Biomedical Microscopic Image Processing by Graphs

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Abstract

The authors present an overview of part of their work on graph-based regularization. Introduced first in order to smooth and filter images, the authors have extended these methods to address semi-supervised clustering and segmentation of any discrete domain that can be represented by a graph of arbitrary structure. This framework unifies, within a same formulation, methods from machine learning and image processing communities. In this chapter, the authors propose to show how these graph-based approaches can lead to a useful set of tools that can be combined altogether to address various image processing problems in pathology such as cytological and histological image filtering, segmentation and classification.

Keywords

Graph-based models, regularization, semi-supervised clustering, filtering, segmentation, classification, cytological and histological image analysis.

Introduction

In microscopic cellular imaging, the objective of segmentation is the extraction of cellular or tissue components. This problem is a difficult problem due to the large variations of the structures' features. There are several strategies for segmenting images and a lot of different segmentation methods can be found in literature. For instance, histogram analysis, pixel classification, region growing, morphological segmentation or methods based on partial differential equations (PDEs) can be mentioned. PDEs-based methods are very effective tools that enable to perform a lot of different image processing tasks under a unified formalism, see for instance (Malladi & Sethian, 1996; Adiga & Malladi & Fernandez-Gonzalez & Solorzano, 2006; Chan & Shen, 2005; Aubert & Kornprobst, 2006) and references therein. Recently, data sets analysis and machine learning methods have received a lot of attention. They are based on graph Laplacian diffusion processes and have been used to perform data sets dimensionality reduction or classification (Belkin & Niyogi & Sindhwani, 2006; Zhou & Scholkopf, 2005; Lafon & Lee, 2006).

In this chapter, we use our recently proposed discrete regularization framework based on weighted graphs (Elmoataz & Lézoray & Bougleux, 2008) to address both the microscopic cellular image segmentation and classification problems. This framework is inspired by continuous regularization and data-dependent function analysis methods. It provides a unified formulation of functionals regularization between PDEs-

based methods from image processing and data analysis from machine learning community. These tools constitute a framework. Within this framework, a large variety of operations can be performed, combined or derived, to produce a specific segmentation scheme for a given problem. This framework leads, on the one hand, to a family of linear and nonlinear filters. On the other hand, it provides label-based diffusion processes for image automatic and interactive segmentation. One of the specificity of the proposed framework is to use graphs as a discrete modeling of images at different levels (pixels or regions) and different component relationships (grid graph, proximity graph, etc.). Working on graphs, our framework leads to a set of flexible tools for image segmentation, image regularization or clusters extraction. The main purpose of this chapter is not to solve a particular class of cytology or histology problems but to show how, with our graph-based methodology, we can address particular image segmentation problems.

The chapter is organized as follows. Next section presents a modeling of microscopic imaging problems in pathology. It also defines the core structure of our approach, the weighted graphs and the discrete calculus performed on such structures. Section called "Graph-based regularization models" introduces our discrete regularization framework. From this formulation, we derive our discrete label diffusion method (semi-supervised clustering) and show how we can improve classical pixel-based semi-supervised segmentation by using region-based graphs. Section entitled "Experiments" presents applications for automatic and interactive color cytological and histological image segmentation and finally compares our approach with other methods. All the experiments or illustrations presented in this chapter are obtained with a standard Linux computer equipped with quadri 2.4 GHz Intel Xeon processors and 16 GB of RAM. Finally, last sections describe future works and conclusion.

Background

Modeling of microscopic imaging problems in pathology

Pathology is roughly composed of two sections: cytology and histology. For both these sections, the visual inspection of cellular specimens and histological sections through a light microscope plays an important part in clinical medicine and biomedical research.

Cytology literally means the study of cells. It studies morphological features of human body fluid cells that are put on a glass slide and stained. The study of the modification of the main cellular components (nucleus and cytoplasm) is the ground of the cytological study. Cytotechnologists and cytopathologists visually evaluate the morphological features of cells and these features involve several notions including size, shape, color, texture and topography. The interaction between nucleus and cytoplasm is also of interest: the position of the nucleus in the cytoplasm, the nucleus-cytoplasm area ratio, the position of nucleols in the nucleus, the color and the granularity of the cytoplasm.

