Automated Leukemia Detection Using Pattern Analysis And Gene Expression Profiling

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ABSTRACT: *Blood cell analysis is an important diagnostic tool because it can help to detect a wide range of diseases. In traditional cancer diagnosis, pathologists examine biopsies to make diagnostic assessments largely based on cell morphology and tissue distribution. However, this is subjective and often leads to considerable variability. On the other hand, computational diagnostic tools enable objective judgments by making use of quantitative measures. Such automated cancer diagnosis facilitates objective mathematical judgment complementary to that of a pathologist, providing second opinion for patients. Newer chromosomal tests and the analysis of multiple genes at a time (also known as gene profiling) may sub-classify the cancer at root level and help determine individual treatment. Instead of analyzing the expression level of one gene at a time, scientists can simultaneously analyze the expression levels of thousands of genes over different samples using DNA microarray, which allows the identification of gene expressions to draw biologically meaningful conclusions for applications that ranges from the genetic profiling to the diagnosis of oncology diseases.*

Keywords: *Pre-processing, Morphology, Feature Extraction, Blood image analysis, DNA microarray, gene expression, image segmentation, DNA chips, gridding.*

I. INTRODUCTION:

Pathologists conduct specific tests on the cancer to determine a number of factors, including the type of cancer cells, the grade of the cancer, and the size of the tumor, the extent the cancer has invaded the surrounding tissue and whether the cancer has spread. This information, compiled in a pathology report, provides patients and their medical team essential information to determine the best treatment [1].

The pathologist first looks at the tissue with the naked eye in a "gross examination." Its appearance and characteristics, such as size, weight, color and texture, are then recorded. Newer chromosomal tests and the

analysis of multiple genes at a time (also known as gene profiling) may sub-classify the cancer at root level and help determine individual treatment [2].

The century-old process of staining biological tissue for examination under a microscope remains the standard assessment tool for pathologists looking for signs of cancer. The new technique provides images that rival or surpass those from histological staining. In addition, the technique is automated, and it provides quantitative data on structures at the cellular level. Blood morphology and staining is used to identify abnormalities in cell shape, structure, and the condition of the cell nucleus [3]. Microscopes equipped with digital cameras are currently the gold standard for analyzing cells.

Cancer is a disease characterized by uncontrolled cell growth and proliferation. For cancer to develop genes regulating cell growth and differentiation must be altered; these mutations are then maintained through subsequent cell divisions and are thus present in all cancerous cells. Blood cancers affect the production and function of the blood cells. Most of these cancers start in the bone marrow where blood is produced. Stem cells in the bone marrow mature and develop into three types of blood cells: red blood cells, white blood cells, or platelets.

Leukemia, a type of cancer found in blood and bone marrow, is caused by the rapid production of abnormal white blood cells [4], [5]. The high number of abnormal white blood cells is not able to fight infection, and they impair the ability of the bone marrow to produce red blood cells and platelets.

There are two main types of leukemia:

- **Chronic leukemia** is often found before there are any symptoms. This type of leukemia usually starts slow but gets worse as the number of leukemia cells increases.
- **Acute leukemia** keeps the white blood cells from working at all. This type of leukemia usually gets worse quickly.

Leukemia can also be described by where it starts in the body:

- **Lymphocytic leukemia** starts in the lymphoid cells, which help make certain types of white blood cells, such as B cells or T cells.
- **Myeloid leukemia** starts in the myeloid cells, which help make red blood cells, platelets, and certain types of white blood cells.

Terms to describe where the cancer starts and how quickly it spreads:

- Chronic lymphocytic leukemia (CLL)
- Chronic myeloid leukemia (CML)

- Acute lymphocytic leukemia (ALL)
- Acute myeloid leukemia (AML)

Gene expression profiling is a technique used in molecular biology to query the expression of thousands of genes simultaneously [6]. The information derived from gene expression profiling often has an impact on predicting the patient's clinical outcome. While almost all cells in an organism contain the entire genome of the organism, only a small subset of those genes is expressed as messenger RNA (mRNA) at any given time, and their relative expression can be evaluated [7]. Techniques include DNA microarray technology or sequenced-based techniques such as serial analysis of gene expression (SAGE). The majority of cells within a tumor will share a common profile of gene expression.

