Automatic Colour Nuclei Segmentation and Classification of Cervical Images using Morphological Operations

S.Anantha Sivaprakasam[1] Dr.E.R.Naganathan[2]

1. Research Scholar, M.S.University, Tirunelveli, Tamilnadu, India.

2. Professor, Department of Computer Science and Engineering, Hindustan University, Chennai, Tamilnadu, India.

Corresponding Author : S.Anantha Sivaprakasam

Abstract :

In this paper, a simple and novel method is proposed for segmenting and classifying the nuclei which are extracted from cervical cytology image. This paper consists of three phases. In the first phase, the background of the cervical cytology image is removed using convolution and contrast enhancement method. In the second phase, the nuclei are segregated from the cytoplasm using Otsu thresholding, regions of interest and morphological operations. In the third phase, the classification of nuclei is carried using the morphological features such as, size, area, eccentricity, diameter and intensity The segmentation and classification accuracy are improved

Keywords : Cervical cancer, Cervical Cytology image, Image Convolution, Otsu thresholding, Morphological operation, contrast enhancement, nuclei.

1. INTRODUCTION

Cervical Cancer is cancer generally found in endocervix, ecocervix and transformation zone. The part close toward the uterus is called ectocervix and is covered by squamous cells and the part next to the vagina is called endocervix and is covered by glandular cells. The place where these two cell meet is called the transformation zone. And most of the cervix cancer starts at this zone [1]. Fig.1 gives the overview of the cervix part.

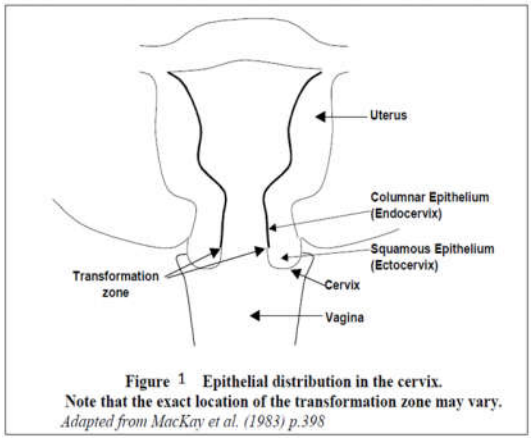


Figure 1 Epithelial distribution in the cervix

Main causes of cervical cancer are

- HPV (Humana Papilloma Virus) infection
- Having multiple sex partners
- Giving birth before the age of 22
- Smoking
- Birth Control pills
- Low socio economic status
- Inadequate intake of folic acid
- Family history of cervical cancer.

Cervical cancer is the second most common cancer affecting women worldwide but at the same time it is one of the most avertable and treatable cancers if it is detected in the early stage. And also it is second deadliest cancer in women. In developing counties and under developed countries, the awareness of the causes and effects of the cervical cancer is far less than developed counties. Every year, this cancer kills 280,000 women. In India, cervical cancer accounts for 27% (77,100) of the total cervical cancer deaths in the year 2008 [2], 33400 women were died of cervical cancer in the year 2010, 16/0.1 million women are affected by this cancer every year.

Cervical cancer can be curable if proper diagnosis is carried at an early stage. Pap Smear test is popular methods for diagnosing the cervical cancer using microscopic cervical cytology images. Features extracted from these images are used to diagnose the stages of the cervical cancer. Manual screening of cervical cytology images obtained from Pap smear test is error prone because of poor contrast, blood stain, uneven dyeing etc. Besides, it is a time consuming process. Differentiation of types of cells such as neoplastic and dysplatic can be automated in order to reduce the human errors and improve diagnosis. Malignant cells of cervical cancer have immature cytoplasm, abnormal features in nucleus, increased nucleus to cytoplasm ratio, multiple number of nucleus in a cytoplasm.

