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Introductory and Literature Review of Cytological Cell Images for Analyzing & Detection of Cancer Cells using Image Processing Techniques

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Abstract: *This research proposal intends to develop an efficient and reliable algorithm for cytological image analysis system and that could be used for analyzing and investigating the pre cancerous cell changes in blood smears at early stage. Thereby classify the smear image slides as benign or having the chance of malignancy.*

1. Introduction

1.1 What is Cancer?

The body is made up of trillions of living cells. Normal body cells grow, divide, and diein an orderly fashion. Cancer begins when cells in a part of the body start to grow out of control. There aremany kinds of cancer, but they all start because of out-of-control growth of abnormalcells.Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells. Cancer cells can also invade (grow into) other tissues, something that normal cells cannot do. Growing out of control and invading other tissues are what makes a cell a cancer cell.

1.2Cytological Cell Image Analysis

Cell image analysis has received much attention with the increasing demands in both bioinformatics and biomedical applications. Cell morphology is essential in identifying the shape, structure, form, and size of cells. Morphological cell analysis is a key issue for abnormality identification, classification and early cancer detection. Morphological cell analysis has been integrated in new methods for biomedical applications such as automatic segmentation and analysis of histological (The study of the form of structures seen under the microscope) tumour sections, boundary detection of cervical cell nuclei considering overlapping (group cells) and clustering and morphological characteristics analysis of specific biomedical cellsetc.[1].Morphological analysis has become a powerful mathematical tool foranalysing and solving cell informatics. Automatic feature quantification is undoubtedly the mostwidely used estimation technique in this topic. Among the variety of developed methods, the main differences and remarkable features can be summarized briefly as shape, geometrical, intensity, and texture. [2].

Digital image processing can help the pathologists to a great extent. The automated detection and segmentation of cell nuclei is one of the most interesting fields in cytological image analysis.In this, nucleus is the very important structure within the cell and its structure represents significant changes when the cell is affected by a disease and thus 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 discrimination of normal and abnormal cells.[2] So the necessity of this study is to design an efficient algorithm for an image analysis system that helps the pathologists for early detection and diagnosis of malignant cancerous cells.

1.3General View of Diagnosis System for Cancer Detection

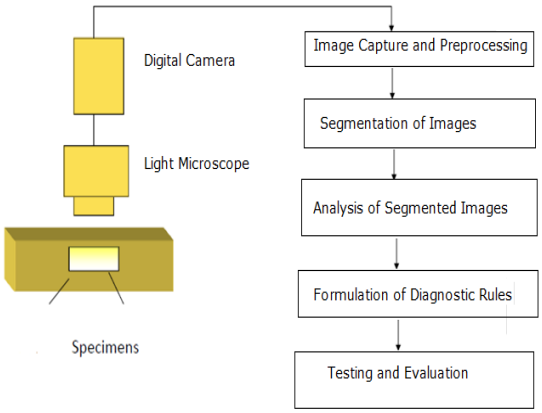

Figure 1. Computer Aided Diagnosis System [3]

A light microscope, also called an optical microscope, is an instrument used to observe small objects and specimens and to see details on them which would not be visible with the naked eye.Using digital camera, image is captured from themicroscope which is analysed and finally tested.To study cell characteristics, detect abnormalities, and determine the malignant degree, pathologists examine biopsy material under a microscope, which is subjective, laborious, and time consuming. Therefore quantitative cell morphology

is studied and computer-assisted systems are presented for diagnostic process.

1.5 Leukemia (Cancer of the Blood)

The word **Leukemia** comes from the Greek *leukos* which means "white" and *aima* which means "blood". It is cancer of the blood or bone marrow (which produces blood cells). A person who has leukemia suffers from an abnormal production of blood cells, generally leukocytes (white blood cells). This abnormality causes the blood cells to grow and divide chaotically. Normal blood cells die after a while and are replaced by new cells which are produced in the bone marrow. The abnormal blood cells do not die so easily, and accumulate, occupying more and more space. As more and more space is occupied by these faulty blood cells there is less and less space for the normal cells and the sufferer will become ill.

