Epithelial Cell Layer Segmentation Using Graph-cut and Its Application in Testicular Tissue

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Abstract

Computerized image processing has provided us with valuable tools for analyzing histology images. However, histology images are complex, and the algorithm which is developed for a data set may not work for a new and unseen data set. The preparation procedure of the tissue before imaging can significantly affect the resulting image. Even for the same staining method, factors like delayed fixation degrade tissue and may alter the image quality. In environmental research, due to the distance between the site where the wild animals are caught and the laboratory, there is always a delay in fixation. In this paper we face the challenging problem of designing a method that works on data sets with different fixation delay and strongly varying quality. Here we suggest a segmentation method based on the structural information of epithelium cell layer in testicular tissue. The cell nuclei are detected using the fast radial symmetry filter. A graph is constructed on top of the epithelial cells. Graph-cut optimization method is used to cut the links between cells of different tubules. The algorithm is tested on five different groups of animals. Group one is fixated immediately, four groups were left at room temperature for 6, 18, 30 and 42 hours respectively, before fixation. The suggested algorithm gives promising results for the whole data set.

1 Introduction

The pathologist analyses tissue slides using light microscopy to detect morphological abnormalities. Statistical quantification of histological aberrations requires evaluation of a large number of slides, which is time consuming. Manual analysis is subjective and there is always a risk that pathologists vary in the assessment of a tissue due to tedious repetition of the work. The histological slides can be digitized, which enables the use of computerized image analysis and machine learning techniques to complement the evaluation of the pathologist. Such computer-assisted diagnosis is receiving the attention of many researchers.

Several reports indicate that compounds found in the environment can effect male reproduction in humans, mammals, birds and fish [6]. Histopathology of testicular tissue is regarded as a sensitive tool for detecting adverse effects caused by chemicals in male reproduction [5]. One of the first steps in computerised analysis of histological images is to identify different structures, like cells, lumen, glands and tubules. Once these structures are segmented, measurements of different features can be carried out to detect adverse morphology. Segmentation will be done by using chromatic information or spatial relationship of different components. The final image quality may vary because of the preparation procedure. It is a challenging problem to propose a method that works correctly, with the same parameters, on any data set even for the same tissue and same staining method. To complicate matters more, the time period between the death of the animal and the placement of the tissue in fixative affects the tissue [4]. This is relevant in environmental research due to the distance between the site were the wild animals are caught and the laboratory. For practical reasons the animal may even be frozen. This generates evaluation difficulties due to autolysis and freeze damages of the tissue. Tissue which is prepared in a perfect lab condition has a normal tubular structure, which is a lumen, surrounded by epithelial cells, however in degraded tissue the lumen may be occupied with cells. The chromatic information may also be affected by the delayed fixation and freezing (see figure 1a and 1b).

In this paper we suggest a segmentation method that uses the structural information of the testicular epithelium marked with GATA-4 antibody. This method can be used on any tissue with similar structure. The spatial relationship of the cells has been used for epithelium classification. Albert *et al*. use a graph theoretical method to study the morphological characteristics of the epithelium [1]. The minimal spanning tree is computed in the three-dimensional (3D) space of the sections with the selected centers of the nuclei as vertices. The average length of all edges in the graph is used for discriminating different specimens. Bilgin *et al*. first use k-means clustering to segment the epithelium and then the cell graph is used for classifying brain tissue samples [2]. Gunduz *et al*. also construct a graph on top of the cells and then compute the graph metrics of the cell graphs, including the degree, clustering coefficient, eccentricity and closeness for each cell to distinguish healthy from unhealthy tissue [7]. Here we also use graphs, but as a segmentation method.

2 Methodology

The proposed algorithm first segments the cell nuclei, which form the vertices of a graph. A graph $g(v,\varepsilon)$ is defined as a set of nodes or vertices v and a set of edges ε, connecting neighbouring nodes. The graph-cut optimisation is used to remove the links between nuclei belonging to different tubules, which thus yields an isolated sub-graph for each tubule. By applying morphological operators on sub-graphs we delineate the outer boundary of the epithelium.

