

Segmentation of Immunohistochemical Staining Of B-Catenin Expression of Oral Cancer

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ABSTRACT

Oral Cancer is any cancerous tissue growth located in the mouth, also called as Squamous Cell Carcinoma . It has been one of the serious cancer that affect the south Asian countries. Oral cancer has variable diagnosis method and the one is biopsy. Histopathological results suffers from considerable inter and intrareader variability even when used by expert pathologists. In order to get both qualitative and quantitative results, we has been developed a system for diagnosis of oral cancer using EM algorithm. The microscopic images of immunohistochemical staining of β -catenin expression are segmented using iterative method of EM algorithm to extract the cellular and extracellular components of an image. The segmentation process of the system uses unitone conversion to obtain a single channel image using PCA that has highest contrast then normalize the unitone image to the [0,1] range. Based on the segmentation process we conclude that β -Catenin expression using EM algorithm is an efficient technique to help the pathologist to evaluate the histological changes on microscopic images of oral cancer.

Keywords – Immunohistopathology, Oral Squamous Cell Carcinoma, PCA, Unitone, EM Algorithm.

I. INTRODUCTION

ORAL cancer is the cancer that starts in the mouth or oral cavity and is especially seen disadvantaged in elderly males. It is one among the 10 most common cancers worldwide, with 2,80,000 new cases of oral cancer found every year. It has been one of the serious cancer that affect the South Asian Countries. In India, it is the sixth most common malignancy reported with high mortality ratio. They are highly curable if found and treated at an early stage. The early detection is expected to increase when the patient's awareness regarding the danger of oral cancer increases. More than 90% of all oral cavity cancers are Oral Squamous Cell Carcinoma(OSCC), and tobacco, alcohol, and betel consumption are the main risk factor for these and many potentially malignant lesions(PML) groups. The adult males who use tobacco and alcohol are the main high risk groups.

Early diagnosis of OSCC makes the dentists speed proceeding to further treatment. For this, the patients to seek an dentist at an early stage. The standard method of revealing PML and OSCC is the conventional oral examination, which

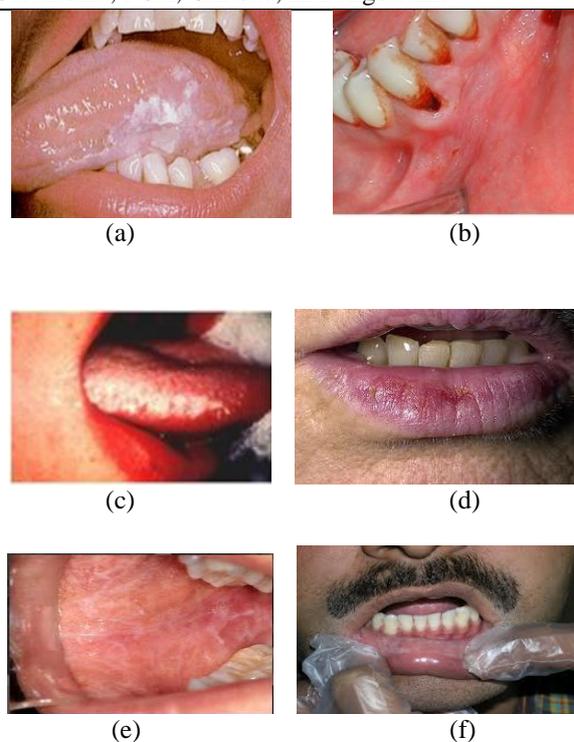


Fig. 1. (a) Carcinoma of tongue,(b) Erythroplakia,(c) Leukoplakia,(d) Actinic Cheilitis,(e) Lichen Planus,(f) Oral Squamous Fibrosis

