Comparison of Segmentation Algorithms by a Mathematical Model for Resolving Islands and Gulfs in Nuclei of Cervical Cell Images

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Abstract: *Cell segmentation from microscopic images is the first stage of the automatic biomedical image processing, which plays a crucial role in the study of cell behaviour which is a very difficult and tedious task because of the variation that exist in illumination and dye concentration of the cells due to the staining procedure. This paper proposes a new method for segmentation of cervical cell nuclei based on a simple mathematical model to eliminate and resolve islands and gulfs which appear in the segmented output of conventional thresholding and region growing methods of segmentation. These components are eliminated and resolved and added to their related cell regions by our proposed mathematical model which first detects the borders of those structures and if it lies within the associated region they are placed within that region. The performance was evaluated and compared with the above mentioned methods. A simple mathematical vision system model to segment and analyze cytological image nuclei is proposed.*

Keywords: *Cervical cancer screening, pap smear images, islands, gulfs, mathematical model.*

Received February 26, 2013; accepted December 24, 2013; published online September 4, 2014

1. Introduction

Cytopathology is a branch of pathology that studies and diagnoses diseases on the cellular level. Pap smear test is an efficient and easy procedure to detect any malformation in cervical cells. Nowadays, the most eminent example in cervical cancer screening in its early stages is through the well-known pap smear test [20]. But human observation is not always satisfying and it is a monotonous task to manually analyze a large number of pap smear images.

Cervical cancer has no noticeable symptoms like pain, lumps at an early stage. At latest stage only, it causes pain in the lower abdominal or back regions. However, most cervical cancer takes many years to develop from normal to risky stages. A single woman dies every seven minutes now and by 2025 it is estimated one death in every 4.6 minutes [4]. Cervical cancer is a preventable disease and it can be easily detected by a routine screening test. Automate pap smear screening interacting with the human technologist, should be a good solution to reduce errors in screening slides. However, automation of the process is challenging due to the tremendous amount of data to be processed. Detecting abnormal cells in a pap smear can be referred as a needle-in-a-haystack problem. It is a very complex and not an easy problem. Cervical cancer is the cancer that forms in tissues of the cervix. (The organ connecting the uterus and vagina). It is usually a slow-growing cancer that may not have symptoms in earlier stages but can befound with regular pap tests (a procedure in which cells are scraped from the cervix and looked at under a microscope).

Cervical cancer develops in the thin layer of cells called the epithelium, which cover the cervix. Cervical cancer usually begins slowly with precancerous abnormalities and even if cancer develops, it progresses very gradually. Cervical cancer is the most preventable type of cancer and is very treatable in its early stages. Regular pap tests and Human Papilloma Virus (HPV) screening can help to detect this disease early.

Currently, cervical smear screening is the most popular method to detect the presence of abnormal cells arising from the cervix. With a small brush, cotton stick or wooden stick, a specimen is taken from the uterine cervix, smeared onto a thin, rectangular glass plate (a slide) and dyed making it easier to examine the cells under a microscope. Furthermore, there exist variances in illumination and dye concentration of the cells due to the staining procedure. Also, there are numerous variables, such as air drying, excessive blood mucus, bacteria or inflammation, which make the recognition of the suspicious cells a difficult task. The purpose of smear screening is to diagnose pre-malignant cell changes before they become cancerous. The visual interpretation of pap smear images is a tedious, time consuming and in many cases an error-prone procedure. This is a

consequence of the fact that the conventional smear exhibits uneven layering, crowding and overlapping of cells. Really it will be a difficult task for a cytopathologist to process enormous amount of data for screening. It is really a tedious task for performing image segmentation on cell images.

The manuscript is organized as follows. Section 2 describes the related works. The proposed methodology is explained briefly in section 3. Section 4 deals with results and discussions and section 5 describes experimental evaluation and in section 6 conclusions and future work are presented.

