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Review Article

Review on Photomicrography based Full Blood Count (FBC) Testing and Recent Advancements

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Abstract

With advancements in related sub-fields, research on photomicrography in life science is emerging and this is a review on its application towards human full blood count testing which is a primary test in medical practices. For a prolonged period of time, analysis of blood samples is the basis for bio medical observations of living creatures. Cell size, shape, constituents, count, ratios are few of the features identified using DIP based analysis and these features provide an overview of the state of human body which is important in identifying present medical conditions and indicating possible future complications. In addition, functionality of the immune system is observed using results of blood tests. In FBC tests, identification of different blood cell types and counting the number of cells of each type is required to obtain results. Literature discuss various techniques and methods and this article presents an insightful review on human blood cell morphology, photomicrography, digital image processing of photomicrographs, feature extraction and classification, and recent advances. Integration of emerging technologies such as microfluidics, micro-electromechanical systems, and artificial intelligence based image processing algorithms and classifiers with cell sensing have enabled exploration of novel research directions in blood testing applications.

Keywords: cell identification, cell classification, deep learning, photomicrograph analysis, lab on a chip, computer vision

Introduction

Rapid and accurate cell analysis is essential in identification of diseases and complications in human body. In this regard, various type of cell analyses are performed on samples obtained from blood, urine, saliva, tissue, and semen [1]. Blood tests are the most accessible method of assessing a biochemistry of a human body. In most cases, a

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full blood count (FBC) which is also known as complete blood count (CBC) or full blood examination (FBE) provides adequate information to identify abnormal conditions in human body. In other words, by testing a human blood sample, a person with a disease, disorder or deficiency is identified based on variations of white blood cell (WBC) count (or leukocytes count), red blood cell (RBC) count (or erythrocyte count), platelet count, hemoglobin concentration, hematocrit, RBC indices, etc. compared to ranges defined for a healthy person [2]. In addition, counts of neutrophils, lymphocytes, monocytes, eosinophils and basophils are also important in identifying various medical conditions such as types of anemia, viral or bacterial infections, tuberculosis, radiation exposure, arthritis, etc. [3]. Although FBC is a medical assessment, by using as a regular monitoring method it is possible to avoid and cure a variety of illnesses and conditions at early stages using abnormal indications provided by the FBC report. Bellan et al. presented a study on predicting in-hospital mortality in COVID-19 based on simple parameters obtained in a CBC [4], Madjid et al. presented the use of components of a CBC as risk predictors for coronary heart disease [5], and Camon et al. studied FBC values to predict poor outcome of pneumonia among patients with HIV infection [6]. Figure 1 shows an overview of cell sensing using photomicrographs with a single cell and multiple cells.

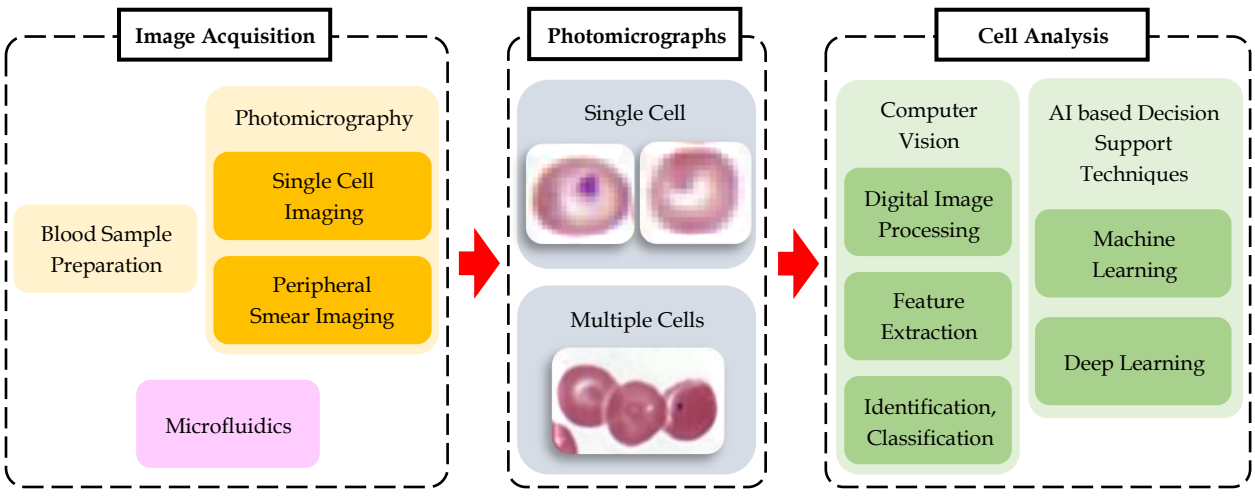

Figure 1. An Overview of Photomicrography based Cell Sensing

In conventional FBC testing, WBC, RBC and platelet counts are obtained by observing an individual blood sample at a time using an optical microscope to identify the cells and manually count different types of blood cells by a skilled hematologist, who is well trained for the task [7]. A hemocytometer is used as a counting chamber to assist manual counting, yet the results depend on the ability and the performance of an individual person who carries out the test [8]. Over the past few decades, semi-automatic and automatic blood testing equipment are developed to overcome disadvantages such as volatility in test results, higher time consumption for an individual sample, errors due to human fatigue, limitations in increasing the productivity of laboratories, and comparatively lower efficiency, that arise when hematologists directly examine the blood sample to identify cells and obtain the count of differentials. At present, hematology analyzers (or automatic cell counters) are the most common high-tech devices used to perform various types of tests on human blood samples. HemaCAM by Fraunhofer Institute for Integrated Circuits IIS, Germany [9], Vision Hema by West Medica, Germany [10], EasyCell by Medica corporation, USA [11], and CellaVision® DM9600 by CellaVision. Sweden [12] are commercially available hematology analyzers. Advantages of hematology analyzers are the ability to handle a large set of samples efficiently, higher accuracy, ability to perform multiple tests in one single platform, and comparatively increased precision [13]. Hematology analyzers are based on cytometry principles and use imaging techniques, Coulter effect, and conductivity based methods to detect blood cells [14]. Imaging flow cytometry (IFC) is a branch of flow cytometry, which is a non-destructive testing method based on photomicrographs of single cells. Measurements of blood cells in IFC are based on scattering and absorption of light by individual cells and measurements in Coulter effect method are based on electrical impedance variation. Therefore, the two principles obtain different properties of cells which have directed to study on combining the imaging techniques with Coulter effect method into a single

