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REVIEW ARTICLE

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Image processing and machine learning in the morphological analysis of blood cells

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Abstract

Introduction: This review focuses on how image processing and machine learning can be useful for the morphological characterization and automatic recognition of cell images captured from peripheral blood smears.

Methods: The basics of the 3 core elements (segmentation, quantitative features, and classification) are outlined, and recent literature is discussed. Although red blood cells are a significant part of this context, this study focuses on malignant lymphoid cells and blast cells.

Results: There is no doubt that these technologies may help the cytologist to perform efficient, objective, and fast morphological analysis of blood cells. They may also help in the interpretation of some morphological features and may serve as learning and survey tools.

Conclusion: Although research is still needed, it is important to define screening strategies to exploit the potential of image-based automatic recognition systems integrated in the daily routine of laboratories along with other analysis methodologies.

KEYWORDS
automatic cell classification, blood cells, image analysis, machine learning, morphological analysis

1 | INTRODUCTION

Peripheral blood (PB), a fluid circulating through the blood vessels of the body, carries blood cells (erythrocytes, leukocytes, and thrombocytes) suspended in plasma. All of them are fundamental for immunity and life. Fortunately, PB is easily accessible and observable by optical microscopy, so that the visual inspection of the cell morphology is a relevant step in the working flow of the hematological laboratories.[1] Over the years, pathologists have been able to identify morphological qualitative features to characterize the different normal cells circulating in PB, as well as different abnormal cell types whose presence in PB are evidence of serious diseases such as leukemias and lymphomas.[2] Morphological analysis of PB smears is extensively used nowadays as a major diagnosis tool, along with other techniques such as immunophenotype and genetics.

Traditionally, PB smear analysis is based on the human inspection, which is time-consuming, requires well-trained personnel and is subject to subjectivity and intra-observer variability. This is particularly true in the case of abnormal cells, and even more when dealing with malignant lymphoid cells and blast cells (BC). Indeed, subtle interclass morphological differences exist for some lymphoma and leukemia cell types, which turns into low specificity scores in the routine screening. Within this scenario, the past 10 years have witnessed an increasing interest of researchers to develop computational image-based methods for automatic recognition of PB cells and the introduction of commercial systems integrated in the daily workload of some laboratories.[3]

In spite of this effort, the following question can be addressed: *is it possible to develop an image-based system that is able to recognize blood cell images in PB among the class of "all" malignant lymphoid cells and blast cells?* Such a question is a still open challenging problem, which if solved, could contribute significantly to the future of the computerized morphological analysis of blood cells. To clarify this question, we replace the term "all" by the following cell classes:

- Abnormal lymphoid cells (ALC) from patients with the following diagnosis:
  - i) CLL—chronic lymphocytic leukemia
  - ii) SMZL—splenic marginal zone lymphoma
  - iii) HCL—hairy cell leukemia
  - iv) MCL—mantle cell lymphoma
  - v) FL—follicular lymphoma
  - vi) PL—B and T prolymphocytic leukemia
  - vii) LGL-T—large granular lymphocyte lymphoma
  - viii) SS—Sézary syndrome
  - ix) PC—plasma cell leukemia/plasma cell myeloma
- Blast cells (BC) associated with both myeloid and lymphoid acute leukemia
- Reactive lymphocytes (RL) related to virus infections
- Normal lymphocytes (NL)

Figure 1 displays an image sample for each cell type, illustrating the variety of morphological appearances. They have been obtained with a conventional Olympus BX43 microscope and a digital camera DP73.

In addition, the microscopic screening of red blood cells (RBC) using PB slides is a common practice of detecting morphological alterations. There is also a relatively new interest in applying digital image analysis to help in this task. Literature reveals works mainly in 2 directions: RBC classification[4] and malaria identification.[5]

To concentrate the focus, this review paper deals only with lymphoma and leukemia cells. The purpose is to discuss on the methods involved in the development of automatic recognition systems, with a view on the underlying concepts, the present literature and the future perspectives, and having lymphoma and leukemia cells as a potential target. The main steps in image-based recognition systems include image segmentation, feature extraction/selection, and classification. Segmentation is the core step in processing the digital images, while features and classification lie within the area of machine learning. Thus, the review will go through these 3 steps.

