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Enhanced CNN Based Electron Microscopy Image Segmentation

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Abstract: *Detecting the neural processes like axons and dendrites needs high quality SEM images. This paper proposes an approach using perceptual grouping via a graph cut and its combinations with Convolutional Neural Network* (*CNN*) *to achieve improved segmentation of SEM images. Experimental results demonstrate improved computational efficiency with linear running time.*

Keywords: *Convolutional Neural Network, SEM image, perceptual grouping constraints via a graph cut, segmentation using a graph cut, graph cut optimization, affinity graph, backpropagation, Maxflow/Mincut algorithm, Neuronal segmentation.*

1. Introduction

Segmentation algorithm is an elegant tool in medical image processing and computer vision. It is the process of partitioning an image to extract objects of interest. Image segmentation has been widely used in image processing applications, such as content based image retrieval, image understanding, object-recognition, and image classification. Recently, the use of image processing applications has spread over web and into our daily lives, and much more thus the speed requirements of the applications are increasing day by day.

Connectomics is an emerging field in neuroscience which deals with the study of function of the nervous system. Neural circuits are central to the nervous system and scientists who have been working on this area must have sufficient knowledge about the existing neuronal patterns and its connections. The high resolution of

Electron Microscopy (EM) images enables accurate capture of components of the neural tissues. However, the modern automated system can acquire tons of data, hand designed analysis of this huge amount of data is a laborious task. SBF-SEM (Serial Block-Face Scanning Electron Microscopy) generates stacks of 2D images in which successive image slices are extremely thin and very well aligned.

Traditional segmentation systems rely on the low-level features, such as brightness, color, and texture of the classifying images. Mid or high level cues can be combined with low level attributes for the hierarchical partitioning of the image into homogenous regions. This combination may need further repartitioning. However, these conventional graph cut approaches are inefficient as they are not taking into account the perceptual grouping of the non-local properties of the images. In this proposed approach we are considering this principle to achieve improved performances.

Organization of the rest of the paper is as follows: In Section 2 we review the related literature briefly. The functioning of Convolutional Neural Network and the learning processes are discussed in Section 3. Section 3 also illustrates the proposed approach of neuronal segmentation. The performance metrics and the results are presented in Section 4. Finally, the paper is concluded by Section 5.

2. Related work

A few of the relevant segmentation approaches employing perceptual grouping, graph cut, and Convolutional Neural Network are briefly reviewed in this section.

In the research of image segmentation, computational models are constructed on the basis of Gestalt rules. Perceptual grouping can extract meaningful information from the uncertain or irregular image. Unlike the output from the low-level vision, structural descriptions from a raw Electron Microscopy (EM) image can be easily obtained from the high-level vision. Perceptual grouping has a profound impact in the field of human visual system. Some of the Gestalt laws of grouping principles are: proximity, colinearity, good continuation, similarity, cocurvilinearity, convexity and closure. Perceptual grouping was defined as the process of grouping components of region of interest to form an individual object. Proximity, good continuation and similarity of edges are computed with the help of local features, such as distance, orientation changes and HSV (Hue, Saturation and Value) histogram difference. Efficiency of these computational models relies on the affinity function. Computation of affinity function and the formation of "best" grouping are the key components of the grouping cues [1]. Cues are a domain-specific function which depends on the assumed properties of the groups of interest.

The importance of perceptual grouping and organization was first pointed out by W e r t h e i m e r [2]. Some of the perceptual grouping principles identified in this work are proximity and similarity, which lead to visual grouping. Other perceptual grouping factors include symmetry, closure, parallelism, etc. However, many of the computational issues of perceptual grouping are still unresolved. Wertheimer's Laws of Grouping: proximity and smoothness are used for the evaluation of saliency maps [3-5], for the grouping of edge points lying on smooth curves [6-8]. Closure is used for grouping edges [9]. Similarity is used for grouping and segmentation [9].

