A Review on Traditional Machine Learning and Deep Learning Models for WBCs Classification in Blood Smear Images

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ABSTRACT In computer vision, traditional machine learning (TML) and deep learning (DL) methods have significantly contributed to the advancements of medical image analysis (MIA) by enhancing prediction accuracy, leading to appropriate planning and diagnosis. These methods substantially improved the diagnoses of automatic brain tumor and leukemia/blood cancer detection and can assist the hematologist and doctors by providing a second opinion. This review provides an in-depth analysis of available TML and DL techniques for MIA with a significant focus on leukocytes classification in blood smear images and other medical imaging domains, i.e., magnetic resonance imaging (MRI), CT images, X-ray, and ultrasounds. The proposed review’s main impact is to find the most suitable TML and DL techniques in MIA, especially for leukocyte classification in blood smear images. The advanced DL techniques, particularly the evolving convolutional neural networks-based models in the MIA domain, are deeply investigated in this review article. The related literature study reveals that mainstream TML methods are vastly applied to microscopic blood smear images for white blood cells (WBC) analysis. They provide valuable information to medical specialists and help diagnose various hematological diseases such as AIDS and blood cancer (Leukaemia). Based on WBC related literature study and its extensive analysis presented in this study, we derive future research directions for scientists and practitioners working in the MIA domain.

INDEX TERMS Blood smear images, CNN, deep learning, medical image analysis, traditional machine learning, WBCs classification.

I. INTRODUCTION

Traditional machine learning (TML) and Deep learning (DL) techniques are widely used for various applications and are extensively applied in the medical image analysis (MIA) domain [1]. In modern healthcare systems, MIA is an essential attribute, assisting medical experts wisely. MIA plays a vital role in diagnosing several diseases such as brain tumors, lung cancer, anemia, leukemia, and malaria. MIA processes various image modalities such as MRI, CT-Scan, Ultrasounds, Positron Emission Tomography (PET), Blood Smear images, and hybrid modalities [2]. In MIA, the image modalities play a vital role in detecting and classifying hard and soft tissues of different body organs for diagnostic and research purposes [3]. MIA has dense contributions for computer vision experts in the investigated topic, where TML and DL play a significant role in leukocyte segmentation, cancer detection, classification, medical image annotation, and image retrieval in computer-aided diagnosis (CAD). The CAD and computer aided-detection (CADx) rely on effective TML and DL schemes because their performance directly affects clinical diagnosis and treatment process [4], [5]. It further assists the doctors in the diagnostic and treatment process, easing their traditional working mechanisms. The recent developments in information technology, such as high-speed computational resources, hardware design, and storage capabilities significantly impact CAD.
Formerly, key application areas of CAD system via TML and DL involve early-stage brain tumor detection in MR images and leukocytes analysis. It provides valuable information to medical experts, helping them diagnose different hematological problems such as AIDS and blood cancer (Leukaemia). The main aim of MIA is to assist medical experts, doctors, hematologists, pathologists, radiologists in the diagnostic and treatment process more effectively and efficiently. In the medical field, it has been perceived that the mainstream human body's diseases are recognized by analyzing leukocytes/WBCs [9]. The increase or decrease of leukocytes/WBCs and their morphological structure, such as size, shape, and color variations in blood smear images, indicate different human body disorders.

There are different types of blood cells, such as WBCs (leukocytes), RBCs (erythrocytes), and platelets (thrombocytes). Leukocytes are further divided into five subcategories: monocyte, lymphocyte, neutrophil, basophil, and eosinophil, as shown in Fig. 1. Various TML and DL techniques have emerged in the last two decades to segment and classify WBCs in microscopic blood smear images. Conventional techniques rely on manual analysis of WBCs in blood smear images, a time-consuming, challenging task, and prone to errors [6]–[9]. Automatic and CAD systems have a crucial role in clinical diagnosis and appropriate treatment [10]–[13].

Therefore, automatic analysis of WBCs in microscopic blood smear images is gaining popularity because it can decrease the workload on hematologists and provide quick, efficient, and accurate results to assist medical experts in the diagnostic process [14]. There are mainly two ways to achieve automated WBCs classification in blood smear images, i.e., TML and DL techniques, which have great potentials to develop such automatic systems that can make medical hematology more efficient [14]–[16]. The General overview of TML and DL Models for WBCs classification in blood smear images is shown in Fig. 2. Different CAD systems can automatically diagnose numerous hematological types, such as AIDS and blood cancer (Leukemia), by analyzing leukocytes [15]. In TML, there are interconnected steps involved, such as segmenting ROI and extracting features followed by optimal classification. A variety of TML techniques are available, i.e., manual, semi-automatic, and automatic segmentation.
techniques to segment ROI from an image [16]. Features extraction is another step in the TML approach. However, selecting an optimal feature extractor is challenging due to varying feature dynamics, such as geometric invariance and photometric invariance. Nowadays, the vast emergence of DL approaches has resulted in high-performance MIA models, especially in clinical hematology using blood smear images [18]–[33].

This research provides a comprehensive survey of the available TML and DL techniques and their medical imaging applications, mainly targeting WBCs classification in blood microscopic images. There have been several surveys on MIA using TML and DL techniques and future trends focusing on MRI, CT, X-rays, but microscopic blood smear is a rarely addressed problem [17], [18]. Therefore, this study intends to fill this gap by analyzing state-of-the-art TML and DL techniques for MIA, particularly leucocytes classification methods in blood smear images. The proposed research’s primary focus is to provide a comprehensive review of the use of TML and DL in MIA.

