GRAPH-BASED MULTI-RESOLUTION SEGMENTATION OF HISTOLOGICAL WHOLE SLIDE IMAGES*

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ABSTRACT

In this paper, we present a graph-based multi-resolution approach for mitosis extraction in breast cancer histological whole slide images. The proposed segmentation uses a multi-resolution approach which reproduces the slide examination done by a pathologist. Each resolution level is analyzed with a focus of attention resulting from a coarser resolution level analysis. At each resolution level, a spatial refinement by semi-supervised clustering is performed to obtain more accurate segmentation around edges. The proposed segmentation is fully unsupervised by using domain specific knowledge.

Index Terms— Breast cancer, Graph, Segmentation.

1. INTRODUCTION

Breast cancer is the second leading cause of cancer death for women. Its incidence increases substantially and continuously while the mortality rate remains high despite earlier detection and advances in therapeutic care. The identification and the use of reliable prognostic and therapeutic markers is a major challenge for decision-making regarding therapy. Proliferation has been shown to be the strongest prognostic and predictive factor in breast carcinoma, especially in patients lacking lymph node metastases. This parameter is daily taken into account by the pathologist for establishing the histopathological grading of breast carcinomas, using enumeration of mitotic figures, through the lens of the microscope. The recent use of immunohistochemical staining of mitosis is able to facilitate their detection. Nevertheless, the visual counting method remains subjective and leads to reproducibility problems due to the frequent heterogeneity of breast tumors. The recently introduced microscopical scanners allow recording large images of the whole histological slides and offer the prospect of fully automated quantification for a better standardization of proliferation rate appraisal. If the advent of such digital whole slide scanners has triggered a revolution in histological imaging, the processing and the analysis of such high-resolution images is a very challenging task. First, the produced images are relatively huge and their processing requires computationally efficient tools. Second, the biological variability of the objects of interest makes difficult their extraction. As a consequence, few works in literature have considered the processing of whole slide images and most of these works rely only on machine learning techniques [1, 2].

In this work, we present a graph-based multi-resolution segmentation strategy for histological breast cancer whole slide images. The proposed strategy is based on a top-down approach that mimics the pathologist interpretation under the microscope as a focus of attention. Therefore, the segmentation performs an unsupervised clustering task at each resolution level (driven by domain specific knowledge) and refines the associated segmentation as the resolution increases. The whole strategy is based on a graph formalism that enables to perform the segmentation adaptation at each resolution.

The paper is organized as follows. Our graph-based formulation for image segmentation is presented in Section 2 and its integration into a multi-resolution segmentation strategy is detailed in Section 3. Section 4 presents experimental results. Last Section concludes.

2. GRAPH-BASED SEGMENTATION

2.1. Preliminaries on graphs

A graph is a structure used to describe a set of objects and the pairwise relations between those objects. The objects are called *vertices* and a link between two objects is called an *edge*. A weighted graph $G = (V, E, w)$ is composed of a finite set $V = \{u_1, \ldots, u_N\}$ of N vertices, a set of edges $E \subset V \times V$, and a *weight function* $w : E \rightarrow \mathbb{R}^+$. An edge of E, which connects two *adjacent neighbor* vertices u and v, is noted (u, v). In the rest of this paper, the notation $v \sim u$ means that vertex v is an adjacent neighbor of vertex u. We assume that the graph G is simple, connected and undirected. This implies that the weight function w is symmetric i.e. $w(u, v) = w(v, u)$ if $(u, v) \in E$ and $w(u, v) = 0$ otherwise. Let $\mathcal{H}(V)$ be the Hilbert space of real valued functions on the vertices of a graph. Each function $f : V \rightarrow \mathbb{R}$ of $\mathcal{H}(V)$ assigns a real value $f(u)$ to each vertex $u \in V$. Similarly, let $\mathcal{H}(E)$ be the Hilbert space of real valued functions

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defined on the edges of the graph.

