Expert Systems with Applications 41 (2014) 2463–2471

Contents lists available at ScienceDirect

Expert Systems with Applications

journal homepage: www.elsevier.com/locate/eswa

Indirect immunofluorescence image classification using texture descriptors

Loris Nanni [a,*], Michelangelo Paci [b,d], Sheryl Brahnam [c]

[a] DEI, University of Padua, viale Gradenigo 6, Padua, Italy
[b] ELT, Tampere University of Technology, Korkeakoulunkatu 3, Tampere 33720, Finland
[c] Computer Information Systems, Missouri State University, 901 S. National, Springfield, MO 65804, USA
[d] BioMediTech, Biokatu 10, Tampere 33520, Finland

ARTICLE INFO

Keywords:
Texture descriptors
Local binary patterns
Support vector machine
Ensemble
HEp-2 cells classification

ABSTRACT

In this work we propose an ensemble of texture descriptors for HEp-2 cell classification. Our system is based on a "pyramidal application" of local binary patterns coupled with a method for handling nonuniform bins. This feature extraction approach is then combined with a support vector machine (SVM) classifier. We test our method on a recent contest dataset (the MIVIA HEp-2 images dataset) using different testing protocols. This dataset is very challenging since the images are characterized by high variability in illumination. Therefore, to obtain good results, it is essential to apply a preprocessing algorithm: we choose the histogram equalization. We found that the best results are obtained when the original intensity images are converted into grayscale images with ten discrete values. Since a training set is provided in the contest dataset, we use it for descriptor selection and for parameter settings. The system built by using the training data is then applied to the testing set. Experiments show that our method outperforms the winner of the recent contest at the 21st International Conference on Pattern Recognition 2012.

Our descriptors and MATLAB code will be available at webpage http://www.dei.unipd.it/wdyn/?IDsezione=3314&IDgruppo_pass=124&preview=.

1. Introduction

Today immunofluorescence (IF) is a common laboratory practice used in diverse microbiological and immunological applications, such as the evaluation of cells and tissues (Soda & Iannello, 2009), the detection of specific proteins, and the identification of the presence of specific antigens (i.e., a substance that induces the production of one or more antibodies in a specimen, such as the serum from a patient's blood).

IF is based on the fluorescence detection of a specific antigen of interest that is accomplished by binding it to an antibody linked to a fluorophore. The IF labeling procedure can be either direct or indirect. In direct immunofluorescence (DIF), the fluorescent antibody recognizes the target antigen and binds to it. In indirect immunofluorescence (IIF), two antibodies are used: a primary unlabeled antibody recognizes the target antigen, and the secondary labeled antibody, usually an anti-immunoglobulin antibody, binds the primary one. Despite the increased complexity of IIF, it has several advantages over DIF: (i) it uses only one type of fluorescent antibody (anti-immunoglobulin antibodies), and (ii) it amplifies the fluorescence due to the multiple bindings of fluorescent secondary antibodies to the primary naked antibody.

IIF is extremely useful in the antibody antinucleus (ANA) test, commonly used in screening rheumatic and nonrheumatic diseases, such as drug-induced lupus, Systemic Lupus Erythematosus, and Scleroderma (Lane & Gravel, 2002). The ANA test basically consists in incubating diluted patient serum on a substrate of rodent kidney or liver cells or, more commonly, on a monolayer of human epithelial-2 (HEp-2) cells Solomon & et al., 2002. If the patient serum contains ANAs, they bind to the nuclei of the cell substrate. After washing, the secondary fluorescence antibody is added, and it binds to the ANAs if they are present. In this way the fluorescent complex can be detected. The HEp-2 cell line is derived from human larynx cancer (Solomon & et al., 2002). Due to their tumoral nature, these cells show high mitotic activity and express a large number of cellular antigens.

IIF slides are examined by a physician using a fluorescence microscope. Diagnosis requires assessment of both the fluorescence intensity and the staining of the pattern (NCCLS, 2012). If the first step can be achieved by assigning a semi-quantitative score with respect to positive and negative controls contained in each slide, the second step, performed on the positive slides, can be difficult for a human observer. This difficulty is compounded by the most widely used dilutions (1:80–1:40), Solomon & et al.,

* Corresponding author. Tel.: +39 3493511673.
E-mail addresses: loris.nanni@unipd.it, loris.nanni@unibo.it (L. Nanni), sbrahnam@missouristate.edu (S. Brahnam).

0957-4174/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.eswa.2013.09.046

2002; Soda & Iannello, 2009, which result in staining patterns that are very difficult to discriminate. Therefore, texture analysis integrated into a computer-aided diagnosis (CAD) system would be of great value for physicians (Sengur & Guo, 2011). Texture analysis would overcome many of the common weaknesses in canonical analysis: interobserver variability, the low level of standardization, the huge amount of data needing processing, and dilution dependence.

The large number of participants in the HEp-2 Cells Classification contest (HEp-2 Contest), hosted by the 21th International Conference on Pattern Recognition 2012 and organized by Prof. Gennaro Percannella (University of Salerno, Italy), Prof. Pasquale Foggia (University of Salerno, Italy) and Prof. Paolo Soda (University "Campus Bio-Medico" of Rome, Italy), confirmed the growing interest in this topic. The aim of the contest was to discover the best pattern recognition system, out of 28 candidates, that was able to classify the presegmented cells provided in the HEp-2 dataset into six classes: (i) cytoplasmatic, (ii) centromere, (iii) homogeneous, (iv) fine speckled, (v) coarse speckled, and (vi) nucleolar. Out of the 28 IIF images in the dataset (containing a total of 1457 cells), participants were provided a training set of 14 images. The winning algorithm was the one that obtained the highest accuracy in cell classification using the training set. The results of the contestant algorithms ranged from ~20% to ~69% accuracy, and only about half of them were able to recognize more than 50% of the samples (Percannella, Foggia, & Soda, 2012).

