Region of Interests Extraction for Analysis of Microscopic Pap Smears Images

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Abstract— This work presents an approach for analysis of Pap smear microscopic images of cervical region. Pap smear test is an efficient and easy procedure to detect any abnormality in cervical cells. But human observation is not always satisfying and it is a tedious task to manually analyze a large number of Pap smear images. Pap smear one of the most interesting fields in biomedical image processing. The proposed system gives a technique related to digital Pap smear microscopic image analysis. The image processing techniques have been used to detect cell's nucleus area and the surrounding cytoplasm. The system was designed to extract each single nucleus belong to a cell individually, and in case of clustered cells (i.e., cancerous cells) the whole cluster area is extracted as one piece.

Keywords—*Pap smear, cervical region, image processing, microscope image*, *biomedical image*

1. INTRODUCTION

The automated detection and segmentation of cell nuclei in Pap smear images is one of the most interesting fields in cytological image analysis as observed by Plissiti et. al. [1]. There is a high degree of cell overlap in such images, the presence of more than one nucleus in a cell and the lack of homogeneity in image intensity. In addition, the nucleus is a very important structure within the cell and it presents significant changes when the cell is affected by a disease and thus the accurate definition of the nucleus boundary is a crucial task. The identification and quantification of these changes in the nucleus morphology and density contribute in the discrimination of normal and abnormal cells in Pap smear images.

The segmentation of nuclei in cytological images has been studied by several researchers [2-8].

In this paper we present a method for analysis of Pap smear images based on Segmentation and ROIs extraction. The nucleus area and its surrounding plasma area will be the main target of the extraction process. This task is the hardest one due to many conflicting factors with the basic main requirements for the successful segmentation (like, cells overlapping, cytoplasm high variance due to Pap smear production sample.

The segmentation task is the major and the most difficult task in the Pap smear images analysis. Many analysis challenges are stimulated due to characteristics may appear in the microscopic Pap smear images, The color variations in different cells regions, this due to in-accurate staining process, The occurrence of cells overlapping areas, The appearance of false regions (i.e., as dark areas) at some of the boundary regions of the cytoplasm areas.

The images used in proposed system are Pap smear prepared and processed at the Pathology Division/ Department of the Central Public Health Laboratory in Baghdad by a newer method (Liquid Based Cytology) (LBC) technique, where the cells are transferred to a preservative solution prior to slide preparation, which yields specimen with less cell overlap, and considerably enhances slide quality, stained cervical cell images for further analysis, acquired through a canon power-shot G5 digital camera adapted to an Olympus optical microscope/model Bx41. The view is magnificated by 400 (high power field) and images were stored in bitmap format and having a size 2000×1600 pixels.

2. PROPOSED SYSTEM

The segmentation and extraction process was designed to consist of the following steps:

I) Color Quantization

As a first step toward making segmentation for the nucleus area (i.e., the ROI) and the surrounding cytoplasm, the segmentation was conducted using modified k-means clustering algorithm. After analyzing large number of pap smear microscope images it was noticed that there are at least 4 types of regions, the most darkest two regions represent parts of the nucleus areas. Since the cytoplasm areas appear with bright color, so the third type of regions was set bright. The white color was chosen as the type because it represents the non-cellular area.

As shown in figure1, since two hue values (i.e., blue and red) may appear as the colors of the nucleus and cytoplasm areas, so the clustering algorithm was applied such that the initialized centroid values {CnB(), CnG(), CnR()} were taken such that they lead to gray colors (i.e., deep dark, dark, bright, and white), and the determination of the new centroid values, during the each round of the k-mean algorithm, is constrained to be not highly deflected from the old values and not be far from the gray.

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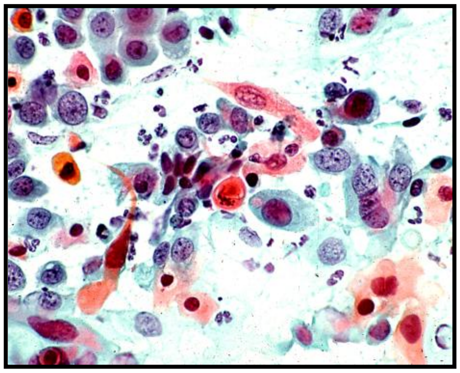


Fig.1 A sample of Pap smear microscope image

This constraint is applied by making selective distribute for the image pixels, where only the pixels that are close, up to some extent, to the nearest centroid are taken into consideration for determining the new set of centroids values, this selective distribute is controlled by two threshold values (i.e., Thr1 & Thr2); the first one is for the allowed deflection between the color components of the tested pixel with the nearest centroid value, while the second threshold is for bounding the allowed deflection of the gray deflection value. Due to the imposed constraints not all the image pixels are distributed among the clusters; only those close to the nearest centroid are nominated as members of the new clusters. Also, the two bounding thresholds values are reduced from one round to the next one in order to avoid the occurrence of large incremental drifts in the colors of centroid value.

