COLOR AND TEXTURE BASED SEGMENTATION OF MOLECULAR PATHOLOGY IMAGES USING HSOMS

Manasi Datar[1]*, Dirk Padfield*[2,3]*, and Harvey Cline*[2]

[1]GE Global Research, Hoodi Village, Whitefield Road, Bangalore, India
[2]GE Global Research, One Research Circle, Niskayuna, NY, 12309
[3]Rensselaer Polytechnic Institute, 110 8th St., Troy, NY 12180

ABSTRACT

Prostate cancer is the most common cancer among men, excluding skin cancer. It is diagnosed by histopathology interpretation of Hematoxylin and Eosin (H&E)-stained tissue sections. Gland and nuclei distributions vary with the disease grade, and the morphological features vary with the advance of cancer. A tissue microarray with known disease stages can be used to enable efficient pathology slide image analysis. We focus on an intuitive approach for segmenting such images, using the Hierarchical Self-Organizing Map (HSOM). Our approach introduces the use of unsupervised clustering using both color and texture features, and the use of unsupervised color merging outside of the HSOM framework. The HSOM was applied to segment 109 tissues composed of four tissue clusters: glands, epithelia, stroma, and nuclei. These segmentations were compared with the results of an EM Gaussian clustering algorithm. The proposed method confirms that the self-learning ability and adaptability of the HSOM, coupled with the information fusion mechanism of the hierarchical network, leads to superior segmentation results for tissue images.

Index Terms— Molecular pathology, Hematoxylin and Eosin staining (H&E), Hierarchical selforganizing maps (HSOM), Color and texture segmentation, Feature extraction, Region merging, prostate cancer, Tissue microarray (TMA), Gleason score, Tumor staging, k-means clustering.

1. INTRODUCTION

Prostate cancer is the most common cancer among men, excluding skin cancer. The American Cancer Society estimates over 220,000 new cases of prostate cancer and nearly 30,000 deaths in the United States per year. A prostate cancer diagnosis is typically established by histopathology using Hematoxylin and Eosin (H&E)-stained tissue sections [1], and pathologists use human pattern recognition to grade prostate cancer. Digital microscopy is becoming increasingly popular in pathology, and morphology from H&E slides obtained with image processing has been correlated with cancer [2, 3]. Tissue microarrays (TMA) are used for high-throughput pathology research wherein multiple tissues are simultaneously processed to remove staining variability and to reduce labor. The tissue cores from different patients are embedded in a paraffin block and sliced to give multiple registered arrays. TMAs are used in drug discovery to test the protein expression in tissues with a range of known outcomes [4]. A first step is segmentation of the image into constituent tissue types, which enables detection of abnormal regions in a pathology slide.

In contrast to the classical methods of image segmentation like thresholding and region merging, unsupervised clustering approaches have the advantages of parallel processing (with appropriate hardware) and adaptability. Hierarchical Self-Organizing Maps (HSOM) provide a framework for unsupervised clustering, and we propose a method for segmentation of glands, nuclei, epithelial tissue, and stroma from molecular pathology images using a 2-stage HSOM. In Section 2, we describe our formulation of HSOMs as applied to microscopy images. In Section 3, we demonstrate qualitative results of the HSOM relative to EM Gaussian clustering. And in Section 4, we state our conclusions and steps for future work.

2. METHODS

The flowchart in Figure 1 describes the various stages in the segmentation process using HSOMs. The elements of the flow chart are described in the following subsections.

2.1. Feature Extraction

Each pixel is represented as a 7-dimensional vector {R, G, B, T_1, T_2, T_3, T_4} of features suitable for training and subsequent classification using the HSOM.

