

A Review of Image Analysis and Pattern Classification Techniques for Automatic Pap Smear Screening Process

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Abstract—Pap smears are a very effective screening test for cervical precancerous. However, hundreds of small windows have to be looked under microscope by a trained cytologist for a single slide from each patient. It makes this process very tedious and erroneous. The automatic analysis of Pap smear microscopic images is one of the most interesting fields in biomedical image processing. This paper gives an overview of the state of the art and currently available literature and techniques related to Pap smear microscopic image analysis. Some techniques are used to detect cell components such as nuclei and cell boundaries. Other segmentation techniques are designed to use in single cell or clustered cell images. Many schemes are proposed for cell classification. The common aim of all these techniques is to develop an automated Pap smear analysis system which can help cytotechnician reducing time spent for slide examination in Pap screening process and save lives.

Index Terms— cervical cancer cell, cell microscopic image, Pap smear, Pap test

I. INTRODUCTION

Cervical cancer, one of various types of cancer found in female, develops in the cervix. Cervix is the cone-shaped lowest part of the uterus that opens into the vagina (birth canal). From the cancer statistical report of the International Agency for Research on Cancer (IARC), in 2004, cervical cancer is the second most common cancer in women in the world. In Thailand, the cervical cancer is the first most common cancer in women. The incidence of cervical cancer indicated that the average number of new cases is about 19.5 per 100,000 persons a year or about 6,300 women. The number of deaths in each year is about 3,000.

A Papanicolaou test, also called Pap smear or Pap test, is a medical screening method that can help prevent cervical cancer. The main purpose of the Pap smear is to detect for cell abnormalities that may occur from cervical cancer or before cancer develops. In Pap smear, sample cells are taken from the

cervix and smeared onto a glass slide. These cells are stained and fixed with a preservative to keep cells from becoming air-dried and distorted. The slides are then delivered to a laboratory where they are screened by a cytologist.

Because the development of cervical cancer takes very long time, 10-20 years, so it can be cured completely in the pre-cancerous stage. This is a reason why Pap smear screening can reduce cervical cancer deaths significantly since its widespread use began in 1950s. In the United States, between 1950 and 1970, the numbers of cervical cancer deaths are decreased 70 percent. However, Pap screening is time-consuming process. In many developing countries where there are still inadequate numbers of cytotechnicians who can examine slides. Therefore, it is urgently necessary to develop the automated Pap smear analysis system that can help cytologists in Pap screening.

This paper reviews several image analysis techniques as well as machine learning methodology used for automatic Pap test screening process and is organized as follows. Section II gives the information about Pap smear microscopic images. The techniques used to segment and classify Pap smear images are mentioned in section III. Finally, the conclusions and future research are in section IV.

II. PAP SMEAR MICROSCOPIC IMAGES

A. The Cervical Cell Microscopic Images

The Pap smear slides usually contain both of single cells and clusters of cells. Most of cells are found with high degree of overlapping. The physical appearance of cells in an image depends how the specimen, which collected from cervix, was smeared, stained, and captured. The stained process makes cells appear in different colors. In image acquisition process, size of cells in an image will be large or small depends on which magnification lens used. The quality of image also depends on the resolution of a digital camera. The sample images which contain normal and abnormal cells are shown in Fig. 1(a) and 1(b), respectively.

Beside of cervical cells, other cells may be found on Pap slides, for example, white blood cells, red blood cells, or even bacteria.

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B. Characteristics of Cervical Cells

Like other cells in human body, a cervical cell consists of two main components. One is nucleus located about the center of cell surrounded by the cytoplasm. Normally, nucleus shape is small and almost round. Its intensity is darker than cytoplasm.

The specimens, which are taken from several areas of the cervix, most often contain cells from columnar epithelium and the squamous epithelium. Between these two is the metaplastic epithelium.