system to deliver a broad result using one blood sample [15]. XN-Series by Sysmex, Japan [16], ADVIA by Siemens, Germany [17], and CELL-DYN Sapphire by Abbott Laboratories, USA [18] use a combination of detection methods to perform blood tests. Although, conventional hematology analyzers dramatically reduce human error factors at an increased efficiency, these devices have disadvantages such as the higher cost, complexity in sample preparation, reduced monitoring capability compared to manual testing, limitations in extracting cell properties, etc. [19]. Therefore, continuous efforts to address aforementioned challenges have led to implementation and integration of emerging technologies such as computer vision, digital image processing (DIP) and artificial intelligence (AI), and microfluidics with conventional testing methods [20]–[22].

Peripheral smear analysis (PSA) is a test which is performed as a part of FBC test or is prescribed following a FBC test if the result indicate abnormal values [23], [24]. Similar to FBC test, a blood sample is observed under a microscope in PSA therefore, photomicrography technique is utilized in obtaining peripheral smear images in which the acquired images contain multiple cells of a single type or a mixture of different types of human blood cells. Integrating an image acquisition device with a microscope provides the capability of obtaining photomicrographs enabling further processing and analysis of blood samples by transferring the photomicrographs to a processing unit and deliver test results based on the measured properties of individual cells and the peripheral smear image as a whole [25]. Therefore, photomicrography is capable of performing FBC test and PSA using the same photomicrographs at once which reduces the tedious task of carrying out two tests on the same sample and increase the comfortability to patients by eliminating the requirement of drawing blood at multiple times [26]. In addition, this adds higher computational capabilities to the system which is essential in introducing alternatives to human operations in observing, identification and analysis tasks using image processing and feature extraction methods followed by decision making

techniques such as fuzzy logic, neural networks, machine learning, deep learning, etc. [27]. Studies toward introducing high-end computer vision based methods into biological assays show a rapid development as a results of outstanding improvements in micro-electromechanical systems (MEMS), optical systems, microelectronics, image processing techniques and algorithms [28]–[30]. Microfluidics is an emerging multidisciplinary field of science which is widely studied towards biomedical applications due to the advantages such as compactness of the systems, less volumetric requirements for biological assays, mobility, avoiding the need of large laboratory facilities, easy access, less time, minimum utilization of resources, etc. [31]. Several microfluidic devices based on Lab-on-a-Chip (LOC), Lab-on-a-Paper (LOP) and Organ-on-a-Chip (OOC) technologies are developed and successfully utilized in applications related to pathology detection, drug development, biological assays, organiods, cancer research, etc. [32]–[35].

Human Blood Cells

Human blood is the medium that transports oxygen absorbed from lungs and nutrients to the whole body. Human blood contains ~55% of blood plasma, ~45% of RBCs, and ~1% of WBCs, platelets and other elements [36] and the Figure 2 shows a photomicrograph of a human blood sample including RBCs, types of WBCs and platelets.

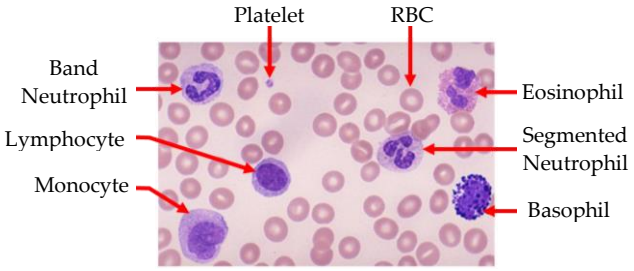

Figure 2. Photomicrograph of a Human Blood Sample [37]

RBCs have a biconcave shape and are dominant among all types of blood cells in terms of the cell count in a human blood sample. WBCs are commonly rounded but sometimes

appear in irregular shapes and are comparatively dominant in individual cell size. Normal range of the RBC count for healthy men varies from 4.7 - 6.1 million cells per microliter and in women from 4.2 - 5.4 million cells [38]. WBCs are responsible for the immune system of the body and consists of five sub-types which are neutrophils (40-60%), lymphocytes (0.5-1%), monocytes (2-8%), eosinophils (1-4%) and basophils (20-40%) [39]. Platelets are comparatively less in number and are responsible of minimizing complications due to heavy blood losses by forming blood clots to stop bleeding. In normal condition, about 150,000 – 450,000 platelets exists in a microliter of human blood. Rapid decrement or an increment of the platelet count indicates that the patient require extensive medical assistance. Platelets are tiny (1-5 – 3 µm) compared to RBCs with a diameter of 6 - 8 µm and WBCs with a diameter of 12 - 15 µm, therefore are the most challenging blood cell type in imaging and identification compared to other cell types [40].