In the proposed study, a novel categorization is employed to find the most common TML and DL methods that are reviewed in separate groups according to the research focus and employed technique. This research also helps identify future research directions by following TML and DL techniques to classify leucocytes in microscopic blood smear images. The followings are some of the significant contributions of the proposed review study:

- The outlines of this paper investigate different applications and uses of TML and DL models in MIA.
- This research study also aims to identify available machine learning techniques for leukocyte classification and analyze the extent of accuracy, applications, and MIA contributions.
- We address the key challenges and requirements of TML and DL models, followed by its future directions and solutions for future research in MIA.

The remaining paper is structured as follows: Section II describes the review methodology and papers scrutinization process in detail. Section III gives a brief introduction about MIA. In Section IV, we present the detailed summary and applications of the artificial neural network and leucocytes classification in microscopic blood smear images. In Section V, the current challenges and requirements are discussed. Future directions of the proposed review study are described in section VI. In the last section VII, we discuss about recent advancements in DL models, followed by conclusions of the proposed review work.

II. REVIEW METHODOLOGY

This section provides a detailed discussion about digital libraries used for conducting a formal research process in the proposed review study. A planned searching procedure is required to find the available literature that fulfills the searching criteria, to utilize the available digital resources purposefully [19]. In the proposed study, we incorporated both manual and automatic searches to get the most relevant research articles by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) model [20], [21].

We performed both manual and automatic searches to fetch the most relevant content. Our searching strategy begins with an automatic search on electronic databases to retrieve relevant data followed by verification of results by MIA and leucocytes classification experts. In the proposed survey, we search for articles from the period of 2014 to 2020. All the included sources are searched automatically as well as manually using the predefined keywords. Keywords for the search are decided by the authors and other research participants (a group of four research students working in the area of MIA). These keywords include “Medical Image Analysis,” AND “Leucocytes Classification,” OR “WBC’s Detection in Blood Smear Images”. Search keywords are defined based on the following steps:

(a) The major terms are derived from research questions
(b) Alternating synonyms or spellings are identified for the major terms.
(c) Keywords are identified from relevant research articles and books.
(d) Boolean operator OR is used for alternating spellings or synonyms.
(e) The major terms are linked by Boolean AND operator and the search string is formed after the analysis of the keywords in order to retrieve the relevant information from the databases.

The above-mentioned keywords and string are checked on each database and its pattern is modified based on relevant results retrieval. Numerous keywords associated with the study’s primary focus is based on the four research questions (RQ) that are designed keeping in view the Patient, Intervention, Comparison, Outcome (PICO) framework [22].

RQ -1: What are the different TML and DL techniques for leukocyte classification in blood smear images?
RQ -2: What are the different applications of TML and DL techniques in medical analysis, especially leucocytes classification?
RQ -3: How are TML and DL techniques used in MIA, particularly leukocyte classification in blood smear images?
RQ -4: What type of machine learning is practical and efficient for analyzing leukocytes in blood smear images?

A. RETRIEVED PAPERS SCRUTINIZATION CRITERION

The initially retrieved papers are subject to inclusion/exclusion criteria by following the PRISMA guidelines. Table I represents the inclusion and exclusion criteria to filter out irrelevant articles. The selection of research articles is completed in three steps. Firstly, duplicate papers are removed. Secondly, the paper title, abstract, and keywords are investigated for relevancy, and finally, the remaining research papers are included after a thorough investigation. The process of exclusion and inclusion criteria is applied to eliminate
conflict analysis and biasedness. A total of 1436 research papers are collected to review the literature based on the research focus during the article selection process. In the initial selection process, manual filtering is incorporated, and the papers are filtered using the relevant title, and 1106 papers are obtained. These 1106 articles are then filtered by observing the abstract and conclusion, finally leaving 922 papers. These papers are filtered by methodology and results in the next step, and 725 articles are obtained. Then the articles are filtered after reading the full contents, leaving 216 articles. We checked the remaining articles’ quality by evaluating the methodology, full-proof results, journal’s impact factor, and citations. After checking all these parameters, 80 papers are picked for the proposed study. After the completion of the paper’s scrutinization process (paper inclusion and exclusion), the quality assessment is performed. Each research article is assessed against the scrutinization criteria. All research articles are reviewed, and the quality of the papers with respect to each research question is assessed. Each of the selected articles is read and analyzed manually by the authors. The publication channels used for the article searching and the stepwise selection process are presented in Fig. 3.

### III. MIA

The process that can provide visual information of the human body to assist the radiologists and doctors in an efficient diagnostic and treatment is called medical imaging [23]. There are many image modalities upon which the doctors and medical experts rely for diagnosing diseases and prescribing treatment. These modalities include CT, X-ray, MRI, microscopic blood smear images, PET, and ultrasound [17], [23], [24]. These imaging technologies play an essential role in MIA; doctors and medical experts can automatically detect and diagnose different chronic diseases by analyzing these images. They can also visualize different body organs for research [37]. The number of research papers explored in this field is shown in Fig. 4. The last two decades have witnessed extensive medical imaging usage in CAD, for instance, in applications such as for leucocytes segmentation and classification, tumor segmentation and classification, detection and classification of breast cancer, image-guided therapy, and medical image annotation [25]–[28]. It has,

| TABLE 1. Criteria for research papers inclusion and exclusion in the proposed study. |
|-------------------------------|-----------------------------------------------|
| **Inclusion criteria**         | **Exclusion criteria**                         |
| The research articles that are published in English language are included. | Research papers published in other than English language are excluded. |
| The articles sufficiently related to the main theme of the investigated topic. | The paper which poorly explain the main focus of the proposed study are excluded. |
| Only the relevant research articles are included, containing clear results and proofs. | The papers which did not contain clear results, adequate comparison. |
| State-of-the-art articles from 2014 to 2020. | Duplicated research articles are excluded. |
A. TML AND DL FOR LEUCOCYTES CLASSIFICATION IN BLOOD SMEAR IMAGES

The literature includes a sufficient number of recently published review articles on TML and DL techniques used in MIA. The most recent and relevant research works about TML and DL methods in medical imaging, particularly for the classification of leucocytes in blood smear images [30], are discussed in the subsequent sections. In the proposed study, the most relevant and recent studies are searched out using keywords “leucocytes detection” or “leucocytes classification” by filtering the recent papers. During searching, we found that there is an exponential research growth of using TML and DL methods for leukocytes analysis in blood smear images. Fig. 5. represents the overall research results of DL and TML techniques for MIA and its exponential growth in the last two decades.