includes biopsy and histopathological examination by confirming clinical suspicion. Currently, a biopsy with histopathology is considered the gold standard for diagnosis of OSCC. However, it is a rather slow process, requiring several days to fix, embed and stain the biopsy specimen before results can be available. It is subject to interpretation of pathologist, and although it can detect cellular and molecular changes if special techniques are employed. OSCC may be preceded by clinically evident PMLs, particularly Erythroplakia (Fig.1.b) and leukoplakia (Fig.1.c). Erythroplakia is rare, and presents as a velvety plaque. At least 85% of cases show frank malignancy or severe dysplasia (precancerous) and carcinomas are seen 17 times more frequently in erythroplakia than in leukoplakia even though leukoplakias are far more common. Leukoplakia is the the most common potentially malignant oral lesion and may also be potentially malignant, the transformation ranging from 3-33% over 10 years [1]. The other potentially malignant lesions or conditions may include actinic cheilitis lichen (Fig1.d), lichen planus (Fig1.e), and oral squamous fibrosis (Fig1.f). Early diagnosis and treatment are the goals. Since the conventional oral examination has undetermined sensitivity and specificity, there is a need for more accurate diagnostic tool that can detect early lesions and determine either potentially malignant or benign nature of the lesion [2]. Traditionally, pathologist use histopathological images of biopsy samples removed from patients, examine them under a microscope, and make judgements based on their clinicopathological acumen. The pathologist typically assesses the deviations in the cell structures and or the changes in the distribution of the cell across the tissue under examination are purely qualitative, and often leads to considerable variability [3]. To circumvent this problem and to improve the reliability of oral cancer diagnosis, it is important to develop a computer aided technique with the advancement of computational technique that help the pathologist to take judgement based on histopathological features. In this paper, a robust, unsupervised, and efficient segmentation technique is analyzed that uses a EM Algorithm to segment the cellular & extracellular Components of image.

II. CHARACTERISTICS OF ORAL CANCER

It is unlikely that oral squamous cell cancer arises from normal surface epithelium. The surface epithelial cells undergo gradual changes from clinically undetectable premalignant lesion to clinically identifiable premalignant lesion. These pre-malignant stages are often reversible and are readily curable. Symptoms of pre-malignant conditions can be identified by screening alone; however most often these remain unnoticed. Patients

report only after the disease advances to an irreversible malignant lesion squamous cell carcinoma developed in the oral mucosa. Oral pre-cancerous lesion has been defined by an International Working Group as 'morphologically altered tissue', which in cancer is more likely to occur its apparently normal counterpart. There are two major clinically visible pre-malignant lesions namely leukoplakia and erythroplakia. Leukoplakia, appears as white plaque, 5mm or more in width, which cannot be attributed to any other disease. Erythroplakia appears as a red plaque. The dysplastic changes may or may not be appearing these stages. It is however universally accepted that squamous



Fig. 2. The β -Catenin expression detected by immunohistochemical staining, (a) Normal Oral epithelium, (b) Oral Leukoplakia without dysplasia, (c) Oral Leukoplakia with mild dysplasia, (d) Oral Leukoplakia with severe dysplasia, (e) Oral Squamous Cell Carcinoma.

cell carcinoma can develop from these premalignant lesions. Leukoplakia is a term expressing clinical disease state, and it occurs in every intra-oral locus and shows various observations. Because a clinician is difficult to be settled with precancerous lesions in these, requires histopathology examination. Leukoplakia diagnosed as epithelial dysplasia, become malignant transformation in progression of the severity in epithelial dysplasia and the mechanism that oral mucosa epithelium constituting leukoplakia becomes malignant through a typical epithelium is not known. There are many reports on β -catenin accumulation into a nucleus of a cancer cell in the epithelial malignant tumor [4]. Localization of β -catenin in the epithelial cell membranes was observed in normal oral epithelium and oral leukoplakia, whereas expression in OSCC was low or totally absent in the cell membrane. The expression of β -catenin in normal oral epithelium was observed on the cell membrane, but not within the nuclei (Fig 2.a). In oral leukoplakia without dysplasia, the expression of β -catenin was observed on the cell membrane and certain portion of nuclei (Fig 2.b). In oral leukoplakia with mild and severe dysplasia, the expression of β -catenin was observed in the nuclei at about 30% and 67% (Fig 2.c, 2.d). In oral squamous cell carcinoma (Fig 2.e), the β -catenin expression was observed in the nuclei at about 80%.

