Original Research Article

Agranulocyte & neutrophil nucleus enhancement through 510nm wavelength of light

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ABSTRACT

Enhanced features can be easily segmented and used for classification algorithms. Light wavelength of 510nm is used as illumination source for bright field microscope. This specific wavelength of light enhances nucleus, granules in White blood Cells (WBCs). Eosinophil and basophil has coarse granules which also get enhanced hence nucleus segmentation gets difficult. This method is effective in enhancing nucleus of agranulocytes viz. Lymphocyte, Monocyte and Neutrophil of normal person. This modification in image acquisition of pathological images will further help in quick segmentation of features.

1. Introduction

Blood smear analysis is fundamental step for every pathologist when blood cell count is abnormal.[1] Any disease reflects changes in count or morphological changes in blood cells.[2] Shape, size of cell, granules size, count of WBCs are primarily affected as it is the 1st defense line of our immune system. Researchers are continuously trying to implement algorithms for pathological feature extraction.[3–5] Segmentation is crucial step in every algorithm as the accuracy of the subsequent feature extraction and classification depends on the correct segmentation of WBCs features.[6]

Research presented in this paper shows that using 510nm wavelength of green light enhances certain pathological features. Illumination source is been modified by many researchers. Light Emitting Diodes (LEDs) are now used for ophthalmic illumination as it generates less heat compared to conventional tungsten and discharge sources.[7] LEDs are cost effective and efficient when used for bright field microscopy.[8] LEDs can be used with condenser for focused beam on microscopic slide.[9] Ilya Ravki has proposed a invention which provides a Multicolor Led light source for transmitted light illumination and is compatible with most of the microscope.[10] Stereo surgical microscopes are now made with color LEDs.[11] Mobile phone microscopy with filters and LED installed is capable of floroscenece imaging.[12]

Light calibration system with compact LEDs is made with intensity knob to control input current to LEDs and with spectral coverage from 550nm to 670nm.[13] LED based three wavelength optical phase microscopy extends axial range of object to be imaged without increasing noise in the phase map.[14] Multiple images are taken with different wavelengths and then results are combined to characterize biological sample.[15]

This paper proposes to use of 510nm wavelengths of light as enhancement tool for microscopic images of WBCs.

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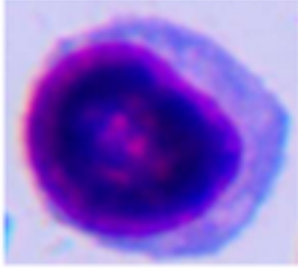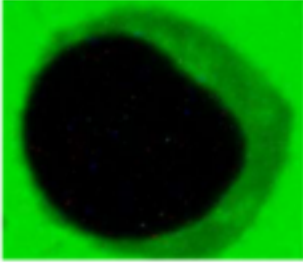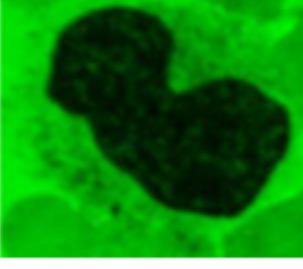*E-mail address*: drnikitamitra.iitbhu@gmail.com (N. S. Mitra).
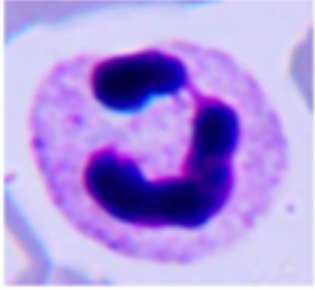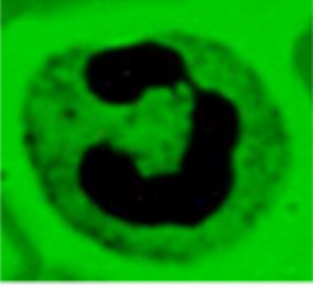
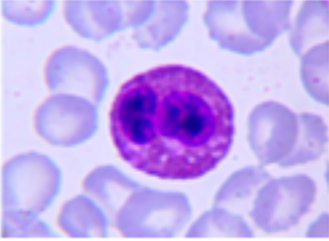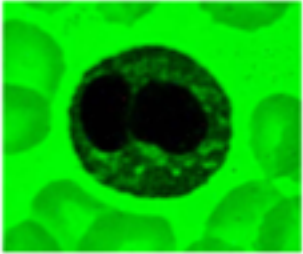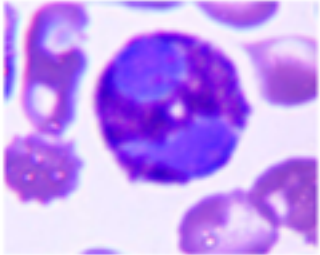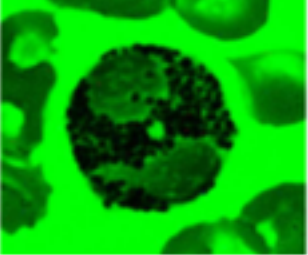|	Standard Bright Field Microscope Illumination	510nm wavelength LED Illumination
Agranulocyte: Lymphocyte		
Agranulocyte: Monocyte		
Granulocyte: Neutrophil		
Granulocyte: Eiosinophil		
Granulocyte: Basophil		

Fig. 1:

2. Methodology

Blood smear stained with stain was used for the experiments. Array of were used to make monochromatic source of light.

Technical specifications of instruments and software used are as follows:

Microscope: Olympus, CH20i

Camera: Magnus Pro, 5MP

Software for Image acquisition: TopView

Storage Format of Acquired Image: image.bmp

LEDs are more effective as source of illumination as it provides monochromatic souse of light, wavelength of narrow band, cost effective and easily available. Illumination source was made with one color LEDs in parallel connection with single potentiometer of 2K∩. 2.5.

DC voltage used to drive different LED sources. Image is acquired with 510nm wavelength of light with the help of LEDs. For image acquisition following steps are implemented:

Switch off the illumination of Microscope.

Remove the condenser.

Illuminate the Green LED array source with DC power supply.

Place it below slide which is just below objective lens.

Software will be used to capture image.

Observe blood smear at 100x oil emersion objective lens.

Observe different types of results.

3. Results

Results of experiments are shown in fig.1. Nucleus, granules of WBCs are enhanced when illumination source of 510nm wavelength is used. Nucleus of agranulocyte are distinctly enhanced. Lymphocyte, Monocyte can be easily seen in the illumination 510nm wavelength of light. In granulocytes, nucleus of neutrophil is also enhanced.

Neutrophil have fine granules for a person in normal condition. When our defense system is activated granule size in neutrophil gets enlarged. In the referred case, exclusive enhancement of nucleus does not happen. 510nm wavelength of light provides contrast of granules along with nucleus.

Eosinophil, Basophil has course granules thus exclusive nucleus enhancement is not possible with this method.

4. Conclusion

510nm wavelength of green light enhances features of Lymphocyte, Monocyte and Neutrophil. These enhanced features can be easily be segmented by image processing algorithms. As the features are already enhanced, time required for segmentation will be reduced, accuracy of algorithm will be high.

5. Source of Funding

None.

6. Conflict of Interest

None.

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