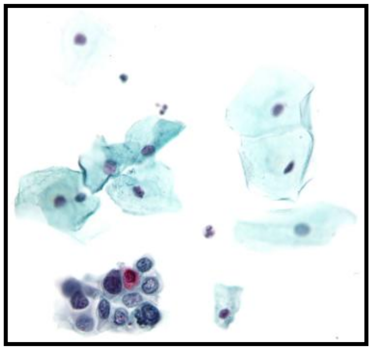
As a last step in the introduced clustering method, a non-constrained clustering round is applied, because all the image pixels should be indexed as members of one of the clusters. The color quantization based on constrained k-means algorithm.

Also in this stage, the color component values of the boundary value separating the non-cellular region from the cellular one is assessed using the following:

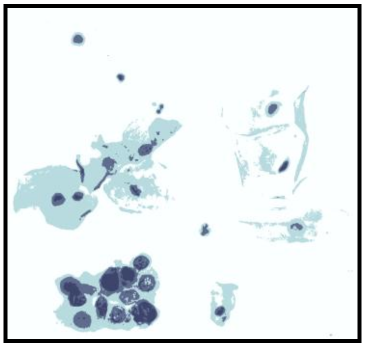
$$C_{bckgrnd}=C(NoCluster-2)+0.6\{C(NoCluster-1)-C(NoCluster-2)\} \quad ,\ldots(1)$$

Where C() represents the color components of the centroids (i.e., CnB(), CnG, CnR()}

Figure2 presents a sample of the color quantization results; the number of clusters was taken 4.



A. The Original image



B. The segmentation results

Fig.2 The results of Segmentation Using Constrained K-Mean Method

II) Binarization for Preliminary Allocation of ROI

In this stage, the nominated region of interest areas (i.e., the nucleolus areas) should be flagged from other areas. Since the nucleolus areas (especially the cancerous areas) appear always dark areas so only the pixels whose gray (or intensity) value is below certain predefined threshold value (i.e., MaxBrightness) are nominated as ROI members. Also, in this stage the boundary color values that separating the white background color (i.e., the non-cellular region color) from the colors of other regions was determined using the following equations:

$$C_{non-nucleus} = C(M) + 0.6\{C(M+1) - C(M)\} \quad , \ldots\ldots.(2)$$

Where C() represents the color components of the centroids (i.e., CnB(), CnG, CnR()}, M is the cluster index value whose members (pixels) indexed as nucleus pixels.

Figure3 presents the output of the binarization stage that applied on the segmented image shown in figure2.

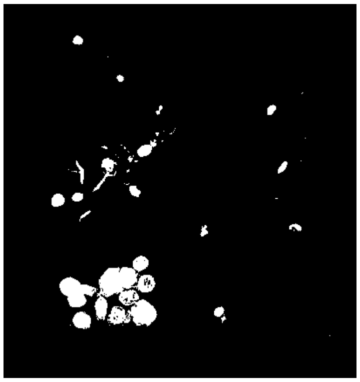


Fig.3 An output of the binarization process

III) Nucleus Areas Extraction

This stage aims to extract the areas nominated as nucleus regions. Due to existence of overlapped areas and false areas (due to pad preparation steps) this stage was developed to make different manipulation operations in order to get as solid as possible

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nucleolus regions that are empty from gaps with possible minimum overlapping.

The input data to this stage is the color values of the input pap smear image {i.e., Blu(), Grn(), Red()}, the determined binary index map array {i.e., Idx()} steps, and the determined non-nucleus threshold value(i.e., $C_{non-nucleus}$). The involved steps of the nucleolus extraction process are the following:

A. Scan the index map image using seed filling algorithm to collect the aggregation on connected nucleolus pixels. The outputs of this step are the arrays {BufX(0..J_f) & BufY(0..J_f)} which hold the column and row number of the collected pixel position, while (J_f+1) represents the number of collected points.

B. For each collected region purify it by removing the collected pixels whose brightness is too high (i.e., Blue, Green, Red) > C_NonNucleus).

