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EFFECTIVE IMAGE PROCESSING METHOD FOR COUNTING OF NUCLEI CELLS

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ABSTRACT

Manual cell counting using Hemocytometer is commonly used to quantify cells, as it is an inexpensive and versatile method. However, it is labour-intensive, tedious, and time-consuming. On the other hand, most automated cell counting methods are expensive and require experts to operate. Thus, the use of image analysis software allows one to access low-cost but robust automated cell counting. This paper proposes a novel approach for automatic nuclei cell counting from histological images, based on effective image processing methods. Current systems are based on color or grayscale images, leading to inaccurate results and limitations. The new techniques include image thresholding, morphological image processing operations, and a connected component algorithm. Experimental results show high accuracy compared to previous works, indicating the effectiveness of the proposed approach in terms of accuracy and F1-Score.

Keywords: Nuclei Cells Counting, Image Processing, Adaptive K-means Algorithm, Histological Images

1. INTRODUCTION

Cell counting is a method of quantifying cells to monitor cell proliferation and viability, enhance and optimise the cell culture condition, and prepare for cell-based assays [1]. It is a standard laboratory procedure to investigate cell density and confluency and has been used for various purposes, including monitoring cell proliferation rates and seeding cells for downstream investigations [2]. Furthermore, it is also commonly employed in medical diagnostics and life sciences research.

The commonly used method of counting cells is via manual counting using Hemocytometer. This method is very versatile and inexpensive as it only uses essential tools commonly available in the laboratory; however, it is very time-consuming, error-prone, and labour-intensive, especially when dealing with medium to high throughput cell counting [3].

Additionally, human interpretation is required in manual cell counting, which makes the method heavily dependent on the operator's expertise. This potentially leads to variability in results between different operators and even by the same operator but at different times, thereby reducing the reliability of the results. Subsequently, different automated methods of counting cells have been developed to surmount the existing challenges of manual cell counting. Many researchers have attempted several methods to automate the cell counting process, including using Hough Transformation [4], gray thresholding, artificial neural networks via classification of cell shapes, automatic segmentation and mathematical morphology. While these methods are more time-efficient and able to reduce errors, they are not easy to follow, expensive and mostly depend on the operator's capability to understand the algorithms behind the automated programs[5].

Developing automatic computer vision systems for visualizing and analyzing microscopic objects are also essential in several bioinformatics applications including morphological cell analysis, diagnosis of diseases, and statistics [6]. They serve biomedical research and bioinformatics to improve their functionality and automate the manual process to minimize the time, effort, and errors. In fact, analyzing and automate microscopic images is critical for solving several issues in biomedical and bioinformatics fields, such as normal cell detection, cancer cell detection, morphological change in nuclei cells, and dynamic change in nuclei cells during procedure of therapeutic. Consequently, nuclei cells counting is considered essential part in histological image analysis. Thus, several methods developed to automate the process of nuclei cells counting for histological analysis instead of using the traditional process. Some research applied the thresholding methods for detection and counting the nuclei cells from histological images [7]. Thresholding method is considered a simple technique for segmenting objects

and regions of interest from images. Other approaches have been introduced to segment histology images using a variety of edge detection methods such as Laplacian of Gaussian (LoG) filter and Laplacian filter. However, using segmentation techniques only for detection the nuclei cells from histological images are challenging tasks, because the histological images have a non-uniform and complex nature and structure.

2. LITERATURE SURVEY

Y. Zhang and X. Zheng, et.al [8] development of deep learning has been greatly promoted, and as the mainstream trend of the development of deep learning, there is a great technological breakthrough in the field of image processing. This paper mainly focuses on the development of image processing technology supported by deep learning algorithm, using particle swarm algorithms, image matching algorithms and deletion strategies to optimize image processing technology, and it is found that each of these methods plays a role in pattern recognition, obtaining deeper meaning of images and deleting unimportant information. Deep learning algorithm enable the processing of a large amount of stored information and ensure the integrity of the image in the process of optimizing image processing.

H. -G. Lee and S. -C. Lee, et.al [9] identify cells in microscopy images with stained nuclei, using the following process: Candidate seeds for nuclei are identified as extrema in a Laplacian-of-Gaussian space, and weak candidates are eliminated from clusters obtained by ellipse fitting; a region of interest for each nucleus is then defined by combining local and global thresholding; and these regions are repeatedly merged and split by modeling the shape of a nucleus and measuring the roughness of the shared boundaries connected nuclei. This method showed superior abilities to detect the nucleus regions and to split the boundaries of connected nuclei. Our experiments show higher scores in comparison with five other techniques in terms of eight evaluation metrics.