Photomicrography

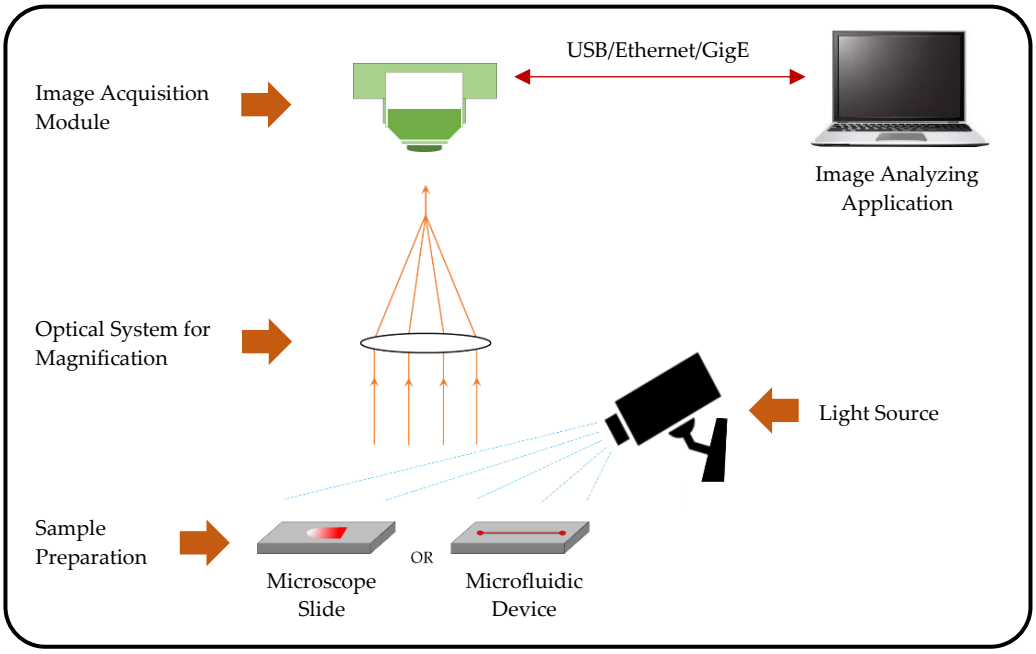

Figure 3. Photomicrography

Based on the imaging technique followed additional hardware requirements arise and variations of the arrangement for photomicrography is observed [41], [42]. The captured digital image is called a photomicrograph or a micrograph. Sample preparation is the important initial step of photomicrography and literature present various methods depending on the cell type at interest as well as the microscopy technique [43]–[45]. Staining is a technique performed in sample preparation to enhance contrast of the image by introducing a substance that penetrates to the cell giving a significant color to the cell which improve the visualization capability of the sample [27], [46]. Novel blood sample preservation techniques such as the method proposed by Chaurasia et al. have enabled new directions towards blood tests [47]. Balanant et al. presented detailed instructions for sample preparation of human RBC for confocal imaging and followed a re-suspension step using a high content of glycerol to obtain high quality by reducing spherical aberration [48].

Hardware development of life science microscopes have enabled new dimensions to biological research and also routine laboratory work. Microscopes are classified under three major categories which are optical microscopes, electron microscopes, and scanning probe microscopes [49]. In terms of applying photomicrography for life science research, microscope is the most commonly utilized optical instrument to magnify the biological sample and the widely used optical microscopes use a set of lenses to refract the visible light beams passing through the sample. In addition, electron microscope type, scanning probe microscope type, confocal type use an electron beam, a probe and a laser beam respectively, to interact with the sample to generate images [50]. Figure 4 shows photomicrographs of blood samples obtained from patients with several anemia types and are captured using an optical microscope and a microscopic camera.

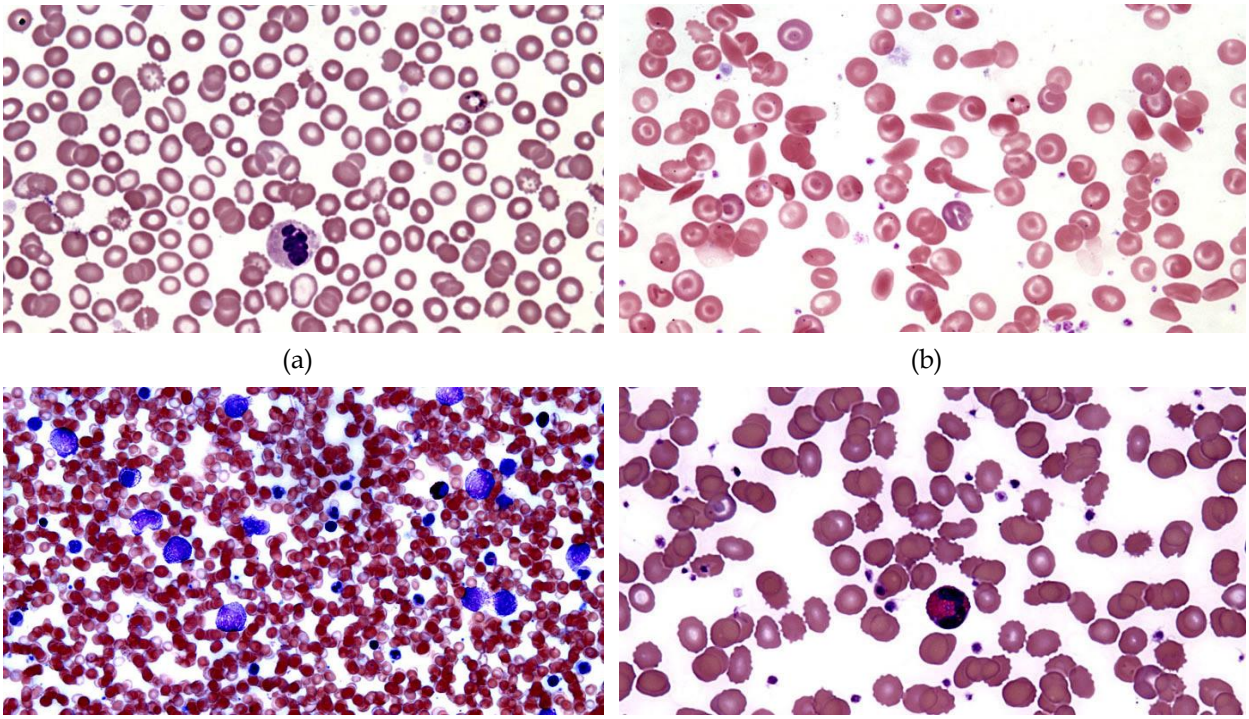

Figure 4. Photomicrographs of a Blood Sample of a Patient with (a) Iron Deficiency Anemia; (b) Sickle Cell Disease (c) Myelomonocytic Leukemia; and (d) Pernicious Anemia [51]