The presence of excess number of blast cells in peripheral blood is a significant symptom of leukemia. So haematologists routinely examine blood smear under microscope for proper identification and classification of blast cells. Manual examination of the slides are subjected to bias i.e. operator experience, tiredness etc. resulting with inconsistent and subjective reports. So there is always a need for a cost effective and robust automated system for leukemia screening which can greatly improve the output without being influenced by operator fatigue [3].

Blood is a red liquid composed of isotonic fluid called as plasma in which various cells are suspended. There are three major groups of these cells and all blood cells are manufactured in bone marrow. The red blood cells called Erythrocytes that contain hemoglobin and carries oxygen to the tissues. Platelets called thrombocytes is responsible for clotting of blood and Leukocytes known as white blood cells , which are the cells of immune system defending the body against infectious diseases and foreign materials.

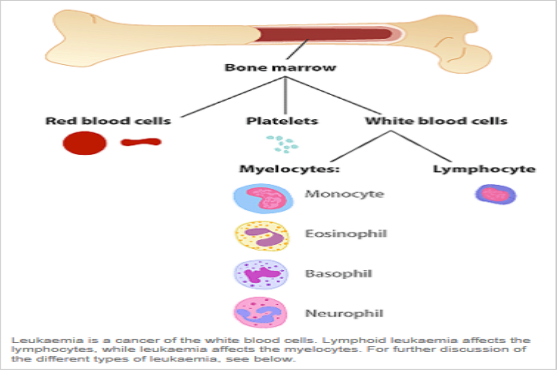

Figure 2. Image from the site http://healthlineinfo.com/what-is-leukemia.html

Our immune system protects us from an extraordinarily large variety of bacteria, viruses, and other pathogenic organisms by identifying and killing them. One drop of blood can contain between 7,000 to 25,000 white blood cells.A Leukemia patient may have as many as 50,000 white blood cells in a single drop of blood.It also constantly surveys the body for the presence of abnormal cells such as tumor cells and virally infected cells, and destroys such cellswhen they are found. Leukocytes are the principal ingredient in the immune system.

They are all produced and derived from a multipotent cell in bone marrow, including granular types such as neutrophils, eosinophils and basophils, and nongranular types such as lymphocytes and monocytes. The number of leukocytes can be used as a reference to identify the disease because the leukocyte number varies with respect to the age for each person.[4,5]

White Blood Cells or Leukocytes

Table 1. Normal Range of White Cell Counts for Healthy Adults and Children [4,5]

per microliter (μL) of blood	**Men**	**Women**	**Children**
White Cells	5,000 to 10,000	4,500 to 11,000	5,000 to 10,000
Red Cells	4.7 to 6.1 million	4.2 to 5.4 million	4.0 to 5.5 million
Platelets	150,000 to 400,000	150,000 to 400,000	150,000 to 400,000

Types of White Blood Cells

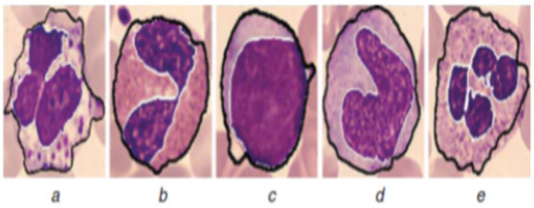

Figure 3. Five Types of WBCs a) Basophil b) Eosinophil c) Lymphocyte d) Monocyte e) Neutrophil [13][14]

Types of WBC	Nucleus Shape	Blood Contains What %?
Basophils	Bi-lobed or Tri-lobed	0.5% - 1%
Eosinophils	Bi-lobed	1% - 4%
Lymphocytes	Spherical	20% - 40%
Monocytes	Kidney Shaped	2% - 8%
Neutrophils	Multi-lobed	55 % - 70%