TML approaches involve interconnected steps, i.e., image pre-processing, segmentation, feature extraction, feature selection, and classification. The pre-processing step includes image enhancement such as contrast adjustment, noise removal, and image sharpening. All these steps are applied to the input image before image segmentation [41]. There are numerous pre-processing techniques such as median filter, low pass filter, high pass filter, and Gabor filter. These are used normally for image contrast adjustment, image sharpening, and noise removal before image segmentation. TML has been addressed by several researchers for leucocytes detection and classification. However, accurate nuclei detection, separation of borders to recover overlapped cells, segmenting ROI, robust features extraction, and best features selection have become challenging and time-consuming using these approaches [31]–[33]. In this approach, after segmenting ROI, the next step is feature extraction. In traditional supervised learning techniques, the classification depends on choosing robust features descriptor and best features selection algorithm [31], which are the most crucial steps towards efficiency and accuracy of the adopted technique. The general overview of TML is shown in Fig. 6.

Many conventional supervised learning methods have been used to classify leucocytes in microscopic blood smear images, such as Support Vector Machine (SVM) [32]–[34], Naive Bayes (NB) [35]–[37], K-Nearest Neighbor (KNN) [38]–[40], and Artificial Neural Network (ANN) [41]–[43]. Some popular WBCs nuclei detection techniques are identified and reviewed, which are presented in Table 2.

B. LEUKOCYTES CLASSIFICATION USING SVM

There are numerous supervised learning techniques available to deal with leucocyte classification, such as SVM, ANN, Naive Bayesian, and Decision Trees. Hegde et al. [70] proposed a novel technique in which the authors first segmented the WBCs and then employed SVM to classify WBC cells into a normal or leukemic cell. Zhao et al. [69] proposed a novel technique to segment and classify Leukocytes in blood smear images. Color co-relation and morphological based segmentation are applied, followed by texture features extraction and classification using SVM to classify WBCs into its five subclasses [90]. Table 3 elaborates on the key contributions and applications of SVM for leucocytes classification in blood smear images.