2.2. Discrete operators on graphs

Let us recall some basic definitions. We consider that a graph $G = (V, E, w)$ and a function $f \in \mathcal{H}(V)$ are given. The *weighted difference* $d_w : \mathcal{H}(V) \rightarrow \mathcal{H}(E)$ of a function f on an edge (u, v) linking two vertices $u, v \in V$ is defined as $(d_w f)(u, v) = \sqrt{w(u, v)}\big(f(v) - f(u)\big)$. This operator leads us to define the *directional derivative* of f, over an edge (u, v), as $\partial_v f(u) = (d_w f)(u, v)$. Then, the *weighted gradient* $\nabla_w f$ of the function f, at a vertex $u \in V$, is defined as $(\nabla_w f)(u) = \big(\partial_{v_1} f(u), \ldots, \partial_{v_k} f(u)\big)^T$. This operator corresponds to the *local variation* of the function f at the vertex u and measures the regularity of f in the adjacent neighborhood $v_1, \ldots, v_k$ of the vertex u. Hence, the $\mathcal{L}_2$-norm of the weighted gradient is $\|(\nabla_w f)(u)\|_2 = \left[\sum_{v \sim u} w(u, v)\big(f(v) - f(u)\big)^2\right]^{1/2}$. Then, the *weighted p-Laplacian* $(\Delta_w^p f)(u)$ at vertex u is defined as

$$(\Delta_w^p f)(u) = \sum_{v \sim u} \gamma(u, v)\big(f(v) - f(u)\big) \qquad (1)$$

where $\gamma(u, v) = w(u, v)\big(\|(\nabla_w f)(u)\|_2^{p-2} + \|(\nabla_w f)(v)\|_2^{p-2}\big)$. Clearly, in the case where $p = 1$ and $p = 2$, we have the definitions of the standard graph curvature $\Delta_w^1 f = \kappa f$ and graph Laplace $\Delta_w^2 f = \Delta f$ operators. More details on these definitions can be found in our previous works [3].

2.3. Discrete regularization framework

To regularize a function $f^0 \in \mathcal{H}(V)$ using the p-Laplacian (Eq. (1)), we consider the following general variational problem on graphs:

$$\min_{f \in \mathcal{H}(V)} \left\{ E_w(f, f^0, \lambda, p) = R_w(f, p) + \frac{\lambda}{2}\|f - f^0\|_2^2 \right\} . \quad (2)$$

The first term, $R_w(f, p)$, is the regularizer and is defined as, with $0 < p < +\infty$: $R_w(f, p) = \frac{1}{p} \sum_{u \in V} \|(\nabla_w f)(u)\|_2^p$. The second term is the fitting term. This optimization problem has a unique solution for $p = 1$ and $p = 2$ [3] which satisfies, for all $u \in V$ [3]:

$$\frac{\partial E_w(f, f^0, \lambda, p)}{\partial f(u)} = (\Delta_w^p f)(u) + \lambda\big(f(u) - f^0(u)\big) = 0 ,$$

which is equivalent to

$$\left(\lambda + \sum_{v \sim u} \gamma(u, v)\right) f(u) - \sum_{v \sim u} \gamma(u, v) f(v) = \lambda f^0(u) .$$

To approximate the solution of the minimization (2), we can linearize this system of equations and use the Gauss-Jacobi method to obtain the following iterative algorithm:

$$\begin{cases} f^{(0)}(u) = f^0(u) \\ f^{(n+1)}(u) = \dfrac{\lambda f^0(u) + \sum\limits_{v \sim u} \gamma^{(n)}(u, v) f^{(n)}(v)}{\lambda + \sum\limits_{v \sim u} \gamma^{(n)}(u, v)} \end{cases} , \qquad (3)$$

where $\gamma^{(n)}(u, v)$ is the γ function (in Eq. (1)) at the iteration step n. The interested reader can refer to [3] for more details on the formulation and the connections with other formalisms. The above algorithm enables to simplify functions living on graphs by a discrete diffusion process.

