Classification of Cervical Cells Based on Labeled Colour Intensity Distribution

Bustanur Rosidi, Noraini Jalil, Nur. M. Pista, Lukman H. Ismail, Eko Supriyanto
Tati L. Mengko

***Abstract*—** Automatic classification system of cervical cells images has been developed by some researchers. The process however takes long time or less accurate. In order to decrease the processing time while maintaining the accuracy of detection, a new method in classification of cervical cancer has been proposed. Before classification, the image is optimized and labeled. This will accelerate the segmentation and classification process. Test result shows the significant reduction of computation time compare to other results, while maintaining the accuracy, specificity, sensitivity and positive predictive value.

***Keywords*—**Cervical cells, classification, processing time, optimized color intensity, accuracy

I. Introduction

After breast cancer, the carcinoma of cervix is the second most common cancer among women worldwide. Statistically, one woman dies every 2.5 minutes because of cervical cancer. It is the most common women's killer in both developing and developed countries. Worldwide around 500,000 cases are counted annually, 75-80% of these are in developing countries [1,2]. Effective screening and adequate treatment can actually prevent women from having cervical cancer [15]. The screening of cervical precancerous lesions is done using Pap smear, a method developed by G. Papanicolou in early 1940's. To do this test cervical specimen are taken with spatula or small brush and smeared on glass slide for examination [3].

Manuscript received August 31, 2011.

Bustanur Rosidi is with the Advanced Diagnostics and Progressive Human Care Research Group, Biotechnology Research Alliance, UTM Skudai, 81310 Johor, Malaysia (phone: +607-553-5273; e-mail: bustanur@utm.my).

Noraini Jalil with the Advanced Diagnostics and Progressive Human Care Research Group, Clinical Science and Engineering Department, FKBSK, UTM Skudai, 81310 Johor, Malaysia.

Nur M. Pista with the Advanced Diagnostics and Progressive Human Care Research Group, Clinical Science and Engineering Department, FKBSK, UTM Skudai, 81310 Johor, Malaysia.

Lukman H. Ismail with the Advanced Diagnostics and Progressive Human Care Research Group, Clinical Science and Engineering Department, FKBSK, UTM Skudai, 81310 Johor, Malaysia.

E. Supriyanto is with the Advanced Diagnostics and Progressive Human Care Research Group, Clinical Science and Engineering Department, FKBSK, UTM Skudai, Johor 81310 Malaysia (e-mail: eko@biomedical.utm.my).

Tati L. Mengko is with the Biomedical Engineering Division, Bandung Insitute of Technology, Indonesia.

Under the microscope, cytologists identify abnormalities of the uterine cervix cells. If there are any abnormalities detected in Pap smear test, it doesn't automatically mean that the patient has a cervical cancer, but it indicates that changes might have occurred in the cervical cells and further action might necessarily to be done.

In developed countries screening with Pap smear had significantly reduced the incidence of cervical cancer and the mortality among women caused by this disease. In countries like USA, cervical cancer is only the eighth most common cancer among women due to a successful, effective and widespread prevention program using Pap smear test. In developing countries however, where the lack of resources exists, cervical cancer remains a serious deadly threat [2].

Infection of cervical epithelium by HPV (Human Papillomavirus) is considered to be the trigger of carcinoma development in cervical cells [5]. There are more than 80 serotypes of this virus that can cause pathologies in human. Seventy percent of invasive cervical cancers in the World are attributed to serotypes 16 or 18 of HPV [5, 6]. During an infection a virus enter the epithelium through a micro fissure to reach basal layer (*stratum cylindricum*) of the squamous cells - the only cell type the virus can enter. Infection occurs in the basal cells which are still competent to replicate and not yet differentiated. The infected cells proliferate and spread in sync with the differentiation process through the overlying cells of the epithelium into the upper cell layer. New viruses are released by the infected superficial layer (*stratum corneum*) when these differentiated cells flake off in the process of skin renewal. Released virions start a new cycle of infection on surrounding cells or other side of the organs [5].

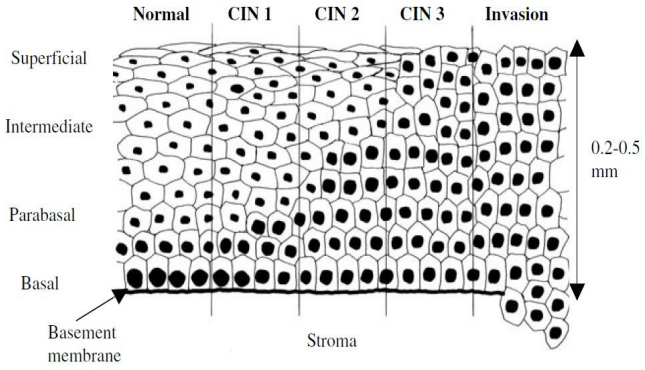


Fig. 1 Progression of Cervical Interepithelial Neoplasia (CIN) in the squamous epithelium of cervix (Source : [4])

It takes usually decades until a viral infection of cervix develops into a cancer. The presence of abnormal precancerous cells in Pap smear test is termed Cervical Interepithelial Neoplasia (CIN) or Squamous Intraepithelial Lesions (SIL). If not early detected or not properly treated this cervical dysplasia can further develop to an invasive carcinoma. The progression CIN is divided into three stages: CIN 1, CIN 2 and CIN 3 (Figure 1) [4, 5]. CIN 1 is considered as a mild dysplasia, the abnormal appearing cells are found in the bottom 1/3 of the epithelium, whereas in CIN 2 already 2/3 or the bottom part of epithelium has been affected. CIN 3 stage is categorized as a severe dysplasia. At this stage the entire epithelial thickness has been affected [4]. According to "The Bathesda System of Terminology" CIN1 is classified as Low-grade Squamous Intraepithelial Lesion (LSIL), whereas CIN 2 and CIN 3 are High-grade Squamous Intraepithelial Lesion (HGSIL or HSIL) [6].