For an accurate contour tracing of the cell membrane, Jacob et al. proposed a semi automated segmentation algorithm. Stephan et al. demonstrated a Boundary Contour (BC) system by considering the perceptual grouping of edges, textures, and smoothly shaded regions. This method is then compared with the probabilistic and artificial intelligence approaches. B e c k [13] proposed perceptual grouping principle to partition a pattern into two regions. This approach is based on the orientation and shape of the pattern. This work is inspired from the neuronal segmentation using the Perceptual Grouping constraints via a graph cut (PG) algorithm put forward by K a y n i g et al. [14]. L u o and G u o [15] proposes Non-purposive Perceptual Grouping (NPG). In this work, small regions are merged to form a large region and then the similar neighboring regions (color or texture) are further merged together to overcome over-segmentation. In this, optimization process of grouping is implemented by a greedy method HCF (Highest Confidence First). Adolfo et al. use unsupervised approach for color image segmentation. Based on the primary perceptual criteria, spatially connected regions are merged. Yingjian et al. demonstrated an effective and efficient perceptual organization method for image segmentation. The region-wise global weights of region adjacency graph uses a global optimization criterion which results in an efficient method. C o x et al. [19] uses a grouping algorithm by minimizing the ratio between the exterior boundary cost and the interior boundary. This is an efficient algorithm for obtaining the globally optimum solution for finding the boundary of a single object. E l d e r et al. [18] demonstrated the simple tangent cycles corresponding to the closed boundaries of the contours in the image.

A wide range of problems uses the Minimum cut algorithms. Some example problems which involve Minimum cut theorem are: image restoration [19], image segmentation [20], and image synthesis [21].

The concept of Convolutional Neural Network (CNN) was motivated from the work of S t e p h e n et al. [22] dealing with hand tracking from video sequences. With the help of CNN, this system, proposed for hand tracking is able to classify whether the hand is closed or open with a minimum error. Also, this system can detect a hand in 99.7% of the test frames. Again, G a r c i a and D e l a k i s. [23] introduced CNN on face detection and reported a perfect face recognition rate. As CNN is partially invariant to rotation, this system can detect highly variable face patterns, like rotated images [23]. Another face recognition algorithm by L a w r e n c e et al. [24] uses CNN and 1-Dimensional Haar wavelet. W a i b e l et al. [25] proposed CNN to recognize the dynamic structure of phonemes. L e C u n et al. [26] has successfully used CNN for perfect hand written character recognizer. In this system, all the variable characters were recognized. J a i n et al. [27] demonstrated that CNN outperforms all other techniques and introduced a new method for image restoration. T u r a g a et al. [28] introduced CNN to generate affinity graph suitable for segmentation.

In spite of the huge amount of literature suggesting various novel and improved approaches for segmentation, newer problems demand the development of still more accurate and fast approaches. The major outcomes of the survey carried out above are the following: perceptual grouping allows efficient segmentation at low computational cost; graph cut is an efficient tool for graph based segmentation; Convolutional Neural Network can produce an affinity graph which is suitable for segmentation.

This paper proposes an efficient segmentation which uses a combined approach of perceptual grouping constraints via a graph cut (PG) algorithm and Convolutional Neural Network algorithm to achieve improved performance.

3. EM segmentation using CNN and PG via a graph cut

3.1. Proposed approach

In this work we use automated machine learning approach for predicting affinity graph using Convolutional Neural Network algorithm. This machine learning algorithm is trained using the training sets of images with "ground truth" segmentations generated by experts. This learned CNN affinity graph can be combined with any of the graph partitioning algorithm which leads to an efficient segmentation result. CNN is adapted to new environment through learning processes, and it is able to automatically extract the salient features to generate an affinity graph. We propose a novel approach for solving the perceptual grouping problem by combining CNN and perceptual grouping constraints via a graph cut algorithm. In this algorithm, classification of single pixels is done by random forest, and it uses graph cut optimization for membrane segmentation. The probability for a membrane is locally estimated by a random forest classifier, while a regular cost function guarantees a global optimum employing graph cuts. The global optimum is efficiently found by graph cut optimization maintaining a good continuation.