Among various features of microscopes, the magnification, contrast enhancement techniques, number of eye pieces, type of lighting and the microscope configuration are considered when discussing vision based microscopic systems. The magnification of the microscope depends on the magnification of the eyepieces which is fixed in most microscopes and the magnification of the objectives which is changed by changing the objective. Microscopes consist of a revolving nose piece which is capable of accommodating several objectives and objectives with different magnifications, numerical aperture, chromatic correction range and image flattening features are available in the market [52], [53]. MPLN series of introduced by Olympus, Japan include MPLN5x, MPLN10x, MPLN20x, MPLN50x, and MPLN100x which has different magnifications [54]. Similarly, other manufacturers such as ZEISS, Germany [55] and

Leica Microsystems, Germany [56] produce objectives which are mainly categorized as achromats, semi-apochromats, and apochromats based the performance against on chromatic aberration. Oil-immersion objectives reduce distortion of light-waves passing through the objectives enabling high resolution microscopic observations, yet require additional steps before the observations to reach the optimum performance of the microscopic system [57]. MPLAPON-Oil Plan Apochromat from Olympus, Japan is an oil immersion objective with high level of chromatic correction and resolution capability [58]. Utilizing advanced manufacturing technologies Olympus has developed a novel proprietary polishing technology that has enabled manufacturing the most recent high-performance series of X Line objectives with improvements in all major three aspects which are numerical aperture, image flatness, and chromatic correction range [59]. Contrast enhancement techniques such as bright-field, dark-field, phase contrast, fluorescence, polarization, confocal laser scanning, and differential interface contrast (DIC) are commonly utilized in optical microscopes to increase the quality of observing sample and increase the clarity of the photomicrographs [60]. Based on the number eyepieces, microscopes are classified as monocular, binocular and trinocular microscopes and the trinocular microscopes consists of an additional observation tube for the camera. The common use of microscopes in life science has transformed from conventional optical microscopes to motorized digital microscopes, which is a combination of advanced mechanical components, optical imaging, electronic detection, and computerized analysis thereby, to provide optimized configurations for cell biology [61].

In general, a microscopic camera consisting an image sensor is used to acquire photomicrographs and two types of image sensors are charge coupled devices (CCD) and complementary metal oxide semiconductor (CMOS) [62], [63]. Olympus DP74, SC180, EP 50 [64], Zeiss AxioCam ERc 5s, Axiocam 208, Axiocam 305 [65], and Leica DMC4500, FLEXACAM C1, EC4 [66] are several microscopic camera models available in the market.

The level of compactness and lightweight compared to performance is the main advantage of microscopic cameras. Due to these aspects, the prices of microscopic camera systems are comparatively higher than general-purpose camera devices [67]. Desai et al. studied towards the use of general purpose digital camera for blood smear imaging as a solution to high-cost of microscopic cameras [68]. Camera resolution typically specified in megapixels (MP) expresses the amount of visual details capable to capture by a camera or the number of data points in a specific image area. For applications where precise monitoring is required, higher resolution camera systems are the most suitable. The frame rate of a camera is specified by frames per second (fps) and it quantifies the number of images captured by a camera in a defined time period. A high-speed camera is capable to reach 10000 frames per second while general cameras captures 30 to 60 frames per second. Therefore, high-speed cameras are capable to precisely observe moving objects. Camera manufacturers such as PHOTRON (Tokyo, Japan) [69], PHANTOM (Wayne, NJ, USA) [70], FASTEC (San Diego, CA, USA) [71] produce series of high-speed cameras suitable for microscopic observations with minimum vibrations and with the ability to mount the camera to the imaging tube of microscopes [72]. FASTCAM Mini AX, FASTCAM Mini UX, Miro C, Miro N, HS, TS, and IL are few examples for high-speed camera models suitable for photomicrography applications [73]. Cameras are developed with the capability to transfer the microscopic images using a wired, wireless or cloud based communication method for further processing and analysis.

Optical imaging, radiography, magnetic resonance imaging (MRI), computerized tomography (CT), and ultrasound (US) imaging are few techniques used in the field of biomedical imaging [74]. Optical imaging methods are widely applied clinically and in research due to the availability of equipment, ability to obtain structural and dynamic features of cells, and the possibility to implement without physical contact [75]. In addition, recent advancements in high-resolution imaging and high-contrast imaging are

important for human cell identification. High-resolution imaging technique capture detailed images of objects in a sample which is beneficial in sensing human blood cells [76]. In cell imaging high-contrast imaging technique is utilized where the density differences are notably distinguished such as in identification of RBCs, different types of WBCs, and platelets. Conventionally, CT technique is a combination of x-ray imaging with statistical data analysis [77] and Lee et al. presented a study on in-vivo blood vessels imaging of a rodent using photoacoustic CT imaging and studied high-contrast optical imaging using 128 unfocused ultrasound transducers together with a laser of wavelength 532 nm and a camera having a CCD image sensor to capture images of the rodent in 360^0 angle at 1.5^0 steps, which are used to generate a 3D volumetric video [78]. In vivo cell imaging based on oblique back-illumination capillaroscopy (OBC) is a non-invasive, label free, phase contrast microscopy based method used to resolve blood cells in veins. McKay et al. studied using a green LED for illumination (Superbright 1W XLamp LED, 88 lm, 527 nm) for OBC imaging and characterized absorption-enhanced and phase-enhanced images, resulting successful visualization of RBC, platelets, WBC and sub-cellular granules of WBCs inside veins [79].