2.LITERATURE REVIEW

HPV (Human Papilloma Virus) causes virus in cervix that starts with pre-cancerous stages to developed stage ie to the cancer stages. Pap Smear test is used to diagnosis the stages of the cervical cancer. There are two types of Pap Smears – Conventional and ThinPrep. Conventional method tool is cytobrush while ThinPrep uses a brook-like device. The merit of using the ThinPrep is that it contains less contaminant and reduces clumping which makes seeing unobstructed cells much easier. Features of cytoplasm and nuclei of cytology images are play a vital role to classify the cell into benign and malignant cells, The level of malignancy is classified into LSIL (Low Grade Squamous Intraepithelial Lesion) and HSIL (High Grade Squamous Intraepithelial Lesion).[16]

Region growing, unsupervised clustering methods, such as, K-means, Fuzzy –c means clustering etc. Morphological based segmentation and watershed segmentation are the various methods available for segmentation of the cervical image into its components. [4] proposed an edge enhancement method that uses Alpha trimmed filter, bi-group enhancer and contour for cytoplasm and nuclei detection. This method is a time consuming one. Besides, it works only on single cell image. Nazahah Mustafa et.al [5] described a Seed Based Region Growing algorithm for automated multi-cells segmentation of Thin Prep Image. In this method, K-means clustering algorithm is used for segregating the image into background, cytoplasm and nuclei. A seed pixel selected using moments is used for region growing. This method is applicable for non-overlapping cell images. K-means clustering methods [6]-[9] and moving K-means clustering methods need an initial value that represents number of colors in the image to determine the regions of the interest. In Pap smear Images, the nature of cells and staining method determine the number of colors in the image.

M..E. plissiti et al [10]-[11] proposed Fuzzy-c manes reconstruction techniques. In this method, Selection of threshold for H-minima transform to eliminate regional minimal plays a crucial role. Geodesic dilation that is an iterative process plays a vital role to create marker image for morphological reconstruction and locate the position of candidate nuclei. The number of iterations of geodesic dilation depends upon the image.. Lipi B. Mahanta et al. [12] proposed a method that segments and diagnosis Pap Smear image using structure and shape. This method is not described the cut off value between normal and abnormal cell. Bustanur Rosidi et al. [13] described the classification of cervical cell based on label intensity. But this method is applicable only for non-overlapping image. Yung-Fu Chen et.al [14] proposed the semiautomatic segmentation and classification of Pap Smear cells. This method is applicable for single cell Pap Smear Image.

This paper employed the automatic segmentation and classification of cervical cell in which the nuclei is segregated using pre-processing an extraction method. The segmented nuclei is classified into normal and abnormal cell based on the morphological features, such as, size, area, eccentricity, diameter and mean intensity. This paper is organized as follows. Section III describes the colour nuclei segmentation and classification methods. Section IV describes the experimental results and analysis. Section V describes the conclusion.

3.Automatic Colour Nuclei Segmentation and Classification from Cervical Image Using Morphological Operation – Overview

The proposed image segmentation technique packed with three phase,

1. Pre-processing
2. Extraction of Nuclei from Cytoplasm.
3. Classification of nuclei

The proposed method procedure is explained through diagram given in the Figure 2. The given input RGB image is smoothened by Gaussian filtering which is used for uniform distribution of intensity. After applying the Gaussian filter, the convolution operation, contrast enhancement and decorreletion operation are applied on the image. Then, Otsu thresholding algorithm plays a vital role to extract the nuclei from the cytoplasm. To remove the unwanted area from the nuclei, morphological operation is applied that retained only the nuclei. Next, criteria selection method is applied on the image to retain only the abnormal cell. Finally, features extraction operation is carried out on the segmented nuclei to extract the morphological features of the cell for classification purpose.