B. Netke, S. Dongre, N. Bhadouria and M. Bhattacharya, et.al [10] demonstrate novel techniques developed to elucidate the effect of electromagnetic fields (EMF) exposure on neuronal cells of post-natal rats as subjects. The brain cell images of the subjects, prior and post exposure to the Electro-Magnetic Fields (EMF) are obtained using the Cresyl Violet (Nissl) Staining method. The images are then subjected to analysis using pre-processing techniques such as average filtering combined with median filtering to denoise the Nissl stained cell images, image enhancement techniques like local enhancement using CLAHE algorithm for abating the pixel intensity variations. The image procured after pre-processing is then segmented using a hybrid segmentation technique that integrates adaptive single seed region growing and threshold based segmentation to obtain separate cells and analyzes them for apoptosis. A novel technique using distance transform with maxima propagation has been developed for automated cell counting. The end results intend to limn the accuracy of the developed cell counting and segmentation techniques over the manual approach. And also, to delineate the impact of electromagnetic fields radiations beyond a threshold value at the neuronal level.

R. Agrawal, S. Satapathy, G. Bagla and K. Rajakumar, et.al [11] proposed method consists of designing and developing an automated system which will assist the medical professionals in correctly diagnosing all the types and sub-types of this disease. In this paper, we have proposed a novel method in which we have taken microscopic blood images as an input image. A dataset of 100 images in which 62 training and 38 testing images is taken.

After that we have converted the image to proper format (YCbCr) for segmentation. For segmenting, we have used the combination of Gaussian Distribution, Otsu Adaptive Thresholding and for clustering we have used K-Means method. Using Gray Level Co-occurrence Matrix (GLCM), the features are extracted and were used for classification using Convolutional Neural Network (CNN). The overall accuracy of the system obtained after processing is 97.3%.

R. A. Khorshed, Q. Yousuf and J. Jiang, et.al [12] explains manual monitoring process of cancer cells development is a subjective, time consuming process, as it typically relies on the visual recognition and experience level of the pathologists. An automated nuclei segmentation and quantification in Immunohistologically stained images have remained a challenging task.

Previous methods used have shown an oversight in the segmentation and counting of the two different types of stained nuclei within the same image (i.e. positive brown stained nuclei and negative blue stained nuclei). Our exclusive method addresses this issue by producing an automated means for the segmentation and counting of nuclei based on the monochromatic characteristics of the different types of stained nuclei objects. Ultimately this could aid pathologists towards more accurate and time efficient diagnosis by considering the affects of protein antibodies inside the nuclei. Our experimental work has proven to produce promising results. This was through the appropriate allocation, segmentation and counting of nuclear contents inside Colonic Cancer of Immuno histologically stained images.

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3. METHODOLOGY

In this Fig.1 block diagram of effective image processing method for counting of nuclei cells are observed.

The proposed approach designed to solve the various issues of detection and segmentation of nuclei cells included several steps such as color channels extraction; image thresholding method; morphological image processing; and connected components labelling. The input of the proposed approach is a color image and the output is the number of nuclei cells in the input image. Image acquisition is the first step in image processing. This step is also known as preprocessing in image processing. It involves retrieving the image from a source, usually a hardware-based source. 3D restoration methods improve the quality of the images reducing noise. When these methods are not employed, other noise reduction techniques must be used. In confocal microscopy images, noise follows a Poisson distribution as image acquisition is based on photon emission.

This step is applied to split the component colors of RGB histological color image into its red, green, and blue channels where each channel has values range between 0 and 255. The aim of this step is to select the most suitable color channel, which illustrates more detailed about spectral content of nuclei cells and reduces the other contents. In fact, color separation is an important and helpful step for segmentation and classification of objects in nuclei cells images. Extracting color channels of the original color image is done by assigning its red, green, and blue pixel values into three independent variables Image thresholding is considered as the most effective approaches used for image segmentation.

A constant threshold value is chosen based on experimental monitoring which used to produce a binary image from the extracted channel color (grayscale) image. An adaptive thresholding method not applied in this work to allow the user with selecting the appropriate threshold value for more appropriate result. Furthermore, the microscopic images are taken mostly in different time with various lighting conditions producing different contrast ratio that made the adaptive thresholding methods not suitable to be applied for all image types. for modifying and extracting the structure of objects and shapes in images. Morphological operations, such as erosion and dilation were demonstrating its usefulness for binary images analyzing. Morphological operations are considered non-linear and commonly used for image edge detection, image filtering, image segmentation, and noise reduction.

Practically, there are two common morphological operations dilation and *erosion*; the other morphological operations like opening and closing are constructed from the combination of dilation and erosion operations. The output of this step is used for counting the number of nuclei cells from binary histological images.

