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Abstract *The analysis of anti-nuclear antibodies in HEp-2 cells by indirect immunofluorescence (IIF) is fundamental for the diagnosis of important immune pathologies; in particular, classifying the staining pattern of the cell is critical for the differential diagnosis of several types of diseases. Current tests based on human evaluation are time-consuming and suffer from very high variability, which impacts on the reliability of the results. As a solution to this problem, in this work we propose a technique that performs automated classification of the staining pattern. Our method combines textural feature extraction and a two-step feature selection scheme to select a limited number of image attributes that are best suited to the classification purpose and then recognizes the staining pattern by means of a Support Vector Machine module. Experiments on IIF images showed that our method is able to identify staining patterns with average accuracy of about 87%.*

1 Introduction

The screening for anti-nuclear antibodies (ANAs) by indirect immunofluorescence (IIF) is a standard method in the current diagnostic approach to systemic rheumatic diseases as well as to a number of important immune pathologies such as Multiple Sclerosis and Diabetes [1]. The test is typically done on cultured cells of the HEp-2 cell line, with the help of a fluorescence microscope: the specialist observes the IIF slide at the microscope (see Fig. 1 for an example), and makes a diagnosis based on the perceived intensity of the fluorescence signal and on the type of the staining pattern.

Specific staining patterns reveal the presence of different types of autoimmune diseases. Therefore, their correct description is fundamental for the differential diagnosis of the pathologies. Examples of six main staining patterns described by literature (homogeneous, fine speckled, coarse speckled, nucleolar, cytoplasmic or centromere) are reported in Fig. 1.

Unfortunately, the visual analysis of HEp-2 staining pattern is extremely dependent on the subjectivity of the specialist, which limits the reproducibility and reliability of the obtained results: studies report very high inter- and intra-laboratory variability (up-to 10%), that can be even higher in case of non-specialized structures [1]. Moreover, visual analysis of large volumes of image data is a tedious and time-consuming operation that requires the time and efforts of highly specialized and trained operators, translating into higher costs for the health system.

The automated classification of the staining pattern based on standardized and quantifiable features of the images, extracted with image processing techniques, may help to solve the issues of repeatability and reliability. Moreover, computer-aided systems are able to analyse large quantities of image data in a fast way, requiring null or minimal interaction from the human operators. With this growing awareness, in the last few years there was an increasing demand for automating the whole IIF process and several tools have been proposed that deal with each step of the test [2, 3, 4, 5]. Nevertheless, the accurate classification of the staining patterns still remains a challenge. Several classification schemes have been proposed: among the others, learning vector quantization (LVQ) [3], decision tree induction algorithms [4] and multi-expert systems [5]. Direct comparison of the results presented by different works is not possible, since they are obtained on different datasets. However, it is worth noting that textural features are generally acknowledged for being the most appropriate for staining pattern classification.

In this work, we present a technique that classifies the cells into one of the six staining patterns addressed by literature. After preprocessing the images, our technique extracts a number of features that describe the textural patterns of the cell; these features are based on statistical measurements of the grey-level distributions as well as on frequency-domain transformations. A two-steps feature selection procedure selects an optimal subset of features that are best suited to the classification purpose. These features are fed into a classification module based on Support Vector Machines.

2 Dataset

For this study we used the dataset provided for the participation to the "Contest on HEp-2 Cells Classification", hosted by the 21th International Conference on Pattern Recognition (ICPR2012). This dataset includes 14 HEp-2 images acquired by means of a fluorescence microscope (40-fold magnification) coupled with a 50W mercury vapor lamp and a a digital camera (SLIM system by Das srl). The camera had a CCD with square pixel of 6.45 μm. The images, stored in BMP format, have a resolution of 1388x1038 pixels and a color depth of 24 bits, respectively (see Fig. 1). The HEp-2 images contained a total of 721 cells. Each cell has been manually segmented and annotated by specialists with both the fluorescence intensity (either positive or intermediate) and the staining pattern. This information was used as ground truth to train and test our classifier. A full characterization of the dataset is reported in Table 1.

