

Original Research Article**Image Analysis and Image Classifier Using Neural Network with Machine Learning to Perform Differential Leucocyte Count****Swaroop Raj B.V.¹, Divya C.², Smitha B.V.³**

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Abstract**Corresponding Author:**

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**(Received on 10.04.2018,
Accepted on 05.05.2018)**

Introduction: A five part differential leucocyte count is provided by a hematology analyser with abnormalities being detected as flags which are then later on reviewed and confirmed by a pathology trainee or pathologist based on the difficulty level of that individual case or knowledge of the particular trainee. Peripheral smear examination is considered the gold standard and is time consuming and subjective.

Methods: Using a self prepared 1500 leishman stained leucocyte image dataset the machine learning programme tensor flow from google using image intensity, histogram and convolutional neural network was trained and this was put to test on 80 random leucocyte images.

Results: Only 65% concordance was obtained on 5 tier leucocyte differential count. However 95% concordance was achieved by using a two tier leucocyte differential classification of polynuclear and mononuclear cells.

Conclusion: Larger dataset of images are needed before image analysis using this model can be used routinely to substitute or as add on to routine peripheral smear examination.

Keywords: Image Classifier; Differential Leucocyte Count; TensorFlow; Peripheral Smear.

Introduction

A five part differential count is routinely provided by a hematology analyser with abnormalities being detected as flags which are then later on reviewed and confirmed by a pathology trainee or pathologist based on the difficulty level of that individual case or knowledge of the particular trainee / pathologist. Differential count by hematology analyser is accurate in most instances, however has its own limitations and hence peripheral smear examination is considered the gold standard for differential counts. Peripheral smear differential count accuracy is largely dependent on the observer and hence its main limitations being subjectivity and level of knowledge of the observer. Studies have been done using neural network based approach using machine learning

in leucocyte classification on leishman stained peripheral smears and have revealed high accuracy and sensitivity of this method [1-6]. Convolutional neural network is a software algorithm which uses deep learning and minimal image processing for image classification and recognition.

Many newer analysers have peripheral smear staining and image analysis of slides for morphological differential count analysis. However these features are only seen in high end and costly analysers and not routinely not available in most hematology analysers.

This study was done to see if image analysis by machine learning using convolutional neural network can provide accurate differential count and remove the subjectivity and inter-observer variability that is usually

associated with manual peripheral smear examination and can this be an alternate cheap solution for laboratories in developing countries where high end analysers with image analysis may not be a viable option.

Objectives

To perform manual leucocytedifferential count and leucocytedifferential count usingtensorflow an open source machine learning framework using image analysis on leishman stained peripheral smears

To correlate the differential leucocyte count between manual and tensorflow machine learning framework.

Materials and Methods

Sample Size Calculation was done using sensitivity of test derived from the study done by Mu Chun Su et al [4]. with sensitivity of the new test as 97%, Precision of 5% and desired confidence level being 99%. A sample size of 80 test images was calculated.

Methodology - EDTA samples received at Department of Pathology, Central Diagnostic Laboratory Services, R L Jalappa Hospital and Research Centre, Tamaka attached to Sri Devaraj Urs Medical College between December 15th – January 15th were included in the study. Peripheral smears were prepared by following standard operating procedures for the same and stained with Leishman stain. Differential leucocyte count was done manually by two pathologists and done simultaneously on same fields by neural network based machine learning algorithm by processing images taken by Axiocam ERc 5s CMOS sensor mounted on Primostar Zeiss microscope using Zen Blue software at 400x magnification. Proforma was filled and corresponding differential leucocyte count by manual and image analysis method was documented.

Statistical Analysis

Sensitivity, Specificity, Positive predictive value and Negative predictive value was calculated forleucocyte differential count using tensor flow an open source

machine learning framework considering manual leucocyte differential count as a gold standard. Concordance percentage was calculated as the accuracy for the test.