Imaging Flow Cytometry (IFC)

IFC is a single cell analysis method which is capable of extracting multi-parametric features of each cell in a blood sample and it is a combination of tradition high throughput flow cytometry with high resolution bright-field fluorescent microscopy [80]. Although, IFC is commonly applied to identify specific types of cells such as in acute leukemia, sickle cell disease, tumor malignancies, bacterial and viral infections [81]–[84], etc., performing IFC on a blood sample deliver parameters related to FBC while providing an in-detail result of the sample [85]. In addition, literature present studies on classification and quantification of erythrocytes, lymphocytes, and platelets in a manner of identifying individual cell types [86]–[88]. Following the basic steps used in flow cytometry,

fluorescence imaging based photomicrography is utilized in IFC and figure 5 shows the steps followed in IFC.

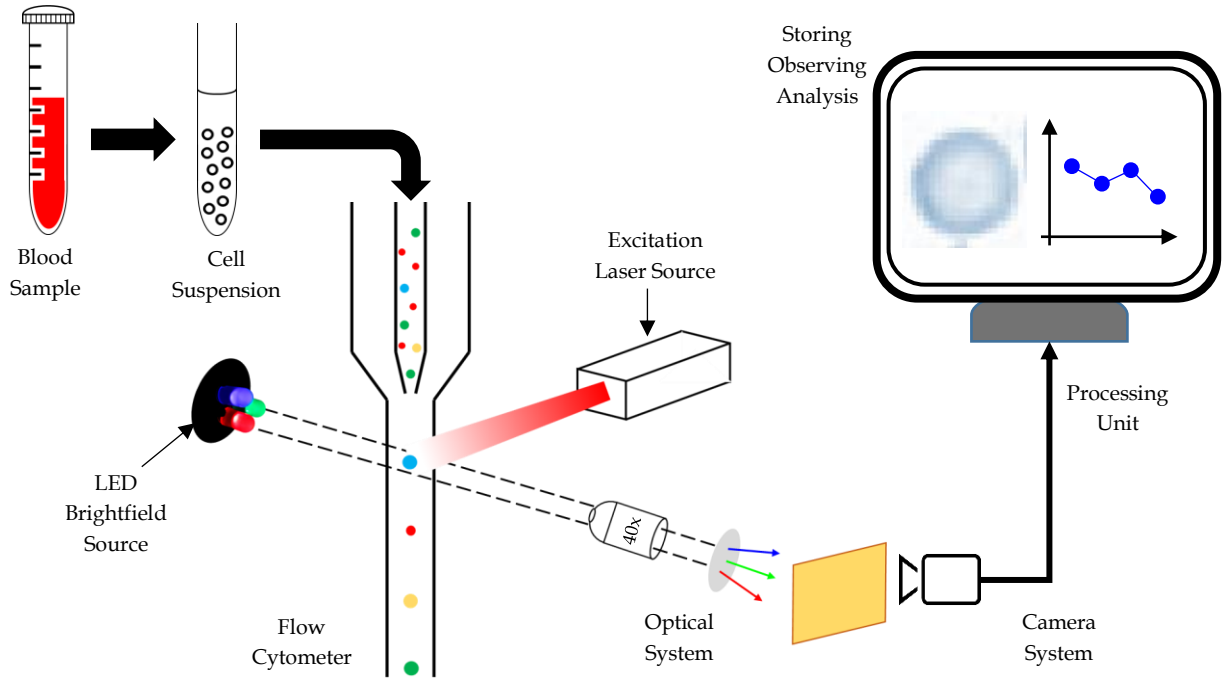

Figure 5. Imaging Flow Cytometry

Computer Vision in Cell Detection

DIP in Photomicrography

In the computer vision approach for photomicrographs based blood testing, image processing is performed to address challenges such as lack of medical images, non-uniform illumination, color shade variations, background noise, deformed shapes, cell overlapping, etc. [89]. Image augmentation, image enhancement and segmentation are the DIP techniques applied to overcome or minimize aforementioned challenges [90]. Use of online available image databases for medical image analysis is a practice followed by researchers to maintain a common ground in comparing performance of algorithms. Raabin-WBC [91], LISC [92], BCCD [93], RBCS [94], and ALL_IDB [95] are few of the online available peripheral blood smear image databases used in literature and lack of

images is a barrier in generalizing image analysis techniques [96]. Image augmentation is a DIP technique performed to increase the number of images in a dataset and color augmentation, cropping, flipping, rotation, translation, occlution, noise injection, random erasing, combination and advanced deformable augmentation are few methods discussed in literature [97]. Image enhancement is commonly done using digital image representation, image restoration, morphological processing, filtering, arithmetic and logic operations, geometric operations, neighborhood processing, convolution and correlation and histogram processing [98]. A digital image is a matrix of pixels and each pixel is represented by bits when storing in a computer memory. Bit values are important in understanding and processing the image because values of each bit represent properties of the image. Binary images (black and white), grayscale images, color images (RGB, CMY, YIQ, etc.), indexed color images, and compressed images (TIFF, JPEG, GIF, etc.) are the types of image representation methods discussed in literature [99], [100]. In a study presented by Deepa et al. the photomicrographs are primarily enhanced by converting the RGB color image to a grayscale image as a preparatory conversion for noise removal [101]. Mahanta et al. presented a study on automated counting of WBCs and platelets using a conversion from RGB image to LAB color space which includes a luminosity layer (L) and two chromaticity layers (A,B) [102]. Following a RGB to LAB color space conversion, Dey et al. extracted chromaticity layers and obtained an image which visualize WBCs and platelets significantly clear compared to RBC [103]. Iqbal et al. presented an improved compression algorithm for JPEG compression to obtain a higher compression ration while maintaining the quality of the images [104]. Image restoration is the process of recovering an image from a degraded version. It can either be a blurred or a noisy image. Image restoration is capable of producing a scene that is relatively closer to the real scenario from an image which often fails to represent the scene adequately [105].