III. PROPOSED SYSTEM

Medical image segmentation techniques can be classified into three broad categories [5,6]: structural, statistical and hybrid techniques. Here, the image data has been segmented using statistical techniques. Statistical techniques are applied on the discrete image data without any consideration for the structure of the region. This technique performs segmentation on the entire data set into different region. The accuracy and quality of segmentation depends on the selection of initial parameters. This technique could be made robust against noise for a particular problem and also tuned to perform segmentation on low contrast image datasets. This paper presents, the system of diagnosis of Oral cancer using EM Algorithm, to extract cellular and extracellular components from an image using statistical technique. This method is advantageous because it can suppress the decision making mechanism as well as the visual quality assessment will easier. This method is implemented by taking β -Catenin expression detected by immunohistochemical staining of three histochemical images: Oral Luekoplakia without Dysplasia (Fig.2.b), Oral Leukoplakia with Dysplasia (Fig.2.d), Oral squamous Cell Carcinoma,(Fig.2.e)

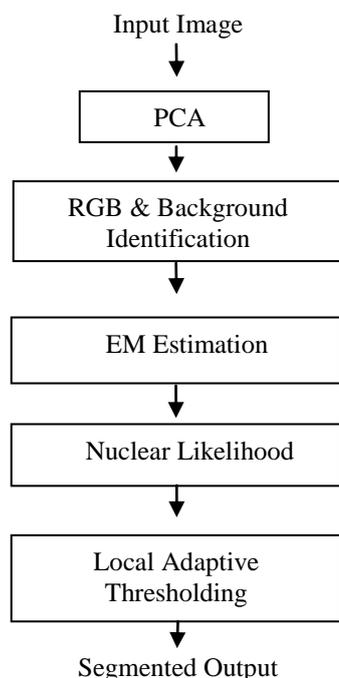


Fig. 3 Segmentation of Cellular Components

Fig. 3 shows the flowchart of the proposed image-analysis system, which mainly consists of five steps: PCA, RGB & Background Identification, EM Estimation, Nuclear Likelihood, and Local Adaptive thresholding.

3.1 High Contrast Image Using PCA

The nuclear and cytoplasmic regions are colored to hues of blue and purple by immunohistochemical stain, while protein-rich collagen structures such as extracellular material is colored into hues of pink. But these images have a considerably limited dynamic range in the color spectrum because, due to the application of chemical dyes[7]. We first convert the input images in the RGB color space onto a 1-D unitone image using the principal components analysis (PCA)[8].The unitone image is computed by projecting the RGB image onto the principle component associated with the

$$m_x = E\{X\} = \frac{1}{K} \sum_{k=1}^K X_k \quad (1)$$

$$C_x = E\{(X - m_x)(X - m_x)^T\} \quad (2)$$

$$X = A^T y + m_x \quad (3)$$

where m_x and C_x are the mean and covariance of the variables (i.e., the RGB components) hence, the resulting unitone image has the highest contrast. We further normalize the unitone image to the [0,1] range.

3.2 RGB And Background Identification

In RGB, all the color appears in their primary spectral components of red, green and blue. We identify the RGB model consists of three components, one for each primary color. All values of R,G and B are normalized in the range [0,1].

3.3 Segmentation of Individual Cells Using EM Algorithm

Modeling the distribution of both cellular and extracellular components with a Gaussian mixture model, we estimate the mixture parameters using the expectation maximization (EM) algorithm [8]. The unknown parameters are $\theta = \{\mu_H, \sigma_H, \mu_E, \sigma_E\}$, where μ_H, σ_H and μ_E, σ_E are the mean and variance of the distributions associated with cellular and extracellular structures, respectively. The EM is an iterative method, which starts with a random initialization. It consists of two steps: expectation (4), which computes the likelihood with respect to the current estimates and maximization (5), which maximizes the expected log likelihood.