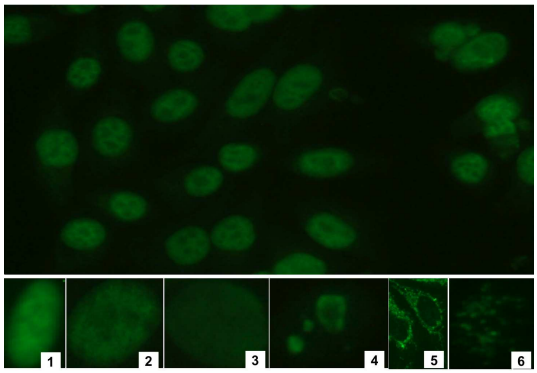

Figure 1: HEp-2 IIF image and examples of staining patterns: (1) homogeneous, (2) fine speckled, (3) coarse speckled, (4) nucleolar, (5) cytoplasmic, (6) centromere.

Table 1: HEp-2 cell dataset.

Pattern	# of samples	intermediate	positive
Homogeneous	150	47	103
Nucleolar	102	46	56
Coarse speckled	109	41	68
Fine speckled	94	48	46
Centromere	208	119	89
Cytoplasmic	58	24	34
tot.	721	325	396

3 Outline of the classification process

3.1 Preprocessing

As the staining pattern information is monochromatic, available color images were first converted to grey-scale. Then all the images underwent contrast and size normalization in order to make the texture information independent from variations of staining intensity and cell size. Contrast normalization was obtained by linearly remapping the intensity values so that 1% of data is saturated at low and high intensities. As for size normalization, all images were re-sampled to a common dimension of 64x64 pixels.

3.2 Texture Feature Extraction

Different staining patterns can be characterized by a limited set of attributes describing the spatial relationships between pixels values and the main image variations occurring in each cell type: this information is generally obtained by means of textural analysis techniques. These techniques can be grouped into two major categories: (i) statistical methods describing the distribution of grey-levels in the image; and (ii) frequency-domain measurements of image variations. In our work both the techniques were exploited in order to extract a large number of textural features able to fully characterize the staining pattern of HEp-2 cells.

GLCM features GLCM is a well established technique that extracts texture information about an image from the spatial relationship between intensity values at specified offsets. More specifically, textural features are computed from a set of grey-tone spacial dependence matrices reporting the distribution of co-occurring values between neighbouring pixels according to different angles and distances [6].

In our work, we grouped intensity values in 16 levels and then we extracted 4 GLCMs for a fixed unitarian neighbourhood distance and a varying angle $\theta = 0^o$,45^o,90^o,135^o. For each of the resulting GLCMs we computed 22 statistical measures (e.g. autocorrelation, correlation, cluster prominence, cluster shade, dissimilarity, energy, entropy, homogeneity, maximum probability, variance, etc.) whose full list and characterization can be found in [6]. We finally obtained a total number of 44 features, represented by the mean and the range value over the 4 GLCMs for each of the 22 statistical measures.

DCT features Besides statistical methods, frequency-domain transformations are largely used to extract relevant textural information for image compression and classification [7].

In our work, we computed the two-dimentional Discrete Cosine Transform (DCT) of the normalized images and then extracted 328 DCT coefficients, which represent different patterns of image variation and directional information of the texture [7]. They include the DC coefficient (top left corner of the DCT matrix), the coefficients describing the vertical and horizontal AC patterns (first row and first column of the DCT matrix) and few other coefficients describing different patterns of texture variation. Fig. 2 shows a 64x64 mask where the black dots represent the selected elements.

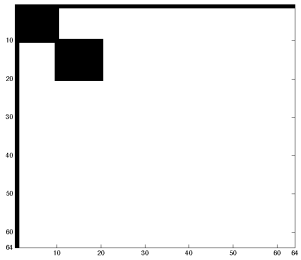

Figure 2: Mask of DCT coefficients.

Combining GLCM and DCT sets, a total of 372 features were used to characterize each sample image.

3.3 Classification

For classification we used Support Vector Machines (SVMs). This is a powerful machine learning method successfully used in many applications, for classification, regression, or other tasks [8]. The classification is based on the implicit mapping of data to a higher dimensional space via a kernel function and on the identification of the maximum-margin hyperplane that separates the given training instances in this high-dimensional space (see [8] for details). Ten-fold cross-validation technique and a grid search were used to optimize the parameters of the SVM radial basis kernel, as suggested in [8].

3.4 Feature Selection

In order to improve the accuracy of the staining pattern classifier, we applied a two-step feature selection (FS) process. The first step is based on minimum-Redundancy-Maximum-Relevance (mRMR) algorithm. This is a well established technique whose better performance over the conventional top-ranking method has been widely demonstrated [9]. The mRMR algorithm sorts, for a given datasets, the more relevant features for characterizing the classification variable by assigning a score to each element of the features vector of a sample. The scoring process aims at selecting a subset of features pointing at the contemporaneous minimization of their mutual similarity and maximization of their correlation with the classification variable.

