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Review Paper Of Conventional Analysis Of Cell Smear Under A Microscope

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Abstract:

Pathologists and cancer biologists rely on tissue and cellular analysis to study cancer expression, genetic profiles, and cellular morphology to understand the underlying basis for a disease and to grade the level of disease progression. Conventional analysis of tissue histology and sample cytology includes the steps of examination of the stained tissue or cell smear under a microscope, scoring the expression relative to the most highly expressing (densely stained) area on a predefined scale for normal, cancer, stromal regions based on the morphology of the tissue, estimating the percentage area of cancer tissue relative of normal and stroma, and multiplying the score by the percentage area of cancer region and converting to another predefined scale for statistical analyses. This paper is an overview of basic concepts of WBCs analysis and methods used for classification.

Keywords: *pre processing, feature extraction, morphology, post processing, qualitative and quantitative analysis.*

1.Introduction

Pathology tests involve evaluation of a small sample of cells under a microscope to determine whether they are cancerous by identifying structural abnormalities. In the microscopic description, the pathologist describes how the cells of the tissue sample appear under a microscope. Specific attributes that the pathologist may look for and describe may include cell structure, tumor margins, vascular invasion, depth of invasion and pathologic stage.

The benefits of microscopes to doctors are numerous. The ability to examine cells and tissues in greater detail can result in an earlier diagnosis and treatment that is tailored to the specific disease of the patient. For example, a microscope can be used to determine the stage of cancer in a patient, as well as whether the cancer (leukemia) has spread to neighboring tissues.

White blood cell composition reveals important diagnostic information about the patients. Substituting automatic detection of white blood cells for manually locating identifying and counting different classes of cells is an important topic in the domain of cancer diagnosis. While white cells contain nucleus and cytoplasm and there are different types of them (fig1). White cells are categorized into five groups: Neutrophil, Eosinophil, Basophil, Monocytes and lymphocyte.

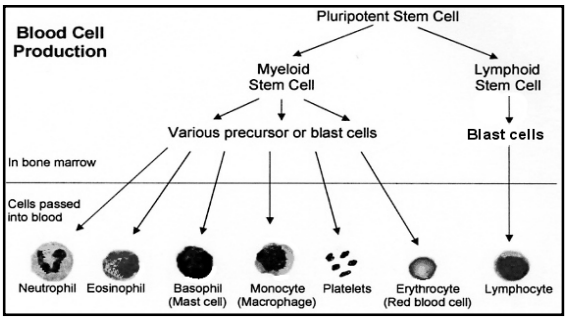


Figure 1: Blood Cell Production and Classification of Blood Cells

The texture, color, size and morphology of nucleus and cytoplasm make differences among theses groups. In laboratories, hematologists analyze human blood by microscope. Their main tasks in this area are: red cell count, white cell count and blood disorder detection. It is tedious task to locate, identify and count these classes of cells.

Due to the importance of these processes, an automated system seems necessary and helpful. White cells are clinically more important than red cells and many of blood

disorders are related to them. Thus, accurate segmentation of these cells is very important.

Microscopy is one of the oldest biological research techniques compared with other biomedical imaging techniques, but it has lagged behind in adopting a statistical, quantitative, and automated approach. Nevertheless, work performed during last years has begun to make microscopic imaging a rapidly growing field of research, making it a suitable tool for a large-scale, systematic, and automated study of biological processes. Important application areas of microscopic imaging include cancer research, toxicity screening, digital pathology, and neuroscience research, requiring an in-depth understanding of the biology, probe chemistry, underlying imaging physics, and associated image analysis.

The abundance, heterogeneity, dimensionality, and complexity of the data generated in modern imaging experiments rule out manual image management, processing, and analysis. Consequently, computerized techniques for performing these tasks have become of key importance for further progress in cell biology.

2.Previous Work

P.S.Hiremath proposed an automated segmentation, identification and classification of Leukocytes (White Blood Cells) namely, lymphocyte, Monocytes and Neutrophil in light microscopic images based on histogram equalization, thresholding and edge detection algorithms.[4]Madhumala Ghosh works aims at classifying five types of WBC (Basophil, Eosinophil, Neutrophil, Lymphocyte and Monocytes) using Bayesian classification followed by preprocessing, segmentation, feature extraction and validation through statistical analysis[5].