Histology is defined as the anatomical study of the microscopic structure of tissues. In a clinical setting, histology is used to analyze disease states at a cellular level by means of light and/or electron microscopy, histochemistry and immunochemistry. Histology studies cells that are grouped in large and complex structures: tissues. The latter cannot be only characterized by the properties of individual cells such as color staining intensity or expression of specific proteins but also by the geometric arrangement of cells and by the topographic relationships between cells.

Image processing methods are of high interest to provide Image Decision Guided Systems (IDGS) to perform prognostic, diagnostic and early detection of cancer (Lézoray & Cardot, 2002). For the case of problems in pathology, image processing can be used for several tasks: quantification of a cellular content (DNA, proteins, color), recognition and sorting of cellular types, extraction of cellular groupings or clusters and of their topographic relations. In terms of image processing problems, the objectives concern segmenting or analyzing objects at different levels: cellular or cellular grouping level. Any cytological or histological images have common properties and can be described by an image object oriented modeling (Renouf & Clouard & Revenu, 2007).

First of all, the images have to be acquired in color. This is essential to follow the exact visual way a pathologist follows to evaluate microscopic images. For a microscopic image, one can always divide it in two parts: the background and the rest of the image, namely the objects to be extracted. The background is always close to a given color and is usually homogeneous even if some debris or artifacts can occur (some mucus for instance). The rest of the image is composed of the elements of interest for the pathologists. In this work, we use an image object oriented modeling of the elements of an image: they can be classified and characterized in terms of size, shape, color and texture. The objectives of the segmentation can appear at two object levels: cellular or cellular grouping. For each one of these levels, different configurations can be established. At the cellular level, one can find isolated, touching or overlapping cells. At the cellular grouping level, one can find groupings (groups of cells) or clusters (groups of groupings). The cellular grouping level can be seen as an upper level of the cellular level: Cells \subset Groupings \subset Clusters.

Weighted graphs

Image segmentation, in particular the case of microscopic cellular images, is an application dependent task and no general scheme or rule can be applied. In this work, we propose a set of graph-based tools to address the microscopic cellular image segmentation problems. One of the specificities of these tools is to use graphs as a discrete modeling of images at different levels. This section recalls basics and discrete calculus on graphs that constitute the basis of our methodology.

Preliminary definitions and graph construction

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 of N vertices, a set of edges $E \subset V \times V$, and a *weight function* $w : V \times V \rightarrow R^+$. 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 (see (Diestel, 2005) for details on these notions). 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 H(V) be the Hilbert space of real valued functions on the vertices of a graph. Each function f of

H(V) assigns a real value f(u) to each vertex $u \in V$. The function f forms a finite N-dimensional space and can be thought as a column vector. Similarly, let $R_w(f, p)$ be the Hilbert space of real valued functions defined on the edges of the graph. These two spaces are endowed with the usual inner products.

Any discrete domain can be modeled by a graph. In image processing, this structure is commonly used to represent digital image. In machine learning community, graphs are usually used to represent data sets and relationships between data points. Many typical structures can be quoted:

- *Grid graphs* (Chan & Osher & Shen, 2001) that are natural structures corresponding to the definition of digital images: vertices represent pixels and edges represent pixel adjacency relationships.
- *Region Adjacency Graphs* (RAG) (Trémeau & Colantoni, 2000) that provide very useful and common ways of describing the structure of a picture: vertices represent regions and edges represent region adjacency relationships.
- *Proximity graphs* (von Luxburg, 2007), for instance the k-nearest neighbor graph, where each vertex is associated with a set of k close vertices depending on a similarity criterion. For a given

vertex $u \in V$, if we consider all the vertices $V \setminus \{u\}$ as the vertex u neighborhood then, the associated proximity graph is the fully connected graph.