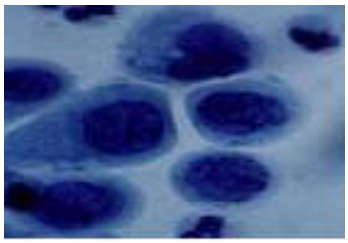


Figure 2. Input image

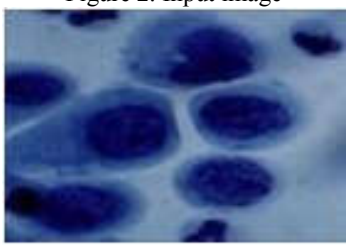


Figure 3 Filtered image

The filtered image is convoluted with the window of fixed size of R*C by a constant H as shown in the figure 5

R/C	R/C	R/C
R/C	R/C	R/C
R/C	R/C	R/C

Figure 5 Convolution kernel of size 3 * 3

--(2)

200	180
56	100

*

2/4=0.5	2/4=0.5
2/4=0.5	2/4=0.5

=

100	90
28	50

200	180
56	100

+

100	90
28	50

=

255	255
84	150

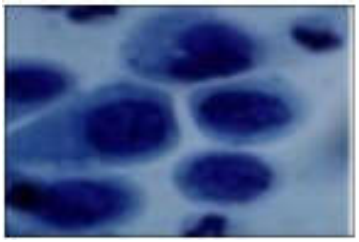
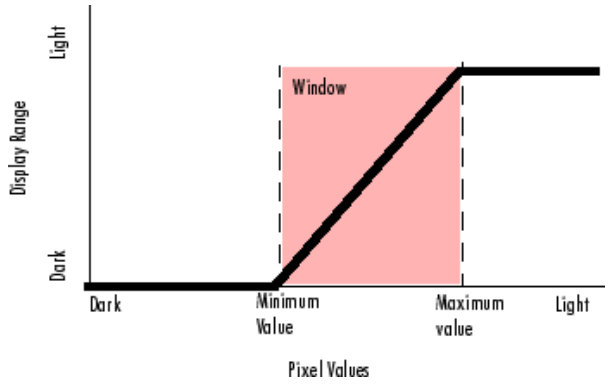


Figure 5 convoluted Image

After applying the convolution, the resulted image is added with the original input image that suppress high values of all three channels of the RGB as shown in Figure.7. The features of the image are enhanced by unsharp masking. The decorrelation stretch method is applied on the image to enhance the colour difference.

3.1.1 CONTRAST ADJUSTMENT

There are no sharp differences between black and white when an image has lack of contrast. Brightness refers to the overall lightness or darkness of an image. To change the contrast or brightness of an image, contrast stretching operation is to be carried. . In this process, pixel values below a specified value are displayed as black, pixel values above a specified value are displayed as white, and pixel values in between these two values are displayed as shades of gray. The result is a linear mapping of a subset of pixel values to the entire range that produce an image with higher contrast.



In this paper, imfilter function is used to enhance the image. This function filers the multidimensional array with the multidimensional filter. This filter computes each element of the output using double precision floating point. This filter truncates output elements that exceed the range of of the given type and rounds fractional value.

3.1.2 DE-CORRELATION STRETCHING

. De-correlation algorithms can be a linear and non-linear algorithm. In image processing, decorrelation techniques can be used to enhance or stretch, colour differences among pixel of an image. This is called as 'decorrelation stretching'. The decorrelation concept is also applied on many fields, such as neuroscience, cryptography, etc. Now in this paper, the cytoplasm and nuclei can be differentiated and image has been pre-processed.

De-correlation [18] stretching enhances the colour separation of an image with significant band-to-band correlation. The exaggerated colours improve visual interpretation and make feature discrimination easier. It can be applied on the image irrespective of the number of colour bands. The original colour values of the image are mapped to a new set of colour values with a wider range. The colour intensities of each pixel are transformed into the colour eigen space of the BANDS-by-NBANDS covariance or correlation matrix, stretched to equalize the band variances, and then transformed back to the original colour bands. To define the band wise statistics, we can use the entire original image, with the subset option, or any selected subset of it

