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Automated Blood Cell Counting and Disease Identification Using Image Processing: Implications for Polymer Composite-Based Biomedical Diagnostic Devices

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Abstract

Accurate quantification of blood cells is central to clinical decision-making and to the performance of emerging polymer composite–based diagnostic platforms. This work presents a cost-effective, image-processing pipeline for automated counting of red blood cells (including overlapping cells), white blood cells, and platelets from Leishman-stained peripheral blood smears, and articulates its relevance to polymer composite microfluidic and biosensor devices. Implemented in Python with OpenCV, the workflow performs grayscale conversion, median/Gaussian denoising, contrast enhancement via CLAHE, edge detection, and Otsu thresholding; overlapping red cells are resolved using a Circular Hough Transform to recover individual instances. The acquisition setup—a compound microscope coupled to a consumer webcam—demonstrates accessibility without specialised haematology analysers. Validation on clinical smear images showed high accuracy and robustness, with automated counts consistent with reference ranges and capable of flagging deviations indicative of anaemia, infection, and platelet abnormalities. Beyond standalone laboratory analysis, we emphasise translation to polymer composite diagnostic devices: robust segmentation and reliable overlapping-cell resolution directly support on-chip quantification, device performance evaluation, and quality control in settings where composite optical and structural properties influence image formation. Overall, the proposed method advances affordable haematology while providing a practical pathway to integrate precise cellular analytics within polymer composite–based point-of-care platforms.

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Keywords: Blood cell counting, image processing, overlapping RBC segmentation, disease identification, polymer composite biomedical devices, microfluidic diagnostics

INTRODUCTION

Recent advances in polymer composite materials have led to their extensive use in biomedical diagnostic devices, including microfluidic chips and biosensors, which require accurate biological sample analysis for effective operation. These devices utilise polymer composites for their desirable mechanical properties, biocompatibility, and manufacturability. Automated blood cell counting methods such as the one presented here are critical for integrating reliable haematological analysis within these platforms. By enabling precise

segmentation and quantification of blood cells, including overlapping red blood cells, the proposed image processing approach bridges crucial gaps between clinical diagnostics and polymer composite device engineering. This interdisciplinary integration aligns well with emerging research trends focused on enhancing polymer composite biomedical devices through advanced imaging and computational analysis.

The paper introduces an automated blood cell counter and analyser using Python and OpenCV with a number of unique features [1]. The system is first cost-saving, utilising a basic webcam and compound microscope instead of traditional, costly Haematology analysers [2]. In particular, a variety of image processing methods like median and Gaussian filtering for denoising, histogram equalisation for enhancing contrast, Canny edge detection, and Otsu's thresholding for segmentation are utilised to properly segment and then count blood cells [3]. One of the most innovative features is the approach to overcome the difficulty of overlapped red blood cell (RBC) counting, which is overcome using the new application of the Circular Hough Transform. In addition, apart from mere cell counting, the system assesses deviations in blood cell counts to determine possible diseases by comparing the counts with set normal ranges [4]. Through supporting the use of a compound microscope combined with a webcam, the research provides an affordable alternative to digital microscopes [5].

The conventional approach for blood cell counting employs the haemocytometer, a chamber developed by Louis Charles Malassez [6, 7]. This apparatus comprises a glass microscope slide with a rectangular indentation forming a precision chamber, overlaid with a laser-etched grid of perpendicular lines [8, 9]. The Neubauer counting chamber, a commonly used variant, consists of a thick crystal glass slide measuring 30x70 mm with a thickness of 4 mm. The central region of the chamber is designated for cell counting, with a grid size of 3x3 mm [10, 11]. For white blood cell (WBC) counting, the corners are utilised, while red blood cells (RBCs) and platelets are counted in the central area due to their higher concentration. The squares within the chamber are further divided, resulting in a central square composed of 400 smaller squares [12, 13]. Sample placement is performed using a micropipette, ensuring a bubble-free sample to maintain accuracy. Subsequent microscopic observation involves counting cells in the grid squares and calculating concentrations, though potential errors are inherent due to pipetting, mathematical calculations, and sample volume [14, 15].