C. Check the size of the collected aggregation of purified pixels: if the size is small (L<MinSize; where L is the number of pixels of the collected pixels, MinSize is a predefined parameter to specify the lowest possible cell size).

D. In case the size of collected aggregation is not too small, then, determine the two dominant colors in the purified aggregation. This step is done by applying the following tasks:

(1) Collect the mean and standard deviation of the color components of the aggregation pixels.

(2) Determine the initial values of the expected eight centroid values using the mean and standard deviation values of the color components, such that:

$$C_{Blue}(k) = M_{Blue} \pm \sigma_{Blue}$$
$$C_{Green}(k) = M_{Green} \pm \sigma_{Green} \quad ,\ldots\ldots\ldots\ldots\ldots(3)$$
$$C_{Red}(k) = M_{Red} \pm \sigma_{Red}$$

Where M denotes the mean value and σ denotes the standard deviation value. The initial centroid values are either the sum or subtraction of standard deviation from the mean value, according to the above equations there is eight probable combinations (i.e., k=0,1,…,7).

(3) Distribute the aggregation pixels among the eight clusters using the city block similarity distance measure.

(4) Choose the most populated two clusters as the initial color values for the most two dominant colors.

(5) Apply k-means algorithm to determine the best centroid values corresponding to the two dominant colors. The stopping conditions for the k-mean iterations are: (i) when reach the predefined maximum number of iterations, (ii) the differences between the new and old centroid values of all color components are small.

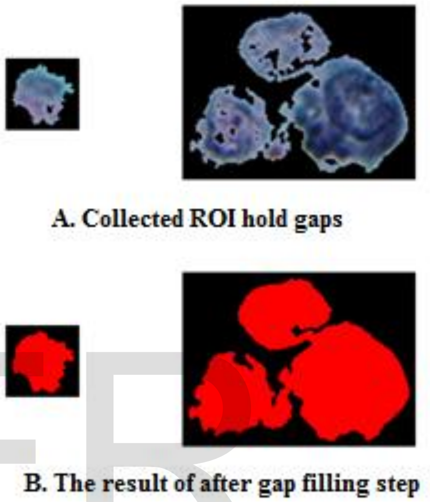
(6) Recalculate the new centroid (i.e., mean values) and the clusters dispersion (i.e., standard deviation).

(7) Determine the bounding color values for each centroid using the following:

$$B_{min}(k)=M_{Blue}(k) - 3\,\sigma_{Blue}\ ,\ B_{max}(k)= M_{Blue}(k) + 3\,\sigma_{Blue}$$
$$G_{min}(k)=M_{Green}(k) - 3\,\sigma_{Green}\ ,\ G_{max}(k) = M_{Green}(k)+ 3\,\sigma_{Green} \quad ,\ldots\ldots\ldots..(4)$$
$$R_{min}(k) = M_{Red}(k) - 3\,\sigma_{Red}\ ,\ R_{max}(k) = M_{Red}(k)+ 3\,\sigma_{Red}$$

E. Re-evaluate the membership of the pixels belong to the collected ROI. If the (R,G,B) color components of the pixel don't lay within any of the bounded range of the two dominants colors then the pixel is removed from the collected ROI.

F. Filling the gaps found inside the collected ROI: in this step the collected segment will convey some gaps inside the nucleus body; as shown in figure (4a). To remove these artificial gaps, the collected ROI is temporarily, established as a white isolated object, then, the algorithm of seed filling is applied to fill the large black hole(s) may exist within the isolated region with white color, as shown in figure (4b).



A. Collected ROI hold gaps

B. The result of after gap filling step

Fig.4 Examples of collected ROI hold gaps and the result of filling it

Since the seed filling algorithm has high computational complexity and to avoid its application on the small gaps, the very small gaps (i.e., consist of few pixels) are handled using tilling mechanism instead of seed filling.

IV) Scan Surrounding Area Using Color Connectivity Criterion

This stage is auxiliary, it is applied to more accurately collect the pixels belong to the extracted ROI but still not considered as members of it. Till this step the thresholding mechanism was adopted decide the region membership. Since the assumption of using two dominant colors to decide the membership of all pixels lay within the area of ROI convey a sort of crisp type of decisions; which may lead to pixels loss especially at the boundaries of ROI because at these place the shading effect play a significant role.