$$Q(\theta, \theta^t) = E[\log P(X, Y|\theta)|X, \theta^t] \quad (4)$$

$$\theta^{(t+1)} = \arg \max Q(\theta, \theta^t) \quad (5)$$

where $x = \{x_1, \dots, x_n\}$ are the observations (i.e., the pixel values) and $Z = \{z_1, z_2\}$ are the latent variables that determine the component from which the observation originates. Once the underlying

distributions are estimated, we compute the posterior probability for each pixel as follows:

$$p(\omega_i | x) = \frac{p(x|\omega_i)P(\omega_i)}{\sum_j p(x|\omega_j)P(\omega_j)} \quad (6)$$

where $i = \{c, ec\}$ indicate cellular or extracellular components, and $p(x|\omega_i)$ is normally distributed as $p(x|\omega_i) \approx N(\mu_i, \sigma_i)$.

3.4 Cellular-Likelihood Image Using Sigmoid Function.

We construct the Cellular –Likelihood image using posterior probabilities and the estimated parameters of the unitone values. We use a sigmoid function which can be controlled with two parameters as follows:

$$f_{Cell_LK}(X) = \frac{1}{1+e^{-\alpha(x-\beta)}} \quad (7)$$

where α controls the smoothness of the s-shaped likelihood curve and β indicates the offset where $f_{Cell_LK}(\beta) = 0.5$. These parameters are tuned adaptively for each image such that $\beta = \mu_H + 2 * \sigma_H$ and $\alpha = -50(\mu_E - \mu_H)$, where μ_H, σ_H are the estimated parameters of the distribution of the unitone values associated with cellular components, and $(\mu_E - \mu_H)$ are proportional to how well these distributions are separated from each other.

3.5 Representation of Binary Image by Adaptive Thresholding

To obtain the binary representation of cell structures we apply locally adaptive thresholding step such that the threshold value is computed differently for each pixel value based on the distribution of likelihood values within its neighborhood as follows:

$$\tau(r, c) = \frac{1}{N_w^2} \sum_{i=-N_w/2}^{N_w/2} \sum_{j=-N_w/2}^{N_w/2} I_{Cell_LK}(i, j) \quad (8)$$

where r, c are the row and column indices, i, j are the offset indices within the local neighborhood, and $N_w = 15$ defines neighborhood window size and I_{Cell_LK} is the likelihood image.

IV. SIMULATION RESULTS

The result of segmentation is based on visual interpretation model and a quantitative evaluation[9]. This method is highly subjective, it accords with the solution for the segmentation of the image. The segmentation results could be easily utilized by medical image application, such as microscopic image classification and information extraction. Here, the accuracy of EM Algorithm depends on the selection of initial parameters. Based on the simulations performed, We conclude that, EM algorithm segmented the cellular & extracellular components of the image very well and the Adaptive Thresholding increases the accuracy, gives better segmentation for visibility. The results are robust, accurate and quantitative.

Simulations were performed on three microscopic images of the β -catenin expression

detected by immunohistochemical staining of oral cancer (Fig 2) for the segmentation of cellular and extracellular components. Oral Leukoplakia without dysplasia, Oral Leukoplakia with Dysplasia and Oral Squamous Cell Carcinoma are selected images for the simulations. For each image we applied the proposed image analysis system.

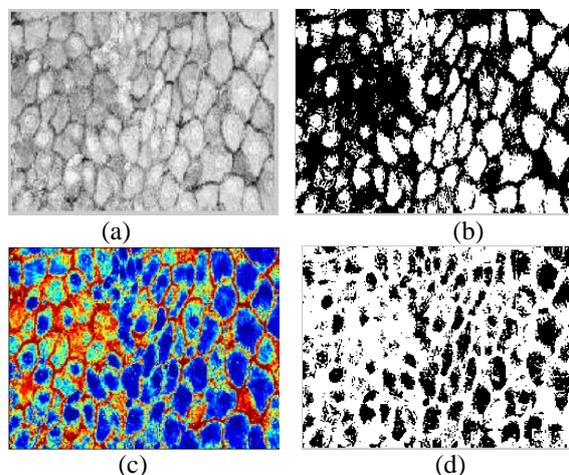


Fig. 3(a) The segmented image of the β -catenin expression detected by immunohistochemical staining of Oral Leukoplakia with dysplasia.(a) unitone image,(b) Segmented cellular & Extracellular image,(c) Cellular Likelihood Image,(d) Adaptive Thresholded Image.