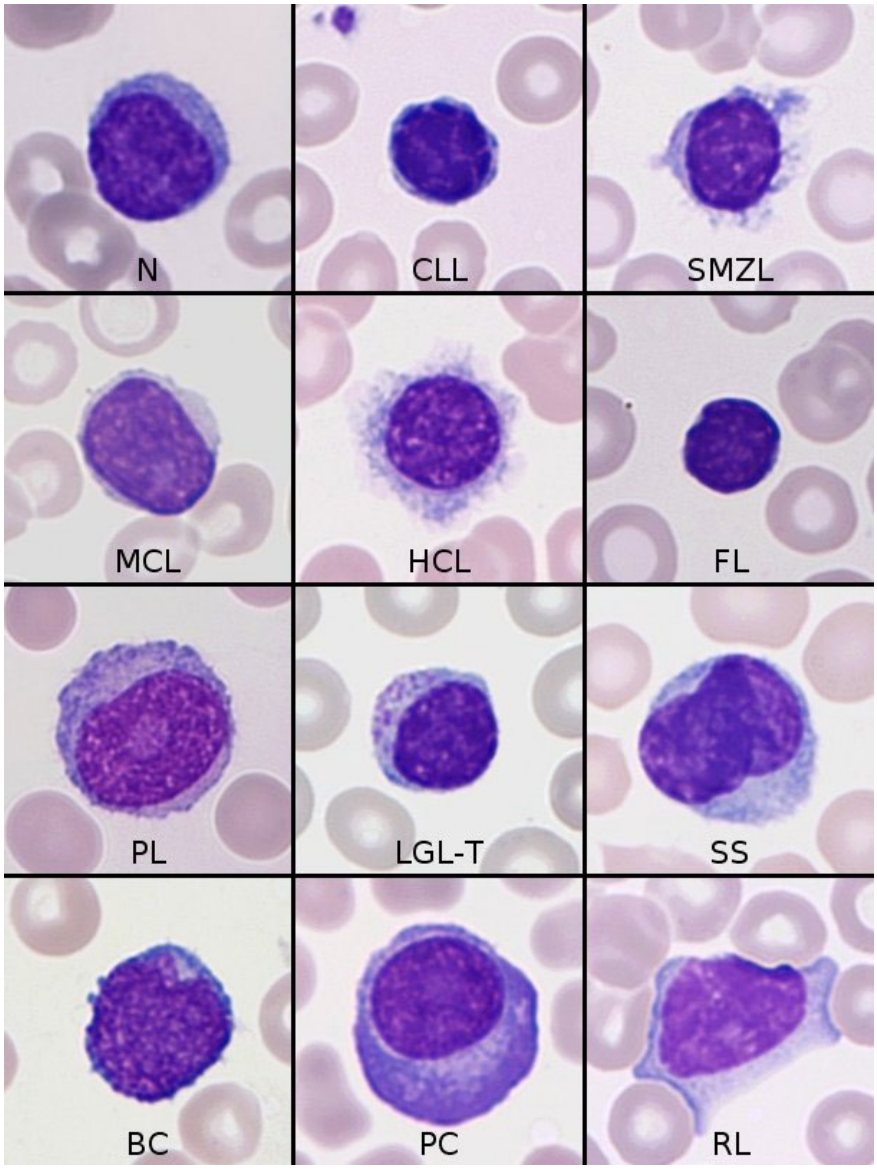

FIGURE 1 A sample image of the 12 cell groups considered as a target in this review, including normal lymphocytes, reactive lymphocytes, blast cells, and 9 types of abnormal lymphocytes

2 | SEGMENTATION

The segmentation goal is to divide an image into different parts without overlapping. These parts are named as region of interest (ROI). While the human vision system segments images on a natural basis, without special effort, the automatic segmentation is one of the most complex tasks in image processing and computer vision. Consequently, a bunch of methodologies and tools have been developed and applied in a wide variety of areas, from industry-related processes to medicine. In the case of PB cells, the segmentation aims to separate the whole cell from the background and separate also their main elements. Most works consider 2 ROIs: nucleus and cytoplasm.