Graph structures are extremely useful and occur naturally while processing digital images. A graph can be associated with any color image representation according to the definition of a distance or a similarity. In that case, processing images is reduced to processing graphs. The weight function measures the similarity between two vertices of the graph. When $w(u,v) \rightarrow 0$, the two vertices u and v are dissimilar. To weight a graph, the following standard Gaussian weight function can be used. For two vertices $u, v \in V$ and an edge $(u,v) \in E$, the edge weight is $w(u,v) = \exp(-|f(u) - f(v)|_2^2 / \sigma^2)$ where σ is a scaling parameter living on the data that depends on the application, and $|\cdot|_2$ is the L2-norm.

The topology of the graph depends on the problem under consideration: grid graphs for image simplification/segmentation, region adjacency graphs for image segmentation/analysis. In this chapter, the proposed experiments are performed with different graph topologies to show the flexibility and the behavior of our methods.

Discrete difference, gradients and p-Laplacian operators

We consider that a graph G = (V, E, w) and a function $f \in H(V)$ are given. The weighted difference operator $d: H(V) \to H(E)$ of a function f on an edge (u, v) linking two vertices $u, v \in V$ is defined as $(df)(u, v) = \sqrt{w(u, v)}(f(v) - f(u))$ (1)

This operator leads us to define the directional derivative of f, over an edge (u,v), as $\partial_v f(u) = (df)(u,v)$. Then, the weighted gradient operator $(\nabla_w f)(u)$ is defined as

$$(\nabla_w f)(u) = (\partial_v f(u))_{(u,v) \in E}.$$

It corresponds to the local variation of the function f at the vertex u and measures the regularity of the function in the adjacent neighborhood of u. The corresponding L2-norm is

$$|\nabla_{w}f(u)|_{2} = \left[\sum_{v \sim u} (\partial_{v}f(u))^{2}\right]^{1/2} = \left[\sum_{v \sim u} w(u,v)(f(v) - f(u))^{2}\right]^{1/2}$$

Then, the weighted p-Laplace operator $\Delta_w^p f(u)$ at vertex u is defined as

$$(\Delta_w^p f)(u) = \sum_{v \sim u} \gamma(u, v)(f(v) - f(u)) \quad \text{where } \gamma(u, v) = w(u, v)(|(\nabla_w f)(u)|_2^{p-2} + |(\nabla_w f)(v)|_2^{p-2}).$$
(2)

Clearly, in the case where p = 1 and p = 2, we have the definitions of the standard graph curvature $\Delta_w^l f = \kappa f$ and graph Laplacian $\Delta_w^2 f = \Delta f$. More details on these definitions can be found in our previous works (Elmoataz & Lézoray & Bougleux, 2008; Lézoray & Bougleux & Elmoataz, 2007a).

Graph-Based Regularization Models

In this section, we present a set of graph-based models for microscopic image processing. These models constitute a set of flexible methods that can be combined or derived to produce a specific segmentation scheme for image segmentation, image regularization or clusters extraction. In the sequel, we present our discrete regularization framework. From this framework, a label propagation method for image automatic or interactive segmentation is derived. Moreover, we also show how we can improve classical semi-supervised segmentation methods by using a simplified version of the original image.

Regularization problem formulation and diffusion processes

The regularization of a function $f^0 \in H(V)$ can be viewed as the following variational problem on graphs:

$$\min_{f \in H(V)} \left\{ E_w(f, f^0, \lambda, p) = R_w(f, p) + \frac{\lambda}{2} |f - f^0|_2^2 \right\}$$
(3)

The first term $R_w(f, p)$ is the regularizer and is defined as, with 0 :

$$R_{w}(f,p) = \frac{1}{p} \sum_{u \in V} |(\nabla_{w}f)(u)|_{2}^{p} = \frac{1}{p} \sum_{u \in V} [\sum_{v \sim u} w(u,v)(f(v) - f(u))^{2}]^{p/2}$$
(4)