The International Conference on Pattern recognition (ICPR) 2012 HEp-2 Contest was won by the Nosaka group, which proposed a solution based on the Co-occurence among Adjacent Local Binary Patterns (CoALBP) Nosaka, Ohkawa, & Fukuim, 2012, a position-invariant operator that extracts features from the auto-correlation matrices of co-occurrence of LBPs (i.e., the spatial relations among the adjacent LBP). Some other interesting methods were those proposed by the second place winner Xiangfei et al. Percannella et al. (2012) and the third place winner Kuan, Zhi, Rui, and Wenyin (2012). The approach taken by Xiangfei was based on Varma's MR8 method (Varma & Zisserman, 2005) for extracting features from a given image. In this method, a set of textons is built from the training set using a K-means clustering approach. Each image in both the training and the testing set is represented as the frequency histogram of textons. The approach taken by Kuan et al. was to combine four texture descriptors: (i) local binary pattern (LBP), (ii) Gabor filters, (iii) discrete cosine transform, and (iv) an appearance statistical descriptor.

In the scientific literature, Cordelli & Soda (2011) recently reported an accuracy rate of 79.3% using the full HEp-2 Contest dataset of 1457 cell images. The authors evaluated many different methods, and their best accuracy rate was obtained using AdaBoost. However, the authors used a different testing protocol than the one used for the contest: they applied a 10-fold cross-validation testing protocol. In this way, certain cells in a given image were included in the training set, whereas other cells in the same image belonged to the testing set. Foggia et al. (2010) and Percannella et al. (2012) have also published results using this dataset, but they used it for a different classification task: mitotic cell detection.

In this paper we report an improved pattern recognition system for IIF image classification based on the algorithm we proposed for the ICPR12 HEp-2 Contest and that originally obtained an accuracy of ~52%. Our improved approach uses an ensemble (Das & Sengur, 2010; Das, Türkoglu, & Sengür, 2009) for handling the HEp-2 cell classification problem. Considering the fact that cells are distributed over two intensity groups, namely positive (brighter images) and intermediate (darker images), we apply histogram equalization to reduce the number of grayscale levels in the original images. For each image, two different preprocessing routines are performed that vary the number of the grayscale levels of the preprocessed image. For each preprocessed image, a set of descriptors are then extracted and used to train a set of Support Vector Machines, which are then combined by sum rule. To represent the images, we use a pyramid multiscale representation (Qian, Hua, Chen, & Ke, 2011) coupled with a multiresolution LBP. In addition, the proposed approach is combined with the method proposed in Nanni, Brahnam, and Lumini (2012) for handling nonuniform bins. Classification results are finally combined by weighted sum rule using the approach proposed in Strandmark, Johannes, and Kahl (2012) for the same problem.

We want to stress that the settings (e.g., descriptors used to represent the images and their combination) of the proposed systems are tuned to maximize accuracy on the training set only. We avoid using the testing set for the system tuning in order to report results comparable with those of the other contest competitors. Our experiments show that our best approach outperforms the winner of the HEp-2 cell classification contest hosted by the 21st ICPR.

2. Proposed system

One of the main problems handling HEp-2 cells is that the cells are classified by their intensity value as either positive (bright) or intermediate (dark). As illustrated in Fig. 1, detecting textures in the samples belonging to the intermediate class greatly increases the complexity of the recognition task.

Since fluorescence light is essentially monospectral, we initially convert the RGB images to grayscale by eliminating the hue and saturation information while retaining the luminance. We also remove the background from each image using the segmentation masks provided in the HEp-2 Contest dataset.

In order to properly manage the intensity issue, we enhance the image contrast by using histogram equalization and by transforming the image into an equalized version with N discrete levels (see Fig. 2). For each original image, we create two processed versions, the first with $N = 10$ and the second with $N = 12$.

We tested many LBP variants for our feature extraction method and found that the best results were obtained using a pyramid multiscale representation of the image. In Fig. 3 we show the pyramid multiscale representation of the cells: for each level, LBP bins are extracted using two different radii (radius = 1 and radius = 2 pixels) (see Section 3 for more details about the feature extraction method).

Although it is well-established that nonuniform patterns perform better than uniform patterns (Nanni et al., 2012), uniform patterns are preferred because nonuniform patterns produce a highly correlated feature vector of high dimensionality. In Nanni et al. (2012) we were able to reduce these problems by using a random subspace (RS) of classifiers, instead of a stand-alone method, and by using fewer bins (i.e., only 100 for nonuniform bins) for our histograms. Classifiers are then trained on these modified training sets and then combined using a decision rule.

RS modifies the training dataset by generating K new training sets ($K = 50$ in this paper) containing only a random subset of k features ($k = 50\%$ of the original features). Building the RS is a three step process:

- *Step 1*: Given a d-dimensional dataset $D = \{(x_j, t_j)|1 \leqslant j \leqslant m\}$, $x_j \in R^d$; $t_j \in C$, where m is the number of training patterns, x_j is the j-th training pattern, t_j is the label of the j-th pattern and C is the set of classes, K new k-dimensional datasets $D_i = \{(P_i(x_j), t_j)|1 \leqslant j \leqslant m\}$ $(1 \leqslant i \leqslant K)$ are created. P_i is a random projection and is obtained by randomly selecting, through the uniform probability distribution, a subset of k features from the whole pool of d features;