To understand the essence of segmentation and the further use of the resulting ROI, we need to see a color digital image as a grid of rectangular pixels, the smallest elements of the image.[6] Color images are decomposed into a number of grayscale images according to a color model. RGB is one of the most typical models, where the components correspond to the classical decomposition of any color into red, blue, and green in appropriate proportions. In a grayscale image, there is no color but a continuous distribution between black and white. Pixels are then quantitatively described by a number, which represents the light intensity in a continuous scale between 0 (black) and a maximum (white). Segmentation techniques are generally based on exploiting 2 basic properties of the pixel intensity values: discontinuity and similarity. Discontinuity looks for abrupt intensity changes, which detects the borders of the parts to be segmented. Similarity identifies regions with pixels having similar values according to prescribed thresholds. The final result of any segmentation method is a set of binary images, commonly known as masks, one for each ROI. Each mask contains the ROI as a bounded white region over a black background.

Segmentation algorithms have been applied in previous works for normal leukocytes and BC from acute leukemia in PB and bone marrow. Authors have used methods such as automatic thresholding,[7,8] color clustering,[9,10] mathematical morphology,[11,12] and active contours.[13,14] Segmentation of abnormal lymphocytes has been scarcely considered, and it has been always limited to a few types of malignancies.[11,12,14-16] This could be due to the complex morphological variants appearing in the abnormal cases. In the case of normality, segmentation may not be so demanding because a number of distinct enough morphological characteristics exists, which may be reasonably easy to capture. However, the case of abnormality requires extracting many specific features from the different cell regions to describe the morphological differences among the variety of subgroups of lymphoid cells. Therefore, segmentation has to be more refined to identify the cell ROI with better accuracy.

In an attempt to extend segmentation to broader classes of malignancies,[17,18] our group has been developing a novel segmentation scheme. As illustrated in Figure 2, it uses the image color information by soft clustering using Gaussian mixture models of different color components (like YCbCr) and the application of the watershed transformation which allows obtaining 3 ROIs: the nucleus, the entire cell, and the peripheral zone around the cell. The segmentation of this external zone has not been addressed in previous approaches, while our work reveals that it is particularly important to extract a new feature to identify cells with hair-like projections. Most recent results[19] addressed the segmentation of the whole 12 cell groups proposed at the beginning of this study. A number of 16 408 images from 374 patients were acquired with the CellaVision DM96 in the Core Laboratory of the Hospital Clinic of Barcelona (Spain), with a resolution of 360 × 363 pixels and stained with May Grünwald-Giemsa. The overall segmentation efficiency was 98.9%, considering that segmentation of a single cell is correct when all the ROIs are well segmented as judged by pathologists. To our knowledge, no previous work has reported such results for the cell groups under the wide morphologic variability addressed in this study.

3 | FEATURE EXTRACTION

Feature extraction is the process of identifying a set of quantitative descriptors for each ROI mask. Three main classes of features are used: geometric, color, and texture. Geometric features have direct intuitive relation with the visual observations and descriptions made by pathologists. Typical descriptors quantify perimeter, area, and shape of nucleus and cytoplasm, among other morphological geometrical characteristics. These features are present in practically any system of morphological analysis.[20]

Color is also closely related to the visual cell appearance. Besides RGB, different authors use other color models to obtain quantitative features.[18,21] The overall idea is to take advantage of the complementary information supplied by alternative colors (cyan, magenta, and yellow in the CMYK model) and attributes such as hue, saturation,

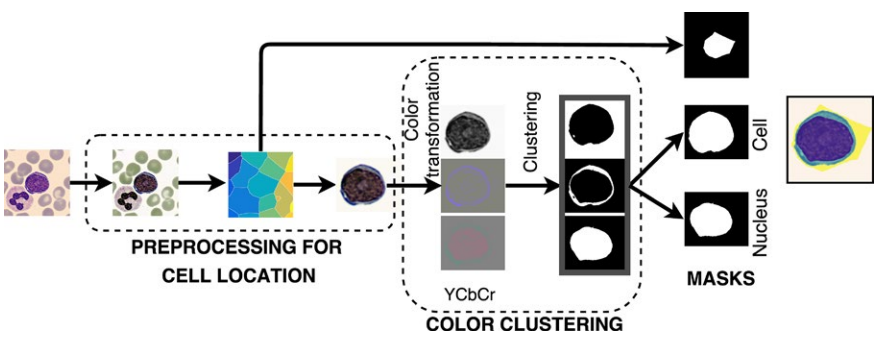

FIGURE 2 A 2 steps image segmentation process to obtain 3 ROIs from a blood cell image. The first step generates the mask of the peripheral zone around the cell. The second one isolates the nucleus and the entire cell