The first three components are R, G, B values for every pixel in the image. There have been efforts to distinguish stain colors using an unmixing algorithm such as [5]. While such algorithms help in quantification and also to compute statistical metrics such as one required for image registration, they may not aid segmentation. The nonlinear nature of the RGB

Corresponding author: Manasi Datar, email: manasi.datar@ge.com

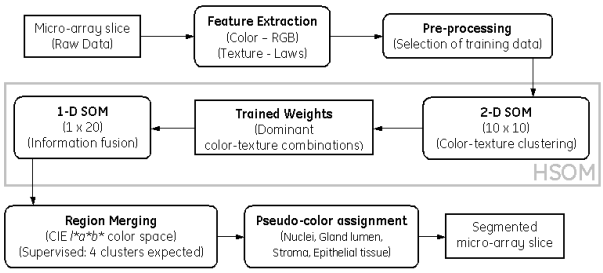

Fig. 1. Flowchart of the proposed method.

color space introduces interdependence between the channels, and any such relationship - reflected in a particular combination of R, G, B values - will aid the classifier. Hence we retain this color space for feature extraction.

The next four components (T_1-T_4) are texture features obtained by applying Laws' filters [6]. Laws developed a set of symmetric and anti-symmetric center-weighted, two-dimensional masks derived from five simple one-dimensional filters. A Principal Component Analysis (PCA) revealed four masks which significantly capture the underlying texture. The image is convolved with each of the four masks to obtain corresponding texture energy images. The choice is justified by the fact that this local convolution approach is computationally simple and is adequate for the application at hand.

2.2. Hierarchical Self-Organizing Map

The hierarchical self-organizing map (HSOM) is a variant of the self-organizing map (SOM), proposed by Kohonen [7]. A SOM usually consists of *M* neurons located on a regular 1- or 2-dimensional grid. Higher dimensional grids are generally not used as they are difficult to visualize.

The training mechanism for the basic SOM is iterative. Each neuron *i* has a *d*-dimensional prototype vector $\mathbf{m}_i = [m_{i1}, ..., m_{id}]$, where $d = 7$ in our case. At each training step, a sample data vector $\mathbf{x}$ is randomly chosen from the training set. Distances between $\mathbf{x}$ and all prototype vectors are computed. The best-matching unit (BMU), denoted here by *b*, is the map unit with prototype closest to $\mathbf{x}$:

$$\|\mathbf{x} - \mathbf{m}_b\| = \min_i \{\|\mathbf{x} - \mathbf{m}_i\|\} \qquad (1)$$

Next, the prototype vectors are updated. The BMU and its topological neighbors are moved closer to the input vector. The update rule for the prototype vector of unit *i* is:

$$\mathbf{m}_i(t+1) = \mathbf{m}_i(t) + \alpha(t) h_{bi}(t)[\mathbf{x} - \mathbf{m}_i(t)], \qquad (2)$$

where *t* denotes time, $\alpha(t)$ is learning rate and $h_{bi}(t)$ is a neighborhood kernel centered on the BMU. The learning rate $\alpha(t)$ and neighborhood radius of $h_{bi}(t)$ decrease monotonically with time.

The hierarchical SOM (HSOM) can be defined as a two-stage SOM whose operating principle is:

1. For each input vector $\mathbf{x}$, the best matching unit is chosen from the first layer map, and its index *b* is input into the second layer;

2. The best matching unit for input *b* is chosen from the second layer map, and its index is the output of the network.

The advantage of using the HSOM is that each high dimensional data vector is mapped to a low-dimensional discrete value so that comparing the values implicitly contains comparison of the original distances.

2.3. Region Merging

The output of the HSOM is, more often than not, an over-segmented image. The colors obtained at the end of HSOM testing stage can be perceived as labels assigned by the classification scheme. Conceptually, the clusters represent neuron states. Hence, such neurons can be merged to arrive at a perceptually consistent segmentation. This is achieved by a homogeneity-based region-merging algorithm similar to [8]. While [8] works with real color values in the image, we are looking to merge similar neurons based on the color characteristics learned by the network.

The RGB color space was used for feature extraction as relationships between the various components due to its nonlinear nature are utilized in the classification process. However, this very characteristic can prove detrimental when used in a distance-based algorithm. Perceptually linear color spaces - where a change in color value is nearly equal in magnitude to a change in visual importance as perceived by humans - are desirable for such computation. Hence, the color characteristics of the neurons are transformed into the CIE $(L^*a^*b^*)$ color space for region merging. The texture components are excluded from due to the lack of a similar perceptually linear space.