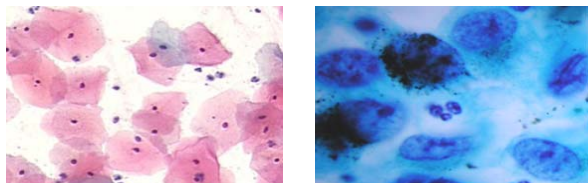


Fig. 1. Sample of cervical cell microscopic images. (a) Normal cells. (b) Abnormal cells. (Courtesy of Udonthani Regional Cancer Center)

The squamous epithelium consists of 4 layers of cells. The cells form at the basal layer and while maturing they move up through the parabasal layer, the intermediate layer, and finally the superficial layer. The cells in each layer are named according to their layer, i.e., basal, parabasal, intermediate, and superficial cells, respectively. While the cells mature and move through the layers, they change shape, color, and other characteristics. When the cells reach the superficial layer they are rejected and replaced by the next generation cells coming from below. In basal layer, cells are small and round with a relatively big nucleus and small cytoplasm. When maturing, the nucleus becomes smaller and the cytoplasm becomes larger. The shape of the cells becomes less round the more mature they are.

The columnar epithelium only contains a single layer of cells containing columnar cells and reserve cells. The reserve cells divide into new reserve cells and new columnar cells. In normal columnar epithelium cells, the nucleus is located at the bottom of the cytoplasm. When viewed from the top, the nucleus seems larger. When viewed from the side, the cytoplasm seems larger.

In dysplastic cells, or abnormal cells, the cell will not grow and divide as it should. This is precancerous cell. The dysplastic cells are divided into mild, moderate, and severe dysplastic. A high amount of the mild dysplastic cells will disappear without becoming malignant, whereas severe dysplastic cells likely will turn into malignant cells. The squamous dysplastic cells generally have larger and darker nuclei and tend to cling together in clusters. In severe dysplastic cells, nuclei are large, with dark granules and usually deformed.

C. Classes of Cells

In Pap smear microscopic image analysis [25], cervical cells are divided into 7 classes categorized by cell appearance, especially in cell nucleus. Since a cell nucleus can present the

significant changes when the cell is affected by a disease, the identification and quantification of these changes contribute in the discrimination of normal and abnormal cells in Pap smear images.

In Fig. 2, cells shown in the first three rows are superficial squamous cells, intermediate squamous cells, and columnar epithelial, respectively. These three types of cells are categorized as normal while cells in other rows are defined as malignant or abnormal cells.

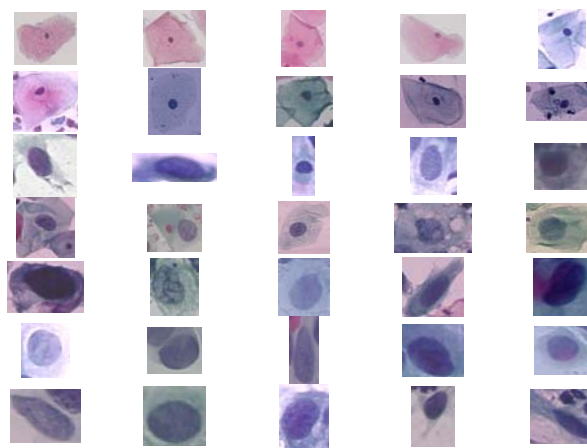


Fig. 2. The seven classes of cervical cells categorized by skilled cytotechnicians and doctors [25]. Cells in the first three rows from the top are categorized as normal cells while the others are abnormal cells.

III. TECHNIQUES USED IN PAP SMEAR IMAGE ANALYSIS

A. Color Models Used

Generally, many papers suggested that the color cell microscopic images should be converted to 8-bit gray levels in pre-processing step before passing to next processes [1]-[19], [21]-[26]. However, another color model such as HSI is also used for cell image segmentation [20].

B. Important Features of Cells

J. Jantzen and G. Dounias proposed several important cell features that are used for cervical cell images analysis and machine learning [25]. These features are:

- 1) Count of nuclei and cytoplasm pixels
- 2) Size of nucleus relative to cell size
- 3) The average perceived brightness
- 4) The shortest diameters of nuclei and cytoplasm
- 5) The longest diameters of nuclei and cytoplasm
- 6) The ratio between the shortest and the longest diameter
- 7) The length of perimeter of nucleus and cytoplasm
- 8) A measure of how well the nucleus is centered in the cytoplasm
- 9) Count of the number of pixels with the maximum/minimum value within a 3-pixel radius

There are many literatures adopted this features and found that the results of cell classification are promising [27]-[29].