Cancerous transformation of cervical cells is associated with an increase of nucleus size and a decrease of cytoplasm size. On Pap smear slide HSIL cells therefore show a significantly higher nucleus to cytoplasm ratio (NC ratio) compared to normal cells [8]. Another feature difference between normal and HSIL cells is the fact that the abnormal cells usually gain darker color after the procedure of fixation and Pap staining. This color intensity difference can be utilized to discriminate between normal and cancerous cells [9, 10].

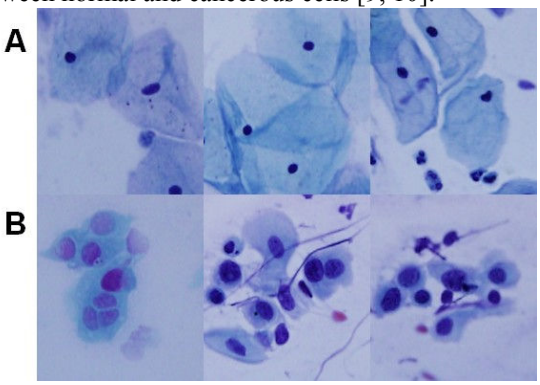


Fig. 2 Example of Pap smear slides. A:normal, B:cancerous cells

In recent years some efforts have been done to improve the analysis of Pap smear slides. The invention of a Liquid Based Cytology (LBC) has significantly helped the work of cytologists due to the introduction of an automation system for sample preparation resulting in cell specimen as monolayer which is easier to be evaluated. The conventionally prepared Pap smear slides show often multilayer cell preparation that makes it more difficult for the analysis.

The LBC system is however not always an affordable technology everywhere in the world. In developing countries the conventional Pap smear technique is still method of choice for cervical cancer screening. To characterize them as normal or abnormal Pap smear slides are carefully examined under the microscope by an expert cytologist or pathologist. Due to the fact that conventional smear often show multilayer, crowding and overlapping cells, the visual interpretation of the microscopic pictures of slide is labor-intensive and requires high level of expertise and experiences by the analyzer. [11, 12, 13]. This requirement cannot always be fulfilled and afforded in developing countries. Additionally, with the increasing number of women gaining access to preventive health care and due to a shortage of skilled and experienced cytologists, the analysis of Pap smear slides becomes highly prone to human error, leading to more inaccuracy of the diagnosis. Driven by this motivation some researchers have in the last few years contributed with ideas to develop an automated system for analyzing such Pap smear images.

A. Existing Work

There are some investigations have been done by researchers to determine whether a cell in Papanicolaou smear images is cancerous or not. They were done due to some problems such as lack of an expert cytotechnologist, a large number slide per day, insufficient lab facilities, procedures becomes time consuming and highly susceptible to human errors. Automatic cervical detection have been introduced by researchers in order to solve the problems [16,23,24].

In order to classify between cancerous and non-cancerous cells, there are few methods have been proposed by researchers. Jeremiah [16] has introduced Pattern Recognition on 2D Cervical Cytological Digital Images. The paper attempts to investigate patterns of cervix cells based on its morphological features, in term of size, shape, and color. Strengthens of this paper, the parameters that will be used for their automated recognition system were cytoplasm to nucleus ratio, color intensity, and wavelet characteristics to enable automatic identification of abnormality in the cervix cells. Algorithm classifiers used by her study are kNN, SVM and NB classifier.

Afterwards, Tanatip [24] proposed a new method which is classification cervical cancer using Fourier transform. The features that used to discriminate between normal and the abnormal cells are calculated based on the mean, variance and entropy obtained from the frequency components along the circle radius centered at the centre of the spectrum and the frequency components along the radial line having an angle θ. Calculating the features were done by using some mathematical formulas and subsequently can give a result whether the Papanicolaou smear images are cancerous or not.

Tanatip also evaluated performance of five classifier in discriminating the normal and abnormal cell. The classifier consists of Bayessian classifier, linear discriminant analysis(LDA), k-nearest neighbour algorithms(KNN), artificial neural network(ANN), support vector machine(SVM). All the classification rates have accuracy higher than 85% , and Support Vector Machine have the best results which 92.65% accurate and the false rate are 7.35% for both positive and negative.

Recently, Rahmadwati [23] carried out classification cervical cancer using histology images. Different from other researchers, the cervical histology images that have been proposed is just not only can discriminate digital images of cervical cancer is cancerous or not, but also can distinguish the stages of cancerous: 1) normal 2) pre cancer and 3) malignant. Besides that, the studies have several important discriminatory features which are a) the ratio of nuclei to cytoplasm b) diameter of nuclei c) shape factor and d) roundness of nuclei. However their studies have shown that N/C feature is very favorable and indicates the most discriminatory power of the feature.