The second term is the fitting term. $\lambda \ge 0$ is a fidelity parameter called the Lagrange multiplier that specifies the trade-off between the two competing terms. Both terms of E_w in (3) are strictly convex function of f (Chan & Osher & Shen, 2001). By standard arguments in convex analysis, this optimization problem has a unique solution for p = 1 and p = 2 which satisfies, for all $u \in V$:

$$\frac{\partial}{\partial f(u)} E_w(f, f^0, \lambda, p) = \Delta_w^p f(u) + \lambda (f(u) - f^0(u)) = 0.$$
⁽⁵⁾

Equation (5) can be viewed as the discrete analogue of the Euler-Lagrange equations. Using the *p*-Laplacian formulation (2) in (5), the optimization problem solution is also the solution of the following system of equations. For all $u \in V$

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

To approximate the solution of the minimization (3), we can linearize this system of equations and use the Gauss-Jacobi method to obtain the following iterative algorithm:

$$f^{(t+1)}(u) = \frac{\lambda f^{0}(u) + \sum_{v \sim u} \gamma^{(t)}(u, v) f^{(t)}(v)}{\lambda + \sum_{v \sim u} \gamma^{(t)}(u, v)} \text{ with } f^{(0)}(u) = f^{0}(u), \qquad (6)$$

where $\gamma^{(t)}(u,v)$ is the γ function defined in (2) at the iteration step *t*. At each iteration of the algorithm, the value of *f* at step (t+1), for a vertex *u*, only depends on two quantities: the original value f^0 and the sum of weighted local variation of the existing values in the neighborhood of *u*. By using different formulations of *w* and different values of *p*, a family of linear and nonlinear filters is obtained. Indeed, when p = 2 and w(u,v) = 1, one obtains the linear diffusion on graphs. When p = 1 and w(u,v) = 1, one recovers the TV digital filter (Chan & Osher & Shen, 2001). The reader can note that this isotropic regularization corresponds to the weighted discrete transcription of the regularization functional in the continuous case. The interested reader can refer to (Elmoataz & Lézoray & Bougleux, 2008; Lézoray & Bougleux & Elmoataz, 2007a). Moreover, in (Ta & Bougleux & Elmoataz & Lézoray, 2007a), we have extended this discrete isotropic regularization to a discrete anisotropic regularization framework for image and data processing.

Through the values of the p parameter, the discrete regularization (5) describes a family of linear and nonlinear filters. This image filtering/denoising can be viewed as an image simplification that can ease for instance a seed extraction step or any image post-processing step. Figure 1 shows a nonlinear filtering on noisy cytological and histological images represented by an 8-adjacency grid graph. This image filtering enhances image components such as edges information, as shown in Figure 1(b)-(d) as compared to original ones in Figure 1(a)-(c). Same effects can be observed on gradient images, Figure 1(f)-(h) as compared to Figure 1(e)-(g). The interested reader can find in (Lézoray & Bougleux & Elmoataz, 2007b) qualitative and quantitative comparisons of the proposed family of filters with other filters that can be found in literature.





Figure 1: Color cytological and histological image filtering. (a) and (c) initial images. (b) and (d) filtered images (nonlinear filtering with p=1). (e) and (g) gradient images from original ones. (f) and (h) gradient images from filtered ones.

Semi-supervised clustering

Numerous automatic segmentation schemes have been proposed in literature and they have shown their efficiency. But, sometimes, automatic segmentation results are not accurate when images are more complex. Meanwhile, recent interactive image segmentation approaches have been proposed. They reformulate image segmentation tasks into semi-supervised classification approaches by label propagation strategies (Wang & Zhang & Shen & Wang, 2006; Grady, 2006; Sinop & Grady, 2007). Other applications of these label diffusion methods can be found in (Zhou & Scholkopf, 2005; Belkin & Niyogi & Sindhwani, 2006). Our previously presented discrete regularization framework can be naturally adapted to address this learning problem for semi-supervised segmentation. The adaptation of our regularization framework leads to a clustering method. In the sequel, we use the terms of semi-supervised classification and segmentation as the same procedure but additional computation steps must be performed to obtain final results. Indeed, a final classification is obtained by class membership probabilities estimation. To obtain the final image segmentation, a labeling of connected image components must be performed.