Since we are looking for four classes, merging is carried out until we have four clusters left. The segmented image thus generated is seen to have more homogeneity within the regions and more disparity between them. Finally, the segmented image is recolored for ease of visualization using the following mapping: nuclei (blue), stroma (red), epithelial tissue (pink), and glands (white).

3. RESULTS

Prostate cancer TMAs were obtained from Cytomyx and Imgenex containing a total of 109 tissue elements consisting of both human prostate cancer and normal controls. H&E stained slides of size 1024*768 were acquired using a 10X objective with a white light microscope (Leica) stored as 24

bit RGB files for image processing. The tissue microarray elements were digitized and processed to segment the different tissue compartments. A normal prostate tissue image at 10X shows glands with an epithelial layer and nuclei arranged along the boundary and stroma in the interior.

Here we present qualitative comparisons of the results of the HSOM to the clustering method using EM Gaussian clustering initialized with a K-means [9] algorithm.

To initialize the K-means clusters, regions of interest were manually selected in the gland lumen, epithelial tissue, stroma and nuclei, and the average intensity in each RGB channel was measured. An iterative K-means procedure was used for each image in the series to find accurate cluster centers after initialization with the measured average intensity. The EM Gaussian algorithm [10] is a maximum likelihood algorithm where the clusters are fit to a mixture of Gaussians wherein the most likely tissue is used to segment the image. Using the initialization from the K-means, a further improvement is estimating the mean μ_m and covariance Σ_m to fit a mixed Gaussian distribution to the sampled pixels. The covariances are then used to estimate the likelihood of each tissue.

The HSOM results (shown in the right column of Figure 2) demonstrate that the nuclei and gland segmentation are similar to the EM Gaussian method. However, the distribution is different for the epithelial and stroma tissues, which are spectrally the most similar classes. The HSOM algorithm is better able to separate these two tissues through the use of texture features in addition to spectral features.

The EM Gaussian method (results shown in the middle column of Figure 2) tends to produce fragmented segments as variation about individual cluster centers is not adequately modeled during training. The HSOM classification has more discrimination. The neuron-merging stage ensures increased homogeneity within individual segments and also provides better discrimination between different segments. This observation also emphasizes the advantage derived by using pixels from the available image for training, as opposed to an extensive set of training images. This is a significant advantage as it is difficult to compose a training set which captures all the variations occurring in clinical data.

4. CONCLUSIONS AND FUTURE WORK

We have presented a method using HSOMs for segmenting H&E prostate TMAs into tissue compartments. The proposed two-stage HSOM approach combines the advantages of unsupervised learning and labeling of the clustered outputs. The HSOM detects dominant color and texture features in the given tissue, which are subsequently used to segment the tissue by pixel classification. We compared the results of the HSOM to those obtained using an EM Gaussian mixture model. The use of texture in the HSOM improves the ability of the algorithm to discriminate among the four classes in the tissue, especially between the epithelial and stroma tissues.

Future work includes extracting discriminate features from the segmentations that can be correlated with the Gleason and tumor staging scores [11]. This will enable automatic staging of tissue images, which can then be used for training new pathologists and can serve as a second reader in the analysis of such images.