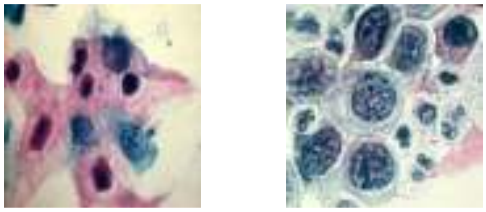
2. Related Works

Thresholding type of segmentation is computationally inexpensive and fast but the results are not reasonable because of the complexities of cell structures due to uneven illumination from inconsistent staining, poor contrast and overlapping cells [19, 27]. Even global approaches like thresholding, clustering, histogram based methods fails due to variable staining even in a single cell [19, 27]. In pixel-classification techniques [19], for the choice of the number of the classes the pixels belong to play a crucial role for the final segmentation result in pap smear images which exhibit great complexity and the number of pixel classes is not clear since, the rough assumption that all the pixels of the image are distributed into two classes, such as nuclei pixels and other pixels, would produce noisy results [1]. The Seeded Region Growing Features Extraction (SRGFE) is used to extract the size and grey level of a certain region of interest on a digital image and also it needs the user to determine the region of interest by clicking the mouse on any pixels in the region and to specify the threshold value, which makes the system impractical [17]. To avoid such problems, when a local method like marker based watershed is used, it is a tough problem to find out a corresponding marker for each object because of the overlapping nature of cells [9]. Many other cytoplast and nucleus morphological segmentation methods based on hand segmentation of images have been proposed in the related literatures [3, 5, 6, 10, 12, 14, 15, 21, 22, 25] which are really a tedious task. Generally, watershed segmentation leads to over-segmentation [23]. A parametric optimal segmentation approach leads to segmentation of non-overlapping cells but it requires a priori knowledge of nuclear characteristics such as cell shape, intensity values etc., [28]. The boundaries of the cells can be obtained by employing methods based on active contours [26], template fitting [7, 13], genetic algorithms [17], region growing with moving K-means [16] and edge detectors [2, 11, 24, 29]. But the unconnected components inside the same region are also detected as false boundaries which makes the visualisation analysis of segmentation a difficult task. Lin *et al*. [14] a genetic algorithm based ellipse detection method is used to segment object cells, but it shows the tendency of false detection when cells boundary are intersected. It also needs much prior knowledge, e.g., the cell size. In our previous method [18], cervical cell image segmentation based on bi-group enhancement and scan line filling was used to identify the areas of the nucleus and the cytoplasm in cervical images that hold only one cell or isolated cells. In our new method processing was performed on raw colour cell images of isolated as well as clustered cell. Hence, our new method can serve as a support for the cytopathologists to serve as a tool for interpretation and diagnosis of images on a cellular level.

In this paper, we propose a novel method for the automated segmentation of cervical cell nuclei in conventional pap-stained cervical cell images, which may contain both isolated cells and clustered cells whose intensities differ rapidly due to the colour staining procedure. The complex problem in the cell image segmentation lies in the vagueness on the boundary of the nuclei.

3. Methodology

The original pap smear images are as shown in Figures 1-a and b. The image shown is very complex in nature due to colour staining and the boundaries of nuclei, cytoplasm is too vague. The segmentation of these types of images is a very tedious task.



a) Pap smear image 1. b) Pap smear image 2.

Figure 1. Original pap smear images for segmentation.

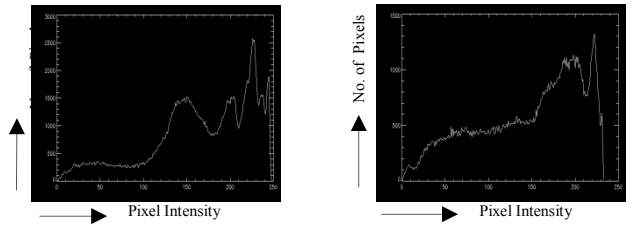
The pre-processing stage prepares the image for further processing, analysis and interpretation. The pap smear images are coloured optical images which are of poor quality due to stains used to colour the cells and uneven lighting across the field of view. The colour information is insignificant compared to the intensity of the image. Hence, in the pre-processing stage, the first step is to convert the RGB image to intensity/gray scale image. RGB image is converted to gray scale by forming a weighted sum of the R, G and B components using the equation:

$$Igray = 0.2989 * R + 0.5870 * G + 0.1140 * B \quad (1)$$

3.1. Conventional Thresholding

Thresholding is much simpler than any other segmentation techniques. The intensity histogram for Figures 1-a and b are as shown in Figures 2-a and b. In the intensity histogram of a complete cell image (i.e., of the cell and parts of the background, possibly also containing fractions of other cells), the range of intensities of interest is delimited at the low end by the lowest occurring intensity. The upper bound is defined

by the intensity at which the valley between the peaks of the background and of the cytoplasm occurs. Although, in the histogram the regions are seen as simply connected regions, their definition implies that they may consist of a number of unconnected components like islands and gulfs in both nuclei and cytoplasm. The threshold is set empirically in the intensity scale of the histogram and the images are segmented to obtain the nuclei output image as shown in Figure 3. The red shaded region implies nuclei. The output images of nuclei imply that they consist of a number of unconnected components like islands and gulfs in both nuclei and cytoplasm.



a) Histogram plot of pap smear image 1. b) Histogram plot of pap smear image 2.

Figure 2. Histograms of original pap smear images.

Figure 3. Gulfs and islands shown after nuclei thresholding.

In the case of conventional histogram thresholding the cell nuclei are not segmented legibly for visual analysis for cell image interpretation. Due to the intensity variation of the nuclei, unconnected components like gulfs and islands segments are found inside the nuclei. The visual analysis becomes very difficult for further analysis of cervical cell images. The drawback of this method can be overcome by constructing a mathematical model to eliminate the islands and gulfs of the previously thresholded images using an iterative procedure.

3.2. Region Growing

Region growing is a procedure [8] that groups pixels or sub regions into larger regions based on pre-defined criteria. The basic approach is to start with a set seed points and from these grow regions by appending to each seed those neighboring pixels have properties similar to the seed. The first order of business is to determine the initial seed points. In our cell nuclei segmentation, the borders of the pixels are declared as seeds. Based on this information we selected as starting points all pixels having values of 255. We choose two criteria to be annexed to a region. The absolute gray-level difference between any pixel and the seed had to be less than the specified threshold., To be included in one of the regions, the pixel had to be 8-connected to at least one pixel in that region. Let R represents the entire image region. We may view segmentation as a process that partitions R into n subregions, $R_1, R_2, ..., R_n$, such that:

$$\bigcup_{i=1}^{n} R_i = R \quad (2)$$

$$R_i \text{ is a connected region}, i = 1, 2, ..., n \quad (3)$$

$$R_i \cap R_j = \varnothing \quad \text{for all } i \text{ and } j, i \neq j \quad (4)$$

$$P(R_i) = \text{True for } i = 1, 2, ..., n \quad (5)$$

$$P(R_i \cup R_j) = \text{False for } i \neq j \quad (6)$$

Here, $P(R_i)$ is a logical predicate defined over the points in set R_i and $\varnothing$ is the null set. Equation 2 indicates that the segmentation must be complete i.e., every pixel must be in a region. Equation 3 requires that points in a region must be connected in some predefined sense. Equation 4 indicates that the regions must be disjointed. Equation 5 deals with the properties that must be satisfied by the pixels in a segmented region. Finally, Equation 6 indicates that regions R_i and R_j are different in the sense of predicate P.

3.3. Eliminating and Resolving of Islands and Gulfs in Nuclei

The mathematical model for eliminating and resolving of unconnected components like gulfs and islands are proposed below. The nuclei output image shown in Figure 4 of the sequential thresholding process serves as the input for our proposed mathematical model for eliminating and resolving islands and gulfs in the nuclei region.