Let $V = \{u_1, \dots, u_N\}$ be a finite set of data, where each data u_i is a vector of \mathbb{R}^m . Let G = (V, E, w) be a weighted graph such as data 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 to estimate the unlabeled data from labeled ones. Let $C = \{c_i\}_{i=1,\dots,k}$ be the set of initial *labeled* vertices and let $V \setminus C$ 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) $f_i^0 : V \to R$, with $i = 1, \dots, k$. For a given vertex $u \in V$, if u is initially labeled then, $f_i^0(u) = +1$ if $u \in c_i$, $f_i^0(u) = -1$ otherwise. If u is initially unlabeled (i.e. $u \in V \setminus C$) then, $f_i^0(u) = 0$. Finally, the vertex clustering is performed by k regularization processes by estimating the resultant function $f_i: V \to R$ for each *i* th class. Using our proposed discrete regularization framework, this is formalized as the follows minimization problem:

$$\min_{f_i \in H(V)} \left\{ R_w(f_i, p) + \frac{\lambda}{2} |f_i(u) - f_i^0(u)|_2^2 \right\}, \text{ where the first term } R_w(f_i, p) \text{ is the one defined in (4).}$$

We use the discrete diffusion process (6) to compute each minimization. At the end of the label propagation processes, the class membership probabilities can be estimated and the final classification

obtained by the following formulation: $\arg \max_{i} \left\{ f_i(u) / \sum_{i} f_i(u) \right\}$, for all $i \in 1, ..., k$ and $u \in V$. Any

other formulations can be used, for instance see (Chapelle & Scholkopf & Zien, 2006). To obtain final image segmentation, a connected image components labeling can be performed on classified elements.

Region-based image semi-supervised segmentation

Most of the classical image semi-supervised segmentation methods are usually based on image pixel diffusion strategies. Nevertheless, if we consider large images, these methods are difficult to apply due to the large mass of data to analyze. To avoid this computational restriction and to provide fast image segmentation, we propose to use a simplified version of the image in place to work with the pixel-based representation (Ta & Lézoray & Elmoataz, 2007b). One possible representation is to construct a fine partition of the image and to consider neighborhood relations between the obtained regions. Many methods have been proposed to construct image fine partition in literature. For instance, see (Ren & Malik, 2003) for methods based on graph cuts algorithm or (Meyer, 2000) for the ones based on morphological operators.

In this work, we propose to use a graph-based method inspired by an approach based on the generalized Voronoi diagram and the energy partition (Arbelaez & Cohen, 2004). This method can be viewed as an image simplification or a graph reduction. Starting from a set of k seeds $S = \{s_i\}_{i=1,...,k} \subseteq V$ over a graph G = (V, E, w), this method associates each vertex to the closest seed depending on a metric function $\delta: V \times V \rightarrow R^+$ that can be defined as

$$\delta(u,v) = \min_{\rho \in P(u,v)} \sum_{i=1}^{m-1} \sqrt{w(u_i, u_{i+1})} (f(u_{i+1}) - f(u_i))$$

where $f \in H(V)$, P(u,v) corresponds to the set of all paths connecting two vertices $u, v \in V$. A path $\rho(u,v)$ is a sequence of vertices $(u_1,...,u_m)$ such as $u = u_1$ and $v = u_m$ with $(u_i, u_{i+1})_{i=1,...,m-1} \in E$.

Then, the influence zone z of a given seed $s_i \in S$ is the set of vertices that are closer to s_i than any other seeds. This influence zone can be defined as $z(s_i) = \{u \in V : \delta(s_i, u) \le \delta(s_j, u)\}$ for all j = 1, ..., k with $j \neq i$. Finally, the energy partition of G for a given set of seeds S and a metric δ is the set of all influence zones.

To obtain the initial set of seeds several methods can be used. In image processing, a common approach is to use the image local minima or maxima in a fixed search window (see for instance (Levner & Zhang, 2007) for another seeds extraction approach).

Figure 2 shows an example of application of this method to simplify a cytological image.