2.4. Discrete semi-supervised clustering

Our previously presented discrete regularization framework can be naturally adapted to address discrete semi-supervised clustering problems. Let $V = \{u_1, \ldots, u_N\}$ be a finite set of data, where each data u_i is a vector of $\mathbb{R}^{\mathrm{m}}$. Let $G = (V, E, w)$ be a weighted graph such that all vertices are connected by an edge of E. The semi-supervised clustering of the set V consists in grouping the set V into k classes where the number of k classes is given. For this, the set V is composed of labeled and unlabeled data. The objective is then to estimate the labels of unlabeled data from labeled ones. Let $C = \{c_i\}_{i=1,\ldots,k}$ be the set of initial *labeled* vertices and let $V \backslash C$ be the initial *unlabeled* vertices (the whole set of vertices except the labeled ones). This situation can be modeled by considering k initial label functions (one per class) $f_i^0 : V \rightarrow \mathbb{R}$, with $i = 1, \ldots, k$. For a given vertex u, if u is initially labeled ($u \in C$) then $f_i^0(u) = +1$ if $u \in c_i$ and $f_i^0(u) = -1$ otherwise. If u is initially unlabeled (i.e. $u \in V \setminus C$) then $f_i^0(u) = 0$. Then, the vertex clustering is accomplished by k regularization processes. This corresponds to estimate functions $f_i : V \rightarrow \mathbb{R}$ for each i^{th} class using the discrete diffusion process (Eq. (3)). At the end of the label propagation processes, the final label of a given vertex $u \in V$ can be obtained by $\underset{i}{\mathrm{argmax}} \left\{ f_i(u) / \sum\limits_i f_i(u) \right\}$.

3. MULTI-RESOLUTION SEGMENTATION

3.1. Principle

Whole slide images (WSI) are usually huge in size. Fortunately, they are stored as a pyramid of tiled images that enables to process them in a hierarchical way [4]. As a consequence, a multi-resolution segmentation method is a natural approach for segmenting whole slide images. Moreover, such a strategy reproduces the analysis done by the pathologists under the microscope: regions of interest are determined at low resolution while cellular classification is performed at high resolution. The proposed multi-resolution segmentation method is based on a top-down segmentation that

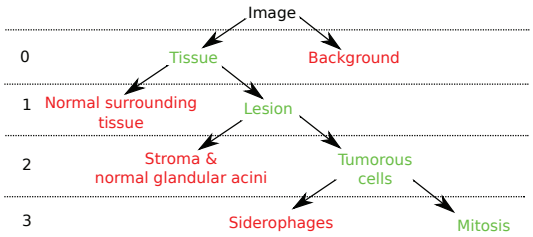

Fig. 1. Illustration of the multi-resolution strategy according to domain specific knowledge.

mimics pathologist interpretation according to specific domain knowledge. Figure 1 illustrates the identification (by a pathologist) of mitosis in breast cancer slides stained with hematoxyline and eosine. Our approach exactly follows this multi-resolution analysis : identification and classification of mitotic figures are restricted to specific regions of interest that are determined at coarser resolutions. At a given resolution, a clustering is performed with the following steps: the image is simplified by discrete regularization (Eq. (3) with $p = 2$ and $\lambda = 0.01$) and clustered by an unsupervised 2-means clustering. A robust version of the k-means algorithm is used that is not very sensitive to initialization [5]. Except for the lowest resolution, the clustering is performed inside specific regions that is segmented at the previous resolution. The obtained clustering is spread by pixel replication at a finer level of resolution and refined in specific regions (according to domain knowledge). Since clustering is performed in a feature space, it does not take into account spatial information, and the obtained segmentation is not accurate around image edges. Moreover, the propagation of the labels across the different resolution levels is performed by simple pixel replication and the segmentation is coarse around edges. To alleviate both effects, each obtained clustering is refined by our discrete semi-supervised clustering (presented in Section 2.4) in a narrow band around the boundaries of the clusters. The whole segmentation strategy can be summarized by Algorithm 1 where I_i denotes an image at resolution level i. For the sake of simplicity, we do not specify how the tiling of the images is taken into account. At level 0, the image is simplified, classified into two classes and the obtained clustering is spatially refined. The simplification of the image is performed by discrete graph regularization with the image modeled as an 8-adjacency grid-graph weighted by a Gaussian kernel. At the next levels, the clustering obtained at a resolution level i is projected on resolution level $i+1$ by simple label replication. Then, the image is simplified, a new clustering into two classes is performed inside one of the classes of the previous level, and the obtained clustering is spatially refined. For levels 0 to 2, the clustering is performed on RGB feature vectors. For level 3, dedicated to mitosis extraction, the clustering is performed on a specific feature vector. Mitosis are characterized by a color close to the brown that can be stressed by chromatic information with the Red-Cyan difference ($2R-G-B$). The feature vector used to represent each pixel is the local entropy of the Red-Cyan difference that is computed in a square neighbordhood around the pixel.