C. Segmentation of Cell Images

Segmentation of cells in cytological images is a fundamental subject of quantitative analysis. Because the malignant or abnormal characteristics of cancer cells are contained in cell nucleus, so the isolation of cell nucleus is an important task of segmentation. The study of cell microscopic image segmentation can be reviewed as follows.

Segmentation of Cell Microscopic Images

There are many types of cytological microscopic images, for example, white blood cells, red blood cells, esophageal cells, or cancerous cells sampled from several parts of human body. Several nuclei segmentation techniques of cell microscopic images have been proposed [1]-[23], [26].

A nucleus segmentation method based on a water immersion algorithm was presented in [1]. An active contour model or snake has also been widely used as a global boundary based technique [2]-[6]. In [2], an unsupervised nucleus segmentation method based on a dual active contour was presented. However, traditional snake has mainly two drawbacks. One is that the initial position of contour should be placed near the real boundary of the object to converge it to the real boundary. The other one is that the process of energy minimization is time-consuming. To overcome these problems, improvements on snake have been proposed [3]-[4]. An improved snake for esophageal cell image based on seed point of the nucleus detected with the ultimate erosion was presented in [5]. The contour points of this improved snake are limited to move along the radial directions which can simplify the computation.

Fig. 3 shows the results of cell nucleus segmentation with different models. Fig. 3(b) and 3(e) are the results of Fig. 3(a) and 3(d), respectively, segmented using snake with conventional gradient-based potential energy and imposing no grow energy. Fig. 3(c) and 3(f) are the results obtained by the improved snake proposed in [5]. The segmentation results of overlapped nuclei are also shown in Fig. 4.

Methods that take advantage of the expected similarity in nuclei shapes and they are based on Hough transform have also been introduced [7]-[8]. Later, the generalized Hough transform [9]-[10] was used to detect elliptical shapes, based on analytical properties of the ellipse.

A combination of the generalized Hough transform and deformable models is used in [11] in order to find a set of templates specific to nuclei shape. A batch form of the Hough transform, the Compact Hough Transform (CHT), using maximum likelihood was presented in [12]. The CHT transforms the input image into one that depicts the likelihood of individual pixels being internal points to structures bounded by convex closed curves. The advantage of the CHT is that no analytical information about the curve is required.

Fig. 5(a) shows the result of the initial search for boundary points around the CHT maxima and Fig. 5(b) is the final result of technique proposed in [12].

A fuzzy logic engine has applied in [13] in order to distinguish the nuclei from the similar color background. A statistical model [7] was derived for the distribution of the features and the fuzzy logic engine was trained according to

the distributions of the features. Furthermore, a fuzzy form of Hough transform was proposed [26], in addition to the properties of the circular and the elliptical transforms, element of fuzzy set theory were used.

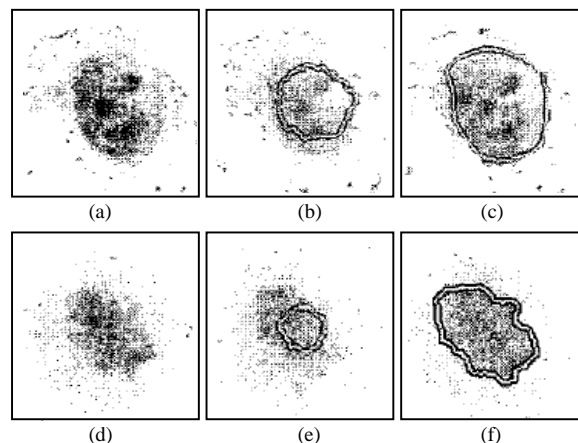


Fig. 3. The segmentation results of cell nucleus. (a) and (d) are the original images. (b) and (e) are the results of (a) and (d) obtained by the conventional snake. (c) and (f) are results obtained by the improved snake presented in [5].

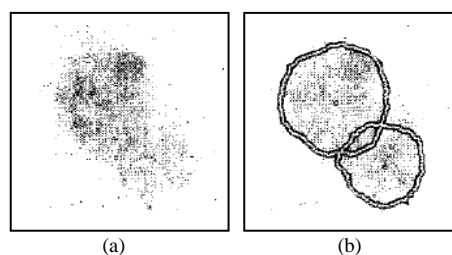


Fig. 4. The segmentation results of overlapped cell nuclei. (a) The original image. (b) A segmentation result obtained by using improved snake presented in [5].