One can note the significant data reduction in term of image components while also respecting image main information. The original image has 134 400 components (image pixels) and the number of obtained zones corresponds to 5% of the original one (in this chapter we use both terms zone or region when referring to the partition components). The reconstructed image is obtained by assigning a model for each region in the partition. A simple model can be a mean or a median value of each zone.



Figure 2: Image simplification. From left to right: initial image of size 480×320 i.e. 134 400 pixels; partition image with 7 132 zones i.e. 95% of reduction as compared to the original one; reconstructed image from partition with region mean color as model. See text for more details.

With this simplified version of the original image, we can compute for instance the Region Adjacency Graph (RAG) by considering the dual representation of the obtained partition: the Delaunay graph (Delaunay, 1934). Each vertex of the RAG corresponds to an image region and the edges to the region adjacency relationships. Any other region-based graph can be constructed such as k-NN graphs, minimum spanning trees, etc. This data simplification can lead to further faster algorithm convergence thanks to the reduced number of vertices to consider.

Figure 3 illustrates the proposed label propagation method. It shows segmentation of cytological image into 3 classes (nuclei, cytoplasm and background) with different graph structures. This example compares the computation time and the segmentation results between a pixel-based grid graph and two region-based proximity graphs (the RAG and the fully connected graph). It also shows the robustness of our approach regarding the initial user input. For the cases of proximity graphs, the computation times include the graph construction itself. Moreover, due to the size of proposed example, the computation time for the partition construction can be ignored.

Figure 3(h) is the semi-supervised segmentation result obtained from the initial labels (Figure 3(d)) and an 8-adjacency grid graph as original image representation. One can observe the number and the precise location of the initial labels, in particular the necessary labels between the two cells. Figure 3(b) is a simplified version (98% of reduction) of the original image (Figure 3(a)). Figure 3(c) is a reconstructed image obtained from the partition where the pixel values of each region of the partition are replaced by the mean pixel color value in the original image of its regions. With this simplified version, we construct two proximity graphs associated with this image: the RAG and the fully connected graph. Figure 3(i) shows the segmentation result obtained from the RAG with the same initial labels as in the grid graph case (Figure 3(e)). We can observe that the two results in Figures 3(i)-(h) are similar but in the RAGbased segmentation case, the computation time is significantly reduced. Figures 3(j)-(k) show the segmentation result obtained from the fully connected graph. If we consider an image as a set of pixels, it is clear that this approach cannot be applied due to the computation time. But, if we consider the simplified version of images, this method becomes an efficient one and we can quote interesting properties to use this structure.

- The graph contains all the image information in the weighted edges and therefore the regularization process only needs a minimal number of iterations to reach the algorithm convergence.
- A minimal number of labels is needed to obtain correct results on the contrary of the grid graph or RAG cases. In Figures 3(f)-(g), only one nucleus and one cytoplasm are marked, and there is no separating label between the two cells.
- An interesting property is that the objects can be quickly labeled in the same class, even if they are not spatially adjacent or close. In Figures 3(j)-(k), the two main nuclei and cytoplasm are segmented even if there are no initial labels. Moreover, the label diffusion process has also found the two pieces of cytoplasm on the left and the piece of cells on the top-left corner of the image.

Moreover, these two examples show the robustness of our approach. Indeed, Figures 3(j)-(k) show similar results with two different input labels (Figures 3(f)-(g)).



Figure 3: Semi-supervised image segmentation with p=2, $\lambda=1$, t iterations for different graph topologies and user input strokes. First row: (a) initial image of size 152×181 , (b) discrete energy partition image, (c) reconstructed image from partition (mean color model). Second row: user input labels. Third row: original image with the obtained segmented regions superimposed: cytoplasm (red), nuclei (green) and regions boundaries (black); the segmentation is performed with the specified iteration steps t and the corresponding computation time. The images (h), (i), (j) and (k) are respectively obtained from label images (d), (e), (f) and (g). Graph topologies used to obtain results (h): grid graph associated with original image, (i): region adjacency graph (RAG) associated with simplified image, (j) and (k): fully connected graph associated with simplified image.