With respective to morphological operations, dilation and erosion are the frequently used methods used in processing photomicrographs of blood cells, additionally opening, closing, boundary extraction and region filling are also discussed in literature [106], [107]. Dilation is adding pixels to object boundaries whereas, erosion is removing pixels in object boundaries [108]. Mahanta et al. studied binary thresholding for masking, opening and dilation for morphological transformation, convex hull optimization to increase smoothness and roundness of the platelets [102]. The results of the study reported an accuracy of 95.59% for the platelet count and 100% for WBCs. Furthermore, in the study presented by Dey et al. binary thresholding, erosion and dilation morphological operations, and area removal is used to isolate the platelets and the results achieved an accuracy of 92.71% for the automated platelet count compared to manual counting for 100 human blood smear samples [109].

In photomicrographs, noises appear due to bad configurations or conditions in image acquisition and biological debris in blood samples such as dead cells, damaged cells, enzymes, etc. Talukder et al. studied on distinguishing between whole blood cells and debris based on far-field pattern of surface Plasmon coupled emission (SPCE) and the authors highlight that SPCE method is performed on images captured only using a camera, without utilizing an optical device for magnification [110]. Filtering or de-noising is a major application discussed in DIP and filters are introduced to improve peripheral blood cell images which contain noises such as impulse, Gaussian, white, colored, blurred, quantization, Poisson, speckle, and photon short noise [111], [112]. Weiner, median, Kuan, normal and Bayes shrink, average, Gaussian filters, finite and infinite impulse response (FIR and IIR), unsharp masking, histogram equalization, linear contrast stretching, Fourier Butterworth, Hilbert transform detail preserving anisotropic diffusion, minimal and band pass are several filters extensively applied on DIP of photomicrographs for de-noising, non-uniform illumination, color shade variations, and

image segmentation [113]–[116]. Pixel details in an individual color band of an image is isolated using color band filters (most commonly in R, G, and B color bands are extracted). And color band filtering is combined with other filters to obtain better performance in de-noising. In [117], each RGB photomicrograph is initially separated to color bands using band filtering, then a median filter of size 3x3 is applied on each plane followed by a Laplacian filter mask iteratively to enhance the image. Equation 1 is the definition of the Laplacian L(x,y) of an image with pixel intensity values I(x,y).

$$L(x,y) = \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2} \qquad \text{Equation 1}$$

In addition, machine learning and deep learning based filters for noise removal and image enhancement are discussed in recent literature [118].

Depending on the application and properties of the images, different image segmentation techniques are presented in literature including edge detection, thresholding, histogram processing, region based techniques, neural networks and compression, etc. [119]–[121]. Edge detection techniques used in DIP are categorized into gradient-based detectors which derives first order polynomials of an image and Gaussian-based detectors which derives the second order polynomial [122]. Sobel operator, Prewitt operator, and Robert operator belongs to gradient-based detectors [123] and Canny edge detector, Laplacian of Gaussian, Gabor filter and Marr–Hildreth detector are Gaussian-based detectors [124]. In a study on blood cell segmentation Savkare et al. implemented Sobel edge detection on enhanced RGB planes of the photomicrograph as a preparatory step to separate overlapped cells [117]. George et al. discussed an efficient method to count RBCs using Canny edge detection to extract RBC sizes and measure the RBC population [125]. In Canny edge detection, a Gaussian filter kernel is convolved with the image for

smoothening and the definition for a Gaussian filter kernel of size (2k+1) x (2k+1) is given by Equation 2.

$$H_{ij} = \frac{1}{2\pi\sigma^2}\exp\left(-\frac{(i-(k+1))^2+(j-(k+1))^2}{2\sigma^2}\right);\ 1 \le i,j \le (2k+1) \qquad \text{Equation 2}$$

A discrete approximation to the Laplacian filter (a convolution kernel which approximates the second derivatives in the definition shown in Equation 1) is convolved with Gaussian filter to form Laplacian of Gaussian filter. Das et al. performed a Laplacian of Gaussian based modified high-boosting operation for edge enhancement, deblurring, and noise removal for a study on efficient blood cell segmentation [126]. The Watershed transformation is a region-based segmentation where each pixel of the images corresponds to a position and the gray levels relative to each of the pixels determining the related altitudes [127]. In a study on WBC identification and counting [128], separation of overlapped WBCs is done using the distance transformation of the Watershed segmentation. Monteiro et al. proposed an improved methodology for detecting and counting blood cells using Watershed transformation solving the cell overlapping during the counts of RBCs and WBCs [129].

Thresholding based segmentation is based on a threshold value in converting a grayscale image into a binary image. A significant presence of a variation of the objects at interest with respective to the background of the image is required to perform thresholding segmentation. Thresholding methods are categorized into global, adaptive and histogram based methods. IdentiCyte, an interface to identify RBCs is presented by Garnier et al. using a manual thresholding method (threshold value is set manually) followed by Watershed method to separate overlapping cells, then noise removal is done on the region of interest (ROI) in the photomicrograph [130]. Automatic threshold segmentation is performed in a study for WBC segmentation using Otsu's method by Salem et al. [131]

and achieved an accuracy of 93.3% compared to manual counting. In addition, performance of Watershed transformation is presented as 91.7% and the results of the study show that thresholding based segmentation is competitive in comparison to Watershed transformation. A dual-threshold method is presented by Li et al. and a combination of RGB and HSV color spaces is studied to overcome weaknesses in individual thresholding in each color space [132]. The study presents an accuracy of 97.85% while exhibiting robustness compared to individual thresholding. Zhang et al. studied on nucleus and cytoplasm segmentation of WBC using a combination of color space decomposition and k-means clustering for cell segmentation [133]. In another study, a novel combination of thresholding, k-means clustering, and modified watershed algorithm is used for segmentation of WBCs, extraction of cell nuclei, and separation of overlapping cells and nuclei [134]. Putzu et al. used a threshold value based on Zack algorithm to apply on image histogram for background extraction for comparatively less computational cost. This method is followed by area opening to obtain a cleaner image [128]. In another study, an adaptive histogram thresholding method is used for the cell segmentation based on the WBC nucleus [135]. Yamin et al. proposed a blood group classification method based on peripheral blood smear images using vertical histograms [136].