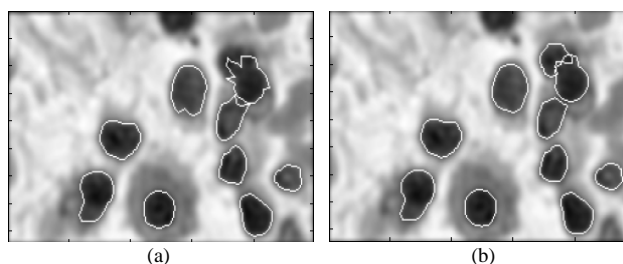


Fig. 5. The segmented images after applying CHT [12]. (a) Segmented image before the boundary optimization. (b) Segmented image after the boundary optimization.

The Genetic Algorithms (GAs) have been widely used in cell segmentation [15]-[19]. The combination of multifractal algorithm [18]-[19], based on computation of singularity exponent on each point, and GA has also been proposed [15]. The multifractal algorithm is used to determine the intervals of singularity exponents of each class, i.e., nucleus, cytoplasm and background, while GA is used in learning step. This

optimization allows to increase the precision at the borders and to decrease confusion between the various classes.

The conventional seed based region growing (SBRG) [22] has been used to detect the edges of certain regions of interest on digital images. The SBRG algorithm offers several advantages over other conventional edge detection algorithms based on gradient decision. It is also very stable with respect to noise. However, the SBRG is considered as a time-consuming algorithm and edge detection results are highly subjective to the user because two parameters, which are threshold value and initial seed point location, must be determined manually. Besides that, SBRG algorithm cannot avoid trapped seed point problem, or disconnected cell boundary, which causes incomplete edge detection process.

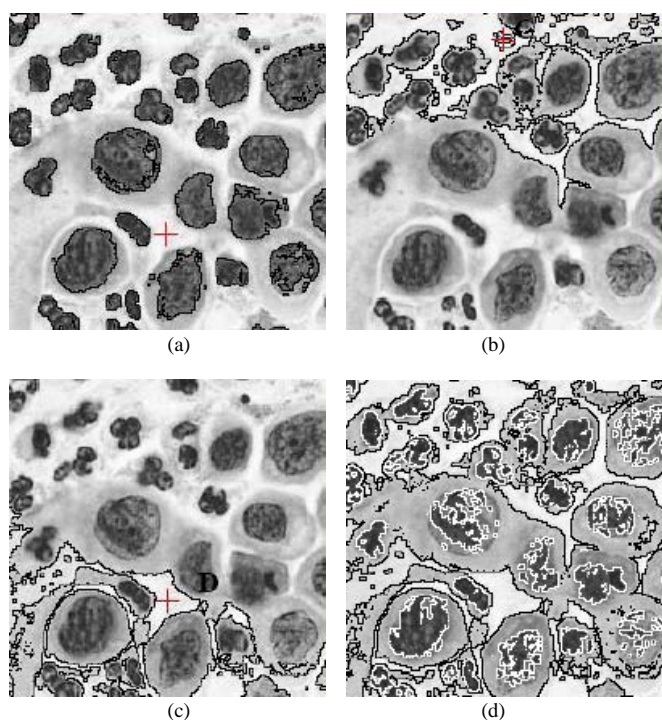


Fig. 6. Pap smear image results after applying various edge detection processes. (a) SBRG (nucleus edge). (b) SBRG (cytoplasm edge 1). (c) SBRG (cytoplasm edge 2). (d) MSBRG technique proposed in [21].

Most of segmentation techniques perform on gray-level images. In [20], the automatic color image segmentation of bone giant cancer cell images is presented. This segmentation technique based on anisotropic diffusion and anisotropic kernel mean shift. The colors of image are split into chromatic and achromatic channels and separately smoothing them through anisotropic diffusion.

Segmentation of Pap Smear Microscopic Images

Most of segmentation techniques applied in microscopic images where cells are extended in a cell grid and there is no overlapping. Some methods are also proposed for the segmentation of isolated cells in microscopic images [1]-[2]. However, cells obtained from Pap test are more difficult to segment because of the diversity of the cell structures contained in the images, the intense variation of background,

and overlapping of cell clusters. This high degree of cell overlapping results in difficult identification of nuclei boundaries.