Leukemia Disease Classification

Doctors divide leukemia into two main groups based on the speed that the leukemia develops. Acute leukemia develops very quickly. Chronic leukemia tends to develop slowly, usually over months or years. For a long time, it may not cause many symptoms. Doctors further divide these groups depending on the type of white blood cell they affect. [6]

Acute leukemia include

- Acute myeloid leukemia (AML), which affects myeloid cells
- Acute lymphoblastic leukemia (ALL),which affects lymphoid cells

Chronic leukemia include

- Chronic myeloid leukemia (CML), which affects myeloid cells
- Chronic lymphocytic leukemia (CLL), which affects lymphocytes

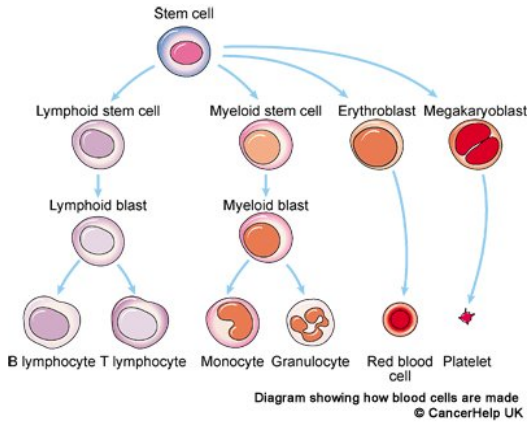

Figure 4. Diagram of Blood Cell Formation

Acute lymphocytic leukemia (ALL) is the most common type of leukemia in young children. ALL can also occur in adults those aged 65 and older. Acute myelogenous leukemia (AML) is a common type of leukemia that occurs in adults than in children and more commonly in men than women. Chronic lymphocytic leukemia (CLL) often affects adults over the age of 55 but almost never affect children. Chronic myelogenous leukemia (CML) mainly affects adults. [6]

Leukemia – Statistics

Facts 2012 (American Cancer Society) includes the estimated numbers of new blood cancer cases and deaths in 2011. An estimated 44,600 new cases of leukemia are expected to be diagnosed and 21,780 people are expected to die from leukemia.

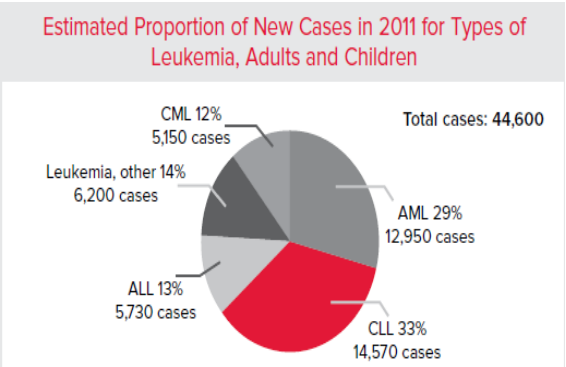

Figure 5. Source: Cancer Facts & Figures, 2011. American Cancer Society; 2011.

Table 2. Information Provided by Leukemia Research Foundation, United States

2012 Statistics	Estimated new cases	Estimated deaths
Acute lymphocytic leukemia	6,050	1,440
Chronic lymphocytic leukemia	16,060	4,580
Acute myeloid leukemia	13,780	10,200
Chronic myeloid leukemia	5,430	610
Other leukemia	5,830	6,710

Leukemia accounts for about 33% of cancer cases in children aged 0-14. An estimated 1,340 cancer deaths are expected to occur among children aged 0-14 in 2012—about one-third of them from leukemia. Leukemia is the leading cause of death by disease in children up to age 14. Leukemia is diagnosed 10 times more often in adults than in children. Due to advances in treatment, there has been a dramatic improvement in survival for people with acute lymphocytic leukemia, from a 5-year relative survival rate of 41% in 1975 to 1977 to 67% in 2001 to 2007 in adults. Survival rates for children with acute lymphocytic leukemia have increased from 58% to 91% over the same time period [7].