Let $\alpha=\{\alpha_i, ..., \alpha_n\}$ represent a set of nuclei, where i= 1, ..., n.

Let $\beta=\{\beta_i, ..., \beta_m\}$ represent a set of non-nuclei, where i= 1, ..., m.

Let the boundary of β_i be γ_i, where $\gamma_i=\{ \gamma_{i1}, \gamma_{i2}, \gamma_{i3}, ..., \gamma_{il}\}$, where '$l$ ' be the number of pixels in γ_i.

If $\gamma_i \subseteq \alpha$, then β_i moved to the set α.

The steps are as follows:

1. Find β from 1-α.
2. Label the regions in β which consists of multiple non- nuclei segments $\beta_i, ..., \beta_m$.
3. Perform dilation operation on the non nuclei segments β_i with a 3×3 structuring element. Let it be denoted as x_i.
4. Find the boundary pixels γ_i of each non-nuclei segment by subtracting x_i from β_i.
5. For every boundary pixel γ_i of non-nuclei segmen β_i, for every 8 neighbour direction, If found a nuclei segment α_i at a distance<δ between any two boundary pixels γ_1 and γ_2 move β_i to α_i.

6. The following matrix shows the discontinuity between any nuclei segments α, whose boundary is not connected. Nuclei [xloc[j]-5: xloc[j] +5, yloc[j]-5: yloc[j] +5].

$$\begin{matrix}
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&\mathbf{1}&1&1&1&1&1\\
0&0&0&0&0&1&1&1&1&1&1\\
0&0&0&0&0&0&1&1&1&1&1\\
0&0&0&0&0&0&0&1&1&1&1\\
0&0&0&0&0&0&0&0&1&1&1\\
\mathbf{1}&0&0&0&0&0&0&0&0&1&1
\end{matrix}$$

7. The output after connecting two boundary pixels is as shown in the matrix below for distance $(\gamma_1, \gamma_2)<\delta$.

$$\begin{matrix}
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&0&0&0&0&0&0\\
0&0&0&0&0&\mathbf{1}&1&1&1&1&1\\
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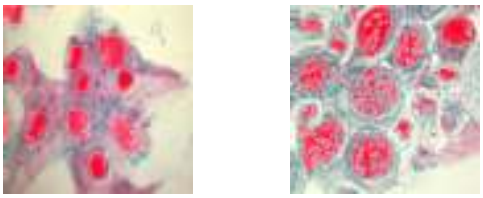
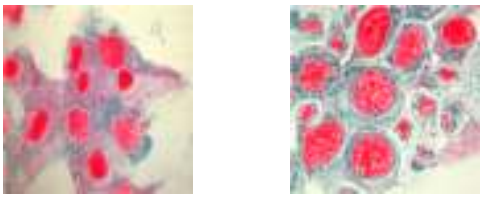
0&0&0&\mathbf{1}&0&0&1&1&1&1&1\\
0&0&\mathbf{1}&0&0&0&0&1&1&1&1\\
0&\mathbf{1}&0&0&0&0&0&0&1&1&1\\
\mathbf{1}&0&0&0&0&0&0&0&0&1&1
\end{matrix}$$

8. Hence, $\gamma_i \subseteq \alpha_i$, then β_i moved to the set α_i. Thus, the islands, gulfs like structures are eliminated and resolved using the proposed method.



a) Conventional thresholding.

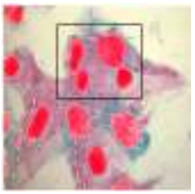


b) Proposed method.

Figure 4. Segmented nuclei images.