Recently, Eric also apply a new method which are classification cervical cells based on Fourier transform infrared spectroscopy (FTIR) absorption data. Eric makes a comparison of the FTIR response of a normal and malignant sample. The infra-red spectra of malignant cervical samples displayed the difference from FTIR spectrum of healthy cervical cells. At some point of frequency, there are some significant changes in intensities of the bands, significant shifts of the peaks normally appears, and an additional band peaking. The infrared spectrum of a sample dysplasia displayed same features as the malignant sample but less significant changed.

From those researches, it was found that the classification of cervical cancer based on colour intensity is the best method, since less time consuming and give higher accuracy. The existing problem by colour intensity classification is the overlapping colour distribution for normal and cancerous cells that produce inaccuracy of classification. Hence, a new technique called labeled colour intensity has been proposed in this work. The idea was to pre process the images before classification including image conversion, segmentation and labelling. This enables the better accuracy of classification while maintaining the short processing time.

II. Material and Method

The images cervical cells were captured from Pap smear slides analyzed using Olympus BX-51 microscope. Images of desired sections of the slides were stored in the computer using imaging software (Olympus). The processing of the images was performed using MATLAB with Image Processing Toolbox and the system's prototype was designed using graphical user interface (GUI). All this data including data bases of patients were collected from The National Cancer Hospital Dharmais, Jakarta, Indonesia.

This experiment applied a systematic methodology consist of five consecutive procedures: image acquisition, image enhancement, feature extraction, image segmentation and image classification, as describes in the following sections.

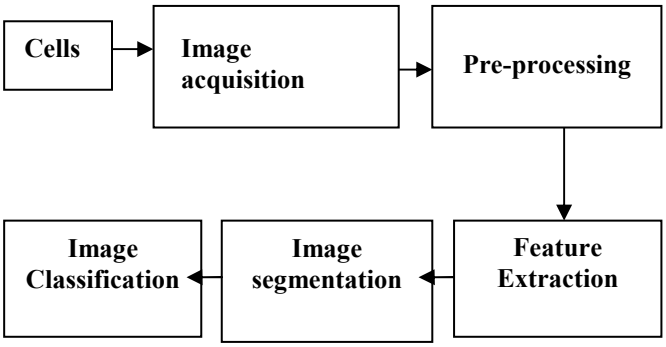
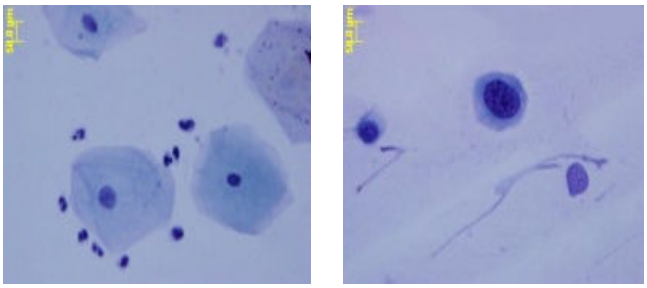


Fig. 3 The proposed Cervical Cancer detection

A. Data acquisition

Image data used in this research are collected from cytological diagnoses of Pap smears performed during 2010 at Dharmais Cancer Hospital, Jakarta, Indonesia. A total number of 100 selected images were kindly provided by the hospital for this experiment after a pathologist has evaluated each of them and categorized it into normal and abnormal one. The cervical cells images were captured from cytological Pap smear slide using Olympus BX-51 microscope under 100X objective lens. All of these data originated from cervical examinations of patients between 17 and 81 years of age. Eighteen women out of 746 patients having undergone the examination in 2010 were positively diagnosed with cervical cancer, whereas 728 of them were normal. Figure 4 shows each a section of Pap smear image samples that have been used to analyze whether someone has cancer or not. The data base of the patients contains information about the number of slides for the Pap smear examination of a certain patient, age, blood type, as well as the pathological medical data.



(a) (b)

Fig. 4 The image of normal cervical cells (a), and cancerous cell (b).

B. Image Pre Processing

In this step the images have undergone several digital image pre processing procedures. These techniques include image conversion and morphological operations. Figure 5 shows the flow chart of the image processing:

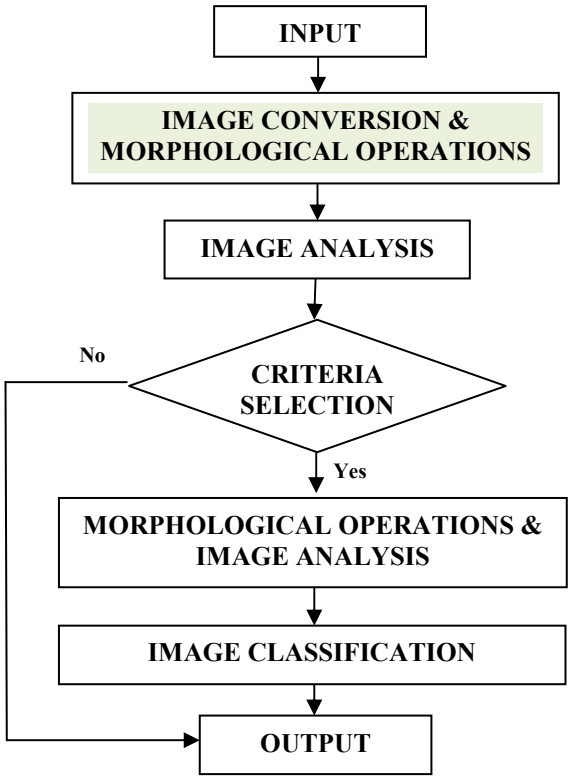


Fig. 5 Image processing techniques

The image processing starts with the loading of a digital image of cervical cells. This image is then converted into gray scale image to binary image. Boundary of all 'elements' in the image are detected and marked using green color line. What are designated as 'Elements' here refer to all features appearing in the Pap smear slide including cervical cells, blood cells, leukocytes and other structures. The next step consists in the measurement of all properties of the elements such as color intensity level and area. Subsequently, criteria selection is performed. This criteria selection refers to the criterion that has been chosen to distinguish between cervical cell and other cells. The criterion used was cells' area and cells' color intensity distribution. This allows only cervical cells to appear in the image and other structures will be eliminated. After this step has been accomplished only cervical cells are left in the image.

The last step of the image processing is the characterization of cervical cells done using a color intensity classification. As mentioned earlier, cancerous cell shows increasing nucleus size but shrinking cytoplasm, resulting in a higher nucleus to cytoplasm ratio (NC ratio) compared to a normal cell. This is a feature that characteristically can be used to distinguish between normal and cancerous cell. The relative size of the nucleus compared to cytoplasm of cancerous cervical cells results in a different color intensity distribution compared to normal cells. Cancerous cells have typically a bigger proportion of darker pixels.

Based on the sample testing of cervical cells, a specific range of color intensity level of cancer cell is calculated. Because the color intensity level of cervical cancer cells is distinctively different from normal ones the characterization of the cervical cell can easily be done.

C. Feature Extraction

In this procedure, the target cell (normal and cancerous cervical cell) is extracted using color intensity classification. Again, cancerous cells and normal cells have distinctively different color intensity distribution. A cancerous cell has relatively larger distribution of darker pixels compared to a normal one. The method used to determine the range of color intensity and area of the cervical cells was *"Variational Level set Formulation of Curve evolution Without Re-initialization"*[10, Chunming Li 2005]. The color intensity level of cancerous cells ranges between 80-100, whereas that of normal cells is between 122-150.

Here is the formulation:

$$\frac{\partial \phi}{\partial t} + F\,|\nabla \phi| = 0 \tag{1}$$

where function F is referred to speed function, while ϕ refers to level set function. Then, re-initialization has been introduced to overcome drawbacks of the traditional Level Set Method. The re-initialization of Level Set equation:

$$\frac{\partial \phi}{\partial t} = sign(\phi)(1 - |\nabla \phi|) \tag{2}$$

where ϕ refers to the function that will be re-initialized and sign is the sign function. Signed distance function must satisfy, $|\nabla \phi| = 1$;

$$P(\phi) = \int_{\Omega} \frac{1}{2}(|\nabla \phi| - 1)^2 \, dydx \tag{3}$$

this is the key role of this method. This integral was used to characterize the distance between function ϕ to sign distance function in $\Omega \subset \Re^2$. Then a variational formulation was proposed.

$$\xi(\phi) = \mu P(\phi) + \xi_m(\phi) \tag{4}$$

where $\mu > 0$ is a parameter that will control the penalizing deviation effect of ϕ and sign function.

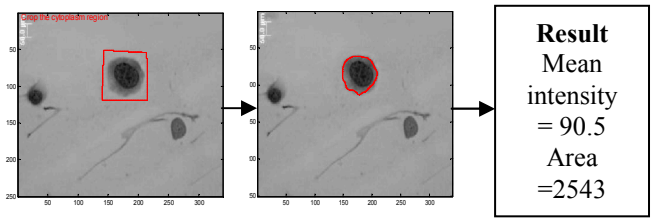


Fig. 6 The implementation of Level Set Algorithm

D. Image Segmentation

After the feature extraction process was completed, the extracted features were undergoing segmentation process.

Segmentation using mathematical morphology (Yeshwanth et al, 2007) was used.

Mathematical morphology involves the use of 'structuring elements' which eventually smooth the original region of the image. Mathematically, the operation is as follows:

$$I_{opened} = I \circ SE \quad (5)$$

where I_{opened} is referred to open image

$$I_{mask} = I_{bin} \Theta SE_E \quad (6)$$

where Θ refers to the erosion operation and SE is refers to structuring element. I_{edge} is used to obtain the object mask. It was first dilated using 4 different angels 0, 45, 90, 135.