A blood cell counting, which involves image processing, is proposed in this paper by Chadha et al. The method utilises colour, texture, and morphology-based classification of blood cell images, employing image processing techniques including texture feature extraction, threshold segmentation, and Hough transformation for counting [16, 17]. Segmentation work by Narjes Ghane et al. presents a novel multi-feature method with threshold, clustering, and watershed [18, 19]. This work addresses the need for efficient and accurate computer-aided diagnosis systems to assist pathologists in diagnosing blood-related diseases based on WBC analysis [20, 21]. The paper by Ahmed et al. provides segmentation methods used in Cellular Image Analysis for measuring Morphological Blood Cell Parameters (MBCPs). The authors discuss metrological aspects that impact the performance of these segmentation methods in clinical diagnosis [22]. The article by Cengiz Beyan and Kursat Kaptan discusses the evolution of automated blood cell counters and their impact on analytical efficiency and information output. The authors also present a case study involving a patient with diffuse large B-cell lymphoma, highlighting abnormal leukocyte and erythroblast parameters as indicators of platelet abnormalities.

In our proposed method, we leverage Python, a high-level, object-oriented programming language, coupled with the widely-utilised OpenCV library for image processing. With a focus on clear and logical coding, Python offers a conducive environment through its integrated development environment (IDLE).

Image acquisition entails retrieving images from diverse sources, marking the initial phase of the process. Pre-processing employs filters such as the median and Gaussian filters to enhance the quality of acquired images. Segmentation, a pivotal step, involves categorising cells into distinct types—RBCs, WBCs, and platelets—based on morphological characteristics such as shape and size.

METHODOLOGIES

A total of thirty patients (Fifteen Males and Fifteen Females) data were used for this study. Ethical approval was obtained from the institutional review board, and written informed consent was obtained from each patient. Venous blood samples were collected from each participant using sterile venipuncture techniques. The blood samples were collected in EDTA-coated tubes to prevent clotting. Immediate preparation of blood smears was carried out by placing a small drop of blood on clean glass slides. Another slide was used to spread the blood evenly and create a thin blood smear. The prepared blood smears were air-dried and fixed with methanol for preservation.

Staining and Microscopic Imaging

The prepared blood smears were subjected to Leishman staining for differentiation of blood cells. The smears were immersed in undiluted Leishman stain for 2 minutes to fix the cells, followed by the addition of twice the volume of distilled water for 10 minutes at 37°C [16, 17]. After staining, the slides were thoroughly washed with distilled water to remove excess stain [18, 19]. Microscopic imaging was performed using a compound microscope with a 15X eyepiece and a 45X objective magnification. A high-resolution web camera (Iballrobo K20) was attached to the microscope's eyepiece for capturing images of blood cells.

Image Processing and Counting of Blood Cells

Before cell counting and analysis, the captured images were pre-processed to enhance their quality. The RGB images were first converted to greyscale to simplify further processing. To reduce noise in the images, both median and Gaussian filters were applied. Equalisation of histograms and CLAHE (Contrast Limited Adaptive Histogram Equalisation) methods were applied to enhance the contrast and visibility of images, with the aim of easier cell segmentation. The algorithm of Canny edge detection was applied for finding the blood cell boundary in images. Through edge finding, cell segmentation was performed. Otsu's thresholding technique was applied to threshold the greyscale images into binary images to distinguish between background and blood cells easily. The WBC and platelet counts were directly retrieved from the segmented images since there was no cell overlapping. Nevertheless, for overlapping RBC counting, the Circular Hough Transform approach was used. This algorithm successfully identified round shapes, allowing accurate overlapping RBC counting in the blood smears.

Figure 1 illustrates representative outputs from the complete image-processing pipeline for female (a–b) and male (c–d) samples. For RBC/WBC panels (a, c), raw Leishman-stained fields are converted to greyscale, denoised with median and Gaussian filters, and contrast-enhanced using histogram equalization/CLAHE, followed by Canny edge detection and Otsu thresholding to obtain clean binary masks. Overlapping RBCs are then resolved via a Circular Hough Transform, yielding instance-level RBC counts despite occlusions. WBCs are segmented by the same thresholding workflow and counted directly. Platelet panels (b, d) show segmentation and direct counting of platelets; overlap was not observed in our data, so no de-overlap step was required. Together, these panels demonstrate how the pipeline produces visually verifiable segmentations and per-class counts that are subsequently compared with reference ranges during validation.

Statistical Analysis and Validation, and Implementation

Descriptive statistics were employed to abstract blood cell counts derived from image processing. Comparisons were made with known normal ranges of RBCs, WBCs, and platelets according to gender. Deviations in the normal range were assessed to determine possible medical conditions like anaemia, leukaemia, or infection. All image processing algorithms and methods were developed using Python as

the programming language and the popular OpenCV library for image processing operations. The Python Integrated Development and Learning Environment (IDLE) served as the coding platform for software development and execution.