4. Results and Discussions

After pre-processing the cell images are segmented by conventional thresholding and region growing. In the both above mentioned segmentation methods the output image suffers multiple unconnected components like islands and gulfs like segments. Hence, in order to overcome this type of drawbacks, the output images were segmented by our proposed mathematical model. The final segmented output images were found to have produced better output for further analysis of the cell images. It is inexpensive and a very fast screening method for cervical cell nuclei when an enormous amount of data to be processed. Figure 4-b shows the segmented output after elimination and resolving of islands and gulfs. In our proposed method, the output of the first example shown in Figure 4 was found merging of the nuclei by the number of iterations of our methodology. Figure 5 shows how the optimal segmentation splitting of merging nuclei evolves with our proposed method when the numbers of iterations are reduced to segment that particular nucleus. Figure 6 shows the segmented nuclei images by region growing technique and our proposed method.



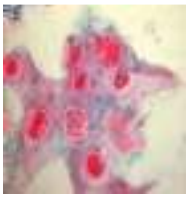
a) Merging nuclei.

b) Five iterations.

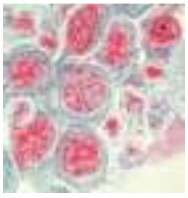
c) Three iterations.

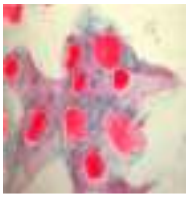
d) One iteration

Figure 5. Examples of iterative segmentation of proposed method.



a) Conventional region growing.





b) Proposed method.

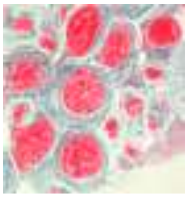


Figure 6. Segmented nuclei images.

5. Experimental Evaluation

For each image segmentation method we record the number of True Positives (TP) the number of pixels that were classified both by the algorithm and the expert as nucleus pixels, True Negatives (TN) the number of pixels that were classified both by the algorithm and the experts as non-nucleus pixels, False Positives (FP) the number of instances where a non-nucleus pixel was falsely classified as part of a nucleus by an algorithm and False Negatives (FN) the number of instances where a nucleus pixel was falsely classified as non-nucleus pixel by an algorithm. From this we can then calculate the sensitivity *SE* (or *TP* rate) as:

$$SE = \frac{TP}{TP + FN} \tag{7}$$

And the specificity *SP* (or *TN* rate) as:

$$SP = \frac{TN}{TN + FP} \quad (8)$$

$$Error\% = \left(\frac{FN + FP}{TotalPixels} \right) * 100 \quad (9)$$

The error percentage is calculated as in Equation 9. The results are tabulated as in Tables 1 and 2.

Table 1. Evaluation of segmentation for conventional thresholding and proposed method.

Images	Conventional Thresholding			Proposed Method (After Removal of Islands and Gulfs)		
	Error in %	Sensitivity	Specificity	Error in %	Sensitivity	Specificity
Pap 1	52.3	0.57	0.44	6.49	0.93	0.94
Pap 2	52.0	0.54	0.48	14.4	0.91	0.84
Pap 3	51.9	0.53	0.47	5.32	0.95	0.95
Pap 4	54.0	0.55	0.44	10.84	0.81	0.91
Pap 5	51.9	0.55	0.48	4.3	0.93	0.97
Pap 6	50.4	0.50	0.50	3.22	0.92	0.97
Average	52.09	0.54	0.46	7.42	0.91	0.93

Table 2. Evaluation of segmentation for conventional region growing and proposed method.

Images	Conventional Region Growing			Proposed Method (After Removal of Islands and Gulfs)		
	Error in %	Sensitivity	Specificity	Error in %	Sensitivity	Specificity
Pap 1	19	0.41	0.95	12	0.97	0.84
Pap 2	15.8	0.38	0.94	14.3	0.94	0.80
Pap 3	9.3	0.42	0.98	5.6	0.94	0.95
Pap 4	14.66	0.42	0.95	13.4	0.91	0.86
Pap 5	25.01	0.38	0.97	6.83	0.99	0.93
Pap 6	4.84	0.43	0.99	4.42	0.90	0.96
Average	14.77	0.41	0.96	9.42	0.94	0.89

Our proposed mathematical model was applied to the above mentioned conventional thresholding and region growing methods and the results were evaluated by calculating sensitivity, specificity and segmentation error percentage.