As mRMR requires categorical and not continuous features variables, we applied features discretization to the input data. In particular, we used CAIM (class-attribute interdependence maximization) algorithm [10], which is best suited to work with supervised data, as it maximises the class-attribute interdependence generating a minimal number of discrete intervals.

However, mRMR algorithm provides only a candidate feature set, which is not necessarily optimal [9]. Therefore, in order to find a compact features set, we applied as second FS step a Sequential Forward Selection (SFS) scheme. In this approach, the subset of optimal features is constructed iteratively. Starting from an initial empty set, at each iteration the feature providing the greatest classification accuracy improvement is added, until no more improvements are obtained.

In our work, the size of the candidate features set selected by mRMR was arbitrarily chosen as 50. The final dimension of the optimal feature set after SFS was found to be 12.

4 Results

The classification results obtained in our experiments have been summarized in Table 2, and organized by staining pattern class. For each of them, we show the accuracy obtained with: (i) the initial 372 elements feature set, (ii) elements candidate set selected by mRMR, (iii) elements candidate set selected by SFS and (iv) the final 12 elements feature vector obtained with combination of mRMR + SFS. In the last table row, the total accuracies in the four cases are shown.

Table 2: Classification results: accuracy rate.

Fluorescence Pattern	no F.S.	mRMR	SFS	mRMR + SFS
Homogeneous	78.66%	84.00%	83.33%	86.00%
Nucleolar	89.22%	93.14%	93.14%	93.14%
Coarse speckled	92.66%	95.41%	94.49%	98.17%
Fine speckled	45.75%	61.70%	69.15%	71.28%
Centromere	84.13%	88.46%	91.35%	87.02%
Cytoplasmic	58.62%	86.21%	81.03%	82.76%
tot.	77.95%	85.58%	86.69%	86.96%

Two main considerations stem from this table:

(i) the average accuracy obtained by the proposed technique in classifying the six different fluorescence patterns was 86.96%, with a maximum per-class accuracy of 98.17% for cells with coarse speckled pattern and a minimum of 71.28% for fine speckled cells. This last result could be expected, since fine speckled texture was extremely irregular.

(ii) as expected, FS significantly improves (+9.01%) the average accuracy of the classifier, proving the implicit feature selection ability claimed by SVM to be very weak. The first step of feature selection based on mRMR improves the per-class accuracy of all the patterns. The application of SFS after mRMR improves the average accuracy but slightly decreases the classification accuracy of two staining patterns (centromere and cytoplasmic). Conversely, the fine speckle pattern, the one with the lowest per-class accuracy, had the best improvement thanks to SFS (+9.58%). A non-uniform behaviour of different staining patterns can be expected, as SFS aims at optimizing the average classification accuracy and not the accuracy of the single classes. The combination mRMR+SFS obtains generally better results than SFS alone for all the classes except centromere (although average classification accuracy of the two strategies is comparable).

Our results suggest that the proposed algorithm is a good solution for the automated classification of immunofluorescence cell patterns. As a matter of facts, the accuracy rate is comparable to the one obtained by the specialists, whose inter-laboratory variability is generally assessed around 10% or even higher [1]. Besides that, differently from human operators our technique provides fully-repeatable results that are based on objective and quantitative features of the images.

5 Conclusion

In this paper we proposed an approach for the automatic classification of staining patterns in HEp-2 cell IIF images, which is critical for the diagnosis of immune diseases. First, texture descriptors based on GLCM and DCT coefficients are exploited to extract a 372-size characteristic vector for each image. Then, a two-step feature selection algorithm, first selecting a reduced candidate feature set with mRMR and then extracting an optimal subset of them with SFS, has been applied to improve the classification accuracies obtained with SVM.

The approach provides an average classification accuracy of about 87%, therefore our results are comparable with those of human specialists. Conversely, they are completely repeatable since our automated technique does not depend on the subjectivity of the operator.

Future work will focus on the improvement of the classifier's performance in discriminating irregular staining patterns, with special regards to the fine speckled class. We believe that a classification scheme taking into better account the inter-class variabilities (e.g. Subclass Discriminant Analysis [11]), will serve to this purpose. Moreover, we plan to combine our pattern classification algorithm with automatic cells segmentation, and apply our method to computer-aided diagnosis (CAD) of autoimmune diseases.

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