$$I_{dilate} = \bigcup \left(I_{edge} \oplus SE_0, I_{edge} \oplus SE_{45}, I_{edge} \oplus SE_{90}, I_{edge} \oplus SE_{135}, \right) \quad (7)$$

$$I_{filled} = \text{Re}\, gion\_fill(I_{dilate}) \quad 8)$$

I_{filled} was used to fill in the holes(black pixels) in the image while 'Region_fill' function fills the black and white boundary with white. The final mask is:

$$I_{mask} = (I_{filled} \bullet SE_E) \quad (9)$$

E. Image Classification

After the result for every cell has been gained through the automatic detection, a binary classification set is applied to measure the performance of the system. The training data was classified into two classes, cancerous or non-cancerous. There are 100 images acquired from Pap smears using Olympus BX-51 microscope. Diagnosis done manually by the pathologist resulted, that out of 100 samples 53 was verified as cancerous, whereas the rest was not. The comparison between the results gained from the output of the automatic system and those of pathologist's diagnosis is shown in Table 1. Some features associated with each extracted object include mean intensity, cell area, nuclear-cytoplasm contrast, and nuclear-cytoplasm radius ratio.

This classification divides the training data into four classes which are true negative (TN), false negative (FN), false positive (FP), and true positive (TP). People positively diagnosed with cancer, and also tested positive are the true positives (TP), while those tested negative are the false negatives (FN). People with non-cancerous diagnosis, but tested positive are the false positives (FP), and those tested negative are true negative (TN). These results were concluded after comparison of the diagnosis using our automated detection system with the diagnosis done by pathologist. We calculated that among 53 non-cancerous samples, 44 were tested true and 9 false. While among the 47 cancerous, 33 tested true and 14 were false. As shown on the Table 1 the values of TP, FN, FP, TN are 33, 9, 14 and 44 respectively. Figure 7 shows the result of comparison of the diagnosis using our automated system and the diagnosis done by the pathologist. From the result of classification, the accuracy, sensitivity, specificity and positive predictive (PPV) value for our system can be measured.

		Characteristics		
		+ve	-ve	
Test	+ve	TP: 33	TN: 44	77
	-ve	FP: 14	FN: 9	23
		47	53	100

Fig. 7 Number of TP (True Positive), TN (True Negative), FP (False Positive) and FN (False Negative)

Table 1. Comparison of cell classifications by software and pathologist for few examples

Cell #	Intensity	Area	Diagnosis (MATLAB)	Time (MATLAB)	Diagnosis (Pathologist)	Time (PAthologist)	Conclusion
1	147	7865	Normal cell	34 ms	Normal cell	5 minutes	TN
29	129.3	485	Normal cell	25 ms	Leukocyte	8 minutes	FN
44	106.8	2478	Cancerous cell	36 ms	Normal cell	7 minutes	FP
47	92.6	2683	Cancerous cell	32 ms	Cancerous cell	8 minutes	TP

III. Result and Analysis

A. Image conversion and labeling.

The objective of the image conversion and morphological operation is to label the connected elements in binary image so that the properties of the element can be measured. At the beginning of the testing, a cytological image of cervical cancer cell was loaded. The image was in RGB format. The RGB image was converted to grayscale image using 'rgb2gray' function and then converted again to binary image using thresholding method. The connected elements in the binary image were then labeled using 'bwlabel' function. It was important to bear in mind that the labeled image may comprise of cervical cells, blood cells, leukocytes and other elements that usually found in cervix smear. The result is shown in the following figure.

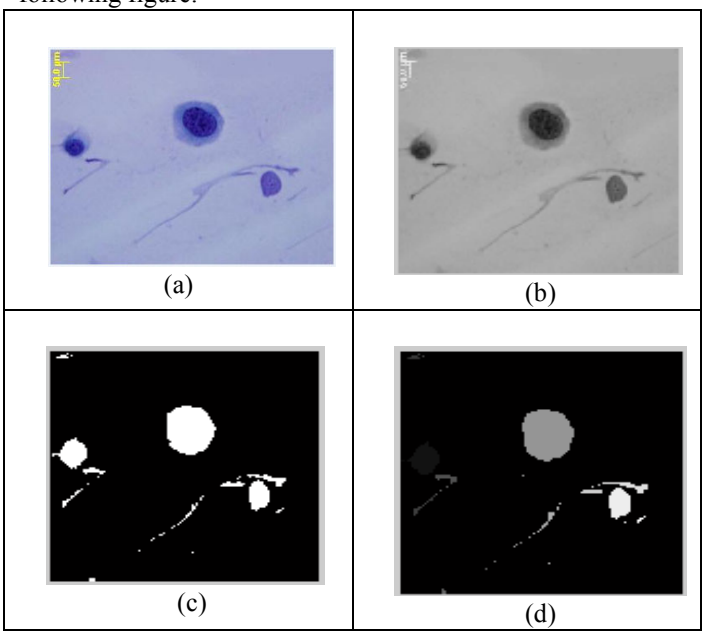


Fig. 8 Image conversion from RGB image (a) to grayscale image (b) to binary image (c) and morphological operation labeled image (d)

B. Image boundary detection and numbering

The objective of this part is to measure the elements properties such as color intensity level, area, and centroid using 'regionprops' function. These properties will determine whether the element is cervical cell or not. Moreover, 'bwboundaries' function is also used to return a cell array, where each element in the image is assigned to a row and column coordinates. The coordinates returned by 'bwboundaries' are then used to plot the borders of all elements in the image. Finally, all elements are numbered from top to bottom, then from left to right. The result is shown in the Figure 9.