2.1 Cell segmentation and vertices identification

The cell nuclei are mostly radially symmetric, several methods are suggested to measure the local symmetry in image. Kuse *et al*. [9] used phase symmetry suggested by Kovesi [8] for segmenting cells, we use the fast radial symmetry filter here to extract them [10]. If pixel p is located on the arc of a circle then the center of the circle is at one radius distance in the direction of the gradient. The Fast radial symmetry filter is calculated at one or more

radii. Loy *et al*. first calculate the Magnitude projection image M_n and orientation projection image O_n which have high response at potential centroids. M_n and O_n in [10] are defined as

$$M_n(P_{\pm ve}(p)) = M_n(P_{\pm ve}) \pm ||g(p)|| \quad , \tag{1}$$

$$O_n(P_{\pm ve}(p)) = O_n(P_{\pm ve}) \pm 1 \quad , \tag{2}$$

where $g(p)$ is the gradient at pixel p and $P_{\pm ve}$ is a "positively/negatively affected pixel" and is calculated using the gradient:

$$P_{\pm ve} = P \pm round(\frac{g(p)}{||g(p)||}n) \quad . \tag{3}$$

The radial symmetry contribution at radius n is

$$S_n = \tilde{M}_n(p)|\tilde{O}_n(p)|^{\alpha} \otimes A_n \tag{4}$$

where α is a scaling factor, A_n is a two-dimensional Gaussian, $\tilde{M}_n$ and $\tilde{O}_n$ are normalized M_n and O_n across different radii, and $\otimes$ denotes the convolution. The result of the filter is the average of the radial symmetry over different values of n. Segmentation of the radial symmetry yields the epithelial cell nuclei, but also other nuclei. We formed a feature vector based on morphology of cells (size, perimeter) and statistical chromatic information (mean, standard deviation, Skewness, minumum value and maximum value of intensity), then applied k-means clustering to cluster cells into two groups. The epithelial cells are used for further steps.

2.2 Edge establishment

In order to establish the edges ε of our graph we need to find the neighbouring cell nuclei centroids. One way is to use the Delaunay triangulation. The Delaunay triangulation of a set of m points corresponds to the dual graph of its Voronoi diagram. The Voronoi diagram divides the space into m polygons $\{P_1, P_2, ...P_m\}$, where P_a correspondes to the point S_a. A point c belongs to polygon P_a if $d(c, S^a) = min_j d(c, S_j)$, where $j \in \{1, 2, ..m\}$ and $d(c, S_j)$ is the euclidean distance between c and S_j. Two points S_a and S_b share an edge in the Delaunay triangulation if their corresponding polygons P_a and P_b share a side in the Voronoi diagram. Delaunay triangulation yields a graph with edges only between adjacent vertices. A sample of such a graph is shown in figure 1c.

2.3 Edge weights

As you can see in figure 1c, the epithelial cells usually form a cluster around a center. The distance between two nuclei in the same tubule is smaller than the distance between nuclei in different tubules. We set the weight of each edge to be inversely proportional to the Euclidean distance between the two vertices that it connects.

2.4 Graph-cut minimization

We use a graph-cut minimization method to remove undesired edges. In graph-cut method we need to specify two special terminal nodes which are called S (source) and T (sink) that

represent object and background labels. Edges between vertices are called n-links, and t-links represent connections between vertices and terminals. All graph edges $e \in \varepsilon$ including t-links and n-links are assigned some non-negative cost w_e. An s-t cut is a subset of edges $C \subset \varepsilon$ such that the terminals S and T become completely separated on the induced graph. The cost of cut is sum of the weights of all the edges it severs which is :

$$|C| = \sum_{e \in C} w_e. \tag{5}$$

A minimum s-t cut, is a cut with minimal cost. Based on combinational optimization a globally minimum s-t cut can be computed efficiently in low order polynomial time. Boykov *et al*. [3] introduced a new version of the max-flow algorithm that outperformed existing techniques for computer vision applications. We need interaction to specify the source and the sinks of the graph.

2.5 Animals and tissue preparation

Thirty healthy, sexually mature minks were collected at the annual culling on a mink farm. The animals were divided in five groups based on time interval between euthanization and fixation. Group one was put in fixative immediately post mortem. Four groups were left at room temperature for 6, 18, 30 and 42 hours respectively, before fixation. Transverse tissue slices from testis were fixed in modified Davidsons fluid for 24 hours at 4°C and embedded in paraffin wax. The samples were cut in 5 micrometer sections and stained with GATA-4 antibody. Digital images of the sections were taken with a Nikon Microphot-FXA microscope using the 10x objective lens.

3 Results

Testicular tissue is composed of tubules. Tubules are formed mainly by the seminiferous epithelium. Toxic damage will affect the cells in the epithelium and alter their morphology. Two sample images of stained tissue of mink testicle, 0 and 30 hours postmortem, are shown in figures 1a and b. As you can see in this figure, the quality of the tissue is very different, but they both have a similar structure, and the epithelial cells of a tubule cluster together.