The principle of good continuation to close the gaps along membranes is used in the segmentation algorithm. It is computed by distance and orientation changes between two edges. The similarity of two edges is decided by the intensities between them. While computing the affinity function of the edges, the principle of good continuation is assured. Output of the edge detection is imperfect and incomplete without the perceptual grouping constraints. This principle is incorporated into the affinity graph generated from the Convolutional Neural Network to segment the raw Electron Microscopy (EM) image using a computationally efficient graph partitioning algorithm and thus a global optimization is guaranteed. In this algorithm, the regular cost function is that of the probabilistic output of a random forest classifier. The aim is to minimize the regular cost.

The CNN and Perceptual grouping constraints via graph cut algorithms are briefly described in the following chapter.

3.2. Convolutional neural network

Machine learning architectures like Convolutional Neural Network is an innovative automated approach for predicting the affinity function. CNN is a MultiLayer Perceptron (MLP) designed specifically to recognize two-dimensional shapes with a high degree of invariance to translation, scaling, skewing, and other forms of distortion. This is the advantage of CNN over fully connected multilayer perceptron. CNN is able to adapt to new situations which are similar to the training set and it is able to detect the patterns which are almost similar to the training set. The advantage of CNN is that it requires little or no preprocessing of images. CNN consists of a hierarchy of series of layers. Each layer contains one or more planes. CNN is able to detect features automatically according to the training set. The performance of these algorithms entirely depends on the choice of the affinity function. In this paper the affinity function is applied to perceptual grouping constraints algorithm.

The use of weight sharing allows implementing the Convolutional Neural Network in a parallel form. Shared weights help to reduce the number of free parameters. The capacity of machine learning is thereby reduced, which in turn improves the machine's generalization ability. The adjustments to the free parameters of the network are made by using the stochastic mode of back propagation learning. Learning of CNN has twofold benefits. With the prior knowledge of the images, it is able to learn complex, high-dimensional, nonlinear mapping. Secondly, it is able to learn the synaptic weights and bias levels. CNN provides partial invariance to translation, rotation, scale and deformation. Three features of CNN are: local receptive fields, shared weights, and spatial sub-sampling.

Convolutional Neural Network may be defined as a directed graph. The nodes of the CNN represent image pixels. The edges represent filters. Weights of the edges are the similarity between the image pixels. Supervised learning consists of acceptance of an input raw EM image, calculation of its output and adjustment of the free parameters by batch processing. Feature extraction, feature mapping are the stages of image processing through the CNN layers. By reducing the spatial resolution of the feature map, a certain degree of shift and distortion invariance is achieved. The CNN will learn the filters for classification directly from data. This machine learning approach will produce an affinity graph which will minimize misclassification edges.

3.2.1. Initialization of CNN

Each layer of the CNN employed in this work contains 6 feature maps with sigmoid nonlinearities. The filters in the CNN are of same size and it is 5×5 in size. This led to an affinity classifier that uses a 17×17 image patch to classify an affinity edge. Select random weights and biases from the elements of filters from a normal distribution of standard deviation [28].

3.2.2. Learning algorithm

In batch processing, for each update, the gradient is computed with respect to the entire training set. This approach is inefficient as the gradient algorithm has slow convergence and the steepest descent increases its speed. Therefore, we used stochastic online learning for this problem. The back propagation algorithm for training the weights in a multi-layer net uses the steepest descent minimization procedure and the activation function used is the sigmoid threshold function.

A Convolutional Neural Network contains alternating sequence operations of linear filtering and nonlinear transformation. The input is an EM image and output layers will contain two images, while intermediate layers (referred to as “hidden” layers) contain images called feature maps. Feature maps are considered to be the internal computations of the algorithm. The CNN used corresponds to the directed graph, where the nodes represent images and the edges represent filters. Through experimentation, it has been proved that three hidden layers are sufficient. Each hidden layer has six feature maps. Each layer of the CNN employed in this work contains 6 feature maps with sigmoid nonlinearities. The filters in the CNN are of same size and it is 5×5 in size. This led to an affinity classifier that uses a 17×17 image patch to classify an affinity edge. Select random weights and biases from the elements of filters from a normal distribution of standard deviation [28].