and brightness (HSV model) or lightness and chromaticity (in Lab and Luv models). The key tool in these features is the histogram obtained for each ROI mask for each color component. Histograms display the number (or proportion) of pixels with intensity values within non-overlapped intervals covering the whole intensity range between zero and a maximum. For each histogram, first-order statistical descriptors are obtained, such as mean, standard deviation, kurtosis, skewness, energy (uniformity), and entropy (variability).

Texture is a term that describes spatial patterns of material, color, or intensity, which can be visually observed but may not be easy to be described quantitatively. Two main approaches for texture analysis are the granulometry and the gray-level co-occurrence matrix (GLCM). Granulometry aims to measure the particle size distribution in an image by means of some operations within the context of mathematical morphology.[6] The application of morphological operations results in the so-called granulometric and pseudogranulometric curves. They represent the size distribution of dark and bright particles. In a similar way as done with the histogram for the color features, statistical measures are obtained from both curves, which form the set of granulometric features. Early applications of mathematical morphology to PB lymphocyte description[11] provided quantitative features (including chromatin density, cytoplasmic basophilia, granulation, and shape) discriminating among several groups of abnormal lymphocytes (CLL, HCL, SLZL, MCL, and FL).

The GLCM[22] has been widely used for texture quantification in medical imaging. An early application in PB cells[23] showed the ability of this matrix to obtain texture features to discriminate between 5 subtypes of normal leukocytes and CLL cells. Most recent studies using GLCM have been performed mainly for differentiating among normal leukocytes[24] and blast lymphoid cells[25] as well as in bone marrow images to distinguish erythrocyte precursor cells stages.[26] However, recent advances have been reported in the definition of texture features using both granulometric and morphological texture features able to discriminate between a wide range of ALCs, blasts, and RL.[13,15,17,21,27]

The reader may find more detailed discussions about cell morphology and quantitative features in.[28]

4 | CLASSIFICATION

4.1 | Classification essentials

At the end of the ROI segmentation and the subsequent feature extraction, each cell image is uniquely described by a set of numerical descriptors. Automatic classification is the process that aims to assign that set of descriptors to a specific class among a set of known classes defined as target. This is a conceptual and practical problem well established within the context of machine learning.[29] In blood cells, the classification objective is to automatically recognize a given cell image in its corresponding group. In the following, we will refer to the *classifier* as the system designed to perform this task. There are a good number of methods and computational techniques to design and evaluate classifiers, but most of them share a common structure and design procedure.[30]

Essentially, a classifier is a mathematical model that has certain structure (functions, equations, relations) and certain parameters, which have to be properly tuned. In the machine-learning arena, the computer, without direct human intervention, performs this tuning autonomously and iteratively. The process that leads to select the optimal model parameters is called *training*. This process requires the availability of a *training set* including a significant number of image cells belonging to the different target groups. These images have to be *labeled*, which means that the model knows the true group to which they belong. This kind of training is called *supervised*. In the cell recognition problem, pathologists have to supply the training set with images truly identified a priori. In practice, it is important that the different groups under study include a relevant number of images with reasonable balances among their relative quantities. The training is usually performed through an iterative optimization algorithm that searches for the parameters that minimize a performance criterion, which considers the error between the training classifications and the true labels.

An important part in any machine-learning system is to combine the training process with a *validation*. The idea is to evaluate the performance of the classifier using a set of new objects (images in our case), which means that they have not been used in the training. The idea is to evaluate to what extent the classifier is able to predict the true class for each image of the validation set and modify the structure of the classifier, or even change the method, until the validation is considered satisfactory. One way to proceed is, before beginning the training, to separate the set of available labeled images in 2 parts: 1 exclusively for training and the other only for validation. This may require a too big number of images. Another way, more practical and effectively used for many designers, is the so-called *cross-validation*. It consists in randomly decomposing the training set into a number of equal partitions, which means subsets that do not share images. Typically 10 or 5 is usual, as illustrated in Figure 3.