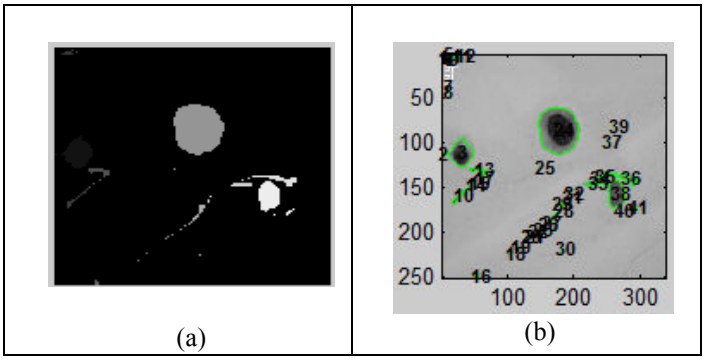


Fig. 9 Image analysis. From labeled image (a) to the image of elements with boundaries (b)

C. Criteria selection

The objective of criteria selection is to detect the cervical cells and make them visible while eliminating other elements. This is accomplished by applying 'ismember' function and cervical cell filter to all of the elements. This filter will only permit elements that meet with the criterion set which is based on the range of the area and color intensity of cervical cell. This operation results in decreasing number of elements appeared in the image.

D. Morphological operations and image analysis

The objective of this operation is to relabel the cervical cells left in the image using 'bwlabel' function. The 'bwboundaries' function was again used to return a cell array,

where each element in the image is assigned to a row and column coordinates. The coordinates returned by 'bwboundaries' were then used to plot the borders of all elements in the image. The result is shown in the Figure 10.

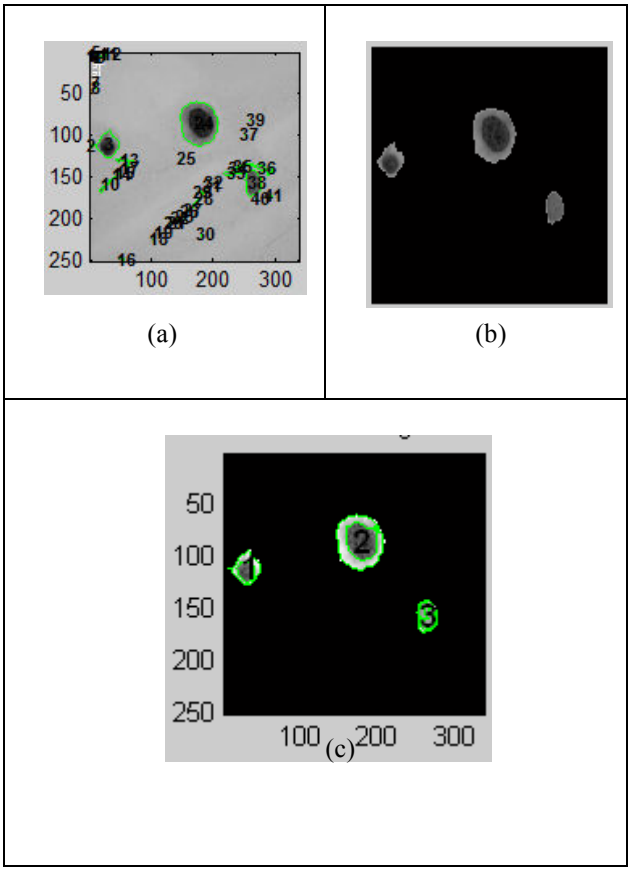
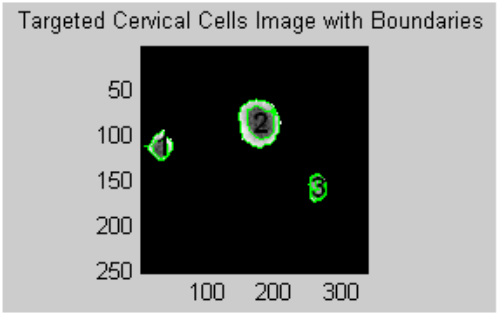


Fig. 10 The result of morphological operation and every cell labeled by number. From image of elements with boundaries (a), to labeled image of cervical cells (b), to cervical cells with boundaries (c)

E. Image classification

The objective of the classification is to categorize the cervical cells into cancerous and non-cancerous cell. At the beginning of this stage, all cervical cells left in the image were looped to number and the cell properties such as intensity, centroid and area were measured. The cells were then categorized into cancerous or non-cancerous cell based on the color intensity classification as mentioned above. Subsequently the measurements of the properties of the cervical cells and their diagnoses were printed on Matlab command. The result is shown in the Figure 11.



n	Intensity	Cell Area	Diagnosis
# 1	98.8	815.0	Cancerous cell
# 2	90.5	2595.0	Cancerous cell
# 3	102.2	645.0	Cancerous cell

Fig. 11 Detection and characterization of cervical cells.

The implementation result of this system is shown in Figure 11. An image of a cancerous cervical cell was used for this implementation. The image was converted from RGB image into grayscale image, and then into binary image. The image was labeled so that the elements' properties can be measured. After applying criteria selection to the image, only cervical cells remained. Then, the image was relabeled again to measure the properties of each cervical cell. By using 'bwboundaries' function, the boundary of cervical cells in the image has been plotted. The measurements of the cell properties were then printed on Matlab screen.