We applied the method outlined earlier to segment the tissue sections into individual tubules. To improve the result, we first applied the bilateral filter. We chose a spatial-domain standard deviation of 3 pixels and an intensity domain standard deviation of 0.2 times the dynamic range of the image. We then applied the fast radial symmetry filter with radii 3, 4 and 5 pixels. The thresholded result gave the nuclei. As we can see in the sample images in figure 1, there were two different cell types in the tissue. We clustered the nuclei in two groups using k-means clustering. The feature vector used contained the mean, standard deviation, minimum and maximum intensity within the nucleus, and the nucleus size. Next we applied Delaunay triangulation to the epithelial nuclei to create a graph. The user was asked to add markers at the center of each tubule to be separated. The markers were grown until they each hit 7 nuclei. These nuclei were taken as sources and sinks of the graph. The max-flow optimisation was run and the links between different tubules were removed.

Figure 1 c and d shows the graphs, overlaid on top of the images. The edges shown in red are those which were cut. The sub graphs associated to every tubule can be used for classification of the tubules; we can extract features based on the morphology of the graphs

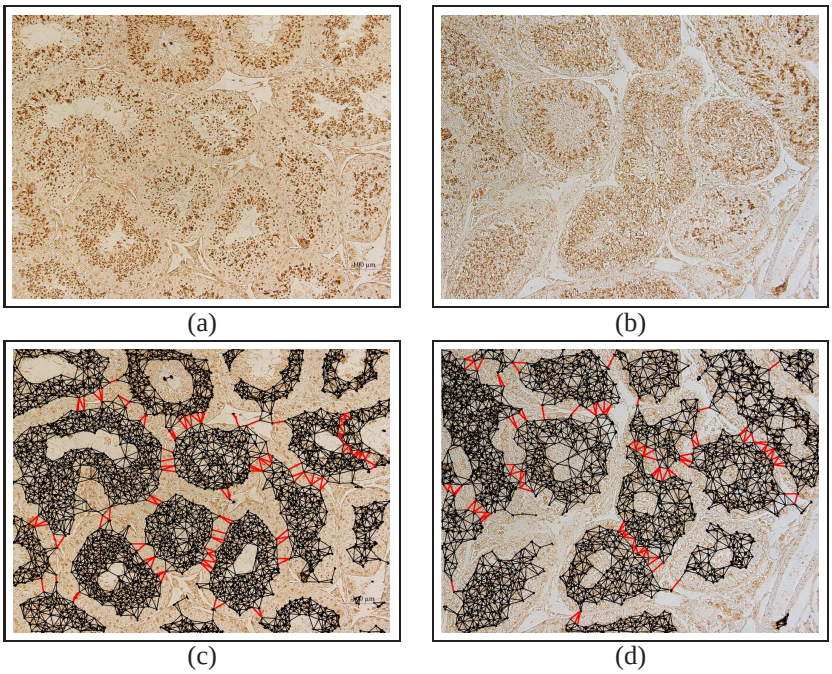

Figure 1: (a) Stained thin section of mink testicular tissue, fixated immediately after euthanasia. (b) Stained thin section of mink testicular tissue, fixated 30 hours postmortem. (c) and (d) Graphs constructed with epithelial nuclei as vertices, for the images in (a) and (b), respectively. Red edges are removed by the proposed method to separate the various tubules.

themselves, or we can use mathematical morphology operators that fill the holes enclosed by the edges of the graph to obtain a full segmentation of the epithelium. We applied our method on 50 images, 10 images per group. A sub-graph associated to a tubule which excludes some of the epithelial cells of the tubule, and a sub-graph which includes some of the cells from neighboring tubules both are considered as wrongly segmented. Similarly, small cell cluster which dose not belong to any tubule is considered as wrong segmentation. For group 1 (zero hour postmortem) 85% of epithelial cell layer of tubules are segmented correctly. For groups 2, 3, 4 and 5, this number is 66%, 71%, 76%, and 72% respectively. As it was expected for the zero hour, in which the structure of the tissue is preserved better, the error is smaller than for the rest of the groups.

4 Conclusion

A method for segmenting the epithelial cell layer was proposed. The result of segmentation can be used to analyse the epithelium structure and establish the postmortem effect of delayed fixation or freezing, and find robust endpoints to detect adverse effects. When analysing material from wild animals, it is important to separate the histological changes caused by delayed fixation postmortem from premortem pathology.

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