Then, 5 iterations are performed in such a way that, in the first one, the first subset is left apart and the remaining are used to train the classifier as described above. Once it is trained, the classifier is applied over the first subset to validate its performance. This training/validation is consecutively repeated until all the subsets have been used. The advantage of cross-validation is that only a training set is needed both for training and validation, while ensuring that all iterative validations are made with images not used for training. Once the cross-validation has been finished, a common practice is to apply again the classifier over the complete training set to obtain the final parameters. A typical measure to evaluate the classifier performance is the so-called confusion matrix and the ROC curves. Overall accuracy (the ratio of images correctly classified in their true category), sensitivity, and specificity are usual quantitative quality indexes to decide whether a classifier is finally ready to be implemented in an operational mode.

Among the methods most used in the classification of PB cells, we find neural networks, decision trees, and support vector machines (SVM).[29,30]

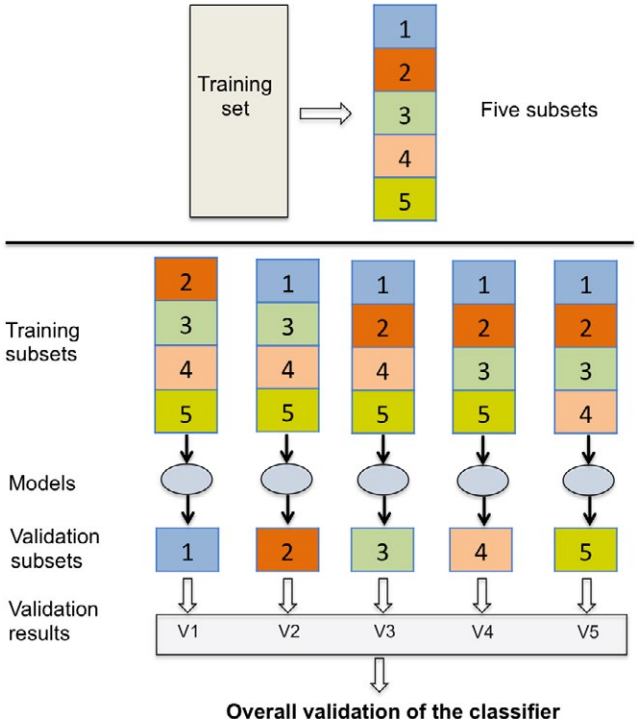

FIGURE 3 Schematic illustration of the cross-validation iterative process to train a classifier. The training set is randomly divided into 5 equal subsets. Five iterations are performed, so that each one of the subsets is separated and the other 4 subsets are used to train the classifier. Then, the classifier is applied to the separated subset to validate its performance. The final performance evaluation is a combination of the 5 validations

4.2 | Literature review for classification of blast and abnormal lymphoid cells

In the case of automatic recognition of acute leukemias, the challenging classification problem is twofold: (i) the differentiation between BC and RL, as both share some morphological similarities, such as nucleoli, basophilic cytoplasm, or diffuse chromatin; and (ii) the distinction between lymphoid or myeloid lineage because they show similar patterns. This problem has been scarcely addressed in the literature in spite that it is well known that automated PB image analyzers tend to underestimate the number of BC, mixing them up with normal or RL.

Authors in[27] have approached this specific problem with a SVM classification scheme, resulting into an optimal classifier with 60 more relevant features. The validation with a set of 220 new independent images, obtained with the DM96 analyzer, got accuracies of 85.11%, 82%, and 73.97% for RL, myeloblasts, and lymphoblasts, respectively. Some previous studies[9,31-33] have presented satisfactory results in the automatic recognition between BC and NL. But morphological differences between these 2 cells are more evident than in the cases approached in.[27] Other works have reported classification of myeloblasts and other myeloid cells at different stages using bone marrow cell images.[34] Other works[14] classified myeloid and lymphoid blast cell images as a single group in contrast to other ALCs.