Results

Training set images for tensorflow software were taken from routinely processed peripheral smears with dataset composed of 300 images of leucocytes classified manually by a pathologist as monocyte, lymphocyte, neutrophil, eosinophil and basophil. The images were rotated and flipped to increase the size of training set to 1500 images. Tensor flow an open source software uses inception architecture formachine learning and runs on python script in command prompt in windows 10 operating system. After training the software using the 1500 image dataset, we ran the 80 images test set and obtained concordant results in 65% images and discordant results in 35% of the test set images. Detailed analysis of the resultsare tabulated below in Table 1.

The average sensitivity, specificity, positive predictive value and negative predictive value for leucocyte differential count using tensor flow was 44.4%, 87.3%, 37.8% and 86.8% respectively. As only 65% concordance percentage and low sensitivity and specificity was obtaing for the five stage classification of leucocytes we modified our leucocyte differential classification to a simpler classification.

Hence we reran the dataset classifying the images as only polynuclear and mononuclear leucocytes considering all leucocytes except lymphocytes and monocytes as polynuclear and lymphocytes and monocytes as mononuclear. Using this classification when we ran the test set thepercentage of concordance results increased to 95% from the previously obtained 65%. Detailed analysis of the results are tabulated below in Table 2.

The average sensitivity, specificity, positive predictive value and negative predictive value for leucocyte differential count using tensor flow classifying images as only polynuclear and mononuclear cells was 93.3%.

Table 1: Sensitivity, Specificity, Positive predictive value and Negative predictive value for 5 type leucocyte differential count using tensor flow

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative Predictive value (%)
Neutrophil	70.5	62	76.6	54.5
Eosinophil	20	96	25	94.7
Basophil	66.6	93.5	28.5	98.6
Monocyte	0	100	0	98.7
Lymphocyte	65	85	59	87.9
Average	44.4	87.3	37.8	86.8

Limitations of this was to obtain similar staining properties of all dataset and test set images, small size of the data set and preprocessing in the data set such as nuclear segmentation and cell discrimination.

The figure 1 below shows the percentage of confidence score for the particular category.

Figure 1 Tensor Flow image classifier classifying the various images and its confidence level with a score of more than 50% considered as significant.

Discussion

Sensitivity and specificity of our study was similar other studies being > 90% when simplified two type leucocyte classification was used. However the 5 type leucocyte differential count had a low sensitivity of 44%.

Our study with a limited dataset of 1500 images obtained an accuracy of 95% with the two step classification of leucocytes when compared to other studies which have all attained an accuracy of more

Table 2: Sensitivity, Specificity, Positive predictive value and Negative predictive value for simplified 2 type leucocyte differential count using tensor flow

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative Predictive value (%)
Polynuclear	96.6	90	96.6	90
Mononuclear	90	96.6	90	96.6
Average	93.3	93.3	93.3	93.3



Fig. 1:

Table 3: Comparison of sensitivity and specificity of leucocyte differential count using machine learning in other studies

	Sensitivity (%)	Specificity (%)
Mu-Chun Su et al ⁴	94.9	99.2
Present Study using 5 type leucocyte differential count	44.4	87.3
Present Study using simplified 2 type leucocyte differential count	93.3	93.3

Table 4: Comparison of accuracy of leucocyte differential count using machine learning in other studies

	Present Study	Choi JW et al ⁷	Mathur A et al ⁸
Accuracy	95 %	97.6 %	92.7 %
Dataset - images	1500	6000	267

than 90%. Studies by Ramesh N et al, Shahih I et al and Rawat et al have all obtained accuracy of more than 90% using more complex machine learning algorithms.⁹⁻¹¹We can see that lower the images in the dataset, lower the accuracy and the only way to obtain higher accuracy was to increase the number of dataset images which trains the computer in assessing difficult images and even classifying at higher level classification such as the classical five level classification which was achieved in other studies [6]. Further studies need to be using more complex image processing and machine learning algorithm using stain normalization, leucocyte segmentation, nuclear / cytoplasmic segmentation and feature extraction to achieve higher accuracy.

Conclusion

The present study yielded satisfactory results with the limited dataset achieving more than 90% sensitivity, specificity and concordance on the two tier leucocyte differential count. However large dataset of training images along with complex image processing will be needed to achieve similar results on the standard 5 type leucocyte differential count and to use real time classification to be used in a more practical situation.

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