Unsupervised HEp-2 mitosis recognition in Indirect Immunofluorescence Imaging

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Simone Tonti, Santa Di Cataldo[1], Enrico Macii and Elisa Ficarra

***Abstract*— Automated HEp-2 mitotic cell recognition in IIF images is an important and yet scarcely explored step in the computer-aided diagnosis of autoimmune disorders. Such step is necessary to assess the goodness of the HEp-2 samples and helps the early diagnosis of the most difficult or ambiguous cases. In this work, we propose a completely unsupervised approach for HEp-2 mitotic cell recognition that overcomes the problem of mitotic/non-mitotic class imbalance due to the limited number of mitotic cells. Our technique automatically selects a limited set of candidate cells from the HEp-2 slide and then applies a clustering algorithm to identify the mitotic ones based on their texture. Finally, a second stage of clustering discriminates between positive and negative mitoses. Experiments on public IIF images demonstrate the performance of our technique compared to previous approaches.**

I. INTRODUCTION AND PREVIOUS WORKS

The antinuclear autoantibodies (ANA) test is a blood exam based on indirect immunofluorescence (IIF) that is able to diagnose a large number of autoimmune disorders using a substrate of HEp-2 cells as markers. The HEp-2 cells have antigens that selectively react with autoantibodies in the patient serum that are held responsible for the diseases, creating a bond that can be observed through a fluorescence microscope. Depending on the quantity and type of the autoantibody, the HEp-2 cells will be characterized by a certain level of fluorescent intensity and by a specific pattern of fluorescence (see examples in Fig. 1 (1,2,3,4)). Hence, these two properties of the HEp-2 images allow a differential diagnosis of the disease.

Besides intensity and pattern, the specialist needs to report on the presence and type of the mitotic cells (i.e. cells undergoing cellular division). The aim is two-fold. (i) The presence of at least one mitotic cell guarantees that the well has been correctly prepared. Hence, slides without mitoses should be discarded. (ii) In the presence of mixed fluorescent patterns, the specific type of the mitoses in the slide, either *positive* (Fig. 1-a,b) or *negative* (Fig. 1-c), helps identifying the autoantibody.

In the last few years, the automatization of the ANA test image analysis has gained more and more attention from the research community, with the aim of improving the repeatability and objectivity of the diagnosis, as well as of limiting the need for specialized personnel to observe the images and, ultimately, of reducing the costs of the test.

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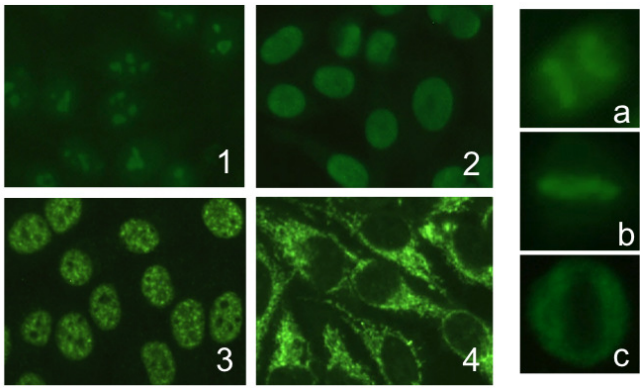

Fig. 1. (1,2,3,4) HEp-2 images with different fluorescent patterns. (a,b) Positive mitoses. (c) Negative mitosis.

A complete decision support system for ANA testing includes the following steps: (i) cell segmentation, to automatically crop the individual HEp-2 cells of the input image; (ii) mitotic cell recognition, to distinguish the cells into mitotic (either positive or negative) and non-mitotic, a.k.a. *interphase*; (iii) intensity classification, to identify the fluorescent intensity level of the image; (iv) pattern recognition, to identify the specific fluorescent pattern of the interphase cells.

The automated analysis of HEp-2 images is a relatively new research area. Even though mitotic cell recognition is a crucial phase of the procedure, so far most of the efforts have been directed towards the implementation of techniques for the classification of intensity [1] and pattern [2], [3] or for HEp-2 cell segmentation [4], [5]. On the other hand, most of the literature on mitosis recognition addresses very different imaging applications, such as time-lapse microscopy [6] or histopathology [7]. Hence, the proposed approaches cannot be easily adapted to the context of IIF-ANA testing.

To the best of our knowledge, there are only three publications on HEp-2 mitotic cell recognition in IIF imaging [8], [9], [10], where [10] extends and improves [8], [9]. Starting from the idea of using supervised learning classification to discriminate between mitotic and interphase cells, the authors in [10] identify the extreme imbalance of these two classes as the main issue to be tackled. Indeed, the interphase cells are always in large majority in a HEp-2 slide. This translates into a major class imbalance of the training sets, which leads most learning algorithms to failure. Hence, the authors propose a hybrid ensemble approach with multi-objective optimization to take account of this problem.