C. ENSEMBLES, HYBRIDS, BAYESIAN, K-NN AND DECISION TREES FOR LEUKOCYTES CLASSIFICATION

In addition to ANNs and SVMs, which have significant contributions to MIA, hybrids, Bayesian, Ensembles, K-NN, and Tree models have also been applied to solve the problems in different sub-domains of medical imaging such as brain tumor detection, lung cancer detection, leucocytes classification, etc. Abdulkadir et al. [71] proposed a hybrid approach for WBC classification in blood smear images. Sajjad et al. [15] proposed a smartphone-based quality health-care system for smart cities, in which an ensemble multi-class SVM is used to classify WBCs in blood smear images.
| Literature References   | Techniques used                      | Remarks                                                                 |
|------------------------|--------------------------------------|--------------------------------------------------------------------------|
| Safuan et al.[41]      | Otsu thresholding and watershed marker| Shape features are used to distinguish between single and group cells     |
|                       |                                      | Optimal threshold value-based image pixels' classification. WBC segmentation accuracy achieved was 96.92%. |
| Huang et al.[44]       | Otsu thresholding                     | Multilevel Otsu threshold-based segmentation cell nuclei and texture and shape features extraction followed by PCA to recognize leukocytes. The overall accuracy for classification using SVM was 81.6%. |
| Danyali et al.[45]     | Fuzzy divergence threshold            | An automatic robust fuzzy divergence-based thresholding technique to segment leukocyte in blood microscopic images with an average accuracy of 98%. |
| Manik et al.[46]       | Adaptive thresholding                 | Intensity maxima is used to enhance the image for optimal threshold values selection. The overall accuracy is 98.9% and differential accuracies are 100% for Eosinophil; 96.7% for Lymphocyte and 100% for Neutrophil. |
| Li et al.[47]          | Dual thresholding                     | Two different optimal threshold values are utilized to segment WBCs. The overall single WBC segmentation accuracy reached 97.85%. |
| Ghosh, et al.[48]      | Region segmentation                   | Image regions are segmented based on image geometric texture features i.e. size, shape, color and texture using fuzzy and non-fuzzy techniques. The sensitivity and specificity of used technique were 96.4% and 79.6%, respectively. |
| Wang et al.[49]        | Spectral and morphologic              | Mathematical morphology is incorporated to extract spatial features followed by a supervised technique to extract spectral information. The experimental results achieved the overall accuracy of more than 90%. |
| Ravikumar, S. (2016).[50]| Adaptive thresholding                 | Color histogram based optimal threshold value to segment leukocytes. ELM, RVM and Fast RVM are compared, Fast RVM achieved higher segmentation accuracy. |
| Sajjad et al.[6, 15]   | k-mean clustering                     | First the input images are converted from RGB to HSV and then color is divided into four clusters using color based k-mean clustering algorithm to segment leukocytes in blood smear images. |
| Sarrafzadeh and Win KY,[51, 52]| k-means clustering & region growing | To segment WBC nuclei k-mean clustering and region growing is employed to separate cytoplasm and nuclei. The accuracy of Precision: 0.90, F-measure:0.89, Recall:0.89, Dice Similarity Coefficient:94% and Piccard Index:89%. |
| Ghane et al.[53]       | Simple thresholding, k-means clustering & modified watershed | Initially, WBCs are segmented in microscopic image using manual thresholding, then nuclei is extracted from leukocytes using k-mean clustering, and then overlapping cells nuclei’s are separated using watershed technique. The similarity measures, sensitivity and precision were 96.07, 92.07 and 94.30% for nuclei segmentation and 97.41, 92.93 and 93.78% for WBCs cell segmentation. |
| Negm et al.[54]        | k-means clustering                    | k-mean clustering algorithm for segmenting of WBCs, normal cells and leukemic cells are classified using SVM. The overall accuracy of technique was 99.517%. |
| Ananthi et al.[55]     | (IVIFS) Interval-valued intuitionistic fuzzy | Logarithmic function is used to calculate the membership of intensities values of each corresponding pixel then IVIFS is constructed using Yager generating function for Non-membership intensities values. The segmentation accuracy on two datasets reached 98% and 96%. |
| Khosrosershki et al.[56]| Simple thresholding                  | Mathematical morphology is used to segment WBCs, followed by fuzzy as a classifier to classify the normal cells and leukemic cells achieving 93.75% degree of accuracy. |
| Khamael et al.[57]     | Edge based geometric active contours  | Three level set forces-based (curvature, normal direction, and vector field) and edge-based geometric active contours are utilized to segment leukocytes. Achieved F-index values of 92.09%, 91.13%, and 90.76%, respectively. |
| Bouchet et al.[58]     | Fuzzy set algorithm                   | hue component in the HSV color space is used to labeled leukocytes pixel, calculated similarity measure, leukocytes is segmented. The accuracy, precision and recall reached 99.32%, 99.41% and 99.24%, respectively. |
| Marzuki et al.[59]     | Snake or active contour               | Snake models are incorporated to detect leukocytes in the blood smear images using a curve. |
| Jha et al.[60]         | Hybrid model based on Mutual Information (MI) | WBCs are segmented by means of active contour and fuzzy C means technique, CNN is used to classify WBCs into its respective categories with accuracy of 98.7%. |
| Liu et al.[61]         | Watershed and Mean Shift Clustering   | Initially, blood smear image is enhanced to detect nucleus, then mean shift clustering technique is used to choose C channel in CMYK color model, finally, the seeds and NMWO are employed determine WBCs and solve the cell overlapping issue. Achieved higher segmentation accuracy and robustness as compared to state-of-the-art techniques. |
TABLE 2. (Continued.) List of various research studies for WBCS nuclei detection in blood smear images.

| Literature references | Methodology | Application |
|------------------------|-------------|-------------|
| Xie et al.[62] | Marker-controlled Watershed | Two marker-controlled watershed and edge gradient-based technique is utilized to segment two types of blood cells i.e. WBCs & RBCs. Segmentation accuracy achieved for WBCs and RBCs were 97.2% and 94.8%. |
| Dai et al.[63] | Graph cuts | Gaussian mixture with improved graph cuts models (GMMs) is used to extract foreground and background objects, weights of each pixel of foreground and then a graph is created from the nodes and edges weights. The experimental results prove that this technique outperforms other state-of-art methods in nuclei detection and segmentation. The average Dice similarity coefficient index was 0.9874, segmentation efficiency and accuracy and is significantly higher as compared to state-of-the-art techniques. |
| Mahapatra, D. (2017)[64] | Graph cuts | SVM is trained with each pixel of the input image, for image segmentation graph cuts and then combined results are obtained from SVM. The dice indices comparison analysis for putamen, caudate nucleus, right kidney, and left kidney segmentation 0.954, 0.9340, 0.9473 and 0.9132, respectively. |
| Zheng et al.[65] | Graph cuts & SVM | To segment WBC self-supervised learning approach is used, SVM classifier is used to classify each pixel of the image. The approach has the lowest OR, ER and RDE (0.69%, 5.24%, 1.16) compared with other techniques i.e., CGS (4.87%, 8.59%, 1.85), FCM (0.85%, 12.41%, 2.44), U-Net (0.83%, 5.39%, 1.25), and SVA (3.32%, 7.09%, 1.68). |
| Zheng et al.[66] | SVM based segmentation | V ogado et al.[74] used a hybrid approach for the classification and segmentation of leukocytes. In their proposed technique, CNN features are used as input to train the SVM classifier. A transfer learning is also utilized for further classification of leukocytes, as comprehensively given in Table 4. |

Tantikitti et al. [72] proposed a computer-aided diagnosing system to diagnose dengue fever disease. A multi-level threshold technique is used to segment leukocytes in blood smear images. This research has two decision tree models for classification. The first model was used to classify the type of white blood cells that are lymphocytes or Phagocytes. The second model is used to classify the dengue virus infection as positive or negative. In [73], a novel technique is proposed in which WBCs nucleus and cytoplasm are segmented using simple thresholding. After segmentation, some morphological operations are performed using ellipse curve fitting, followed by feature extraction. For feature selection, the sequential forward selection technique is incorporated, and finally, a naïve Bayes classifier is used to classify WBCs. Vogado et al. [74] used a hybrid approach for the classification and segmentation of leukocytes. In their proposed technique, CNN features are used as input to train the ANN for leukocytes classification.