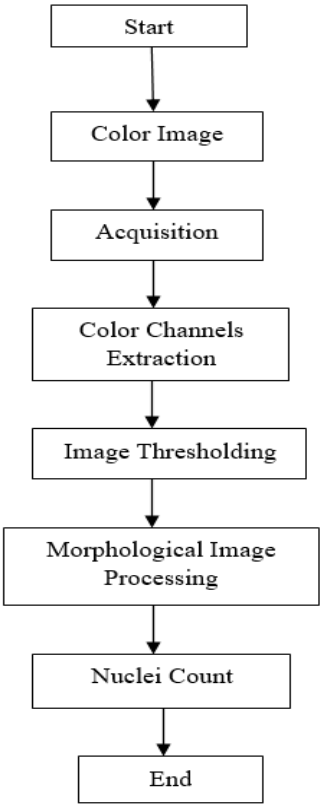

Fig.1: Block Diagram Of Effective Image Processing Method For Counting Of Nuclei Cells

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4. RESULT ANALYSIS

In this section, performance analysis of effective image processing method for counting of nuclei cells is observed.

Table.1: Performance Analysis

Parameters	Watershed Transform	Image Processing
Accuracy	89.3	98.8
F1-Score	81.6	90.5

Fig.2 accuracy comparison graph for watershed transform and image processing.

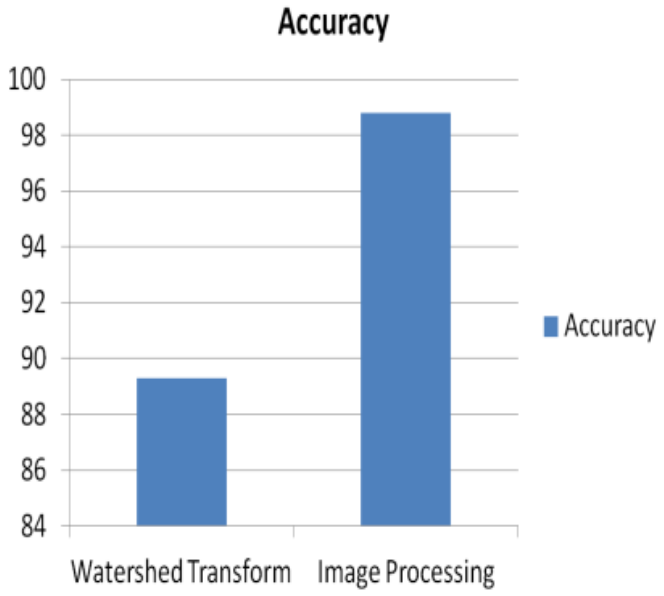

Fig.2: Accuracy Comparison Graph

F1-Score graphical representation for watershed transform and image processing is observed in Fig.3

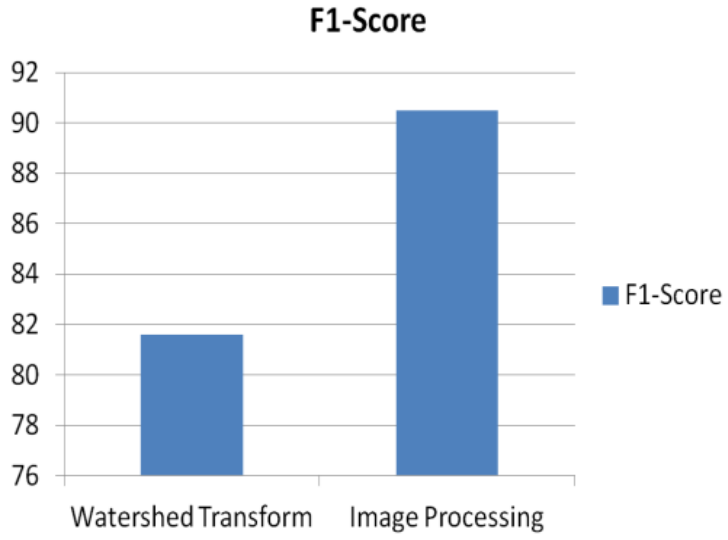

Fig.3: F1-Score Comparison Graph

5. CONCLUSION

In this section effective image processing method for counting of nuclei cell is concluded. The proposed approach focuses on improving segmentation by isolating the color channel and using appropriate thresholds. It differs from existing methods in step orders and effectiveness, enabling practical problem-solving. The new approach demonstrated higher accuracy and effectiveness in detecting and counting nuclei cells automatically. This research demonstrated the approach's effectiveness in detecting nuclei cells with higher accuracy compared to previous similar work.

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