A DNA microarray (**DNA chip** or **biochip**) is a collection of microscopic DNA spots attached to a solid surface [8]. DNA microarrays are generated by either printing pre-synthesized cDNAs (500–2000 bases) or synthesizing short oligonucleotides (20–50 bases) onto glass microscope slides or membranes. cDNAs for microarrays may include fully sequenced genes of known function or collections of partially sequenced cDNA derived from expressed sequence tags (ESTs) corresponding to the messenger RNAs of unknown genes.

DNA microarray image processing is used to measure and image the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. In general, thousands of gene-specific probes are arrayed on a small matrix, and this matrix is probed with labelled nucleic acid synthesized from a tissue type, development stage, or other condition of interest [9], [10]. The substrate of a microarray consists of a piece of glass, or a silicon chip, similar to a microscope slide. Onto this substrate, thousands of patches of single-stranded DNA are fixed which are called probes [11].

The chip is then scanned for the presence and strength of the fluorescent labels at each spot representing probe-target hybrids. The level of fluorescence at a particular spot provides quantitative information about the expression of the particular gene corresponding to the spotted cDNA sequence [12]. Due to lowering costs, RNA-Sequencing is becoming more common as a method for cancer gene expression profiling. It is superior to microarray techniques due to not having the bias inherent in probe selection. Some of the commercial software packages include ScanAlyze [13], [14], GenePix [15],

Dapple [15], [16], ImaGene [17], [18], QuantArray [18] etc. for automated microarray image analysis.

The goal of microarray image analysis steps is to extract intensity descriptors from each spot that represent gene expression levels and input features for further analysis. Biological conclusions are then drawn based on the results from data mining and statistical analysis of all extracted features [19]. The main objective is to use DNA microarray image processing as a tool to diagnose a complex disease (e.g. cancer) at its genetic level by expression level of the genes.

Table 1: List of Genes based on Leukemia Type (courtesy: Leukemia Gene Database)

Type of Leukemia	**Gene Name**
Acute Lymphoblastic Leukemia	MLLT2, MYC, ZNFN1A1,LAF4
Acute Myeloblastic Leukemia	ARNT
Acute Myelogenous Leukemia	IRF1, RGS2, GMPS
Acute Myeloid Leukemia	AF10, CBFB, NUP98, NUP214, HOXA9, CREBBP,ARHGEF1
	2,CDX2, LCP1, CEBPA, DEK, FUS, RUNX1
Acute Promyelocytic Leukemia	PML, THRA, NPM1
Acute Undifferentiated Leukemia	SET
B-cell Chronic Lymphocytic Leukemia	BCL3, BTG1
B-cell (acute) Leukemia	PBXP1
Chronic Lymphocytic Leukemia	DLEU1, DLEU2
Chronic Myelocytic Leukemia	AXL
Murine Myeloid Leukemia	EVI2A, EVI2B
Myeloid Leukemia	CDC23, CLC
pre B-cell Leukemia	PBX1, PBX2, PBX3
T-cell Leukemia	TCL6, TCL1B, TRA@, MTCP1, LDB1
T-cell Acute Lymphoblastic Leukemia	NOTCH1, NOTCH3,LYL1,HOX11, BAX, LMO1, LMO2,TAL1, TAL2

T-cell Leukemia (in lung carcinoma)	CAV1
Cutaneous T-cell Leukemia	NFKB2
Human Monocytic Leukemia	ETS1
Mast cell Leukemia	KIT
Mixed Linkage Leukemia	MLLT3, MLL3
MLL	LAF4

II. REVIEW OF EARLIER WORKS

Authors suggested several methods to segment nuclei of white blood cells via techniques that can be categorized into color-based methods. These methods are simple but are not capable of segmenting the white blood cells nucleus accurately. In addition, cytoplasm is colorless in most cases. Thus, its boundary is not detectable and cannot be segmented by these methods. Methods based on imaging techniques generate superior results. For example, the method proposed in [14] obtained more acceptable results using multi-spectral imaging techniques. In this method, intensity of each pixel in different spectra is used to construct the feature vectors and a support vector machine (svm) is used for classification and segmentation. In spite of efficacy of this method for segmenting white blood cells components, this system's implementation is costly and thus cannot be used widely at all laboratories. Cytoplasm and nucleus segmentation via mathematical and contour models is the third method and also the most important one. In this field, some methods such as region growing, watershed [10], parametric active contour deformable models, and also combination of the watershed technique and a parametric deformable model are introduced in the literature [1-7]. These methods are more complex and require more processing time in comparison with the first group of methods. However, their advantage is subtle more accurate segmentation.