Algorithm 1 Multi-resolution WSI segmentation

$I_0^s = \text{Regularization}(I_0)$
$I_0^c = \text{2-MeansClustering}(I_0^s)$
$I_0^r = \text{SpatialClusteringRefinement}(I_0^c)$
for $i = 1$ to 3 **do**
  $I_i^p = \text{ReplicatePreviousResolutionClustering}(I_{i-1}^r)$
  $I_i^s = \text{Regularization}(I_i)$
  $I_i^c = \text{2-MeansClustering}(I_i^s)$ inside one class of I_i^p
  $I_i^r = \text{SpatialClusteringRefinement}(I_i^c)$
end for

One key-point of our proposal is the spatial refinement of the clustering obtained at a given resolution level. This procedure is detailed in the following.

3.2. Clustering spatial refinement

Let $V^i = \{u_1^i, \ldots, u_N^i\}$ denote the set of vertices of a grid-graph $G^i = (V^i, E^i, w)$ associated to an image at a given i^{th} resolution level. Each vertex is associated with a cluster label $l : V \rightarrow \mathbb{N}$ previously obtained by k-means clustering. The set of vertices associated to one cluster j is: $V_j^i = \{u \in V^i : l(u) = j\}$ where $j = 0, \ldots, C$ with $C+1$ the final number of classes. The aim of the clustering spatial refinement is to modify the labels assigned to vertices in order to have a clustering that is better delineated along its boundaries. Therefore, we use our discrete semi-supervised clustering formulation. In this case, all the vertices are initially labeled and their label can be modified by the regularization process (Eq. (3)) with λ set to zero. Moreover, since the spatial refinement has to be performed only around the boundaries of objects, we consider a specific grid-graph that is a subset of the whole grid-graph. The set of vertices that corresponds to the boundaries between two different clusters at i^{th} resolution level is defined by : $\partial V^i = \{u \in V^i : \exists v \in V^i \text{ with } (u,v) \in E^i \text{ and } l(u) \neq l(v)\}$. The set of vertices that belongs to a narrow band of size $2\delta+1$ around the set ∂V^i is defined by: $\partial^+ V^i = \{u \in V^i : \exists v \in \partial V^i \text{ with } d(u,v) \leq \delta\}$ where $d(u,v)$ is the distance of the path $\{u = v_1, v_2, \ldots, v_n = v\}$ with $\forall i\ (v_i, v_{i+1}) \in E^i$. The set of edges E^{i+} is defined as the subset of edges in E^i that connects two vertices of $\partial^+ V^i$: $E^{i+} = \{(u,v) \in E^i : u \in \partial^+ V^i \text{ and } v \in \partial^+ V^i\}$. The clustering spatial refinement is then accomplished by k regularization processes on the graph $G^{i+} = (\partial^+ V^i, E^{i+}, w)$ as described in Section 2.4. The clustering spatial refinement with increasing resolutions is illustrated in Figure 2.