Hardware Setup

The system integrated a compound microscope, a web camera, and a computer with the necessary Python environment for executing the developed image processing software. The collection of blood samples was performed through standard venipuncture techniques using clean syringes. To prevent clotting, the blood was collected in tubes coated with ethylene diamine tetraacetic acid (EDTA). Immediate blood smear preparation was carried out right after the collection to ensure the preservation of cell morphology. Blood smears were then air-dried and fixed using methanol. For differentiation of blood cells, Leishman staining was employed. The prepared blood smears were subjected to an undiluted Leishman stain for 2 minutes to allow for fixation. After fixation, the addition of twice the amount of distilled water to the stain and incubation for 10 minutes at 37°C were carried out. Subsequently, the slides were thoroughly washed with distilled water. To capture blood cell images, stained smears were examined under a microscope with a 15X eyepiece and 45X objective, using a 10.2 MP iballrobo K20 web camera for image acquisition. Proper focusing and pre-processing, including conversion to greyscale, application of Median and Gaussian filters for noise reduction, and use of histogram equalisation and CLAHE for contrast enhancement, were crucial. The canny edge detection algorithm and Otsu's thresholding facilitated segmentation and differentiation of RBCs, WBCs, and platelets. The Circular Hough Transform technique was employed for accurate counting of overlapping RBCs, overcoming traditional methods' limitations. Counts of WBCs and platelets were straightforward due to the absence of cell overlap. The system's accuracy was confirmed by comparing counts with normal ranges based on gender, identifying potential diseases from abnormal cell counts.

Statistical processing was offered in table and graph form, using Python and OpenCV. The research, following ethical principles, consisted of 10 patients, giving accurate counts for WBCs and platelets and counting overlapping RBCs correctly, validating the system against the conventional method, and helping with potential disease diagnosis.

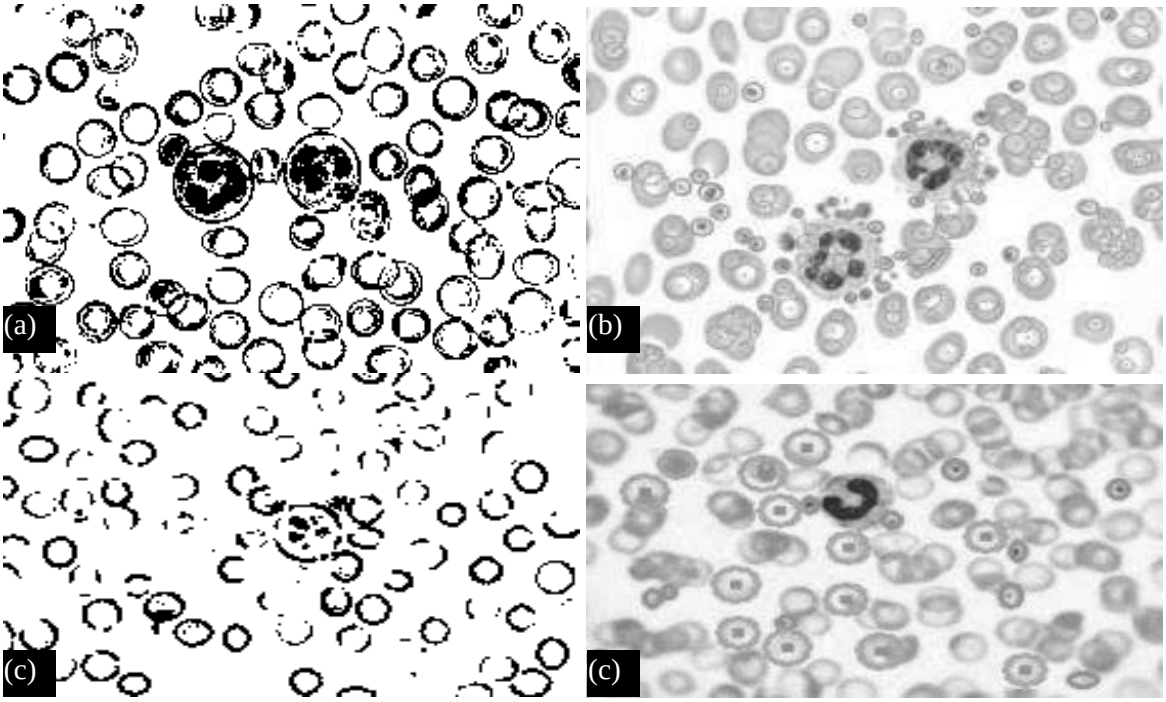

Figure 1. Output obtained after processing for (a) Female RBC and WBC, (b) Female Platelets, (c) Male RBC and WBC, and (d) Male Platelets.