Experiments

In this section, we illustrate the abilities of the presented graph-based methods to segment automatically or interactively color cytological and histological images. Finally, we compare and evaluate our approach with other methods proposed in literature.

Automatic image segmentation by label propagation on a minimum spanning tree

In this experiment, the considered image is from a database of digitized cells images (serous cytology) collected from pleural and peritoneal effusions with different pathologies (Lézoray & Elmoataz & Cardot, 2003). In this class of images, the cytoplasm and the nuclei are respectively colored in green and blue. Both cytoplasm and nuclei have to be segmented.



(a) 26 000 pixels (b) 936 zones (96% of re- (c) Original + Labels duction)



Figure 4: Automatic cytological segmentation by label diffusion processes based on a MST. (a) initial image. (b) reconstructed image with partition and region mean color value. (c) original image with labeled seeds superimposed; the seeds were classified by k-means (k=3) algorithm. (d) and (f), obtained regions map with the specified λ parameter. (e) and (g) original image with the obtained regions boundaries superimposed in white.

Figure 4 shows automatic color cytological image segmentation by label propagation on a MST. First, from the original image (Figure 4(a)) we compute a simplified image (Figure 4(b)) and construct a regionbased Minimum Spanning Tree (MST). A MST is a well-known structure in graph theory and gives a connected graph with no cycles and it is also sparse (for N data points, it has only N-1 edges). We use the set of seeds (used to obtain the image partition) as initial labels of our label diffusion approach. Seeds are classified into 3 classes by a classical k-means algorithm (based on region color features). Result of this seeds classification is shown in Figure 4(c). One can note that this classification is not perfect and some nuclei seeds have been classified as cytoplasm ones. Figures 4(d)-(e)-(f)-(g) show respectively the obtained regions maps and the regions boundaries for different values of λ parameter of the proposed label diffusion algorithm. When λ is positive value, the label diffusion is highly oriented by initial labels. When λ is null, the algorithm has the ability to modify initial labels classification. Figure 4(f) shows label modification effects on classification results. Bad initial cytoplasm labels are changed to background label and conversely. This interesting property resides in the graph topology and in our label diffusion formulation. There are differences between a simple region merging algorithm and our automatic classification scheme. Indeed, the proposed method does not need any merging criterion to assign a label to a region: it is implicitly done by the weighted graph representation. Moreover, our method does not need any stopping criterion: the final result is obtained when the algorithm reaches convergence.

Interactive image segmentation by label diffusion processes on a region adjacency graph

Classical color histological images contain background and cellular objects. The segmentation problem of this class of images consists in extracting clusters of abnormal nuclei. In this experiment, the considered image is from a database of breast tissues digitized images, collected from different pathologies and marked with a Ki67 marker.



Figure 5: Semi-supervised (interactive) color histological image segmentation with pre-processing step. (a) initial image of size 555×770. (b) filtered image from original. (c) initial of reconstructed partition with mean color value. (d) initial image with initial labels superimposed. (e) initial image with the obtained regions superimposed (green for the clusters and black for clusters boundaries). (f) user additional labels. (g) initial image with the obtained final regions superimposed (green for the clusters boundaries).

Figure 5 shows interactive color histological image segmentation by label propagation on a RAG. This application illustrates the advantage of user guideline segmentation. When images become much more complex, automatic segmentation results are not always accurate and a user's correction becomes necessary to obtain the desired result. In this application, first we filter the original image (Figure 5(a)) by our regularization method on an 8-adjacency grid graph to ease the seeds extraction for the partition construction. The image filtering result is shown in Figure 5(b). With this simplified image, we construct a partition to obtain a significant reduced version of the original image (Figure 5(c)). A RAG is constructed with this reduced image where each vertex corresponds to an image region with region mean color as model. Our label diffusion process is performed with this graph and the initial user labels (Figure 5(d)). The obtained segmentation is shown in Figure 5(e). One can note that the segmentation is not correct. The initial labels are not enough precise to separate the clusters. Figure 5(f) shows the user additional labels to correct the segmentation and Figure 5(g) shows the corrected final classification result.