IV. ANN FOR LEUCOCYTES CLASSIFICATION

ANN is a supervised learning technique inspired by the biological nervous system of the human brain. It involves input, output, and hidden layers that are linked together via weighted connections. The performance of any ANN technique depends on these weights, which are numerical values. The output layer generates results given the inputs based on weights, error function, and neurons in the hidden layer. Several research studies have applied ANN in the context of MIA due to its enormous applications, including leukocytes classification, brain tumor classification, breast cancer detection, and lung cancer detection. Some notable contributions and applications are summarized in Table 5.
TABLE 4. Notable contributions and applications of bayesian, ensembles, hybrids, K-NN and trees models for leucocytes classification in blood smear images.

| Research reference | Research Contributions | Application |
|--------------------|------------------------|-------------|
| Sajjad et al.[15]  | Cloud-assisted resource-aware framework for WBCs localization and classification using Ensembles multi-class SVM within microscopic blood smear images, with average accuracy 98.6% | Resource aware Health care system for smart cities. |
| Gautam et al.[36]  | A simple Otsu thresholding is used to segment leucocytes, some morphological processes are applied to the segmented image, features are extracted and naïve Bayesian classifier is incorporated to classify leucocytes with 80.88%, accuracy in an average time of 22 s per image. | Automatic classification of leucocytes |
| Tantikitti et al.[72]  | WBC counting and decision tree is used to classify dengue viral infections of patients from blood smear images. 92.2% accuracy with 167 cell images while dengue classification modeling technique having 72.3% accuracy with 264 blood cell images. | Dengue virus diagnosing system |
| Prinyakupt J, P,C.[73]  | Leucocytes are segmented using thresholding then best features are extracted from the ROI, features selection is achieved using sequential forward selection, followed by naïve Bayes classifiers to classify WBCs. The dice similarity of 98.9 and 91.6% for segmented nucleus and cell regions, and the overall correction rates in the classification were 98 and 94% for naïve Bayes models and linear, respectively. | Computer aided detection system for WBCs classification |
| Vogado et al.[74]  | Hybrid approach to classify leucocytes, CNN based extracted features were then used as input to train SVM classifier with 99.20% accuracy. Furthermore, classification of WBCs with transfer learning is performed. | Computer-aided systems for leucocytes classification |
| Rawat et al.[75]  | WBCs were segmented, then some textures features are extracted from the segmented region to train PCA-SVM based hierarchical classifier to classify the acute lymphoblastic leukemia into its sub-categories. The overall classification accuracy 94.6%. | Computer aided diagnostic system |
| Shaikhina et al.[76]  | Decision tree and Random forest classifiers are used to predict high-risk kidney transplantation, by observing different parameters of donor and recipient during transplantation process i.e. levels of antibodies, number of leucocytes with 85% accuracy. | Decision support tool predicting outcomes of kidney . |
| Abdeldaim et al.[77]  | This study presents a Computer aided diagnostic system for Acute Lymphoblastic Leukemia. In the proposed study, first the input WBC image is converted from RGB to CMYK, then Zack thresholding technique is used to segment WBC, followed by features extraction including color, texture, and shape and K-NN classifier is used to classify into ALL and non-ALL with 98.6% accuracy. | Automatic acute lymphoblastic leukemia detection |
| Mathur et al.[78]  | Naïve Bayesian classifier based automatic segmentation and classification system for WBCs is proposed, that can assist healthcare specialist, speed up diagnosis process and eliminate human errors in disease diagnosing stage. An overall accuracy of 92.45% and 92.72% achieved over the training and testing set respectively. | Automation of Differential Blood Count (DBC) |

A. LEUKOCYTES CLASSIFICATION BASED ON DEEP LEARNING

DL allows us to define a system in which the feature extraction is not designed by human engineers but learned from data using a general-purpose learning procedure [79]. In the field of MIA, deep learning achieved satisfactory performance and relatively easy to build an end-to-end network using CNN [80]. TML models are trained on manually extracted features, or they learn features via other simple machine learning techniques to perform different classification tasks. Therefore, DL techniques have attracted the researcher’s attention and motivated them to explore DL’s benefits for WBCs classification. Currently, DL has become a powerful research tool in artificial intelligence, speech analysis [81], natural language processing (NLP) [82], and medical imaging [83]. DL’s use is also becoming an essential aspect as a pattern recognition tool in the field of MIA [84]–[86]. According to a recent review on DL based MIA [87], DL algorithms and particularly convolutional networks, have become a choice for many for analyzing medical data. These methods are particularly suitable to those areas where human-like intelligence is required to analyze large amounts of data. Additionally, good knowledge is needed to extract rich features from a massive raw data volume [88]. However, this task is challenging and time-consuming when a vast collection of data is to be handled efficiently. DL provides end-to-end learning and eliminates all extra overheads of selecting feature descriptors and feature selection, as shown in Fig.7. DL methods’ significant advantage is learning and automatically extracting semantically rich...
features from the raw data [82]. This is the main difference between TML and DL models. DL’s unmatched benefits have attracted a large research community and industries to use DL-based approaches for MIA.

DL models can be classified into different categories such as convolutional neural networks [95], deep belief networks [96], Long short-term memory networks [97], Recurrent Neural Networks (RNN) [98], and deep auto-encoders [99]. Convolutional neural networks (CNNs) is widely used in medical imaging [17].