TABLE I
SUMMARIZATION OF REVIEWED SEGMENTATION TECHNIQUES

Technique	Pros	Cons
Water immersion algorithm	Ensures closed boundaries detection	Fails in detecting of overlapped cells
Traditional active contour model	Unsupervised global boundary based segmentation	- The initial position of contour should be placed near the real boundary - The process of energy minimization is time-consuming
Improved active contour model	Takes advantages of traditional active contour model	Simplifies the computation
Hough transform	Detects expected similarity in nuclei shapes	Shape of an object should be round or almost round
Generalized Hough transform	- Takes advantages of traditional Hough transform - Can detect elliptical shapes	Fails in detecting of overlapped cells
Compact Hough transform	Does not require analytical information about the curve	Fails in detecting of overlapped cells
Fuzzy logic engine	Can handle uncertainty in data values (color, circularity, and object dimension) well	Fuzzy logic rules are fixed and cannot be adapted to changing conditions
Genetic Algorithm	Efficiently searches the hyperspace of segmentation parameter	Slowness
Seed based region growing algorithm	- Detects the edges of certain regions of interest in an image - It is stable with respect to noise	- Time-consuming algorithm - Edge detection result are highly subjective to user defined parameters - Cannot avoid trapped seed point problem or disconnected cell boundary
Moving k-means clustering	Can find the threshold values automatically	Cannot separate overlapped cells
Modified seed based region growing algorithm	The seed point locations and threshold values are determined automatically	Cannot separate overlapped cells

The fully automated segmentation of cell nuclei in Pap smear images was present in [14]. A deformable model [6] is used to define the nuclei boundaries in conventional Pap stained cervical cell images. The initial estimation of the deformable contour is obtained automatically and no user interaction is required.

An automated edge detection technique for Pap smear images using moving k-means clustering [23] and modified seed based region growing algorithm (MSBRG) is proposed

in [21]. The MSBRG employs the advantages of conventional SBRG in detecting the edges of certain regions of interest. In this study, k-means clustering algorithm is used to find the threshold values and these values are then used in MSBRG to detect the edges of the regions of interest automatically. The results after applying SBRG and MSBRG edge detection techniques are shown in Fig. 6. In conventional SBRG, there are 3 steps needed for cell edge detection: nucleus edge, cytoplasm edge 1, and cytoplasm edge 2, while MSBRG done in only one process.

The segmentation techniques reviewed above can be summarized their pros and cons as in Table I.

D. Classification of Cervical Cells

The classification process for cervical cell image requires that single cells in the slides can be automatically isolated and analyzed. Several classification techniques have been proposed [24]-[25], [27]-[29].

A prescreening instrument for cervical smear images using computerized image processing and pattern recognition techniques is proposed [24]. This system used a dual wavelength method for isolation of the cytoplasm and cell nuclei. It consists of four major stages: segmentation, feature extraction, cell classification, and smear classification. In segmentation stage, image is scanned at 4 μm resolution to do coarse segmentation. Then, coordinates of suspected atypical cells are stored. This procedure will be repeated over the specimen. Next, suspected object will be scanned at 0.5 μm resolution at two wavelengths and isolated to make sure it is a free cell.

In feature extraction, three different groups of parameters are extracted from cells. Density-oriented parameters are derived from the gray-level histogram calculated either over the nucleus or over the cytoplasm area. Shape-oriented parameters are calculated from the boundaries, and texture-oriented parameters are calculated from the gray-level image but only over the nucleus area.

In cell classification, cells can be classified into two groups: normal or atypical. Linear discrimination analysis was used in order to find a linear transformation. The results described in this stage were achieved by the nucleus definition failure logic. The nucleus detection failures were distributed according to cell categories as follows: 1) surface cells; 2) intermediate cells; 3) parabasal cells; 4) cylinder cells; 5) dyscaryotic surface cells; 6) dyscaryotic intermediate cells; 7) dyscaryotic parabasal cells; and 8) other dyscaryotic cells.