Peter Bajcsy [19] presented an overview of microarray technologies, overall microarray data processing workflow and management, microarray layout and file format, image processing requirements and existing spot variations, and image processing steps. Luca Sterpone [20] proposed a new FPGA-based edge detection system for the gridding of DNA microarray images. Eleni Zacharia [21] proposed 3-D spot modeling for automatic segmentation of cDNA microarray images. Dimitris Maroulis [22] presented an original and fully automatic approach for accurately locating a distorted grid

structure in a microarray image which relies on a genetic algorithm. Radhakrishnan Nagarajan [23] investigated correlation of the pixels comprising a microarray spot to segment the microarray spots. Antonis Daskalakis [24] combined unsupervised clustering with restoration filters for boosting the performance of microarray spots segmentation & improving the accuracy of subsequent gene expression. Emmanouil Athanasiadis [25] proposed new methodology for segmentation of cDNA microarray images proposed based on combination of GMM with GVF active contours.

Angulo J. [26] proposed automatic analysis of DNA microarray images using mathematical morphology. Basim Alhadidi [27] presented a new algorithm that achieves an automated way for applying grid in the cDNA microarray images through the determination of spots position in the cDNA microarray. Rezaul Karim [28] reported a review of image analysis techniques for gene spot identification in cDNA microarray images. Zhang Yao [29] proposed statistics-adaptive method for gridding of cDNA microarray images. Antti Lehmussola et al. [30] evaluated performance of nine microarray segmentation algorithms- Fixed circle (FC), Adaptive circle (AC), Seeded region growing (SRG), Mann–Whitney (MW), k-means (KM), Hybrid k-means (HKM), Markov random field (MRF), Model-based segmentation (MBS) and Matarray (MA).

Many authors have suggested different algorithms for gridding and spot segmentation [31-37]. There are automated algorithms for these preliminary and very crucial steps for further processing. But the methods available at the moment for analyzing the results of microarray experiments are often far from being satisfactory. Many improvements are needed for the already existing algorithms in order to make them more accurate and reliable.

III. METHODOLOGY

Over the last two decades, a tremendous amount of research work has been conducted for automated cancer diagnosis. This is partly because automated cancer diagnosis holds great promise for large-scale use in the advanced cancer treatment and partly because automated cancer diagnosis is not a straightforward task, with a number of challenges to be overcome.

The automated cancer diagnosis consists of three main computational steps: preprocessing, feature extraction, and diagnosis. The aim of the preprocessing step is to eliminate the background noise and improve the image quality for the purpose of determining the focal areas in the image. Features are extracted either at

the cellular or at the tissue-level. The cellular-level feature extraction focuses on quantifying the properties of individual cells without considering spatial dependency between them. For a single cell, the morphological, textural, fractal, and/or intensity-based features can be extracted.

DNA microarray technology has a high variation of data quality. Therefore, in order to obtain reliable results, complex and extensive image segmentation methods should be applied before actual DNA microarray information can be used for biomedical purpose. Thus, the aim is to solve the main issues in spot validation that arises during an automatic DNA microarray analysis procedure and also to extract intensity descriptors from each spot that represent gene expression levels and input features for further analysis. Biological conclusions are then drawn based on the results from data mining and statistical analysis of all extracted features.

The aim of the diagnosis step is (i) to distinguish benignity and malignancy or (ii) to classify different malignancy levels by making use of extracted features and (iii) to detect a complex disease (e.g. cancer) at its genetic level by expression level of the genes. This step uses statistical analysis of the features and machine learning algorithms to reach a decision.

Computational Steps in Automated Cancer Diagnosis:

i) Morphological & Statistical Analysis of WBCs

1. Background elimination of input image using thresholding.
2. Segmentation of WBCs using colour segmentation.
3. Extraction of nucleus from segmented WBCs by binarizing the input image.
4. Computation of desired statistical parameters of WBCs.
5. Verification of results for diagnosis of leukemia.