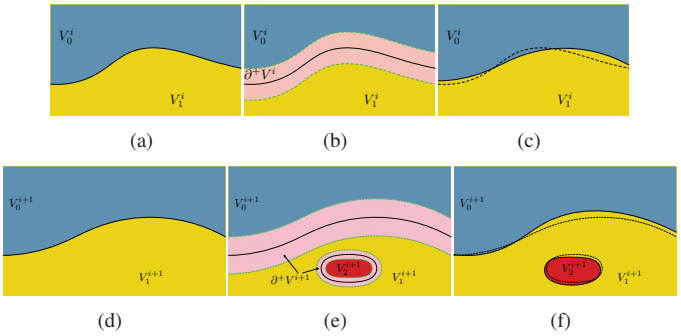

Fig. 2. Illustration of the multi-resolution clustering. First resolution level analysis: (a) 2-means classification, (b) superimposed narrow band of (a), (c) clustering spatial refinement of (b) with the original boundary of (a) superimposed and dotted. Second resolution level analysis: (d) labeled image by label replication of factor 2 of (c), (e) 2-means classification inside one class of interest with superimposed narrow band, (f) clustering spatial refinement of (d).

4. RESULTS

As stated by Figure 1, our multi-resolution segmentation adds one level of analysis at each resolution level. We illustrate the powerfulness of our proposed method on three whole slide images of breast carcinoma immuno-stained for mitosis highlighting. Image were obtained with a ScanScope digitizer (Aperio Tachnologies, Vista, CA) at 20x magnification scale and stored with JPEG compression (75% of quality). The images at full resolution are up to 30000px$\times$40000px large. The three other resolution levels correspond to a linear resolution decrease of factor 4. Figure 3 illustrates the processing for each image at the first three levels: one discrimination is performed at each resolution level. Since the final objective of the processing is the enumeration of mitotic figures for proliferation estimation, an illustration of mitosis detection is also provided. The whole multi-resolution segmentation process takes 30 minutes on a standard computer (2GHz with 1 GB of RAM) without any multithreading or multicore optimization.

5. CONCLUSION

In this paper, a multi-resolution image analysis strategy for automatic enumeration of mitotic figures on whole slide images is proposed. The whole classification process begins with the lowest resolution image and moves to higher resolution into regions of interest gradually identified. Graph-based regularization provides a unified formalism for both image simplification and spatial cluster refinement. Contrary to methods than can be found in literature, our method is totally unsupervised and has the advantage of reducing the amount of data to be processed at each resolution level by selecting regions of interest.

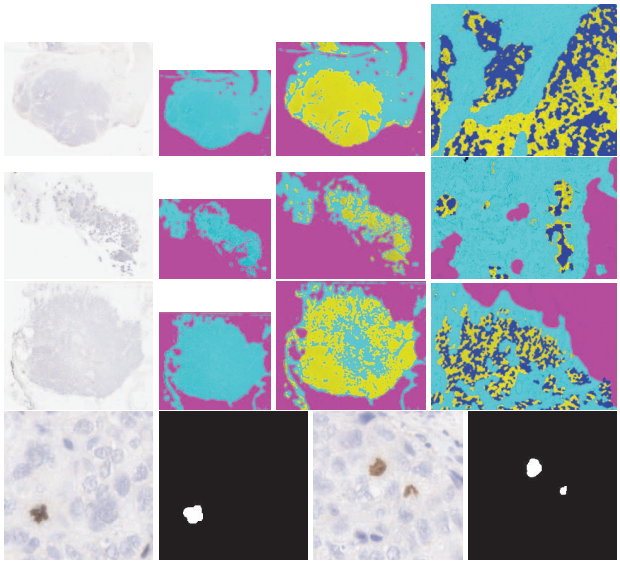

Fig. 3. Illustration of the multi-resolution clustering process. Three first rows (from left to right): initial image, first resolution segmentation result (background in pink, tissue in cyan), second resolution segmentation result (lesion in yellow), third resolution cropped segmentation result (tumor cells in yellow, stroma in dark blue). Last row: mitosis extraction on two tiles at full resolution.

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