An article from Indian Journal of Medical and Paediatric Oncology, 2012 stated that 65% to 85% of all leukemia reported is ALL. From a TOI (Times of India) article dated 28the March 2012: India officially recorded over half a million deaths due to cancer in 2011 – 5.35 lakhs as against 5.14 lakh (2009) and 5.24 lakh (2010). UP recorded 89,224 deaths due to cancer, while Maharashtra saw 50,989 fatalities. The Union health ministry says there are about 28 lakh cases of cancer at any given point of time in India, with 10 lakh new cases being reported annually. World Health Organization (WHO) says the estimated cancer deaths in India are projected to increase to 7 lakh by 2017.

2. Literature Survey

The objective of the literature survey was to acquire an understanding of the current state of knowledge in the field in which this research was undertaken and to identify key research groups, seminal authors and forums where such research was presented. The survey process involved an examination of numerous texts, refereed journal publications, conference proceedings, Internet-sourced publications and trade journals.

M. E. Plissiti, C. Nikou, and A. Charchanti [8] proposed a method for segmentation of cell nuclei based on the watershed transform that can be applied to the cell images containing both isolated and clustered cells. In this centroid of regional minima of the images are detected through morphological reconstruction and boundaries of the nuclei are defined with watershed transform. Then clustering is performed using Fuzzy C means algorithm. As a future work, performance of clustering algorithm has to be improved with the selection of different nuclei features.

Shys-Fan Yang-Mao et al., [9] presented an edge enhancement nucleus and cytoplast contour (EENCC) detector to enable cutting the nucleus and cytoplast from a cervical smear cell image. A mean vector difference enhancer is presented to suppress the gradients of noises and also to brighten the gradients of object contours. In addition to cervical smear images, these proposed techniques are also useful for and can be deployed on the object segmentation of other kinds of images as well. In their future work, they intended to develop a system for detecting whether a cervical smear cell is normal or abnormal based on the size, shape, and texture of its cytoplast and nucleus.

SitiNoraini Sulaiman et al., [10] proposed a technique for the separation of overlapping cells in which it integrates edge detection process and pseudo color technique with color space extraction. The proposed technique is capable to distinguish each cervical cell from overlapping cervical cell images. But still research can be extended by focussing on the images that have been corrupted with certain degree of noise.

BH Willis,P Barton et al., [11] ," Cervical screening programmes: can automation help? Evidence from systematic reviews, an economic analysis and a simulation modelling exercise applied to the UK" concluded as the available evidence is still insufficient to recommend implementation of automated image analysis systems and suggested further research.

Henry C Kitchener, Roger Blanks et al.,[12], An article " Automation assisted versus manual reading of cervical cytology (MAVARIC): a randomised controlled trial", 2010 - Lancet Oncology. The main finding of the MAVARIC trial was that sensitivity of automation-assisted reading was inferior to manual reading of cervical cytology. Their review suggested that an automated reading is less sensitive than manual reading in the detection of cervical intraepithelial neoplasia grade 2 or worse and grade 3 or worse and cannot recommend the adoption of automation-assisted cervical cytology in well organised screening programmes.

BabakSokouti [13] proposed a technique that integrates Edge Detection process and Pseudo-Color technique, with Color Space Extraction employed at preprocessing stage. First, the color space concept is applied to extract the original image of Pap smear into red plane, green plane and blue plane. Then the Seed Based Region Growing technique is applied to find boundaries of the cells. Pseudo-color technique is then embedded to the demarcated region to determine each part of the cell; nucleus, cytoplasm and background. The proposed technique is capable to distinguish each cervical cell from overlapping cervical cells image.For the future work, they recommended artificial neural networks for classification.

LimHuey Nee, MohdYusoffMashor, Roseline Hassan[3] proposed a method for WBC segmentation for acute leukemia that consist of gradient magnitude, thresholding, morphological operations and watershed transform to perform cell segmentation and they obtained qualitatively good result but drawback of their method is incomplete localization of the cells of interest including nucleus and cytoplasm as a whole.