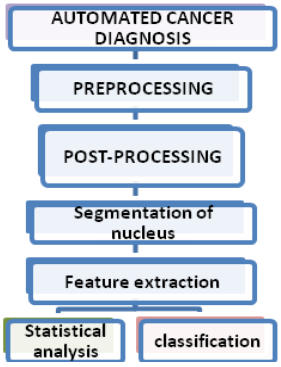


Figure1: Block Diagram of Morphological & Statistical Analysis of WBCs

ii) Microarray image analysis for gene expression profiling

1. Removal of the image background morphologically from the intensity profile of the input image.
2. Location of centres of the spots by extracting the centroids.

3. Segmentation of spots using global and local thresholding to classify cell-pixels as foreground (spot-pixels) or background.
4. Calculation of ratios of red to green fluorescence intensities for the foreground and background respectively, which gives the expression levels of the genes.
5. Compare the ratio of the intensity for one sample to the other to measure whether a gene is up-regulated or down regulated.

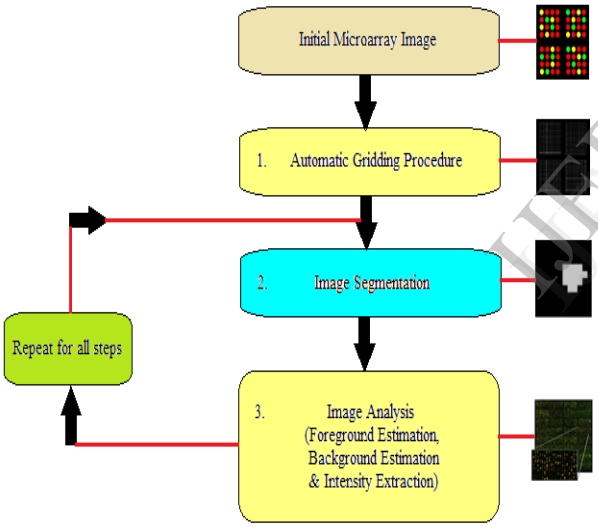
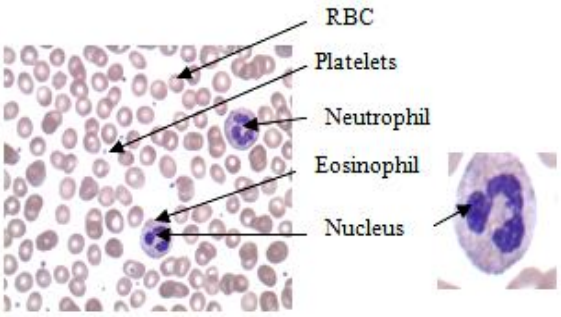


Figure2: cDNA Microarray Image Analysis

IV.RESULTS & DISCUSSION

1. Morphological & Statistical Analysis of WBCs



a) Original Image b) Cropped Image

The most important part of pattern recognition in image processing is feature extraction. When the input data to an algorithm is too large to be processed and it is suspected to be redundant then the input data will be transformed into a reduced representation set of features.

Nucleus segmentation of WBC

c) Extracted Nucleus d) Nucleus Perimeter

e) Segmented nucleus for Monocyte, Neutrophil and Basophil

Table 2. Shape Parameters of WBC Nucleus

TYPE	BASOPHIL	EOSINOPHIL	NEUTROPHIL	LYMPHOCYTE	MONOCYTE
Area	19368.83±3915.8	13664.35±2977.3	12777. 18±976.95	15158.96±2730.3	27092.00±2786.3
Perimeter	641.31±118.3	640.68±103.86	669.25±103.71	466.65±45.19	831.57±147.17
Compactness	21.41±2.78	30.85±7.41	35.63±9.59	14.48±0.54	28.24±6.66
Eccentricity	0.54±0.01	0.80±0.09	0.73±0.11	0.47±0.13	0.55±0.12
Orientation	52.73±5.99	0.71±47.91	3.59±49.79	-9.76±37.91	-39.21±34.8
Solidity	0.92±0.02	0.80±0.07	0.80±0.06	0.98±0.01	0.94±0.03
Form factor	0.63±0.08	0.44±0.11	0.37±0.12	0.86±0.04	0.61±0.14
Roundness	0.83±0.02	0.48±0.14	0.51±0.08	0.86±0.07	0.68±0.14