The normal group is very inhomogeneous with respect to cytoplasm area, a very important cell characteristic. This group includes both of large surface cells and intermediate cells as well as the relatively small cylinder cells. Therefore, a 3-group discriminant analysis was performed with the surface cells and the intermediate cells in one group, parabasal and cylinder cells in a second group and the malignant cells in a third group.

In the smear classification, the last stage, the smear is classified as normal or suspected malignant on the basis of the single cell classifications, namely, based on counting the number of atypical cells among normal cells.

However, there are some limitations using this approach. The input cells are scanned within a specific wavelength and the criteria of cell selection is based on specific thresholding which is not much flexible to use.

Another Pap smear classification approach, an analysis of Pap-Smear image data, is presented in [25]. This study also provides a Pap smear benchmark database which has reliably examined data for comparing classification method. They proposed a basic data analysis that provides numerical measure indicating how well the classes are separated. The data analysis consists of three main steps: 1) manual classification of cell classes, 2) features extraction, and 3) cell classification. In the first step, the sample images are carefully classified by cytotechnicians and doctors and then they are distributed unevenly in seven classes: superficial squamous epithelial, intermediate squamous epithelial, columnar epithelial, mild squamous non-keratinizing dysplasia, moderate squamous non-keratinizing dysplasia, severe squamous non-keratinizing dysplasia, and squamous cell carcinoma in situ intermediate. The first three cell types are defined as normal while other types are abnormal cells.

In the second step, feature extraction, twenty features are extracted from images of single cells. These features are nucleus area, cytoplasm area, N/C ratio, nucleus brightness, cytoplasm brightness, nucleus shortest diameter, nucleus longest diameter, nucleus elongation, nucleus roundness, cytoplasm shortest diameter, cytoplasm longest diameter, cytoplasm elongation, cytoplasm roundness, nucleus perimeter, cytoplasm perimeter, nucleus position, maxima in nucleus, minima in nucleus, maxima in cytoplasm, and minima in cytoplasm.

Features extracted in previous step are then used as input data in cell classification step. The distance between each class center is measured and compared with the variation to indicate the degree of class overlap. They compared the results of three advanced classifiers against a simple minimum distance classifier. However, it is not quite possible to separate classes linearly. Cell types such as mild, moderate, and severe dysplasia (abnormal form) have unclear cell boundaries even to professionals.

This study concluded that some features such as N/C ratio, nucleus brightness, and cytoplasm brightness are generally the most capable for discriminating classes in both 2-class and 7-class problems. Nucleus longest diameter and nucleus area are the next two most important features.

A problem of Pap smear classification consists of using important features of cells, usually described by a large feature vector, to induce a model that classifies cells into defined classes. Selecting the right set of features for classification is one of the most important problems in designing a good classifier. A Pap smear classification technique based on the nearest neighbor classification rule [30] and the Tabu search [31]-[32] is proposed in [27]. Features describing each cell are obtained by [25]. Different nearest neighbor classification rules, which are the 1-nearest neighbor, the k-nearest neighbor and the wk-nearest neighbor, are used for classification problem. The Tabu search approach is used for the feature selection problem. Another approach for the feature subset selection is presented in [29]. In the

classification phase, a number of variants of the nearest neighbor classification method are used. A Genetic Algorithm is used to reduce sets of features in the feature selection phase. Genetic Algorithms offer a particularly attractive approach for problems like feature subset selection since they are generally quite effective for rapid global search of large, non-linear and poorly understood spaces. The Ant Colony Optimization is also used in feature subset selection problem [29].

The obtained results of [27]-[29] indicate the high performance in searching for a reduced set of features with high accuracy and in achieving excellent classification of Pap-smear cells both in 2 classes and in 7 classes.

IV. CONCLUSIONS AND FUTURE RESEARCH

Most of prominent digital image analysis techniques for cervical precancerous screening using Pap test are reviewed in this paper. This review consists of three sets of information. Characteristic of Pap smear images are reviewed. Pap smear images cell segmentation, feature extraction and classification are also reviewed. This review may help researchers in this field to see the problem and the state of the art techniques and also have a good start to get off the ground solving it. There are still some weak points for the techniques reviewed, such as, low accuracy of classification in some classes of cells, which needs to be improved.

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