Extracting Cell Properties using Photomicrographs

At present, feature extraction and classification techniques are at an intensive development in DIP perspective, as a reason of rapid advancements in computer vision technology [137]. Feature extraction is the key to the cell identification which directly affects the diagnosis accuracy of the cell detection systems [138]. Tavakoli et al. studied WBC classification based on detecting cytoplasm by initially obtaining the convex hull of the nucleus [139]. In this study, a group of shape based features which are solidity, convexity, circularity (see Equation 3, 4, and 5 respectively) and another group with four

newly defined color characteristics based features using components of RGB, HSV, LAB, and YCrCb color spaces (48 color features) are extracted to improve the segmentation accuracy of cytoplasm detection. In defining the color based features, section of the cytoplasm detected inside the convex hull is considered as the representative of the cytoplasm (ROC).

$$\text{Solidity} = \frac{\text{Area of Nucleus}}{\text{Area of Convex hull}} \quad \text{Equation 3}$$

$$\text{Convexity} = \frac{\text{Perimeter of convex hull}}{\text{Perimeter of Nucleus}} \quad \text{Equation 4}$$

$$\text{Circulariy} = \frac{(\text{Perimeter of Nucleus})^2}{4\times\pi\times(\text{Area of Nucleus})} \quad \text{Equation 5}$$

Cheng Lu et al. studied automated detection of pathological cells and the extracted cell features are categorized under four major categories as shown in Table 1 which are shape-based, intensity-based, gradient-based and texture-based [140].

Table 1. Classification of Cell Properties in Feature Extraction

Shape-based	**Intensity-based**	**Gradient-based**	**Texture-based**
Area, Perimeter, Form factor, Eccentricity, Solidity, Major/ minor axis length, Compactness, Roundness, Extent	Mean, Standard deviation (SD), Maximum/ Minimum intensity value, Entropy	Mean/SD/Entropy of Gradient Magnitude, Mean energy of the gradient magnitude, Ratio of edge pixels and other pixels	Three Tamura textures (coarseness, contrast, direction), Twenty two Haralick textures in four directions

In recent studies, AI based feature extraction of blood cells and classification techniques are extensively studied and are presented in literature towards cell sensing [141].

AI based Decision Support Techniques

Research towards algorithm development for decision support reduce tedious task of examining the blood sample to extract cell properties and identify abnormalities [142]. With advancements in AI techniques, deployment of AI based approaches in DIP of photomicrographs are utilized for cell identification, feature extraction and analysis [143]–[145]. Neural network based decision support methods are widely described in literature [146]. Grochowskey et al. studied a learning based system for blood smear analysis and an Eigen faces based method to extract features from photomicrographs and train the neural network based classifier resulting 96% accuracy and 90% sensitivity in classifying RBCs compared to medical specialists [147]. Machine learning is a branch of AI, and in terms of image analysis machine learning require human intervention in the process of feature extraction. Machine learning is widely applied in blood cell analysis related studies and several machine learning approaches discussed in literature are artificial neural networks (ANNs), decision tree algorithm, support vector machine (SVM), Naive Bayes classifier, linear discriminate analysis, multi-layer perceptron, etc. [148], [149]. Supervised machine learning algorithms require labelled data, which a major challenge in medical imaging and unsupervised techniques used algorithms to analyze and cluster data. SVM is a supervised machine learning algorithm which is presented in several studies and it performs pattern classification based on labelled data performing linear and non-linear classification [150]. Jianyi et al. studied microscopic image segmentation based on SVM and revealed that soft interval linear classifier performs better than kernel function in segmentation and achieved 99.13% accuracy in segmentation [151]. The machine learning approach proposed by Tavakoli et al. to classify five different types of WBCs performs manual feature extraction and the significance is that a convolutional neural network (CNN) which is generally applied in deep learning,

is used as the classifier for the presented machine learning approach to increase performance and reduce ambiguous feature extraction property of deep learning [139].

Compared to machine learning, deep learning technique exhibits the major difference of automated feature extraction [152]. Automatic feature extraction is beneficial in applications where the parameters to extract are not fully defined such as in photomicrographs of human cells but extracting unknown features from an image also present the disadvantage of complexity in understanding and optimizing the learning model [153]. Deep learning models require large dataset, therefore unique data augmentation techniques such as dropout, batch normalization, transfer learning, pre-training, ones-hot learning and zero-shot learning for better performance of deep neural network architectures [154]. Alexnet, VGG, GoogleNet, ResNet, Highway nets, DenseNet, ResNet, SENet, NASNet, YOLO, GANs, Siamese nets, U-net and V-net are several deep learning architectures discussed in literature [155]. CNN is one type of a feed-forward ANN which is commonly used in DIP applications [156]. Novoselnik et al. presented development of a CNN with three alternating convolution layers and three pooling layers [157]. The presented CNN use an RGB images with a size of 300 x 300 pixels and Python is used to program the CNN resulting an accuracy of 90.62% in identification of WBCs. Mudugamuwa et al. presented a study on classification of healthy RBCs and sickle cells based on KERAS open-source Python library and Tensorflow backend using RStudio software which is an open source tool for R language [158]. Figure 6 shows the deep learning model designed which include four convolutional layers, two pooling layers, three dropout layers, a flattening layer and a fully connected layer in addition to the input and output layers.