The resulting blood cell counts were also compared to the known standard normal values for each parameter. The results clearly indicated that some subjects had abnormalities in their blood cell counts, which could be an indication of illness. For instance, one male patient had an elevated WBC count, which is normally an indication of infection or inflammatory diseases. Another female patient had an abnormal RBC count, which could be an indication of anaemia. The results demonstrate the clinical utility of the proposed automated system in early diagnosis and detection of some blood disorders. Of special interest to medical applications is the cost-effectiveness and efficiency of the developed blood cell counting system as a replacement for conventional manual methods and expensive haematology analysers. Proper and timely analysis of blood smears can assist in the early diagnosis of diseases like anaemia, leukaemia, and infection, which would allow for early medical intervention. In terms of engineering, the combination of image processing methodologies and the usage of the Python programming language and the OpenCV library worked well for obtaining accurate blood cell counting output. The use of modular design in the software makes it easy to scale up as well as possibly integrate sophisticated machine learning algorithms for disease categorisation and more accurate cell segmentation in the future.

From a scientific perspective, the use of image processing methods in medical diagnostics is an important step forward. The capability to analyse and process large numbers of blood smear images over a relatively brief time frame provides researchers and clinicians with useful information about cellular morphologies and abnormalities. The accuracy and reproducibility of the cell count by the software illustrate the potential for future developments in computer-aided diagnosis and customised medicine.

While the current study emphasises image processing for blood smear diagnostics, the underlying methodology is directly adaptable for real-time analysis within polymer composite-based microfluidic and biosensor devices. The optical and structural characteristics of these polymer composites influence image acquisition and processing, making robust segmentation and classification techniques essential. The novel overlapping RBC counting algorithm, in particular, offers practical value for improving the accuracy of cellular analysis integrated into polymer composite diagnostic platforms, supporting device performance evaluation and quality control.

RESULTS AND DISCUSSION

The findings of the present study provide a clear view of blood cell counts in a heterogeneous population and highlight possible correlations between blood cell counts and diseases. The data were collected from 30 people, 15 males and 15 females, and their blood samples were analysed using automated haematology analysis. The mean count of RBC in men was 5.04million cells/mcL, whereas in women it was 4.30million cells/mcL. This has been in accordance with past knowledge, as males tend to have higher counts of RBC compared to females. Interestingly, RBC counts play a central role because they indicate the ability of blood to carry oxygen. Variations of established norms were seen among disease patients. Anaemia, which involves lower RBC counts, was particularly evident in two instances, cutting across gender. Conversely, viral infections that normally result in an immune response were associated with rising WBC counts, whereas platelet counts fell. Notably, heavy menstrual bleeding was found mostly in females and caused reduced RBC as well as platelet counts, suggesting the possible physiological effect of such bleeding episodes on blood constitution.

Table 1. Summary of blood cell count ranges.

Blood Cell Type	Gender	Normal Range	Increased Range	Decreased Range
RBC (million cells/mcL)	M	4.5-5.5	>5.5	<4.5
RBC (million cells/mcL)	F	4.0-4.9	>4.9	<4.0
WBC (cells/mcL)	Both	4,500-11,000	>11,000	<4,500
Platelets (cells/mcL)	Both	150,000-450,000	>450,000	<150,000

The range of normal WBC count is (5300 to 8550 cells/mcL) in males and (4600 to 9600 cells/mcL) in females. The fluctuation in WBC counts may be due to a response of the body to infection, inflammation, and other physiological reactions. The viral infections were observed to have increased WBC counts, reflecting the immune response against infection. The results support the idea that WBC counts are a significant reflection of the defence mechanism of the body.

Platelet count, responsible for blood clotting and wound healing, ranged from 1,55,000 to 3,80,000 cells/mcL in males and from 1,60,000 to 3,80,000 cells/mcL in females. The decreased platelet count observed in viral infections could be due to platelet activation during infection. Menorrhagia in women was found to be accompanied by a significant decrease in platelet count, which could be due to excess blood loss during menstruation. The relationship of iron deficiency with blood cell counts was also studied. Iron deficiency, a common nutritional disorder, was found to be related to low RBC count and high platelet count. The low RBC count agrees with the well-known role of iron in the synthesis of haemoglobin, which is responsible for RBC function. The high platelet count could be an adaptive response to compensate for the reduced oxygen-carrying capacity due to the low RBC count. Figure 2 is a graphical portrayal of Blood Cell Count, which shows RBC and WBC variation for various patients.