The average segmentation error was found to be 52.09% before removal of islands and gulfs. When our Mathematical model for eliminating and resolving islands and gulfs were used, the discontinuity due to theunconnected components in the region nuclei vanished and the segmentation error was decreased from 52.09% to 7.42%.The sensitivity i.e., *TP* rate and specificity i.e., *TN* rate graph for conventional thresholding and proposed method are as shown in Figure 8. The sensitivity and specificity of the images was found to have an average of 0.54 and 0.46 for conventional thresholding. But our proposed method showed an improved average sensitivity as 0.91 and specificity as 0.93.

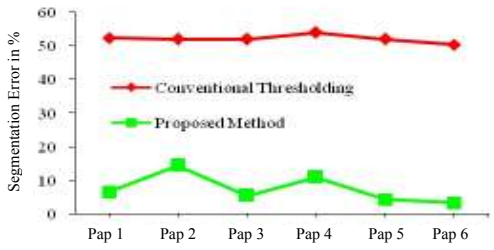


Figure 7. Comparison of segmentation error for conventional thresholding and proposed method.

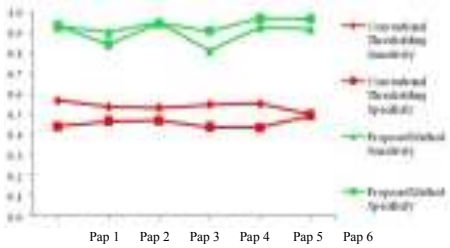


Figure 8. Comparison of sensitivity and specificity for conventional thresholding and proposed method.

Table 2 shows the evaluation performance metrics of segmentation error, sensitivity and specificity for region growing and our proposed method. There was a decrement in average error percentage from 14.77 to 9.42. The sensitivity of the images i.e., *TP* rate average had a shift from an average of 0.41 to 0.94. The specificity of the images i.e., *TN* rate average has been slightly decreased from an average of 0.96 to 0.89. The line graph for comparison of segmentation error, sensitivity and specificity for the conventional region growing and our proposed method are as shown in Figures 9 and 10.

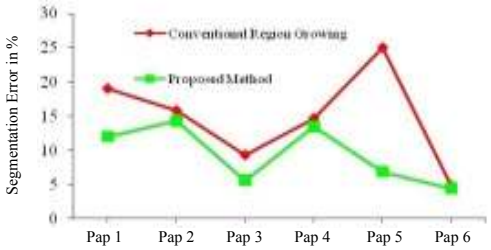


Figure 9. Comparison of segmentation error for region growing and proposed method.

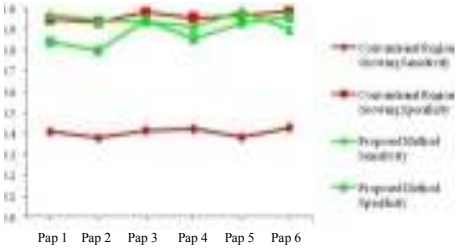


Figure 10. Comparison of sensitivity and specificity for conventional region growing and proposed method.

The output images serves as a basis for further analysis of cell images. We can make good diagnosis based on cell morphology i.e., the deviations in the cell structures. The method was evaluated for both single cell and cluster of cells. The future work can be extended for analysis in overlapping cells and splitting of nuclei in more complex clustered cells. This method can be adopted for finer segmentation where there are unconnected components like islands and gulfs.

6. Conclusions and Future Work

The task of detecting abnormal cells in a pap smear is a very complex and challenging problem. The complexity and variability of the anatomy that is being imaged is itself a difficult task and to develop a model

to visualize it is still more a multifaceted issue. Conventional thresholding and region growing techniques are drastically improved by the elimination and resolving of islands and gulfs by our simple mathematical model for healthy segmentation of cervical cell images. The same technique can be extended to cytoplasm region also.

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