Statistical analysis leads us to make the correct decisions based on the collected feature data space. Basically statistical analysis guides us with logical accuracy towards the decision making in classification approach. In this approach eight shape features are extracted from the nucleus of WBC and a ratio between the area of nucleus and cytoplasm is also taken into account. Shape features are area, perimeter, compactness, eccentricity, orientation, solidity, form factor and roundness. General shape features are extracted using the standard procedure present in the MATLAB Image Processing Toolbox.

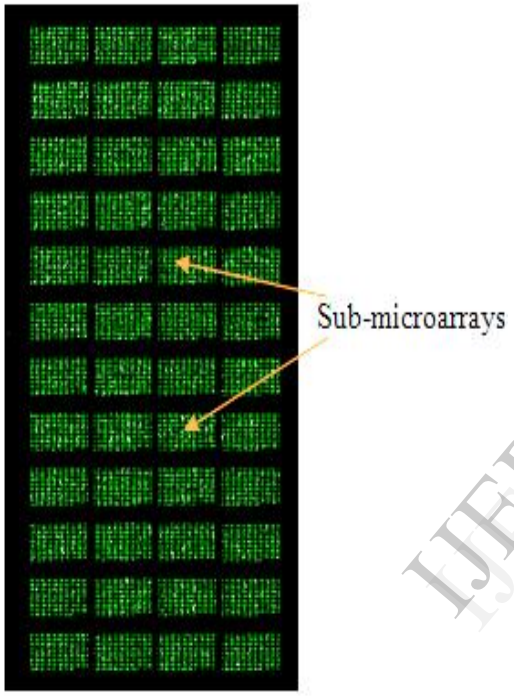
2. Microarray image analysis for gene expression profiling

Input microarray image is an RGB image, where the green component is given by Cy3, the red component is given by Cy5 and the blue component set to zero. The image consists of 48 sub microarrays, arranged in a 4 by 12 grid and each sub microarray contains 24 columns and 18 rows of gene spots. A sub-microarray is cropped from the input image for further processing. To identify where the centres of the spots are and where the gaps between the spots can be found, the average of rows and columns of sub-microarray has to be calculated.

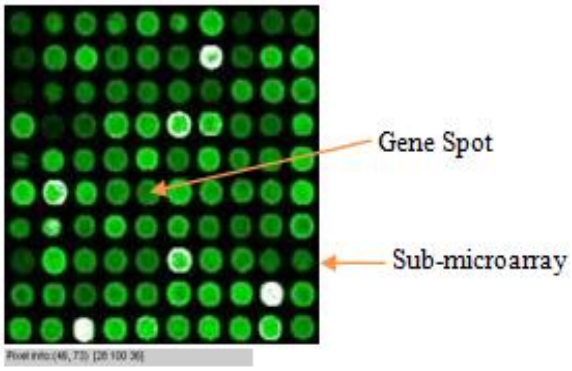
MATLAB software is used to implement the algorithm. Image segmentation is performed in MATLAB with Image Processing Toolbox, which provides image segmentation algorithms, tools and a comprehensive environment which supports a wide range of image processing operations, such as image analysis and enhancement, region of interest operations, linear filtering and filter design.

Spots are segmented from background with thresholding by applying a single threshold level to the whole image so that all spots are detected equally. To detect the weaker spots local thresholding is applied.

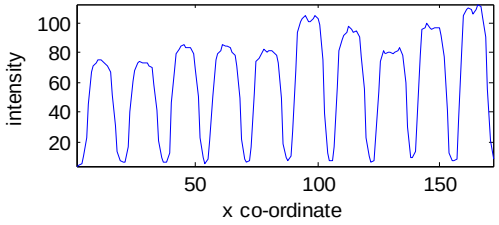
The pinholes are filled to solidify the spots. Then, the spot intensity & relative expression level are measured from two color intensities using a simple log-ratio measurement. Further, the background can be removed; measurements can be calculated again and compared.