Tom´aˇs Kazmar works on two different levels of abstraction. First, applied statistical learning to learn 6 different types of different local cellular texture features and partition the image. At the same time generate initial seeds for cell centers using two different methods: radial symmetry decomposition (RSD) and blob-like key point detection [6]

Kaustav Nandymade an approach for improved segmentation, measuring more features of segmented objects and replacing the stacked classifier with an artificial neural network (ANN). [3]

Chanho Jung and Changick Kim apply the distance transform to the clustered nuclei. A marker extraction scheme based on the H-minima transform is introduced to obtain the optimal segmentation result from the distance map. In order to estimate the optimal h-

value, a size-invariant segmentation distortion evaluation function is defined based on the fitting residuals between the segmented region boundaries and fitted models. Ellipsoidal modeling of contours is introduced to adjust nuclei contours for more effective analysis. [7]

Magudeeswaran Veluchamy works on segmentation of blood cells by morphological operations such as thresholding, erosion and dilation to preserve shape and size characteristics. These features are extracted from segmented blood cells by estimating first, second order gray level statistics and algebraic moment invariants. In addition geometrical parameters are also computed. The analysis of extracted features is made to quantify their potential discrimination capability of blood cells as normal and abnormal. [1]

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.

Seyed Hamid Rezatofighi [12] design a new system based on image possessing methods to classify five major groups of white blood cells in peripheral blood. Therefore, at first, segmentation of white blood cells nuclei [1, 14, and 17] is carried out via Gram-Schmidt method [11, 15]. Then, distinguishing basophils from the other samples are performed using features extracted from nucleus areas. As cytoplasm edge is unobservable, a snake algorithm is used after some preprocessing procedures in order to segment the

cytoplasm. The features elicited from the nucleus and cytoplasm areas in both steps are categorized into color, morphological, and textural features. Two groups of textural features attained by the Local Binary Pattern (LBP) and the co-occurrence matrix are evaluated. The feature selection step is adjoined to this process for ameliorating the classifier performance and expediting the program trend. Finally, the performance of two different classifiers, SVM and ANN, when using different sets of features is compared. [18, 19]

3.Steps For Wbc Analysis

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 first challenge is the noise elimination in the task of determining the focal areas in the image. The noise arises from staining the biopsy samples; uneven distribution of stain usually causes problems in processing the stained material. In the case of focusing on the properties of nuclei/cells in the image, the second challenge is the nucleus/cell segmentation. This is challenging because of the complex nature of the image scenes (e.g., touching and overlapping cells) and the noise (e.g., stain artifacts). The third challenge is the feature selection to represent a cell/tissue in the task of cellular or tissue-level property quantification. The features should provide distinguishing quantitative measures to automatically diagnose the cancer. The last important challenge is the system evaluation in the task of diagnosis.

The automated cancer diagnosis consists of three main computational steps: preprocessing, feature extraction, and diagnosis (fig 2). 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. This step also comprises nucleus/cell segmentation in the case of extracting cellular-level information. The preprocessing becomes the most important yet difficult step for a successful feature extraction and diagnosis.

After preprocessing 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.

The tissue-level feature extraction quantifies the distribution of the cells across the tissue; for that, it primarily makes use of either the spatial dependency of the cells or the gray-level dependency of the pixels. For a tissue, the textural, fractal, and/or topological features can be extracted.

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. This step uses statistical analysis of the features and machine learning algorithms to reach a decision.