B. LEUKOCYTES CLASSIFICATION USING CONVOLUTIONAL NEURAL NETWORKS (CNN)

CNN consists of multiple convolutional, pooling, and fully interconnected layers with activation functions. It is trained using gradient descent and backpropagation as any standard ANNs (see Fig. 8) [100]. Typical CNNs generally have a successive convolutional and pooling layer followed by a fully connected layer. A Softmax function is used at the output nodes to classify WBC’s into its five respective categories, i.e., monocyte, lymphocyte, neutrophil, basophil, and eosinophil. Banik al. [101] proposed a novel CNN model for WBCs classification by fusing the features of first and last convolutional layers using the BCCD database. Choi et al. [102] proposed a CNN model with eight layers for WBCs classification. Karthikeyan et al. [103] presented an LSM-TIDC method to classify WBCs in blood smear images. Firstly, images are pre-processed, then texture and geometrical features are extracted using a multi-directional model. Finally, the extracted features are fed as a feature vector to deep convolutional networks for efficient and early detection of WBCs in blood smear images. In [14], the authors proposed a Regional-Based CNN using transfer learning approaches to classify WBCs in peripheral blood smear images. The overview of some recent articles using DL for leukocyte classification is shown in Table 6.

V. CURRENT CHALLENGES AND REQUIREMENTS

In this extensive literature review, we found the major research challenges and requirements, several key features, their applications, and advantages of TML and DL techniques for MIA, particularly for WBC’s classification in blood smear images. In the last few years, there are certain standard and powerful TML and DL models developed for MIA, such as brain tumor localization and classification from MRI, leukocytes detection and classification in blood smear images, and lung cancer detection in CT images [124]. Still, there exist some significant challenges that the research community either has to accept or try to overcome. These challenges include the unavailability of publicly available large and good quality datasets, dedicated medical experts, and lightweight TML and DL techniques. Some of the challenges are related to the mathematical and theoretical underpinnings of many DML techniques [123], [124]. To overcome these challenges, unsupervised or semi-supervised systems are required [83]. The proficiency of semi-supervised and unsupervised methods in MIA will be compromised to avoid these issues. It is also challenging to move from supervised learning to unsupervised learning approaches without affecting the system’s accuracy and efficiency. MIA applications and systems employing TML and DL methods are still far from perfect, leaving significant space for improvements.
FIGURE 8. Building blocks of general CNN architecture for leukocytes classification. A slight modification of a figure in [17].

A. UNAVAILABILITY OF PUBLICALLY AVAILABLE DATASETS
The major problem in the field of medical image analysis is the unavailability of publicly available datasets. To address this issue, the researchers need to encourage health organizations to make their medical data available; it can be interesting if quality data is publicly available for researchers. Moreover, initiatives that encourage open data from different health institutions worldwide are encouraged; some operation are also necessary (e.g., data from hospitals and conditional access to datasets). In all these cases, incentive mechanisms can be related to financial return, entertainment, or services to these institutions while providing quality data. The topic becomes more interesting for research when the data is available in massive amounts, just like other fields (e.g., video summarization [125], IoT [126], energy management [127], and so on.). It is vital to collect extensive and quality datasets with ground-truth labels for specific MIA applications. Moreover, such datasets can be used for benchmarking as well as hosting different competitions.

B. TRAINED PREDICTOR GENERALIZATION ABILITIES
The key issue with MIA and leucocytes detection and classification is to train a predictor. An ideal learning technique with a better balance of generalization ability and a computationally efficient heuristic model is required to overcome this problem. A learning paradigm that uses true or random labels and provides effective tools to deal with available datasets and efficient training algorithms are needed to train a model with remarkable generalization abilities. Learning with deep neural networks has enjoyed huge empirical success in recent years across a wide variety of tasks in the field of MIA, i.e., brain tumor detection, lung cancer, breast cancer detection, and leucocytes classification. Despite being a complex, non-convex optimization problem, simple methods such as stochastic gradient descent (SGD) can recover reasonable solutions that minimize the training error. More surprisingly, the networks learned this way exhibit good generalization abilities [128], even when the number of parameters is significantly larger than the amount of training data [129]. During model training, only minimizing the training error is not enough. Picking the wrong global minima can also lead to bad generalization behavior for the predictor. In such situations, generalization behavior depends implicitly on the algorithm used to minimize the training error. Different algorithmic choices for optimization, such as the initialization, update rules, learning rate, and stopping condition, will lead to different global minima with different generalization abilities.

C. TRUST-WORTHY METHODS TO BE FUNCTIONAL IN REAL-WORLD ENVIRONMENTS
The existing TML and DL techniques are not good enough to be trusted without medical expertise to function in real-world health diagnosis systems [130]. There must be an expert as well as technical skills to train a learning model for MIA and leucocytes classification. We need to explore such precise and trustworthy methods which do not need health experts and are implementable in real-world health applications.

VI. FUTURE RESEARCH DIRECTIONS
Considering the major challenges encountered by the MIA community outlined in section V, extensive work is demanded from the biomedical industry and research community to contribute to MAI and especially leukocytes analysis in blood smear images.