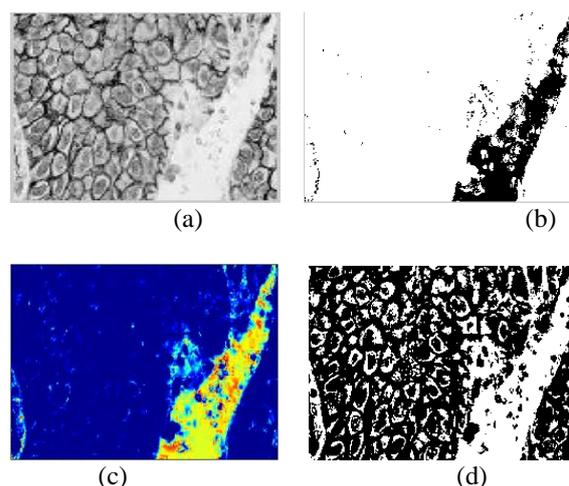


Fig. 3(b) The segmented image of the β -catenin expression detected by immunohistochemical staining of Oral Squamous Cell Carcinoma.(a) Unitone image,(b) Segmented cellular & Extracellular image,(c) Cellular Likelihood Image,(d) Adaptive Thresholded Image.

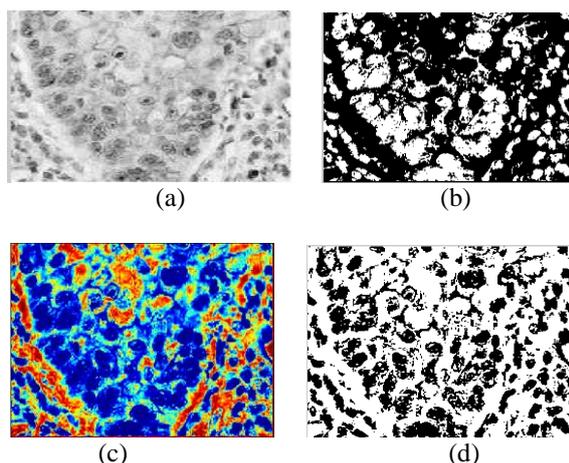


Fig. 3(c) The segmented image of the β -catenin expression detected by immunohistochemical staining of Oral Leukoplakia with dysplasia.(a) unitone image,(b) Segmented cellular & Extracellular image,(c) Cellular Likelihood Image,(d) Adaptive Thresholded Image.

Fig. 3(a) shows the segmented image of the β -Catenin expression detected by immunohistochemical staining of Oral Leukoplakia without dysplasia using PCA, EM algorithm and Local Adaptive Thresholding. Here, the performance of EM Algorithm is very poor in segmenting Cellular & Extracellular Components. In Fig.3(b), the staining of dysplasia of the oral leukoplakia is segmented by the above three methods, here the β -Catenin accumulated in the nucleus of the cell are clearly seen. The Performance of EM algorithm, and Local Adaptive Thresholding is good in Oral Squamous Cell carcinoma shown in Fig.3(c).and gives the promising results.

V. DISCUSSION AND CONCLUSION

The proposed system demonstrates the feasibility of robust segmentation of individual cells in the tissue image by EM algorithm, Principal Component Analysis (PCA) and Local Adaptive Thresholding for finding the outline of β -catenin expression detected by immunohistochemical staining of Oral cancer. The Simulation results provide promising performance by supplement the decision-making mechanism and increase the visual quality assessment. Finally we conclude that detection of β -Catenin expression using EM algorithm is an efficient technique to help the pathologist to evaluate the histological changes on microscopic images of oral cancer.

VI. ACKNOWLEDGEMENTS

I would like to thank my guide Mrs. K. Srividhya., Assistant professor, who guided me to complete this project and also thank everyone those who supported me to achieve my target.

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