Cytological analysis of normal and cancerous cells reveals some characteristically different features that can be utilized as parameters to discriminate these two cell types from each other. In this experiment we applied the differences of color intensity distribution between cancerous and non-cancerous cervical cells to classify them. The transformation from normal cervical epithelium cells into dysplasia and finally to cervical carcinoma is associated with an increased nuclear-cytoplasmic ratio, loss of the layer of flattened cells close to the surface and an increase in the volume of extra cellular space [21 and 22]. These cytological differences lead to a different 'behavior' of normal and cancerous when processed in the fixation and staining with Pap stain reagents. Due to bigger size of the nucleus and generally a shrunken cytoplasm cancerous cells appear with lower color intensity (darker) then normal cells.

To evaluate the validity of the detection system and to assess the system performance a performance testing was done. Four types of performance testing are calculated by formula (10) to (13) and the result is shown in Table 2. The result shows an acceptable performance however some influence factor should need to be considered to improve the accuracy, specificity, sensitivity and PPV. These include the presentation of the cells on Pap smear slides that usually in multilayer form and tend to clump together, which makes the system unable to detect the cell boundaries. Additionally the specimen staining method contributes also to the changes of mean intensity of cells. Improvement needs still to be done in

order to achieve more precise results with higher accuracy. The results show that the develop system is able to be used for cervical cancer detection based on labeled color intensity distribution.

$$specificity = \frac{TN}{TN + FP} \times 100\% \quad (10)$$

$$PPV = \frac{TP}{TP + FP} \times 100\ \% \quad (11)$$

$$sensitivity = \frac{TP}{TP + FN} \times 100\% \quad (12)$$

$$accuracy = \frac{TP + TN}{TP + TP + FP + FN} \times 100\% \quad (13)$$

Table 2. Result of performance testing

Type of Performance Testing	Percentage (%)
Accuracy	77.0
Sensitivity	78.6
Specificity	75.9
Positive Predictive Value	70.2

IV. CONCLUSION

A new technique for cervical cell classification has been developed. The classification is done based on differentiation of labeled color intensity distribution. This method has been tested on cervical cell images from 746 patients and compared with manual classification done by experienced pathologist. Besides, the method also compared with other similar methods developed by others researchers. Test result shows the method able to classify the cervical cells with accuracy up to 77% in few milliseconds processing time, while manual pathologists require few minutes and other methods consume few seconds. This proofs that the developed method improve the existing color intensity classification method and can become a potential candidate for cervical cancer rapid screening.

ACKNOWLEDGEMENT

This work is fully support by Ministry of Science, Technology and Innovation (MOSTI) Malaysia under grant of Science Fund Vot No. Q.J130000.7125.03J24. The authors would like to thank to National Cancer Hospital Dharmais, Jakarta, Indonesia, Research Management Centre (RMC), Biotechnology Research Alliance (RA), Faculty of Heath Science and Biomedical Engineering, Universiti Teknologi Malaysia (UTM), and School of Electrical Engineering And Informatics, Institut Teknologi Bandung for their support.

References

[1] D.M. Parkin, F. Bray, J. Ferlay and P. Pisani, Global cancer statistics, 2002. *CA: a cancer journal for clinicians*, 55, 2005, pp 74–108.

[2] X. Castellsagué, S. de Sanjosé, T. Aguado, K.S. Louie, L. Bruni, J. Muñoz, M. Diaz, K. Irwin, M. Gacic, O. Beauvais, G. Albero, E. Ferrer, S. Byrne, F.X. Bosch, Ed. (2007) HPV and Cervical Cancer in the World 2007 Report *Vaccine*, Volume 25, Supplement 3, 1 November 2007

[3] G.N. Papanicolaou and H. F. Traut, Diagnosis of Uterine Cancer by the Vaginal Smear, *The Commonwealth Fund*, New York, 1943, pp. 19–45.

[4] D.C. Walker, B.H. Brown, A.D. Blackett, J. Tidy and R.H. Smallwood, A Study of the Morphological Parameters of Cervical Squamous Epithelium, *Physiol. Meas., IOP*, 24, 2003, pp 1 – 15

[5] R. J. Greenblatt, Human papillomaviruses: Diseases, diagnosis, and a possible vaccine, *Clinical Microbiology Newsletter*, 27(18), 2005, pp. 139–145.

[6] B.S. Apgar, L. Zoschnick, T.C. Wright Jr., The 2001 Bethesda System terminology, *Am Fam Physician*., 68(10), 2003, pp. 1992-1998.

[7] J.M. Agosti, S.J. Goldie, Introducing HPV vaccine in developing countries - key challenges and issues. *N Engl J Med,* 356, 2007, pp1908–1910

[8] J.P. Thiran, J. P., Macq, B. & Mairesse, J. , Morphological Classification of Cancerous Cells, *IEEE Proc. of Int. Conf. on Image Processing*, 1994, 3, pp 706-710.