A. DATA AUGMENTATION TECHNIQUES TO FILL THE DATASETS DEFICIENCY
In this study, we have focused on the most frequently mentioned problem of unavailability of datasets in the field
### TABLE 6. Some notable key contribution of different deep learning models for leukocytes classification.

| Reference                | Key contributions                                                                 | Model used                                                                                          |
|--------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| Wang et al.[104]         | CNN based leukocyte recognition is done using Single Shot Detector (SSD) and an Incremental Improvement Version of You Only Look Once (YOLOv3) Detector. The method achieved 90.09% mean accuracy and 93.10% mean average precision while the inference time is 53 ms per image on a NVIDIA GTX1080TI GPU. | CNN architectures SSD & YOLOv3                                                                  |
| Kutlu et al.[14]         | A computer-aided system has been proposed, which is based on DL that can easily locate and classify WBCs types in blood smear images. | Neural Networks (R – CNN)                                                                           |
| Fan et al.[105]          | Fist leukocyte-mask is used on pixel-level prior to the network training, and then to segment region of interests (ROI), finally the trained model is used to classify leukocyte in blood smear image with 0.99 precision and 0.98 dice on dataset-1 and dataset-2. | Residual Network (ResNet50)                                                                         |
| Hegde et al.[106]        | In this paper, a comparative study of traditional learning and CNN based features extraction is organized to classify. The average sensitivity and accuracy of 99% was obtained for of white blood cells classification. | Pre-train CNN Model AlexNet                                                                         |
| Acevedo et al.[107]      | The main contribution of this research is a classification scheme based on a trained CNN to classify eight classes of blood cells circulating in microscopic blood smear image with overall classification accuracy of 96.2%. | Pre-trained CNN model                                                                                |
| Qin et al.[108]          | Leukocyte classification using fine-grained technique, which is based on deep residual learning theory to classify leukocytes microscopic blood smear images. During training the top-1 accuracy of 77.80%, top-5 accuracy of 98.75% and testing average accuracy was 76.84%. | Deep residual neural network                                                                       |
| Tiwari et al.[109]       | The authors proposed a fine-tuned CNN technique, that can automatically classify blood smear image into its subtypes of blood cells with average accuracy of 0.97. | Double Convolution Layer Neural Network                                                               |
| Hung J, Carpenter A.[110]| In this paper, a two-stage DL based technique is proposed that can automatically detect and classify RBCs and other cells including infected and WBCs. The total accuracy of the non-difficult infected cells is 72%. | Faster R-CNN & AlexNet                                                                               |
| Imran Razzaq M, Naz S.[30]| An efficient contour aware CNN and faster R-CNN based segmentation approach to segment RBC and WBC whereas for classification they used CNN based extreme machine learning to classify WBCs an accuracy of 94.71% and 98.68% for RBC & its abnormalities detection and WBC, respectively. | Contour Aware CNN & Faster R-CNN                                                                    |
| Zhao et al.[69]          | An automatic method is proposed to segment and classify WBCs from peripheral blood images into their respective subclasses using CNN features and random forest classifier with accuracy of 92.8. | CNN with random forest classifier                                                                  |
| Vogado et al.[74]        | CNN architecture is incorporated with transfer learning approach to extract features and then SVM classifier is used to classify blood cells into normal and leukaemic with an accuracy of 99.20%. | Transfer learned CNN model with SVM                                                                 |
| Habibzadeh et al.[111]   | Classification of WBCs using a consecutive proposed DL framework, to classify WBCs into their four primary types including Neutrophils, Eosinophils, Lymphocytes, and Monocytes an accuracy rate obtained 99.46% and 99.84% with ResNet 101 and ResNet V1 152, respectively and fine-tuning all layers with 3000 epochs. | ResNet & Inception                                                                                 |
| Song et al.[112]         | Super pixel and CNN based technique is proposed to segment cervical cancer cell, using region of interest detection approach. The experimental results demonstrated an accuracy of 94.50%. | Convolution neural network (CNN)                                                                    |
| Özyurt, Fatih.[113]      | Pre-trained CNN models including VGG-16, AlexNet, GoogleNet and ResNet are used as feature extractors, the extracted features on fully connected layers are then combine, MRMR features selection followed by extreme leaning machine is incorporated to classify WBCs for which 99% and 93.7% accuracy is reported. | CNN-MRMR-ELM                                                                                       |
| Rehman et al.[114]       | A novel technique is proposed for ALL classification in stained bone marrow images. First lymphocytes are segmented using simple threshold method then the segmented lymphocytes are passed via DL techniques based on CNN to classify into L1, L2, L3 and normal lymphocyte achieved 97.78% accuracy. | Deep convolutional neural network                                                                  |
| Bani-Hani et al.[115]    | This study proposed a CNN to classify WBCs into its respective five categories: eosinophils, lymphocytes, monocytes, neutrophil and eosinophil. The overall accuracy of 91% is achieved for the validation set and 99% for the training set. The specificity and sensitivity of 97% and 91% are reported, respectively. | Deep CNN with Optimized Genetic technique                                                           |
| Di R uberto et al.[116]  | A new technique is proposed to recognize WBCs in microscopic blood smear images using blob detection and then DL approach to classify them into normal and leukemia cell an accuracy of 100% for SMC-IDB, the IUMS-IDB datasets and 99.7% for the ALL-IDB in white cells detection and 94.1% in leukemia classification. | Pre-trained CNN model AlexNet                                                                     |
TABLE 6. (Continued.) Some notable key contribution of different deep learning models for leukocytes classification.