In this stage additional scanning is implemented to perform the following steps:

1. Check if the pixel doesn't belong to ROI or not, if check result is yes leave the pixel and jump to go to the next one. While if check result is yes then continue to apply the following steps.
2. Check whether the ROI pixel is at the boundary of the region or not. This check is done by counting the number of

ROI pixels surrounding it. If the number of counted pixels is less more than 6 then the tested pixel is left and the scan goes to test the next pixel. Otherwise the system goes to the next steps.

3. Open a window (3**x**3) around the tested pixel and count the local mean and standard deviation for each of the 3 color components (Blue, Green, Red). The application of bounding condition of the standard deviation values, such that they should not be too small or too large.
4. Within the window's region test each pixel that flagged as not part of the pre-collected ROI using the following gradient criteria:

If $|Pixel.Blue-M_{Blue}|/\sigma_{Blue}<R$ & $|Pixel.Green-M_{Green}|/\sigma_{Green}<R$ & $|Pixel.Red - M_{Red}|/\sigma_{Red}$ Then "The Pixel is considered as part of ROI"

Where M represents the mean value, σ represents the standard deviation, and R represent the gradient value (in this work, its value is set 1.25).

5. Apply above step for certain number of times under the stopping condition "the iteration is stopped when no surround non-ROI pixels are changed to be part of ROI, or the number of iteration reach a pre- defined number"

V) Removal of Small Artificial Gaps & Pores

This stage is, also, auxiliary, it is applied to remove some small artificial pores or gaps may due to gradient connectivity step. So, in this stage the segments of connected pixels (whether its index values is 0 or 1) are counted, if count values is small then the collected pixel are removed.

VI) Scan the Surrounding for Cytoplasm Collection

This stage is the final one; it is designed to do the following tasks:

1. Apply seed filling upon the index map array to collect the flagged pixels as ROI points. These collected points are part of the nucleus area.
2. Determine the horizontal and vertical extents of the collected area, then, establish a sub image area to establish the ROI using the collected pixels' coordinates.
3. Scan the sub-image to allocate the border (boundary) pixels; then for each found border pixel make a local scan around it, if any pixel not flagged as nucleus pixel and its intensity below the background threshold (BckCBlu, BckCGrn, BckCRed) then pixel is considered as asurrounding cytoplasm pixel.

The use of a combination consist of seed filling scanning followed by raster scanning is aimed to make fast extraction of the nucleus area with the surrounding cytoplasm.

3. TEST & RESULT

The test results indicated that the ROI extraction process is nearly between 86-88%, the failure is mainly occurred in two ways:

1. The appearance of small internal or as a marginal gaps. This artifact will reduce the discrimination power of some geometrical features in comparison with textural features,
2. Some non-cellular dark areas are considered as ROIs areas. To handle this problem an additional class called "Artificial ROI" was added to the list of ROI classes. And, depending on the utilization of some local texture features (like, concurrence or roughness) this class could be signified from other classes due to the existence of high local correlation between the adjacent pixels".

REFERENCES

[1] Plissiti M.E., Charchanti A., Krikoni O. and Fotiadis D.I., "Automated segmentation of cell nuclei in PAP smear images", ITAB Proceedings International Special Topic Conference on Information Technology in Biomedicine, Greece, Ionnia, 26-28, October 2006.

[2] Bamford P., Lovell B., "Unsupervised cell nucleus segmentation with active contours", Signal Processing 71(2), pp. 203-213, 1998.

[3] Bamford P., Lovell B., "A water immersion algorithm for cytological image segmentation", Proceedings of the APRS Image segmantation workshop, pp. 75-79, University of Technology Sydney, Sydney 1996.

[4] Mouroutis T., Roberts S. J., "Robust cell nucleisegmentation using statistical modelling", IOP Bioimaging, 6, pp. 79-91, 1998.

[5] Garrido A., Perez de la Blanca N., "Applying deformable templates for cell image segmentation", Pattern Recognition 33, pp. 821-832, 2000.

[6] Lee K.M., Street W.N., "Learning shapes for automatic image segmentation", Proc. INFORMS-KORMS Conference, pp. 1461-1468, Seoul, Korea, June 2000.

[7] Begelman G., Gur E., Rivlin E., Rudzsky M., Zalevsky Z., "Cell nuclei segmentation using fuzzy logic engine", International Conference on Image Processing, Vol. 5, pp 2937-2940, October 2004.

[8] M. Lipi, N. Dilip and N. Chandan, "Cervix Cancer Diagnosis from Pap Smear Images Using Structure Based Segmentation and Shape Analysis", Journal of Emerging Trends in Computing and Information Sciences, Vol. 3, No. 2, February 2012.

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