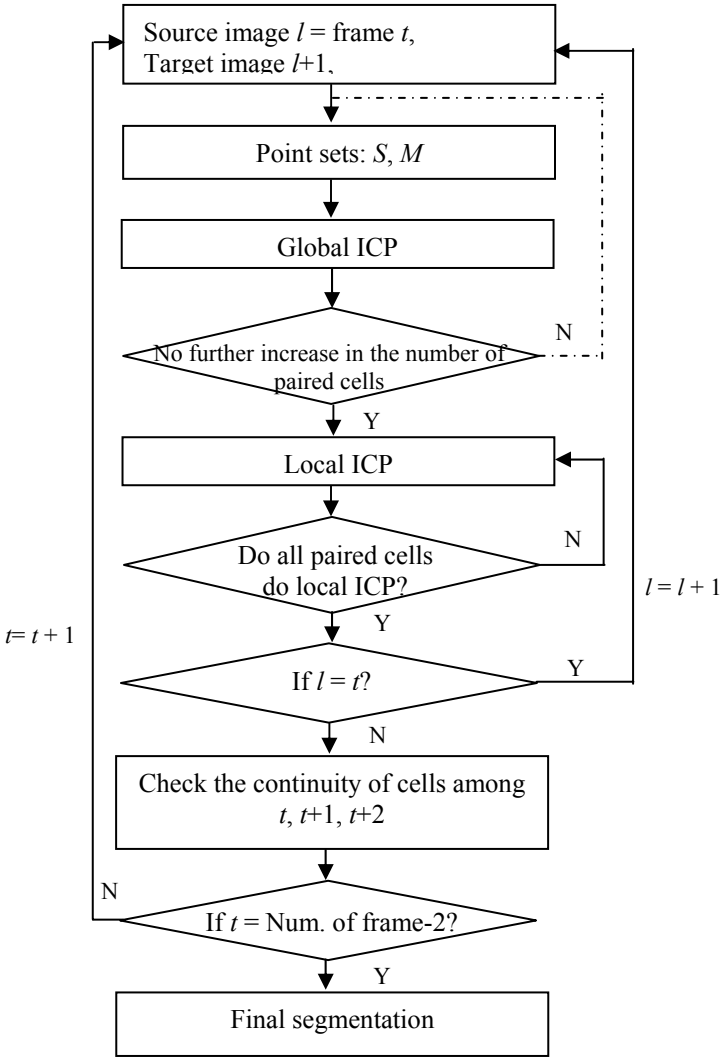

Fig. 2. Flowchart of registration-base segmentation.

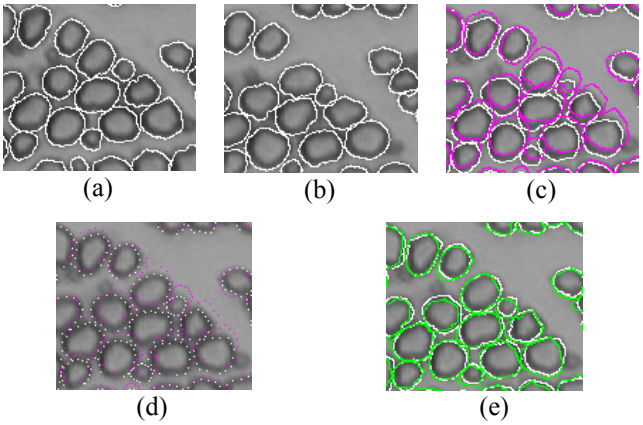

Fig. 3. (a-b) frames t and t+1; (c) registration result after global ICP; (d) selected sample points for local ICP; (e) registration result after local ICP.

(a) frame 1 (b) frame 2 (c) frame 3

Fig. 4. The problem: missing cell #2 in a series of three micrographic frames.

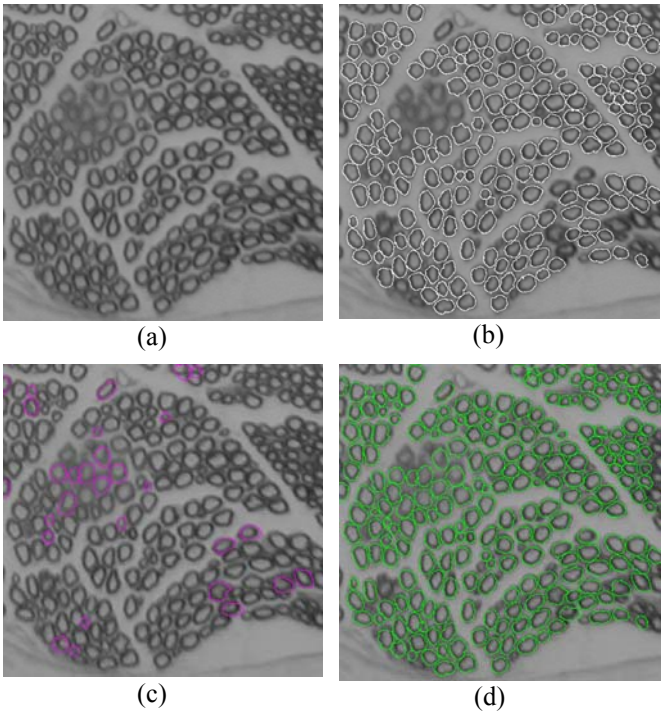

Fig. 5. Experimental result. (a) original image (b) the segmentation result in the earlier study (c) cells marked in purple are obtained by the proposed registration-based segmentation (d) final segmentation result.

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