Several works have focused on the classification of lymphoid neoplasms (LN), like in the early content-based image retrieval (CBIR) system for clinical pathology diagnosis support system,[35] where a classifier for 3 LN (CLL, FL, and MCL) and a fourth group of NL was trained with a 10-fold cross-validation scheme. Results were satisfactorily compared with human experts. SVM classification of 5 types of normal leukocytes and only 1 group of abnormal lymphoid cells (CLL) was reported in.[36] Although average accuracy was around 94%, CLL accuracy was 88%. Within the CBIR framework, authors in[11] developed a method to classify AL from 6 LN (CLL, HCL, SMZL, FL, MCL, and T-PL) using decision trees. Authors in[14] classified among 4 types of malignancies (CLL, MCL, FL, and acute leukemia) and NL using SVM by combining shape, area, and texture features, obtaining classification rates of 84.6%. A related work[37] presented another 10-fold cross-validation classification test with a new and independent smaller database but with the SVM trained with the old bigger database, resulting in 87.2% of accuracy due to that the new interclass similarities and intraclass variations were never seen during the training.

Our group has been working in the classification of a wide range of abnormal lymphoid cells (AL). Combining geometric, color, and texture features, a first method was presented[17] for the automatic recognition of 4 AL types (HCL, CLL, MCL, and B-PLL) together with NL, obtaining an accuracy of 85.3%. In a further work,[18] abnormal cells from FL and RL were added to the previous study. A SVM classifier was trained with 10-fold cross-validation, obtaining an overall 90.3% classification accuracy for the 7 groups. It is interesting to note that, in this work, the classifier training is performed in parallel with a process that iteratively reduces the number of initially extracted features. Selection of a reduced number of descriptors is an important issue in machine-learning algorithms to reduce complexity and computation time. Figure 4 shows a block diagram of the combined feature selection and training. In this particular case, a number of 2464 descriptors were initially extracted from the ROI and, at the end of the iterative process, 150 descriptors were selected and ranked according to their maximum relevance and minimum redundancy. Note that only 6 descriptors were geometric, while 144 described color and texture. The final classifier is optimized to operate with the selected features only.

In a recent study, this classification scheme has been combined with the image segmentation approach displayed in Figure 2 and applied for the first time to classify the 12 cell groups illustrated in Figure 1. A set of 9395 images was analyzed, obtained from PB smears of 218 patients, and captured by a conventional microscope (Olympus BX43) equipped with a camera DP73. Figure 5 summarizes the main results, showing the confusion matrix. This is a common way of evaluating the performance of an automatic classification. Rows show the true cell types and the columns the types predicted by the classifier. Values are normalized (in percentage) with respect to the total number of true numbers (last right column). The diagonal represents the proportion of true positive for each class, ranging between 81% and 96%. The overall accuracy is the mean value of these rates: 88.3%. Out of diagonal values give the rates of wrong classifications. For example, the fourth row indicates that 85% of the 938

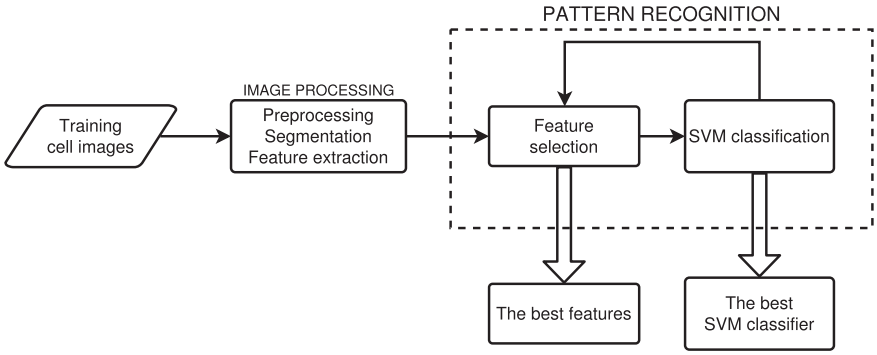

FIGURE 4 Block diagram with the steps to train the classifier. In the first block, a set of quantitative features is obtained after segmentation of the blood cell images of the training set. The second block includes a SMV classifier whose input is a reduced number of selected features, which are the most relevant and the less redundant according to a prescribed criterion. The training includes the cross-validation as shown in Figure 3