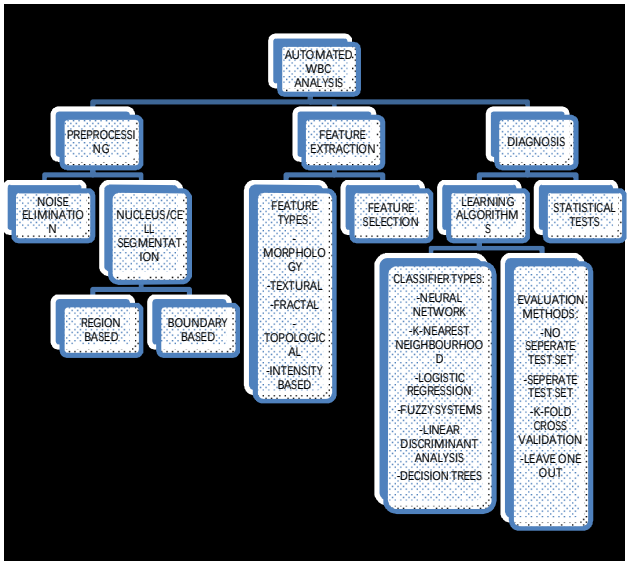


Figure 2: Block Diagram of Automated WBC Analysis

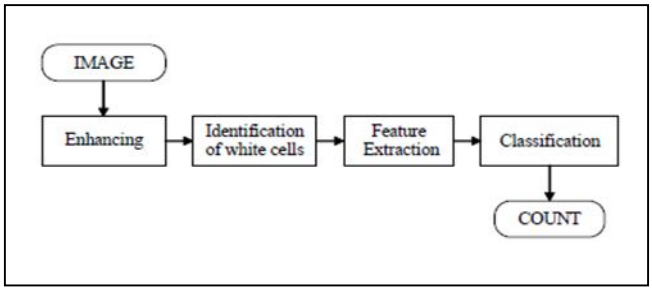
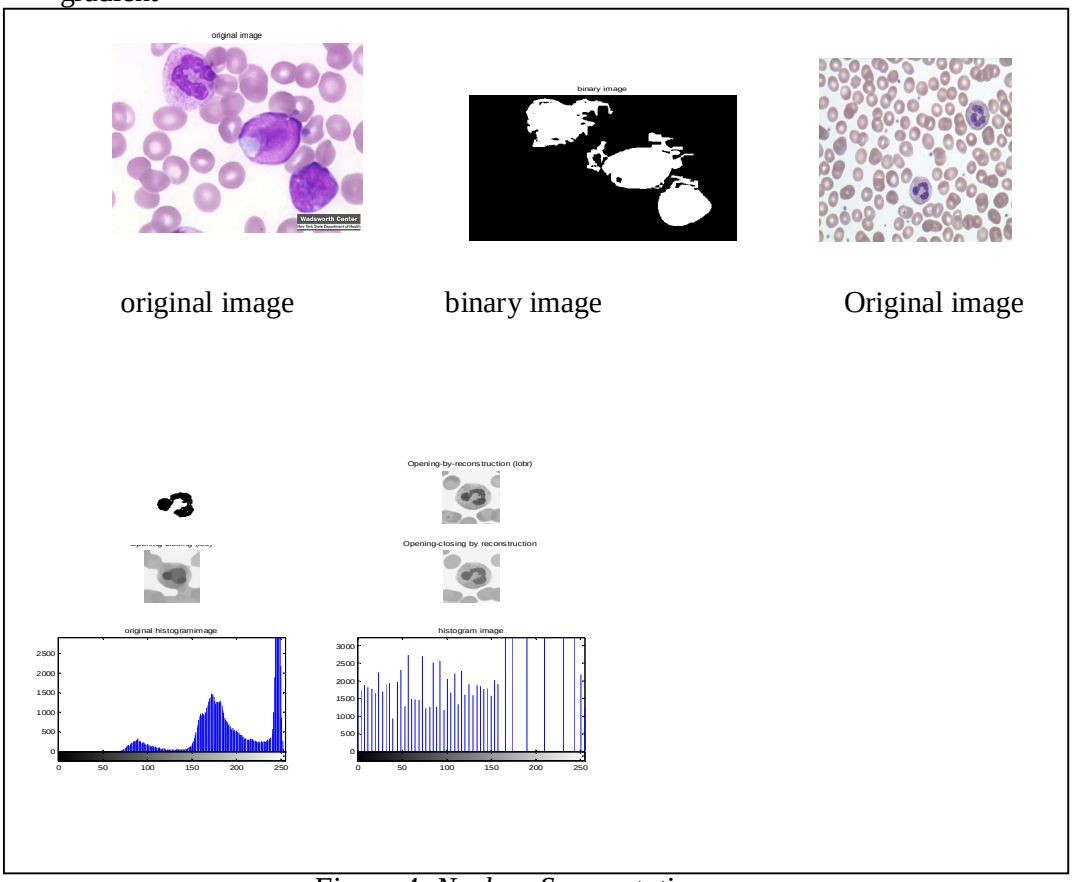