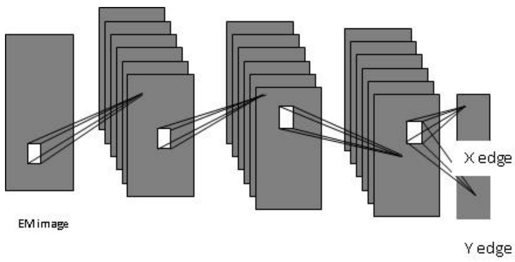


Fig. 1. Convolutional neural network

The input of the Convolutional Neural Network is the raw input image and the features of the image are automatically detected by CNN. CNN is learned using online stochastic gradient descent learning algorithm and it is adapted to new environments. The output of the CNN gives two images, *x* edge and *y* edge. Using an affinity function, the affinity graph is constructed.

3.3. Perceptual grouping constraints via a graph cut

The images are represented by a graph, $G=(V,E)$ where the nodes (V) correspond to the image pixels, $p \in P$ of the image and edges (E) connect the pair of similar nodes. Similarity (intensity, texture, etc.) between the image pixels are considered as the weights of the edges. The goal of the segmentation is to partition the nodes of the graph into disjoint sets, which minimize the cost function. Such partition is known as graph cut. The cost of the cut is the sum of weights corresponding to the graph edges which produces segmentation. Construct a graph such that the minimum cut of the graph also minimizes the global or local regular energy. Add two special nodes, source, s and sink, t. Then, $V = p \in \{s,t\}$. The set of undirected edges contains two types: *n*-links (neighborhood links) and *t*-links (terminal links). Two terminals, s and t are connected to each pixel $p \in P$. Each neighboring pixel pair is linked by an *n*-link.

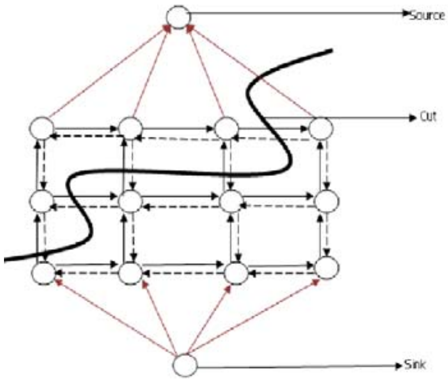


Fig. 2. Graph cut

Labeling of pixels is done in such a way that optimizes the energy function $E(y)$. $E(y)$ is formulated as [13]:

$$E(y) = \sum_{p \in P} E_d(y_p) + \lambda \sum_{p \in P, q \in N_2(p)} E_s(y_p, y_q) \tag{1}$$

where $E_d(y_p)$ is a data term and $E_s(y_p, y_q)$ denotes the smoothness term. P is the set of all pixels in the raw EM and $N_2(p)$ is the set of all pixels adjacent to a pixel p in the 2D image plane. However, for efficient segmentation we need to modify this terms.

The global minimum of $E(y)$ can be computed by max-flow/min-cut algorithm. In order to reduce the discontinuities in the segmentation for neighboring pixels, E_s is redefined as [13]:

$$E_s(y_p, y_q) = \exp\left(-\frac{(x_p - x_q)^2}{2\sigma_s^2}\right) . \frac{\delta(y_p, y_q)}{\mathrm{dist}(p, q)} \tag{2}$$

where x_p is the gray value of the image at pixel p and $\mathrm{dist}(p,q)$ is the distance between the neighboring pixels.