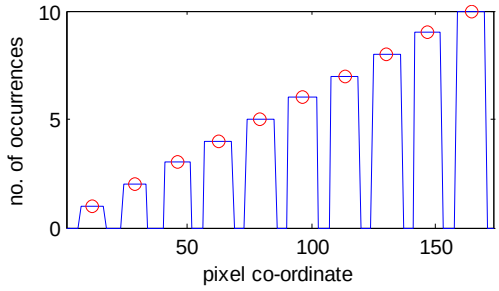
a) Input Microarray image



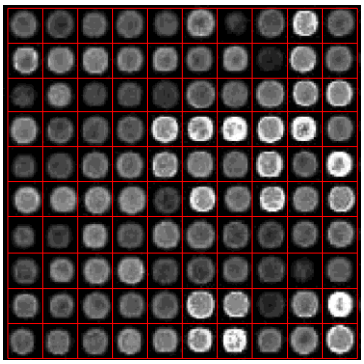
b) Cropped image



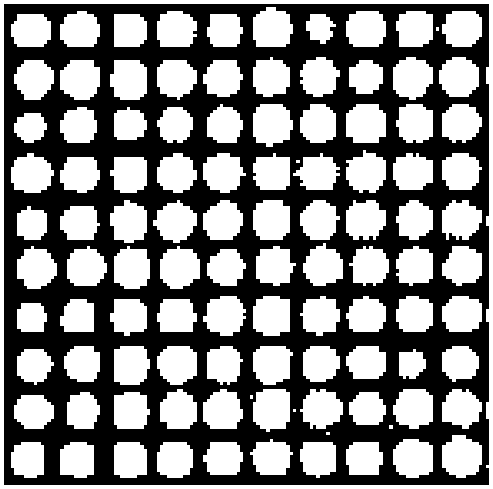
c) Horizontal profile



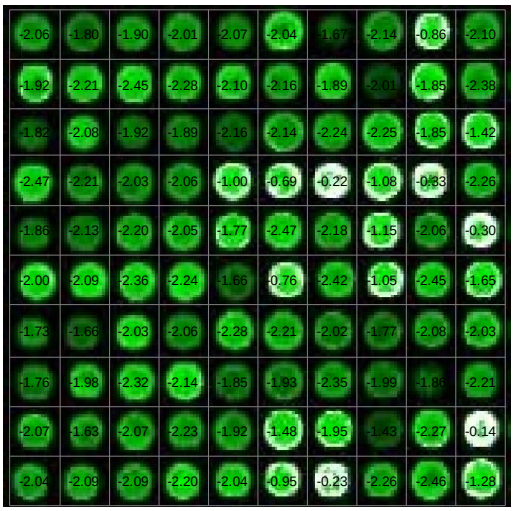
d) Spot centres



e) Gridding



f) Spot segmentation



g) Intensity extraction of spots

V. CONCLUSION

Pathology report is a critical component for diagnosis of many diseases. A pathologist will examine the blood sample under a microscope to determine if it contains normal, pre-cancerous or cancerous cells based on specific attributes like cell structure, tumor margins, pathologic stage etc. As the cancer progresses from one stage to another, the expression level of the genes responsible for controlling cell growth changes drastically. These gene expression changes can be correlated with cancer treatment outcomes and this facilitates objective mathematical judgment complementary to that of a pathologist, providing second opinion for patients. Automated blood sample and microarray image analysis gives a better visualization of the results than those obtained from microscopic examination.

The DNA and cell patterns of cancer can be compared with the patterns of known types of cancer to see if they match. They can also find some translocations involving parts of chromosomes too small to be seen under a microscope. This type of advanced testing can help to classify some leukemia's, sarcomas and carcinomas. These tests are also useful after treatment to find small numbers of remaining leukemia cancer cells that may be missed under a microscope. Tests of the DNA of each cell's antigen receptor genes are a very sensitive way to diagnose and classify cancers.

Image analysis is an essential aspect of pattern analysis and gene expression profiling. For analysis of some images, useful features are changes in gray levels, areas with irregular borders, changes from previous images of same patient. As in all feature extraction, the selection of image features will be influenced by the goal of the classification system. The numerical comparison of different studies is important to identify and avoid misleading results. A large body of literature exists in this area, but problem remains partially solved. Many improvements are needed

for the existing algorithms in order to make them more accurate and reliable.

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