Table 1 summarises the range of blood cell counts that provide important information regarding the normal, elevated, and low levels of various blood cells.

For Red Blood Cells (RBC), the normal range for males is 4.5-5.5 million cells/mcL. Values above 5.5million cells/mcL in men show an increased level, which may imply states like polycythemia. Counts less than 4.5million cells/mcL show a drop, possibly implying anaemia or other disorders of the blood. For females, a normal range of RBC count is 4.0-4.9million cells/mcL. Values above 4.9million cells/mcL represent an increase, while values below 4.0million cells/mcL represent a drop.

In Figure 3, RBC, WBC, and Platelet counts are illustrated, reflecting the variation exclusively among the female patient cohort. Moving on to White Blood Cells (WBC), both men and women usually exhibit a normal range of 4,500-11,000 cells/mcL. WBC levels over 11,000cells/mcL could reflect infections, inflammatory diseases, or leukaemias. Levels below 4,500cells/mcL could indicate immune deficiencies or marrow disorders.

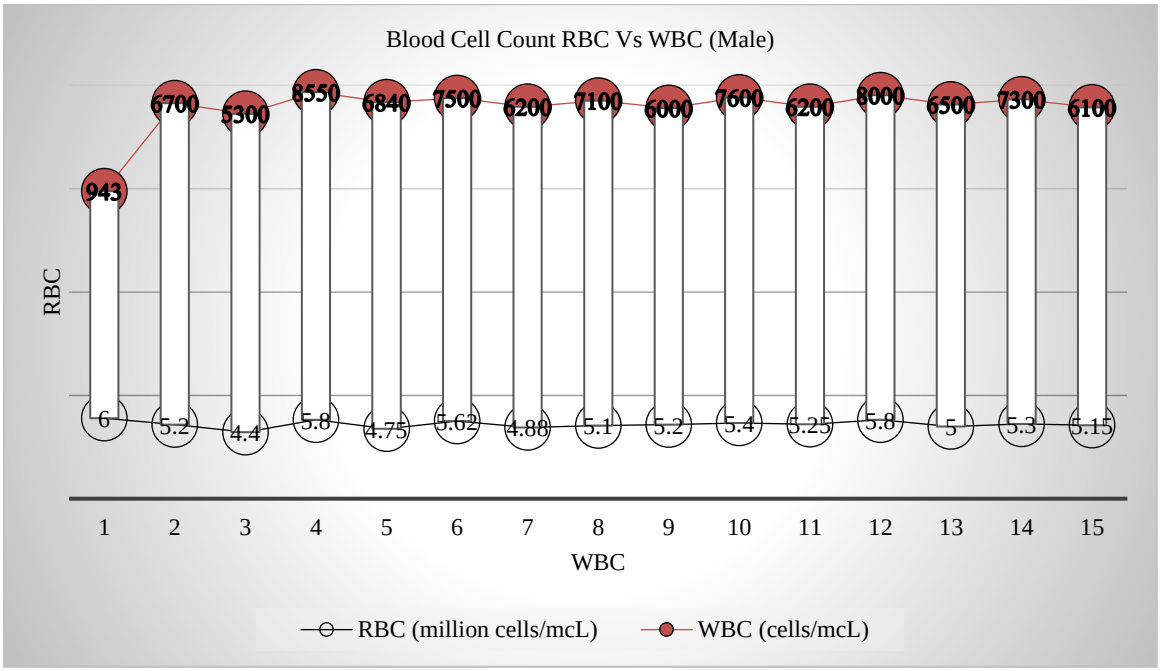

Figure 2. Blood Cell Count graph for RBC and WBC.

For Platelets, the reference range for both males and females is between 150,000 and 450,000cells/mcL. Platelet counts more than 450,000cells/mcL could indicate thrombocytosis, which could be caused by some infections or bleeding disorders. Platelet counts below 150,000cells/mcL could indicate thrombocytopenia, which might cause heightened tendencies towards bleeding or poor clotting.