Figure 3: Steps for Analysis of WBCs (19)

Algorithm 1 Nucleus segmentation procedure

- # IMREAD an input image I;
- # create a binary image, Ib, by thresholding;
- # Compute morphology for Ib, to eliminate background, Is;

- #: compute the watershed transform using Ib as markers and Is to compute the gradient



original image binary image Original image

Figure 4: Nucleus Segmentation

Algorithm 2:

Cytoplasm segmentation procedure (18)

- # Read an input image I;
- # compute the granulometric function to obtain the size distribution of the RBC, sd;
- # create a binary image, Ib, using thresholding to separate background and objects;
- # Compute an opening on Ib with a structuring element of size sd;
- # discard components that do not intersect with a previously segmented nucleus.

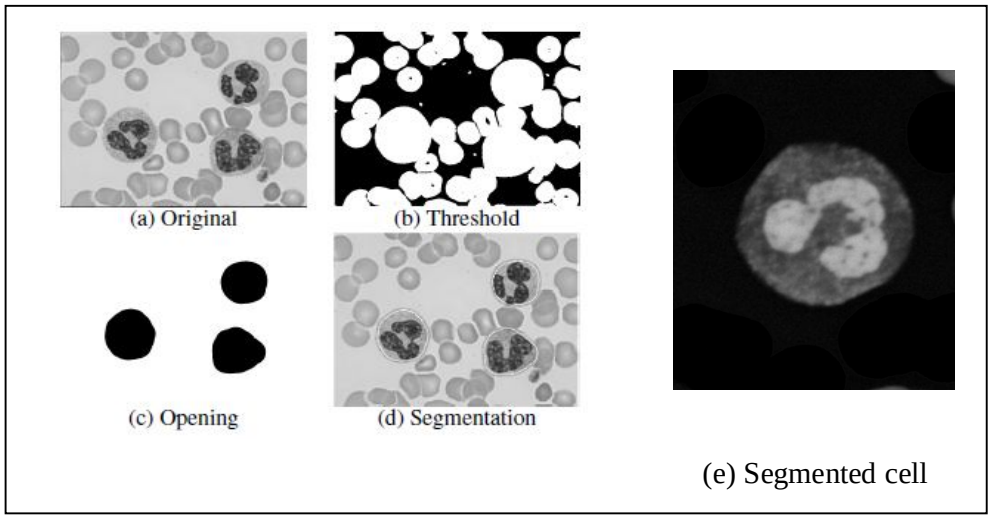


Figure 5: Cytoplasm Segmentation

Next is the feature extraction step. This step quantifies the properties of the biological structures of interest, extracting features either at the cellular-level or at the tissue-level. While cellular-level features focus on capturing the deviations in the cell structures, tissue-level features focus on capturing the changes in the cell distribution across the tissue. The features can be grouped into five based on the information they provide; the list of these features are given below. The important challenge in this step is to find the most proper cell/tissue representation(s) and select a subset of the features extracted from this representation(s).

- The morphological features provide information about the size and the shape of a nucleus/cell.
- The textural features provide information about the variation in the intensity of a surface and quantify properties such as smoothness, coarseness, and regularity.
- The fractal-based features provide information on the regularity and complexity of a cell/tissue by quantifying its self-similarity level.
- The topological features provide information on the cellular structure of a tissue by quantifying the spatial distribution of its cells.
- The intensity-based features provide information on the intensity (gray-level or color) histogram of the pixels located in a nucleus/cell.

Since morphological and textural features are the features which are elicited from white blood cells by a hematologist, many papers such as [1], [10], [11] use feature extraction methods on the basis of these features. For classification, Bayes classifier [12], different

types of artificial neural networks (ANNs) such as feed-forward back-propagation [5] and [6, 7], local linear map [15], fuzzy cellular neural network [15] are often used in the literature.

4.Conclusion

Image data present additional difficulties. Some of the classic problems in pattern recognition have dealt with the recognition and classification of images. Feature extraction in images consists of identifying some aspect of image that allows it to be recognized. In complex images, this phase can be quite involved. A large body of literature exists in this area, but problem remains partially solved. 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.

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