Evaluations

In this section, we compare one of our proposed schemes with other segmentation methods proposed by literature. The chosen scheme is the interactive image segmentation. This scheme is compared with the following methods.

- Two approaches based on k-means and Bayesian classifications proposed by Lézoray et al. (2002).
- Two approaches based on pixel and subwindow random tree classifications proposed by Dumont et al. (2007).

For more details on these methods, the interested reader can refer to the corresponding articles. The test set is composed of ten images from serous cytology (Lézoray & Cardot, 2002). Cells are colored by the international standard coloration of Papanicolaou. Two classes are considered: nuclei pixels and other pixels. Figure 6 shows three examples of the test set with the expert manually segmented ground truth (second row) and the segmentation results of our interactive scheme (third row). To compare classification schemes, we use four common classification rates based on well-classified pixels.



Figure 6: Three examples of the test dataset images. First row: initial images. Second row: expert ground truth. Third row: segmentation results with our interactive scheme.

Table 1: Global classification rates and comparison with Dumont et al. approaches (2007).

	Pixel based	Subwindow based	Our scheme
R_0	95.93%	96.39%	98.31%

Table 2: Classification rates and comparison with Lézoray et al. approaches (2002).				
	<i>k</i> -means	Bayesian	Our scheme	
R_1	88.7%	97.53%	92.31%	
R_2	98.65%	95.40%	99.15%	
R_3	93.67%	96.47%	95.73%	

Tables 1 and 2 show classification rate comparisons between our interactive scheme and the above mentioned pixel-based schemes relying on random decision trees proposed by (Dumont & Marée & Geurts & Wehenkel, 2007), and two classification models (k -means and Bayesian) proposed by (Lézoray & Cardot, 2002). Best accuracies are face bolded. Classifications rates R_0 , R_1 , R_2 , and R_3 are expressed as follows:

 $R_0 = \frac{\text{number of pixels well classified}}{\text{number of pixels of the image}},$

 $R_1 = \frac{\text{number of nuclei pixels well classified}}{\text{number of nuclei pixels of the image}},$

 $R_2 = \frac{\text{number of background pixels well classified}}{1}$

number of background pixels of the image

$$R_3 = \frac{R_1 + R_2}{2}$$

Results in Table 1 show that our approach has the best classification accuracies as compared to methods proposed by (Dumont & Marée & Geurts & Wehenkel, 2007). Table 2 presents classification accuracies per class for the three methods under consideration. Our approach clearly outperforms k-means and Random Decision Trees but is less accurate than Bayesian classification for nuclei pixels. These results can be interpreted as follows. One of the advantages of our interactive scheme is that even if the segmentation is not correct, the user can modify his input labels until obtaining the desired results. The consequence is that we can obtain better results as compared to an automatic scheme since rates are very close. Regarding the background pixel and the nuclei pixel classification rates, one can note that Bayesian classification tends to over segment the nuclei and our approach tends to under segment them.

Future Research Directions

The proposed graph-based framework constitutes useful tools to address several image processing problems such as filtering, segmentation or classification. Moreover, this framework uses arbitrary graphs as an underlying representation and a unified formulation. This strong specificity implies that our graphbased tools can be easily adapted to be used with any discrete domain that can be represented by a graph. Hence, it leads to an interesting ongoing work: to use the same tools and formulation to analyze, categorize or visualize segmented cellular objects in image databases.

Conclusion

In this chapter, we have considered a framework of graph-based tools for microscopic images segmentation. These tools (filtering, simplification, clustering, segmentation and classification) can be combined altogether or in addition with classical approaches to obtain schemes for the segmentation of a particular class of cytological and histological images problems in pathology. Moreover, the proposed framework is sufficiently general to be applied to any type of microscopic images. We have proposed efficient automatic and interactive user guideline segmentation techniques. Moreover, our formulation works with graphs of arbitrary structure. This point provides on one hand, fast segmentation algorithms via image simplification and/or graph topologies and on the other hand, the interesting property to segment nonlocal (non spatially close or adjacent) image components.

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