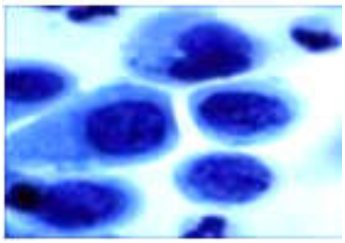


Figure 8 Enhanced image

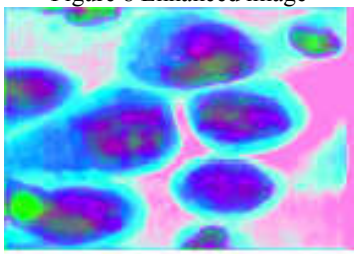


Figure 9 ecorrelated image

3.2 EXTRACTION OF NUCLIE

Further, the output image obtained from the pre-processing step is binarized with OTSU thresholding algorithm to extract the nuclei from the cytoplasm. Otsu's method [17] is iterative used to automatically perform clustering based image thresholding. It is used to minimize the interclass variance. It is expressed in term of equation

$$\sigma^2_a = \sigma^2 - \sigma_\omega(i) = \omega_1(i)\,\omega_2(i)\,[\mu_1(i) \quad \mu_2(i)]^2 \quad \text{- (3)}$$

Figure 10 Output image which contains only nuclei after applying OTSU algorithm

After getting the binary image, isolated interior pixel should be filled. For that purpose, morphological fill operation is applied on the binary image.

Binary Image	Morphology Fill operation
1 1 1	1 1 1
1 0 1	1 1 1
1 1 1	1 1 1

Using the regional properties of the image, small and large areas of the nuclei are identified. After identifying, the smallest nuclei areas are removed from the image to retain only the nuclei which size is greater than normal cell. The binary nuclei mask is enlarged using morphological operator , dilation and we get the dilated Nuclei mask. Morphological dilation is applied with structuring element D on a binary image G as represented in the equation

$$G \oplus D = \{z \varepsilon I \mid (D\varepsilon) \varepsilon Z \cap G = \theta \quad \text{- (4)}$$

Where D^ε is a structuring element and defined by

$$D^\varepsilon = \{ x \quad I \mid x \quad D\} \quad \text{--.(5)}$$

Where I is integer grid.

Figure. 11 Enlarged nuclei.

Every nuclei has morphological features , such as, size, perimeter, eccentricity, etc. These features are used to classify the nuclei into normal and abnormal cell. The features here we considered, are size, perimeter, eccentricity, equiv diameter and mean intensity of the cell. These features are extracted from the image using region properties method.

a) Area

The area of the each nuclei is one of the feature to classify the cell into normal or abnormal cell. Besides, the number of normal and abnormal cell in the cervical image is a good decisive factor for identifying the abnormality. We calculated the area of each nuclei in terms of pixels.

b) Shape and size analysis

Shape is another important feature for classifying the cervical cell. In general,, the shape of cell nuclei are round and elongation occurs when abnormality occurs. The change of degree in shape is a good decisive factor to analyze the shape of the nuclei. This paper takes the following factors into account

Perimeter : It is a scalar value that specifies the distance around the boundary of the region. The perimeter can be calculated by means of calculating the distance between each adjoining pair of pixels around the border of the region. In case if the image contains the dis-contiguous regions, perimeter may contain the undefined value.

Eccentricity : The cell nuclei are in round in shape in the normal condition.. It is essential to calculate the semimajor and semiminor axes' lengths. Then the eccentricity for each nucleus is obtained using the following formula

$$= \{(a2 - b2)/b2\}^{1/2} \quad - (6)$$

where a and b are the semimajor and semiminor axes of the ellipse respectively. The eccentricity value is 0 in normal condition It deviates from the zero in the abnormal condition.

Diameter: It is a scalar value that specifies the diameter of a circle with the same area as the region. It is computed as follows

$$\text{Diameter} = \text{sqrt}(4*\text{Area}/\text{pi}) \quad - (7)$$

Mean Intensity : It specifies the mean of all intensity values in the region. EKo Supriyato et. al. [15] described color intensity level of normal cell and cancerous cell rages. The following table described the intensity level of the different cell types.

Table 1. Range of Mean Intensity and Area of the cancer stages

Mean Intensity Range	**Area Range**	**Stage of the cancer**
Above 100	**Below 150**	**Normal cell**
Above 80 – below 100	**Above 150 and below 400**	**Cancerous cell (Mild)**
Above 50 – below 80	**Above 400 and below 600**	**Cancerous cell (Moderate)**
Below 50	**Above 600**	**Cancerous cell (Severe)**

Steps of the Proposed Method : The proposed method process is given below step by step.