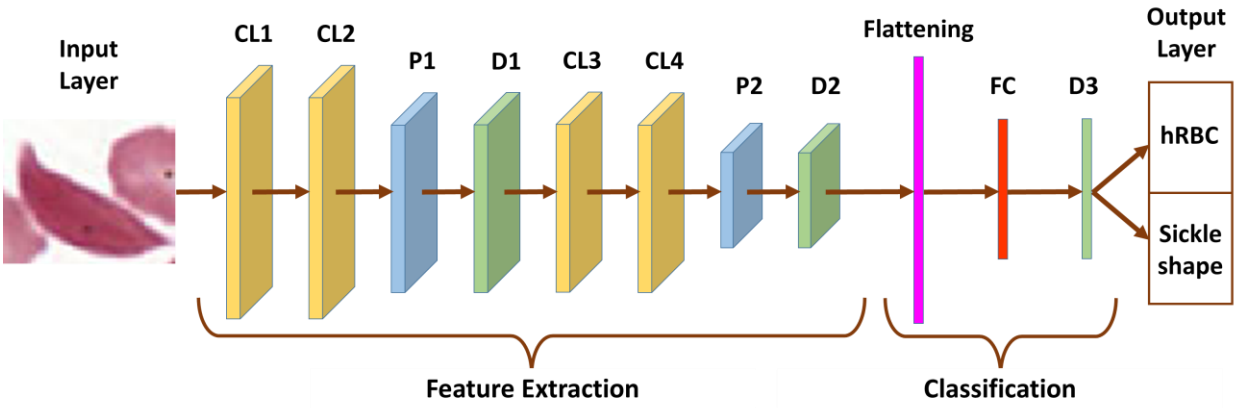

Figure 6. Deep Learning based CNN Model

Deep learning transformers models are developed on sequence-to-sequence architecture and the attention mechanism used in transformers are described by Equation 6 [159]. Q is a matrix that contains the query (vector representation of an image in the sequence), K are all the keys (vector representation of all the images in the sequence) and V are the values which are again the vector representation of all the images in the sequence.

$$\text{Atention}\,(Q, K, V) = \text{softmax}\left(\frac{QK^T}{\sqrt{d_k}}\right)V \qquad \text{Equation 6}$$

YOLO (you only look once) is a widely applied real-time deep learning algorithm and Jiang et al. presented Attention-YOLO algorithm by adding channel attention mechanism and special attention mechanism to the standard YOLOv3 in which Darknet-53 is used as the feature extraction network [160]. In comparison with the standard YOLO, Attention-YOLO demonstrated an improvement in recognition accuracy by 6.70%, 2.13%, and 10.44% for RBCs, WBCs, and platelets, respectively and the mean average precision is improved by 7.10%. In a study to classify lymphocytes, monocytes, eosinophils, and neutrophils of WBCs, a deep learning based hybrid architecture using Alexnet, GoogleNet and SVM is presented successfully by Çınar et al. [161].

Future Directions

Label-free imaging techniques are at interest of research work towards DIP of photomicrographs and Kurochkin et al. presented a study towards cell detection using adaptive spatial filtering and adaptive Niblack filtration to detect moving RBCs in a blood vessel [162]. Recent advancements in microscopy have enabled new dimensions to photomicrography based research. Light sheet fluorescence microscopy (LSFM) is capable of providing high three-dimensional spatial resolution, high signal-to-noise ratio, and fast imaging acquisition rate [163]. Autofluorescence is a label free cell detection technique explored towards cell detection and has potential for blood cell classification application using IFC, microscopy and fluorescence lifetime imaging (FLIM) [164]. Non-invasive capillaroscopy is an emerging technique and Bourquard et al. studied towards detecting severe neutropenia optical imaging of naifold microcirculation [165]. OBC is an optical imaging technique in which improvements towards phase contrast microscopy, optimizing illumination wavelength and polarization, applying AI algorithms are at interest in the development of automated real-time human blood cell detection and analysis systems [166]. Microfluidic capillary tissue phantom developed by McKay et. al in [167] provides an insight of the promising future towards MEMS based devices towards FBC testing. In recent years, research on integrating microfluidics with blood tests have proven success specially in blood sample preparation, showing a promising future towards increasing the efficiency, testing capacity and also towards advanced Point-of-Care (POC) diagnostics enabling real-time detection of blood cells [168]. Zhao et al. studied towards a high-throughput microfluidic device for real-time individual RBC analysis based on photoacoustic detection [169]. A lens-less shadowing imaging technique is proposed by Fang et al. using a CMOS image sensor, LED light source and image processing software tool [170]. The study presented a very low mean error of percentage compared to a commercial hematological analyzer for the three WBC types at

interest neutrophils (3.45%), monocytes (6.04%) and lymphocytes (6.7%) which shows the high potential to be developed as an on-chip device with further study towards imaging and identification. Recent work towards deep learning methods have provided a new direction towards classification and counting micro and nano scale objects such as blood cells, pathological cells such as cancer cells, tissue cells, virus, bacteria, etc. [171]. Gaobao liang et al. presented a study on successful combination of CNN with recursive neural network (RNN) for blood cell classification [172]. Therefore, it is identified that combining different AI techniques for blood cell analysis also demonstrate high potential in future.

Conclusion

DIP based identification and classification is a top most utilized technology in computer vision and is used for a wide range of biomedical applications. Identification of individual cells and counting the number of cells in a photomicrograph are the directions studied towards blood cell sensing and have produced promising results. At present, photomicrography based blood cell observation is studied towards single cell analysis such as in image flow cytometry and also multiple cell analysis as the vision based approach provides the ability to extract and analyze a number of features of cells such as the cell size, shape, constituents, deformations, etc.. Image acquisition, image enhancement, image segmentation, feature extraction and classification are the key areas in applying DIP to blood testing. Challenges in photomicrographs based blood testing are approached by recent developments in computer science, microfluidics and sophisticated mechanical systems. Automated and semi-automated equipment and systems are also proposed to enhance the efficiency, productivity, and accuracy of the blood testing procedures. Microfluidic imaging flow cytometry and AI based classifiers are examples for such novel multidisciplinary directions proposed in literature. In addition, literature discusses the specific advantages integrating computer vision in

testing blood samples such as remote monitoring, keeping a digital track of medical information, reusability, non-destructive nature of the techniques, and application of adaptive algorithms for decision support which enables multiple future directions for the research.

Conflicts of Interest

Authors declare no conflict of interest.

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