Kronecker delta function ensures that the energy term is regular. Probabilistic output of the random forest classifier is used for membrane detection. In order to minimize the computational cost, histograms over the context region are taken. In order to eliminate the false positive of the segmentation, principle of good continuation to close the gaps along membrane is used. A directional energy is introduced to avoid the shortcomings of the gradient flux so that smoothness in labeling is ensured. E_{gc} is formulated as [13]:

$$E_{gc}(y_p, y_q) = |\langle v_p, u_{pq} \rangle| . \exp\left(-\frac{(x_p - x_m)^2}{2\sigma_s^2}\right) . \frac{\delta(y_p, y_q)}{\mathrm{dist}(p, q)} \tag{3}$$

where u_{pq} is the unit vector with orientation of the straight line between pixels p and q, and v_p is a vector directed along the membrane. The smoothness along the direction of the membrane pixels are overcome by E_{gc}.

To incorporate information from adjacent sections into the segmentation, E_{na} is defined as [13]:

(4) $$E_{\text{na}}(y_p, y_q) = m_q \,|\langle v_p, v_q \rangle|\,.\frac{\delta(y_p, y_q)}{\text{dist}(p,q)}$$

where m_q is the probability of pixel q on the membrane and v_p is the largest eigenvector of the Hessian at pixel p multiplied by the corresponding eigenvalue.

Since the effect of gradient flux is minimum (false positive membrane segmentation) due to the texture in the image, we omit the gradient flux and the final energy term is formulated as[13]:

(5) $$\begin{aligned} E(y) = {} & \sum_{p \in P} E_{\text{rf}}(y_p) + \lambda \sum_{p \in P, q \in N_2(p)} E_s(y_p, y_q) \\ & + \lambda_{\text{gc}} \sum_{p \in P, q \in N_2(p)} E_{\text{gc}}(y_p, y_q) \\ & + \lambda_{\text{na}} \sum_{p \in P, q \in N_3(p)} E_{\text{na}}(y_p, y_q) \end{aligned}$$

where $E(y)$ is the energy term. The first term denotes the energy term of the probabilistic output of the random forest classifier for every pixel $p \in P$. The second term denotes the directional energy term based on good continuation. $N_2(q)$ denotes the set of all pixels adjacent to a pixel q in the 2D image plane. The third term denotes the energy of the smoothness term. $N_3(q)$ is the set of neighboring pixels in adjacent section (3 dimension). The fourth energy term incorporates images from the adjacent sections [13].

Convolutional Neural Network constructs the affinity graph which is segmented using perceptual grouping constraints via a graph cut. In the first phase, affinity graph is generated using CNN. The second phase of the proposed method consists of two steps: construction of a graph from the perceptual grouping, and then partition the graph according to max-flow min-cut theorem. The first step includes a mapping of the perceptual grouping problem to an energy function. A cue evaluation algorithm for each feature pair is computed. The second step uses graph cuts to minimize the cost function to find the “preferred” perceptual groups. The probability output of a random forest classifier is used in a regular cost function, which integrates the information available from gap completion via perceptual grouping constraints. The segmentation is posed as a minimization of the regular cost function involving the image intensity and the flux of the intensity gradient field, and graph cut is used to compute the globally optimal solution.

The computation of the minimum cut can be efficiently performed by the max flow algorithms. In past studies, Minimum cut algorithms principle has been applied to a wide range of problems. The energy term is regular and can be globally optimized using max-flow/min-cut computation. Label each pixel in $p \in P$ with $\{0,1\}$ such that the labels for all pixels minimizes the Energy term $E(y)$ defined in (5) (see Algorithm 1).

Algorithm 1. Perceptual grouping constraints via a graph-cut [13]

Step 1. Given an image, construct a graph $G=(V,E)$ and the weights of the edges are the similarity between pixels.