Modified forms of supervised classification, such as the one presented in [10], are indeed appropriate to handle extremely imbalanced sets. On the other hand, applying supervised learning to the specific context of mitotic HEp-2 cell recognition has two major issues: (i) the need of collecting and annotating a significant number of representative images to train a classifier; (ii) the necessity of re-designing the learning stage of the classifier at every variation of the imaging conditions (for example, in case of interphase cells with fluorescent patterns not represented in the original training set). These issues make the use of supervised mitotic recognition techniques utterly inconvenient in a standard clinical practice.

In this paper, we approach the problem of mitotic cell recognition in a completely different way. Similarly as previous works, we recognize mitotic cells through the analysis of their texture. Nevertheless, we carry out this analysis on a per-image basis, applying an unsupervised technique that does not need to be trained with labeled data. This approach has two main advantages. First, it is less severely affected by class imbalance. Second, it does not leverage on the characteristics of a fixed set of representative images. Thus, it is more robust to unexpected variations of such characteristics, which is a typical issue of biological systems.

The performance of our algorithm is demonstrated on public datasets of IIF images (including the one used by [10]), and experimentally compared with the previous approaches.

II. PROPOSED METHOD

In this work, we focus solely on mitotic cell recognition, which as discussed in Section I is one of the least addressed steps of a decision support system for ANA testing. Hence, consistently with previous works on this topic, we assume of receiving the cropped images of the individual HEp-2 cells directly as input of our algorithm.

The main steps of our proposed technique are outlined in Fig. 2. (i) The first stage, *selection of candidate cells*, takes the cells of a HEp-2 slide as input and provides a set of candidate cells as output. Ideally, such set includes all the mitotic cells of the HEp-2 slide and only a limited number of the interphase cells. (ii) The *mitosis detection* step takes the candidate cells as input and identifies the mitotic cells among them. (iii) Finally, the mitotic cells are categorized into *positive* and *negative* by the *mitosis categorization* step.

A. Selection of candidate cells.

As can be observed from Fig. 1, mitotic cells are distinguishable from other cells in the image by their distinctive fluorescent pattern. Depending on the specific agglomeration of the chromosomic mass during cell division, this pattern may assume one among the forms shown in Fig. 1, where (a,b) are two phases of positive mitosis and (c) is a negative one. More specifically, a IIF image will contain either positive or negative mitoses, which are mutually exclusive.

The *selection of candidate cells* is a preparatory step to mitosis detection that automatically filters the input cells, based on their resemblance to a set of a-priori models of

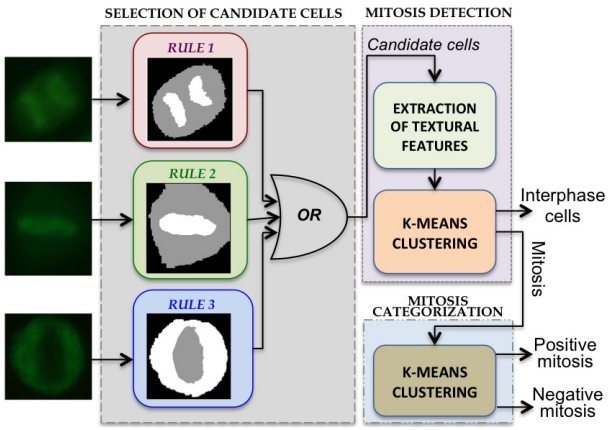

Fig. 2. Outline of the proposed technique.

mitosis. The aim is two-fold: (i) to exclude from the mitosis detection process the cells that are most surely non-mitotic; (ii) to decrease the number of interphase cells undergoing classification, thus softening class imbalance.

The selection of candidate cells is implemented as a set of rules, each modeling the morphological characteristics of a specific mitotic phase. For example, with reference to the scheme of Fig. 2, a HEp-2 cell will be included in the candidate set if it fulfills any of the following:

Rule 1: dark body with two bright internal regions
Rule 2: dark body with one bright internal region
Rule 3: bright body with one dark internal region

where dark and bright regions (respectively, grey-colored and white-colored areas in Fig. 2) are distinguished by applying Otsu thresholding algorithm locally to the intensity histogram of each individual cell.

The end result of this step will be a candidate set possibly containing all the mitotic cells of the input image and a fraction of the interphase cells. Even though the interphase cells are still prevailing at this stage of the algorithm, class imbalance is considerably reduced, which facilitates the following *mitosis detection* step.