[9] N. Mustafa, N. A. Mat Isa, M. Y. Mashor and N. H. Othman, Capability of New Features of Cervical Cells for Cervical Cancer Diagnostic System Using Hierarchical Neural Network, *IJSSST*, Vol. 9, No. 2, May 2008, pp 56-64

[10] I. Richter, *Lehrbuch für Heilpraktiker: medizinische und juristische Grundlagen*, Elsevier, Urban & Fischer Verlag, 2009, p. 669

[11] N.H. Othman, M.C. Ayub, W.A.A.Aziz, M. Muda, R. Wahid & S. Selvarajan, Pap Smears – Is It An Effective Screening Methods for Cervical Cancer Neoplasia? – An Experience with 2289 Cases, *The Malaysian Journal of Medical Sciences.* 4(1), 1997, pp. 45-50.

[12] S.K. Tay, *Cervical Cancer: Its Causes and Prevention*, Singapore: Times Book Int., 1996.

[13] T.G. Hislop, P.R. Band, M. Deschamps, H.F. Clarke, J.M. Smith, & V.T.Y. Ng, Cervical Cancer Screening In Canadian Native Women: Adequacy of The Papanicolaou, *The Journal of Clinical Cytology and Cytopathology,* 38(1), 1994, pp. 29-32.

[14] C. Li, C. Xu, C. Gui, and M. D.Fox., Level Set Without Re-initialization: A New Variational Formulation, *Proceedings of the 2005 IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR'05),* 2005.

[15] H.Z. Noorani, A. Brown, B. Skidmore, & G.C.E. Stuart, Liquid-Based Cytology and Human Papillomavirus Testing In Cervical Cancer Screening, *Canadian Coordinating Office for Health Technology Assessment*: Technology Report, 2003.

[16] J. Suryatenggara, B.K. Ane, M. Pandjaitan and W. Steinberg, Pattern recognition on 2D cervical cytological digital images for early detection of cervix cancer, *2009 World Congress on Nature and Biologically Inspired Computing*, *NABIC,* 2009 - Proceedings, art. no.5393710, pp. 257-262.

[17] J. S.-J. Lee, J.- N. Hwang, D. T. Davis, A. C. Nelson, Integration of Neural Networks and Decision Tree Classifiers For Automated Cytology Screening in *Proceeding of International Joint Conference on Neural Networks Seattle,* Vol. 1, July 1991 pp. 257-262

[18] N. Lassouaoui, L. Hamami and N. Nouali, Morphological description of cervical cell images for the pathological recognition (2005) *Proceedings - WEC'05: 3rd World Enformatika Conference*, 5, pp. 49-52.

[19] D.C. Wilbur, and Bibbo, 2008, Automation in Cervical Cytology,

Chapter 34 in *Comprehensive Cytopathology,* 2008.
[20] Y. Srinivasan, S. Yang, B. Nutter, S. Mitra, B. Philips and R. Long, Challenges in Automated Detection of Cervical Intraepithelial Neoplasia, in *Medical Imaging 2007: Computer-Aided Diagnosis*, edited by Maryellen L. Giger, Nico Karssemeijer, *Proc. Of SPIE* Vol. 6514, 65140F, 2007
[21] F.H. White and K. Gohari, Alterations in the volume of the intercellular space between epithelial-cells of the hamster cheek-pouch—quantitative studies of normal and carcinogen-treated tissues, *J. Oral Pathol. Med.* 13, 1984, 44–54
[22] F.B. Sorensen, P. Bichel and A. Jakobsen, Stereological estimates of nuclear volume in squamous-cell carcinoma of the uterine cervix and its precursors, *Virchows Archiv A Pathol. Anat. Histopath.* 418, 1991, 225–233
[23] Rahmadwati, Golshah Naghdy, Montse Ross, Catherine Todd, E. Norachmawati, Classification cervical cancer using histology images*, 2010 Second International Conference on Computer Engineering and applications,*pp.515-519.
[24] Thanatip Chankong, Nipon Theera-Umpon, Sansanee Auephanwiriyakul, Cervical cell classification using Fourier transform, *ICBME 2008*, proceeding 23, pp. 476-480, 2009.
[25] Yanxiu Liu, Tong Zhao, Jiayong Zhang, Learning multispectral texture features for cervical cancer detection.
[26] Eric Njoroge, Stephen R. Alty, Member , IEEE,Mahbub R. Ghani, Member, IEEE, Maha Akatib, Member, MRCOG, Classification of cervical cancer cells using FTIR data.*Proceedings of the 28th IEEE,EMBS Annual International Conference,New York City,USA,Aug 30-Sept 3,2006.*pp. 5338-5341

Bustanur Rosidi is a microbiologist. He has been working for three years in the diagnostics research group, research alliance biotechnology, Universiti Teknologi Malaysia. He obtained his PhD degree in microbiology from the University of Bochum, Germany. His research areas are cancer, genetic engineering and biomedical science. He has more than 20 international publications in the area of microbiology and medicine.

Prof. Supriyanto is a biomedical engineer. He is head of diagnostics research group, Universiti Teknologi Malaysia. He obtained his PhD degree in medical electronics from University of Federal Armed Forces, Hamburg, Germany. His research interest is engineering application in medicine. He has more than 100 international publications in the area of medical electronics, medical image processing and medical computing. He has 18 national and international innovation awards and more than 10 patents of biomedical products.