Step 2. For all nodes $p \in P, N_2(p)$

$$E(y) = \sum_{p \in P} E_{\mathrm{rf}}(y_p) + \lambda \sum_{p \in P, q \in N_2(p)} E_s(y_p, y_q)$$
$$+\lambda_{\mathrm{gc}} \sum_{p \in P, q \in N_2(p)} E_{\mathrm{gc}}(y_p, y_q)$$
$$+\lambda_{\mathrm{na}} \sum_{p \in P, q \in N_3(p)} E_{\mathrm{na}}(y_p, y_q).$$

Step 3. Check whether entire labeling y for all pixels minimizes the energy function; if yes – terminate, otherwise go to Step 2.

Although this energy term incorporates information from adjacent sections, the main focus of the segmentation is two dimensional. This is due to the fact that the resolution of SEM images is high (about 5 nm per pixel), but along the vertical direction of the image stack, the resolution is limited by the section thickness of the sample [13].

3.4. The algorithm proposed

The Perceptual Grouping constraints via a graph cut (PG) algorithm reproduced above from [13] is combined with Convolutional Neural Network to improve the performance of segmentation. The energy of the affinity graph obtained from CNN is optimized using perceptual grouping constraints via a graph cut. CNN algorithm generates an automated affinity graph which is very efficient. But it is inefficient to detect the smooth positive boundaries. Since in PG, smoothness information from adjacent sections, etc. is considered, the performance of the proposed algorithm is improved.

Algorithm 2. Proposed method

Phase1: CNN

Step 1. Input the raw SEM image and the ground truth image into the CNN.

Step 2. Update the weights in the network until the *x*-edges and *y*-edges are correctly classified.

Step 3. Construct a graph from the above edge image.

Phase 2: PG

Step 1. The output of CNN is considered as the input of PG algorithm.

Step 2. Cost function is optimized as (5).

Step 3. Output of this algorithm is the segmented image.

4. Performance evaluation and results

4.1. Performance evaluation

The performance of various schemes suggested is evaluated using the popular precision, recall, true positive rates, false positive rates, accuracy and *F*-score measures, whose standard definitions are as given below:

Precision (*Pr*)**.** This is the ratio of the true positives to the sum of true positives and false positives:

$$\mathrm{Pr} = \frac{\mathrm{TP}}{\mathrm{TP+FP}}.$$

Recall (*Re*)**.** This is the ratio of true or actual positives to the sum of actual positives and false negatives.

$$\mathrm{Re} = \frac{\mathrm{TP}}{\mathrm{TP+FN}}.$$

These two measures, precision and recall, quantitatively represent the quality of segmentation algorithms irrespective of whether the image is over segmented or under segmented.

Precision tells us what fraction or percent of the algorithm detected boundary pixels are the boundary pixels in the ground truth. This measure is sensitive to over segmentation. Recall tells us the fraction or percent of the ground truth boundary pixels that are detected by the algorithm. This percent is sensitive to under segmentation. *F-score* is defined as the harmonic mean of the precision and the recall.

$$F-\mathrm{Score} = \frac{2\times \mathrm{Pr}\times \mathrm{Re}}{\mathrm{Pr+Re}}.$$

Other related measures are *False Positive Rate* (FPR), *True Positive Rate* (TPR) and *Accuracy*. *FPR* is defined as the ratio of the false positives to the sum of false positives and false negatives. This measure denotes the correctly classified portion of the positives events.

$$\text{FPR}=\frac{\text{FP}}{\text{FP+TN}}.$$

TPR is defined as the ratio of true positives to the sum of true positives and true negatives. It denotes the positive outcome fraction of the total absent events.

$$\text{TPR}=\frac{\text{TP}}{\text{TP+FN}}.$$

Accuracy is defined as the ratio of the misclassified pixels to the total number of pixels.

$$\text{Accuracy}=\frac{\text{TP+FN}}{\text{TP+FN+TN+FP}}$$

where TP, FP, TN and FN respectively denote the number of true positive, false positive, true negative and false negative predictions.