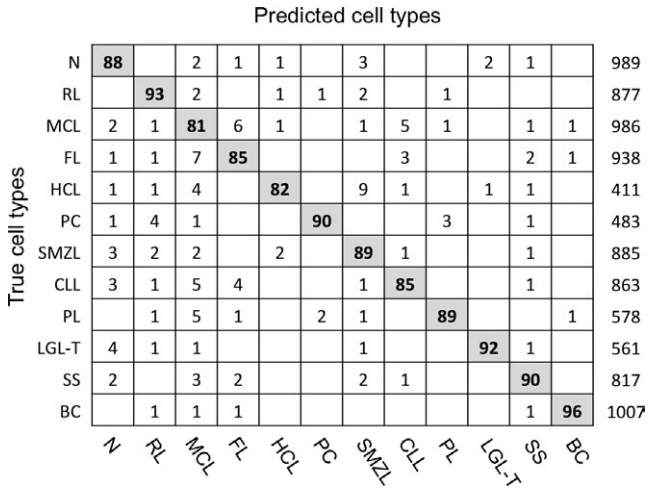

FIGURE 5 Classification results by means of the confusion matrix for a group of 12 cell image types. Rows represent the true cell type and the columns the predicted type given by the classifier. Values are given in percentages over the total number of cells in each type (outer right column). Diagonal values are the true positive rates for each class. The overall accuracy is the mean value of these rates: 88.3%. Out of diagonal values give the rates of wrong classifications

FL cells have been correctly classified, while, reading the remaining numbers in the row, we see how many true FL images have been misclassified in the other cell groups.

5 | CONCLUDING REMARKS

This paper has started with a question that could be arguable. In our opinion, seeking for a wider scope classification system is conceptually appealing. Adding more target cell classes helps to motivate more refined image processing and segmentation techniques, to propose new quantitative features for qualitative morphological characteristics, and to design more efficient classification algorithms. Indeed, these developments supply tools that may help the cytologist to perform efficient, objective, and fast morphological analysis of blood cells. They may also help to better interpret some morphological features and serve as learning and survey tools.

Research is still needed as technologies evolve faster and new methods and computational tools appear within the context of machine learning, like for instance the deep learning paradigm.[30,38] An idea to advocate would be the organization of an extensive database of cell images, with contributions of a diversity of hospitals and laboratories, covering the wide spectrum of cell classes. It would be a truly helpful instrument and common reference for researchers and developers and a good initiative to be promoted through an organized cooperation among interested partners.

From the point of view of laboratory diagnosis, automatic morphological recognition of ALCs and BC is not practically resolved by commercially available equipment. The key question is how to integrate these advances into a practical computerized image-based system. Regarding classification, the last step in the workflow, most research works end up with a classifier whose performance is validated using sets of images and measuring the accuracy over the whole set. To approach an implementation stage, classifications should be tested with a patient-based perspective,[18] in such a way that the input for the classification system should be the set of images contained in a PB smear of an individual patient. Then, the classification output would be the separation of the images within the different cell groups selected as targets. Under this perspective, it may be desirable to concentrate target cells into prescribed target groups. For instance, a reasonable classification strategy may be performed in two steps. First, all the abnormal lymphocytes may be considered as a single group with the idea of discriminating "abnormality" from normal or reactive cells. Then, a second classification may be done to discriminate the cells within the group of abnormal lymphocytes. It is important to define screening strategies to exploit the potential of a wide scope recognition methodology and design realistic and systematic proof of concept validations in laboratories.

In addition, there are some practical issues that have to be considered in a development stage, like the influence of the staining and illumination, among other aspects related to the smear origin. Robustness of the classification methodology against such variability sources should be enhanced in view of a practical implementation. As pointed out in some recent reviews,[3] integration in daily routine is the final target. Advanced image-based automatic recognition systems could be integrated as new modules with existing analyzers, or brand new systems could be built, and altogether combined with other well-established systems such as flow cytometers.

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