R. Adollah, M.Y Mashor, E.U Francis, N.H Harun,[14] proposed a method called multilevel thresholding based on the intensity gray level histogram of the image in order to segment the object of interest. This method is utilized to segment WBC from its complicated background but this proposed method is suitable only for normal images and not suited for dark or bright images.

Der-Chen Huang, Kun-Ding Hung [15] contributed a method to segment the nuclei from the smear images and the method nucleus enhancer can enhance the nucleus region and suppress the non-nucleus region. They combined PCA (Principle Component Analysis)and genetic based K means clustering to classify the type of leukocytes.But still improvement has to be done to enhance the nucleus and cytoplasm region using different clustering algorithms.

SubrajeetMohapatra,SushantaSamanta, DiptiPatra, and Sangamitra Satpathi[16]. A fuzzy based two stage color segmentation strategy is employed for segregating white blood cells from other blood components. Hausdorff dimension and countor signature is implemented for classifying cell nucleus. SVM is used for classification. But still improvement has to be done to enhance the classification of lymphoblast into various sub types

and also to investigate differentleukemia type classification.

SubrajetMohapatra[17] ,in this a two stage color segmentation strategy is employed for segregating leukocytesor white blood cells (WBC) from other blood components. Discriminative features i.e. nucleus shape, texture are used for final detection of leukemia. Two novel shape features i.e., hausdorff dimension and contour signature is implemented for classifying a lymphocytic cell nucleus. Support Vector Machine (SVM) is employed for classification.As afuture work, classification of lymphoblast into various subtypes and alternate techniquescan be investigated for stain independent blood smear image segmentation and leukemia type classification.

Wei-Liang Tai1et al., [5] segmented the stained blood cell images and extract the geometric features to identify and to classify different type of blood cells. In certain cases the cytoplasm of some neutrophils had a complex texture and this were mistaken for eosinophils. They suggested that SVM classification with hierarchical multi class SVM could improve the performance of classification.

H.T. Madhloom et al., [18] proposed a method that focus on white blood cell nucleus segmentation that can be used to separate the nucleus from the whole cell body by using the combination of automatic contrast stretching supported by image arithmetic operations, minimum filter and global threshold techniques. Work can be extended with larger data sets and also to enhance the classification techniques by making use of better classifiers.

N.H. AbdHalim et al., [19] used a global contrast stretching and segmentation based on HSI (Hue, Saturation, Intensity) color space are used to improve the image quality. In their future work , they suggested to use the same technique for extracting the features from the blood slide images.

NorHazlyna and MohdYusofMashor [20] method successfully segmented and distinguished most of the acute leukemia blood cells from its background. Furthermore size and the shape of the acute leukemia cells are closely preserved. The work can be extended for other classifications of leukemia.

RaminSoltanzadeh et al., [21] introduced an algorithm for detection of candidate zone of nucleoli located in nuclei as a new tool for automatic exploring inside WBCs. This method is based on curvelet transform that is an appropriate tool for extraction and amplification of detailed information of cells such as nucleoli. Although the mentioned methods are able to generally segment WBCs, more advanced techniques are required forexploring insideWBCs.

J. M. Sharif, M. F. Miswan et al.,[22] The main idea in this research is the using of masking and morphological operation function to eliminate unwanted objects. This research has used the technique from the combination of pixel based, region based and morphological segmentation and it is hoped that a better mix methods can be developed from a variety of methods. The improvement need to be done for both segmentation and overlapped cell handling to obtain better result in the future.

Zhonghua Lin and HongfeiYu , [23] they presented a cell image segmentation andclassification method based on OTSU method and connected region labeling. The experimental results show thatthe method is practical and effective and it has good robustness andensures the accuracy.When the boundary of a cellimage is very fuzzy, the segmentation and classification arenot very good. To solve this problem they have to improve the preprocessing methods.

Sheng-Fuu Lin and Yu-Bi Hong, [24] they presented a simple method to ease the influence of un-uniform stain and extract the white blood cell nucleus for further analysis. Furthermore, they presented a feature oflobes to distinguish the nucleus granulocyte (neutrophil, eosinophil, and basophil) from others. Future work could be done on the improvement of the accuracy classification rate by enhancing the efficiency of the algorithm.