| Model Authors | Model Description | Accuracy (%) | Deviation |
|---------------|-------------------|--------------|-----------|
| Loey M, Naman M, Zayed H | Firstly, blood smear image is pre-processed then a pre-train CNN model AlexNet is used to extract features and a fine-tuned AlexNet is trained to classify blood cells into normal and abnormal cell with 100% accuracy. | Pre-trained deep convolutional neural network AlexNet | 96.86% |
| Ma et al | In this paper, the authors proposed a new classification framework for blood cell image which is based on deep convolutional generative adversarial network (DC-GAN) and a residual neural network (ResNet) with accuracy achieved 91.7%. Furthermore, transfer learning is incorporated to improve model accuracy. | DC-GAN with ResNet | 96.86% |
| Baydilli YY, Atila Ü | A novel DL technique is proposed to classify WBCs, first data is augmented using different augmentation techniques to increase data then DL based capsule network is used to classify WBCs into its five subclasses achieving accuracy of 96.86%. | Capsule networks | 96.86% |
| Tobias et al | Deep learning based technique Faster R-CNN is proposed detect WBCs and RBCs in order to achieve the goal to assist medical expert in diagnosing different hematologic disease automatically. Accuracy for RBCs is 83.25% and for Eosinophils 99%. The lowest testing accuracy does not go lower than 66%. | Faster R-CNN | 96.86% |
| Wang et al | Developed a hybrid approach by combination of FPM and SO-YOLO DL architecture for WBC detection. FPM, technique is used to get high resolution WBCs blood smear images, then SO-YOLO detect WBCs in blood smear images and get 100% recall rate and 100% precision rate. | FPM and SO-YOLO DL architecture | 96.86% |
| Alzubaidi et al | This research proposed lightweight DL models that can classify the RBCs into three classes: elongated (sickle cells), circular (normal) and other blood content | Deep CNN Models | 96.86% |
| Kassani et al | A hybrid DL-based method is proposed to classify immature leukemic blasts and normal cells with 95.17% sensitivity, 98.58% specificity and overall accuracy of 96.17%. | Hybrid DL Architecture | 96.86% |

of MIA and leucocytes classification. An extensive data augmentation technique and transfer learning models are recommended to improve MIA and WBC’s detection classification in blood smear images. There are several data augmentation techniques used to extend the existing data, i.e., classical image transformations like rotating, cropping, zooming, Gaussian blur, sharpening, edge detection, histogram-based methods, and finishing at Style Transfer and Generative Adversarial Networks.

B. MEDICAL EXPERTISE AND TECHNICAL SKILL ARE REQUIRED

In the future, computer-aided MIA-based diagnostic applications can benefit from the recent advances in TML and DL models. These models are already available on multiple open-source platforms such as Tensorflow, Caffe, and Keras [131]. However, selecting and training an appropriate machine learning model for a specific MAI problem is challenging due to limited medical expertise and clinical knowledge.

C. RESOURCE CONSCIOUS DL MODELS FOR LEUKOCYTES CLASSIFICATION

In recent developments, DL, i.e., GAN’s (Generative Adversarial Networks), R-CNN, Fast R-CNN, faster R-CNN, and deep fusion of TML and DL techniques models have achieved higher performance in brain tumor detection, leukocytes classification, breast cancer detection, and other MIA tasks. However, their primary concerns are high computational cost and high memory requirements. So, computationally efficient and energy-friendly TML and DL models need to be explored for leukocytes analysis in blood smear images. Furthermore, such light weighted models can be easily implemented over resource-constrained devices.

D. END-TO-END LEUCOCYTES DETECTION AND CLASSIFICATION MODELS

Traditional learning techniques can be replaced by a deep neural network (DNN) based models. With the recent advancement of CNNs [132], end-to-end models are also gaining in popularity due to simplified model-building processes and the ability to classify leukocytes into its five categories. These models are based on data-driven learning methods and competition with complicated MIA models based on DNN. Different end-to-end architectures for leucocyte detection and classification in blood smear images, such as attention-based methods [133], [134] and CNN based model are also prominent.

E. UNIVERSAL EVALUATION FOR TML AND DL IN MIA

In MIA, the research community mainly relies on subjective evaluation techniques. However, this task is challenging, time-consuming, and can be prone to errors. Thus, further research is required to explore universal evaluation techniques that can automatically measure the performance of TML and DL models for MIA from different perspectives.

VII. DISCUSSION AND CONCLUSION

This study provided a comprehensive review of TML and DL techniques used for leucocyte classification in blood smear images. We reviewed different TML and DL approaches to classify WBCs in blood smear images. The data are collected from primary studies published during 2014 to 2020. The current study’s literature identifies 80 primary studies (articles published in journals, books, conferences, and online materials) defining TML and DL techniques for leucocytes classification in blood smear images and its applications in medical diagnosis. While reviewing the articles, we found
that both TML and DL approaches have performed equally well with overall contributions in MIA. This study is focused on identifying different applications of TML and DL in MIA and leucocytes classification in blood smear images. The objective of this study is to gain insight into complex details of TML and DL by accumulating and analyzing the knowledge provided in the literature in order to facilitate further research in the field of MIA. This study shows that much work is still needed to investigate the use of TML and DL techniques for useful MIA and leucocytes classification in blood smear images. This study also aimed at identifying applications of advanced DL models other than leucocyte classification. However, it is found that almost all other medical diagnosis applications are either directly or indirectly related to TML and DL. The accumulation of all this information in this study will benefit the research community by identifying where they need to start in further research on TML and DL models for MIA.

In future these techniques will have tremendous contributions in the development of medical imaging, natural language processing and speech analysis. Beside WBCs, TML and DL techniques are also used for the detection and classification of different MIA domains i.e., MRI, CT, X-ray, Ultrasound images analysis. In the current study, we reviewed different TML and DL techniques such as SVM ANNs, Ensembles, Bayesians, neuro-fuzzy, hybrids, DL and CNNs which are used to analyzed blood smear image [15], [72]–[78]. In MIA, blood smear images are the emerging domain that achieved great attention by the research community since last three decades. Standard contributions and applications of TML and DL in MIA are presented in this study. Furthermore, we also identified the current challenges, future directions and solutions for the advancements of TML and DL models in the field of MIA and particularly for WBCs classification in blood smear images. In future, we aim to extend our survey by considering various MIA domains such as MRI, CT, Ultrasound, X-ray images by utilizing the potentials of TML and DL techniques.

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