- Read RGB as input I(x,y)
- Apply the Gaussian filter to remove the noise F(x,y) = smooth(I(x,y))
- Apply convolution operation with kernel h(x,y) with average C(x,y)
- Original image is added with convoluted image R(x,y) = I(x,y) +C(x,y)
- Apply image contrast enhancement technique using unsharp mask
- Apply the De-correlation technique D(x,y)
- Apply OTSU method on the binarized image D(x,y)
- Apply Morphological fill operation to fill the interior holes
- To get the binary nuclei mask, the small areas are identified using regional property.
- Small area are identified and removed from D(x,y)
- Apply Morphological dilation to enlarge the segmented nuclei with fixed structuring element of size 2 with disk shape
- Further, the binary nuclei mask D(x,y) is complemented D'(x,y)
- Finally, Feature extraction is carried out on the image D'(x,y) using regional property and identify the normal and abnormal cell using different morphological features, such as, size, perimeter, eccentricity, Diameter and mean intensity.

4. EXPERIMENTAL ANALYSIS AND RESULTS

Five morphological features, such as, area, perimeter, eccentricity Diameter and mean intensity values were taken into consideration for classifying the cervical cell. Here, more than 30 pap smear cervical cytology images were taken as experimental samples. As far as abnormal or cancerous cell is concerned, its area, perimeter equivdiamter value were in increased manner and its intensity value is ranging from 1 to 100. As far as eccentricity is concerned, the normal nuclei have a minimal proportion between the width and height and have greater roundness and its value is normally zero. Uncontrolled growth of the nuclei does not keep this uniform proportion and as result their eccentricity deviated farther away from Zero. In case of normal cell, its area, perimeter, equivdiamter values are in decreased manner and

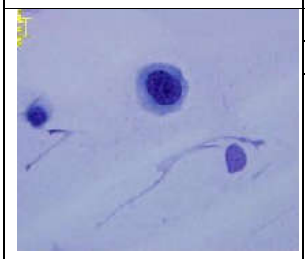
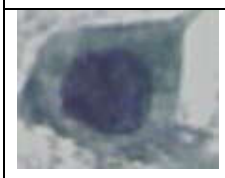
5. CONCLUSION

The proposed method segregated the nucleus from the Pap Smear cervical cytology images using pre-processing method , OTSU thresholding algorithm and morphological operation.. The result obtained from this method is found to be satisfactory. The empirical results show that this method gives good segmentation and classification of nuclei. The accuracy of the classification is improved because we took five morphological features for classification. Further work will be carried out by attempting to separate cytoplasm from the image, analyzing other morphological features and analyse the stage of the cancer very accurately.