5. REFERENCES

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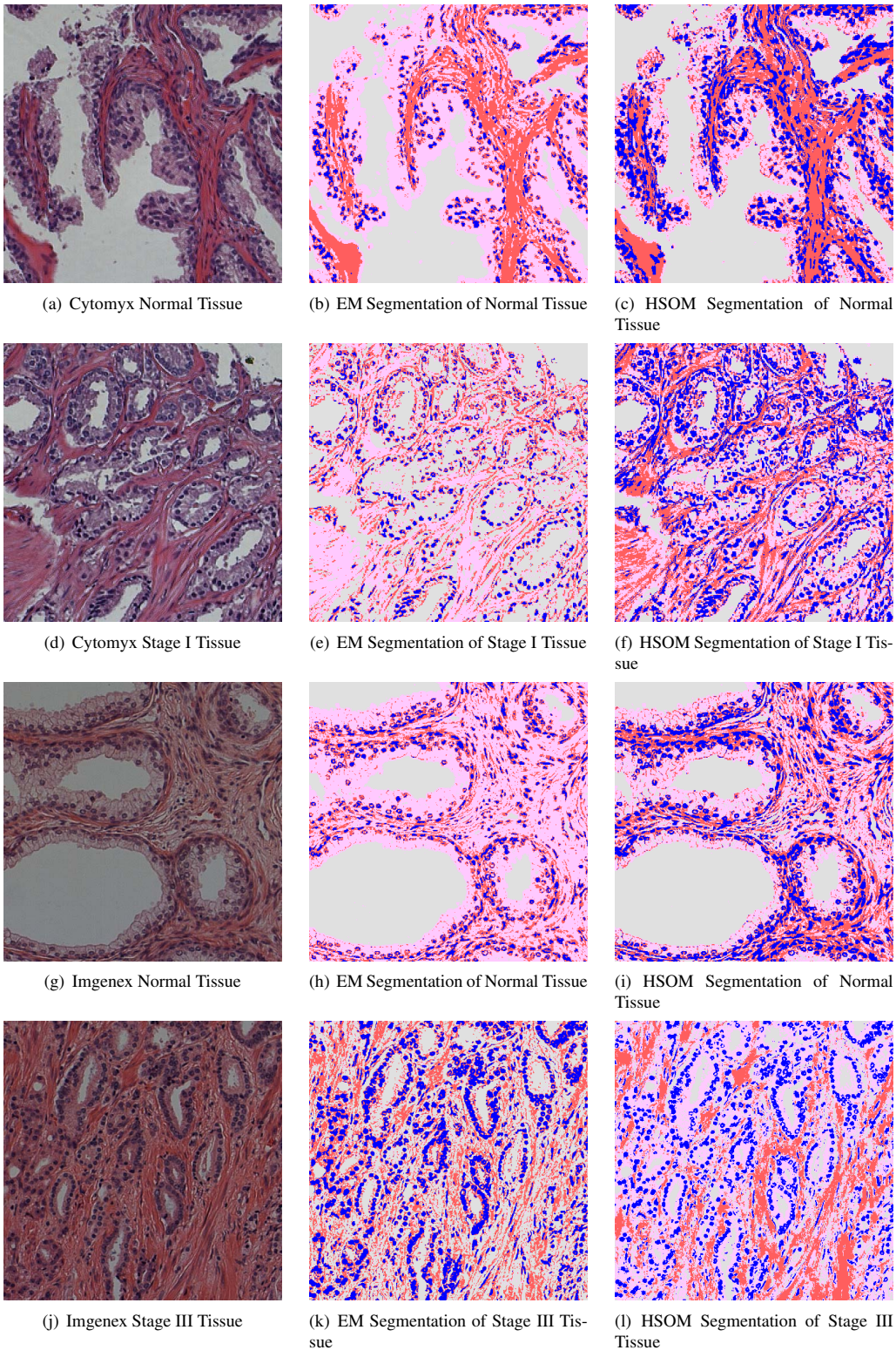

(a) Cytomyx Normal Tissue (b) EM Segmentation of Normal Tissue (c) HSOM Segmentation of Normal Tissue

(d) Cytomyx Stage I Tissue (e) EM Segmentation of Stage I Tissue (f) HSOM Segmentation of Stage I Tissue

(g) Imgenex Normal Tissue (h) EM Segmentation of Normal Tissue (i) HSOM Segmentation of Normal Tissue

(j) Imgenex Stage III Tissue (k) EM Segmentation of Stage III Tissue (l) HSOM Segmentation of Stage III Tissue

Fig. 2. Comparison of segmentation results on Cytomyx and Imgenex datasets. The images in the left column are the original images, in the middle column are the EM segmentations, and on the right are the HSOM segmentations. The first and third rows give examples of normal tissues, and the second and fourth rows give examples of various tumor stages. Because of the use of texture, the HSOM method is better able to separate the spectrally similar stroma and epithelial tissues.