Comparison of the blood cell count in a group of 30 patients was informative in terms of the type and pattern of the count by gender. For RBC, the mean count was 5.15million cells/mcL for men and 4.57million cells/mcL for women. The standard deviations were 0.73 for men and 0.47 for women. White blood cell count had means of 7103cells/mcL and 6170 cells/mcL for men and women, respectively, with standard deviations of 1496 and 1947 for men and women, respectively. Platelet count had mean values of 222300cells/mcL and 217666 cells/mcL for men and women, respectively, with higher standard deviations of 57432 male 84105 for women, respectively in Table 2.

The Implications of these findings remain in support of gender-specific patterns in differences in blood cell counts. The comparatively higher RBC count in men compared to women may be due to hormonal or physiological differences. The same mean WBC counts of both sexes suggest an equal distribution of immune response. Notably, the higher variability in platelet counts might signify a broader range ofpotential clotting and bleeding tendencies. Clinical considerations emphasise that abnormal RBC counts are indicative of conditions like anaemia, potentially associated with iron deficiency. Elevated WBC counts often suggest the presence of infections or underlying immune disorders. Deviations in platelet counts could serve as indicators for bleeding or clotting disorders. Furthermore, the data underscores gender-associated trends in disease occurrences. The rate of viral infections is impressive in both sexes, whereas anaemia and deficiency of iron are more prevalent in females.

Table 2. Blood cell count statistical analysis.

Gender	RBC (million cells/mcL)	WBC (cells/mcL)	Platelets (cells/mcL)
Male	Mean: 5.15	Mean: 7103	Mean: 222300
	Median: 5.20	Median: 6840	Median: 220000
	Std Dev: 0.73	Std Dev: 1496	Std Dev: 57432
Female	Mean: 4.57	Mean: 6170	Mean: 217666
	Median: 4.65	Median: 5425	Median: 215000
	Std Dev: 0.47	Std Dev: 1947	Std Dev: 84105

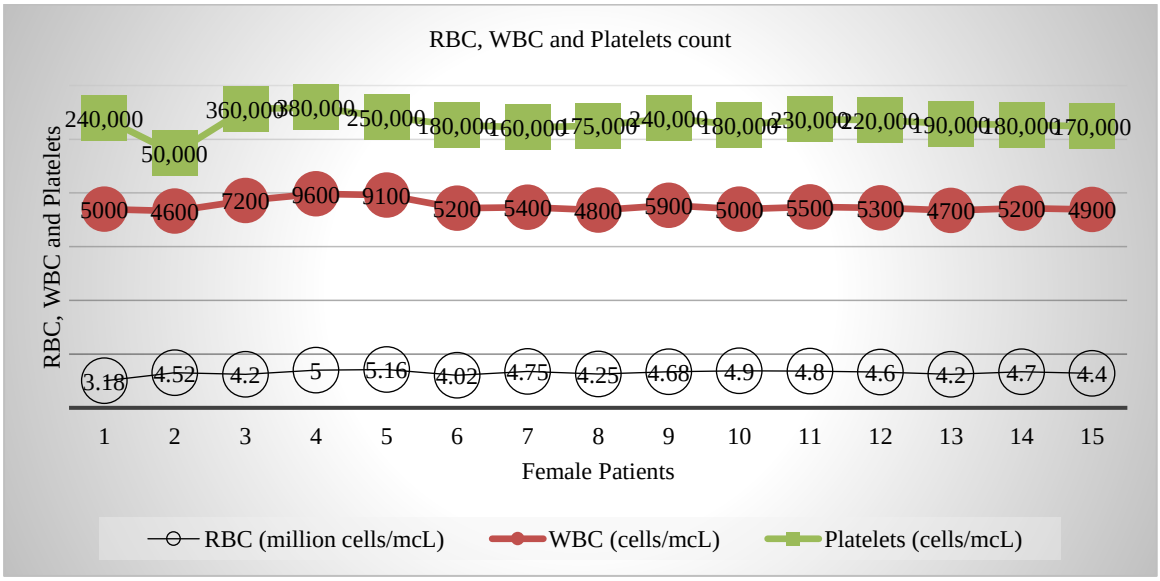

Figure 3. RBC, WBC and Platelet count variation for female patients.

CONCLUSIONS

This work introduces an effective image processing approach for automated blood cell counting and disease identification, validated with high accuracy on clinical blood smear images. Beyond its core clinical importance, the methodology presents significant potential for integration with polymer composite-based biomedical diagnostic devices, enabling enhanced cellular analysis within advanced microfluidic and biosensor platforms. Future work will aim to adapt and validate the technique in polymer composite device settings, thereby fostering innovation at the nexus of biomedical imaging and polymer composite materials science.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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