REFERENCE

1. [1] American standard society, “Cervical Cancer”.
2. http://www.cancer.org/healthy/findcancerearly/cancerscreeningguidelines/american-cancer-society-guidelines-for-the-early-detection-of-cancer, Last accessed on September 24, 2012.
3. [2] Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. (2011), Global cancer statistics. *CA: A Cancer Journal for Clinicians*, 61: 69–90. doi: 10.3322/caac.20107
4. [3] *Mahanta et al.,* Analysis of Malignant Cervical Cells Based on N/C Ratio Using Pap Smear Images. *International Journal of Advanced Research in Computer Science and Software Engineering 2 (11), November- 2012, pp. 341-346*
5. [4] Shys-Fan Yang-Mao, Ynng-Kuan Chan and Yen-Ping Chu, Edge Enhancement Nucleus and Cytoplast Contour Detector of Cervical Smear Images, IEEE Transactions on Systems, Man and Cybernetics – Part B: Cybernetics, Vol 38, No.2, April 2008
6. [5] Nazah Mustafa, Nor Ashidi MAT ISA and Mohd Yusoof MASHOR. Automated Multicells Segmentation of Thinprep Image Using Modified See Based Region Growing Algorithm. Biomedical Soft Computing and Human Sciences. Vol. 14. No. 2 pp 41-47(2009)
7. [6] Chang Wen Chen, Jiebo Luo and Kevin J. Parker. Image Segmentation via Adaptive K-means clustering and Knowledge-Based Morphological operations with Biomedical Applications, IEEE Transactions on Image Processing, Vol. 7, No. 12, December 1998.
8. [7] R. Ghafar, N.A. Mat Isa, U.K. Ngah, MY. Mashor, N.H. Othman. Segmentation of Stretched Pap Smear Cytology Images using Clustering Algorithm.
9. [8] Ghafar, R. Mashor, M.Y. & Othman, N.H. 2002. Segmentation of Pap Smear Slides Images using K-means clustering, In Proceedings of Kuala Lumpur International Conference on Biomedical Engineering, 41-43, Kuala Lumpur, Malaysia
10. [9] S.Anantha Sivaprakasam and E.R.Naganathan.. Combined approach on Cervical cytology image segmentation. International Journal of Softcomputing, 10(2),163-168, 2015
11. [10] M.E.Plissiti, E.E Tripoliti, A. Charchanti, O. Krikoni and D.I. Fotiadis. Automated Detection of Cell Nuclei in PAP Stained Cervical Smear Images using Fuzzy clustering.
12. [11 Marina E.Plissiti, Christophoros, Nikou and Antonia Charchanti. Automated Detection of
13. Cell Nuclei in Pap Smear Images using Morphological Reconstruction and Clustering, IEEE Transactions on Information Technology in Biomedicine, Vol.15, No. 2, March 2011.
14. [12] Lipi B. Mahanta, Dilip Ch. Nath, Chandan Kr. Nath. Cervix cancer Diagnosis from Pap Smear Images using structure based segmentation and shape analysi. Journal of Emerging Trends in Computing and Information Sciences, Vol.3. No. 2, February 2015
15. [13] Bustanur Rosidi, Noraini Jalil, Nur.M. Pista, Lukman H. Ismail, Eko Supriyanto, Tati L. Mengko. Classification of Cervical Cells based on Labeled Colour Intensity Distribution. International Journal of Biology and Biomedical Engineering. Issue 4, Vol. 5, 2011
16. [14] Yung_Fu Chen, Po_chi Huang, Ker_cheng Lin, Hsun-Hung Lin, Lin-En Wang, Chung_chuan Cheng, Tsung_Po Chen, Yung_Kuan Chan, and John Y. Chiang. Semiautomatic Segmentation and Classification of Pap Smear Cells. IEEE Journal of Biomedical and Health Informatics, Vol. 00, No. 00 2013.
17. [15] Eko Supriyanto, Nur Azureen M. Pista, Iukman Hakim Ismail, Bustanur Rosidi, Tati Latifah Mengko. Automatic Detection System of Cervical Cancer Cells Using Color Intensity Classification. ISBN: 978-1-61804-019-0. Recent Research in Computer Science 2011.
18. [16] Kurman.R.J, D. E. Henson, A. L. Herbst, K. L. Noller, and M. H.Schiffman, “Interim guidelines of management of abnormal cervical cytology,”J. Amer. Med. Assoc., vol. 271, pp. 1866–1869, 1994
19. [17] Naveed Abbas and Dzulkifli Mohamad, **Automatic Color Nuclei Segmentation of Leukocytes for Acute Leukemia,** Research Journal of Applied Sciences, Engineering and Technology 7(14): 2987-2993, 2014
20. [18] Dr.E.R.Naganathan, S.Anantha Sivaprakasam and V.Saravana Kumar. Enhanced Colour Image Segmentation on Cervical Cytology image. Proceedings of the International Conference on Applied Mathematics and Theoretical Computer Science - 2013

Table 2 Classification Result

Image	cell No.	Area	Mean Intensity	Perimeter	Eccentricity	Equiv Diameter	Stages of the cancer
	1	207	51.245	49.4568	0.631313	16.2345	CIN2
	2	918	46.6786	108.042	0.685814	34.1882	CIN3
	3	151	93.3475	44.287	0.711893	13.3987	CIN1
	1	1512	76.21	173.79	0.158	43.88	CIN2