We demonstrate the training of Convolutional Neural Network (CNN) to produce an affinity graph from raw EM images. We are able to correctly predict the affinity graph when perceptual grouping constraints via a graph cut (PG) is combined with the output of Convolutional Neural Network. Then, in the post processing step, contour of the image is detected and it is added to the image. *F*-score of this proposed work is obtained as 77%.

4.2. Data set

We use the data set of the Drosophila first instar larva Ventral Nerve Cord (VNC) with the Serial section transmission Electron Microscopy (SEM) [20]. The images from SEM have a resolution sufficient to distinguish the components of the complete neuronal tissues. One image stack contains several thousands of images and the voxel measures 2×2×1.5 microns approximately, with a resolution of 4×4×50 nm/pixel [29]. Non linear lens distortion of the electromagnetic lenses of the microscope needs to be corrected before processing. The multiple images taken from the microscope are then stitched together and perform *z*-alignment of images. As a preprocessing step, the image distortion correction plugin for ImageJ [30] is used for correcting the distortion. TrakEM2 is an ImageJ plugin for three dimensional modeling and stitching [29]. The images are manually segmented by human experts using TrakEM2, a free plugin of ImageJ.

4.3. Experimental results and discussion

The input image is given in Fig. 3. Ground truth Image in two forms is as shown in Fig. 4. The output of perceptual grouping via graph cut (PG) algorithm is shown in Fig. 5a, and the output of proposed approach (PG combined with CNN) is given in Fig. 5b.

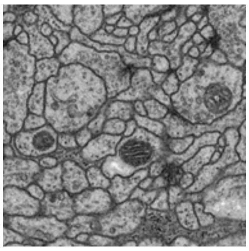


Fig. 3. Input image

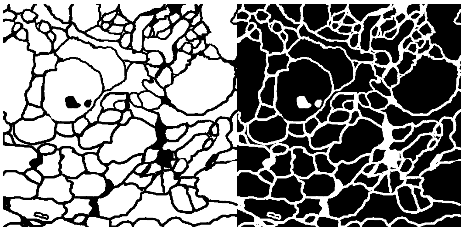
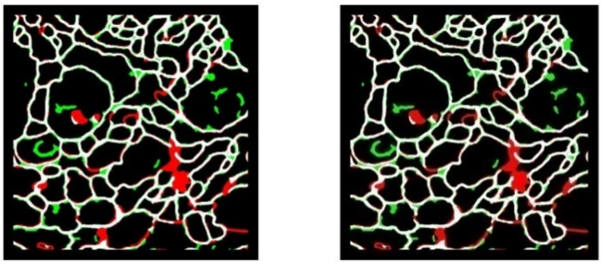


Fig. 4. Ground truth



a – Output of PG b – output of PG+CNN

Fig. 5. Results of segmentation

The image shows the color overlay of the segmentation with good continuation and the ground-truth label. False positives are shown in green and false negatives are shown in red.

The results in the form of quantitative measures of evaluations are given in Table 1 for PG and PG+CNN.

Table 1. Comparison of results

Method	Precision	Recall	Accuracy	False positive	True positive	f-score
PG	0.5752	0.9200	0.8146	0.2194	0.9200	0.707844
PG+CNN	0.7481	0.8099	0.8870	0.0881	0.8099	0.777787

The segmentation performance measures of the proposed approach, CNN+PG, are compared with the perceptual grouping constraints via a graph cut (PG) approach. The results demonstrate that the precision and the *F*-score are highest for the proposed approach, and the other measures are also improved in the proposed approach. The experimental studies demonstrate also that the computational time is reduced as compared to the purely manual tracing algorithms.

5. Conclusion

In this paper we have presented a new approach for image segmentation using the combination of perceptual grouping constraints via a graph cut algorithm and Convolutional Neural Network (PG+CNN). The system proposed considers the perceptual grouping of the non-local properties of the images; which is absent in conventional graph cut methods. This proposed approach of segmentation is able to optimize in the aspects of precision. A series of experiments involving four different features and execution time were conducted. The experimental results demonstrate that our proposed approach is capable of improving the *f*-score value and computation time.

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