ShraddhaShivhare, Rajesh Shrivastava, [25] the differential counting of white blood cell provides invaluable information to doctors for diagnosis and treatment of many diseases. Manually counting of white blood cell is a tiresome, time-consuming and susceptible to error procedure due to the tedious nature of this process. So an automatic segmentation technique for microscopic bone marrow white blood cell images is proposed. They investigated whether information about the nucleus alone is adequate to classify white blood cells and the features using nucleus alone can be utilized to achieve aclassification rate of 77%. Future workcould be done on the improvement of classification rate by enhancing the efficiency of the algorithm.

P.S.Hiremath et al.,[26] The objective of their study is to develop an automatic tool to identify and classify the white blood cells namely, lymphocytes, monocytes and neutrophil in digital microscopic images. They have proposed color based segmentation method and the geometric features extracted for each segment are used to identify and classify the different types of white blood cells. They suggested that the work could be improved further by better pre-processing methods and feature sets.

Ling Li1, Guitao Cao et al.,[27] proposed a new method about localization of immature precursor cells in pathological images of bone marrow. They modified OTSU method to binarize the image and then they processed the image with morphological operations, combined with their own characteristics and the sizes of cells and finally theyobtained the right results of cellular localization. This proposed method depends on the quality of

images and is vulnerable to noise. So their future work will focus on improving the methods of detecting cells .

Prof. Samir K. Bandyopadhyay et al.,[28] they proposed a method to segment nucleus and cytoplasm of white blood cells (WBC). They segmented cell images with varying background and illumination conditions. The results of segmentation show the better performance in comparison to the conventional methods. They used simple morphological operators and explore scale-space properties of a toggle operator to improve the segmentation accuracy. The proposed scheme has been successfully applied to a large number of images, showing promising results for varying cell appearance and image quality and encourage future work.

Prof. Samir K. Bandyopadhyay et al., [29] presented a method for WBC segmentation into nucleus and cytoplasm which helps in the diagnosis of several diseases. Their method successfully segments WBC images into nucleus, cytoplasm and background and is more reliable, computationally less expensive and the proposed algorithms are better to detect the cells. Future work will focus on classification of the resulting contours, as well as improving culling measures to filter out more incorrect contours.

3. Conclusion

Although cytological image analysis has been used for the estimation and diagnosis of the diseases, some problems still exist in its application. During mass screening programs there will be huge number of samples [30] to be analysed and diagnosed and current manual screening methods are time consuming to diagnose more samples in short time. This screening process is very important, which alert the specialist to check further, if abnormal cells were detected. As per the study of the Literature Survey, the following observations are made:

- Reliability and accuracy of the calculated cell strongly depends on the input image. Noise statistics, localization and good approximation of the result strongly depends on the pre-processing techniques used.So suitable methods should be explored to improve the reliability and accuracy.
- Conventional methods are failed to provide accurate and précised results due to the limitations in selecting and extracting the features. So advanced feature extraction techniques can be used to improve the performance.
- Separation of overlapped or grouped cells is a tedious task in cell image analysis. Overlapping and connected cluster is still a key problem in cell image segmentation. If overlapping cells are precisely segmented, we can achieve significant improvement in the system. So there is a need to tackle these issues to improve the efficiency of the system.
- Classic machine learning models such as support vector machines (SVM), Bayesian network (BN) , Artificial Neural Network (ANN) models etc. can be used to improve the classification. So integration of the morphological cell analysis with some artificial intelligence methods such as fuzzy logic, neural networks and genetic algorithm helps to improve the efficiency of the system for better detection and classification.
- Recent systematic review commissioned by the HTA (Health Technology Assessment) concluded that reliable conclusions about automated screening could not be drawn owing to the lack of sufficiently rigorous evaluations and trials. So further high quality primary research is required.

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