B. Mitosis detection.

The fluorescent pattern of mitotic and interphase cells can be conveniently described by means of textural analysis. In our technique, the textural characteristics of the candidate cells are quantified by means of a well-established method based on the gray-level co-occurrence matrix (GLCM [11]), which was successfully applied to other tasks of IIF analysis, such as the automated classification of HEp-2 fluorescent patterns [12], [3]. GLCMs are grey-tone spacial dependence matrices that characterize texture by calculating how often pairs of pixels with specific values and in a specified spatial relationship (represented by a neighborhood distance and angle) occur in the image. In our experiments, this type of features demonstrated the best discriminative performance compared to other models of textural analysis such as GABOR, SURF and several formulations of Local Binary Patterns (details in Section III).

In our technique, each candidate cell (as obtained in Section II-A) is assigned a 12-element feature vector containing second-order statistical measures derived from the GLCMs[1].

The feature vectors are fed into a k-means clustering algorithm with $k = 2$, which partitions them into two separate clusters so as to minimize the within-cluster sum of squared Euclidean distances. In order to avoid local minima, the clustering algorithm is replicated ten times with random starting points. Envisioning the case of HEp-2 images with no mitotic cells, the algorithm also supports convergence to only one cluster (i.e. with no elements belonging to the second cluster).

After convergence, the majority and the minority (or empty) cluster are assigned, respectively, to the class of the *interphase cells* and to the class of the *mitotic cells*.

C. Mitosis categorization.

The final step is aimed at the categorization of mitoses into *positive* and *negative*. Positive mitoses are characterized by a weakly fluorescent body and a bright chromosomic mass, negative mitoses by the opposite situation.

Again, these two categories can be distinguished by means of textural analysis. In our technique, the textural feature vectors of the *mitotic cells* (as obtained in Section II-B) undergo a second round of k-means clustering, which partitions them into two sub-clusters. The sub-cluster containing the highest prevalence of cells fulfilling *Rule 1* or *Rule 2* is labelled as *positive mitosis*, while the sub-cluster containing the highest prevalence of cells fulfilling *Rule 3* is labelled as *negative mitosis*

III. RESULTS AND DISCUSSION

To assess the performance of our technique, we run experiments on two publicly available datasets of IIF images.

The first dataset (referred to as *dataset 1*) is commonly used as a benchmark for the validation of IIF image analysis techniques, including the ones for HEp-2 mitosis recognition. It consists of 28 HEp-2 images of six different fluorescent patterns (homogeneous, fine speckled, coarse speckled, nucleolar, centromere and cytoplasmic) and two different intensity levels (intermediate and positive). The dataset provides cell segmentations performed manually by a specialist as well as ground truth about mitotic (including positive/negative categorization) or interphase label of each cell. The cells are 1527 in total, 70 of which are mitotic. 25 images out of the 28 include interphase cells and also few mitotic cells (from a minimum of 1 up to a maximum of 7 per image). Remaining 3 images contain only interphase cells. For a complete characterization of the dataset, the interested reader can refer to [3], [10].

If we interpret mitotic and interphase as, respectively, the positive and negative class of a classical binary classification problem, mitosis recognition accuracy can be quantified as:

$$acc = \frac{TP + TN}{FP + TN + TP + FN} \tag{1}$$

[1]12-element feature vector is obtained computing contrast, correlation, energy and homogeneity on three GLCMs extracted with unitarian neighborhood distance and varying angle $\{0°, 45°, 90°\}$.

where TP (true positives) and TN (true negatives) are, respectively the number of mitotic cells and of interphase cells correctly identified, while FP (false positives) and FN (false negatives) are the number of interphase and of mitotic cells incorrectly assigned to the opposite class.

In presence of strong imbalance of the positive and negative class (as in our specific case, where less than 5% of the cells are mitotic) the classical formulation of acc is not meaningful, because it tends to account only for the classification accuracy of the majority class [10]. Hence, the *geometric means of accuracies* can provide a more effective measure of the system performance. This metrics is calculated as:

$$gacc = \sqrt{acc^+ \cdot acc^-} \tag{2}$$

where acc^+ and acc^- are the recognition accuracy over, respectively, the positive and the negative class. $gacc$ has the advantage of equally penalizing poor results in any of the two classes.

Indeed, the performance evaluation is much more meaningful when acc and $gacc$ are considered jointly. As demonstrated in [10], the ideal performance case on imbalanced datasets occurs when the values of acc and $gacc$ are high and well-balanced, which is well characterized by the following measure, defined in the $[0, 1]$ range:

$$bm = \frac{\sqrt{(1 - gacc)^2 + (1 - acc)^2}}{2} \tag{3}$$

The lower bm, the closer to the ideal the system performs.

The left section of Table I reports results obtained by our proposed technique on *dataset 1*, in terms of acc, $gacc$ and bm. Besides GLCM, already discussed in Section II-B, we tested different types of textural features, including the ones that are generally used for HEp-2 pattern classification (see second column of Table I). For a characterization of these descriptors, the interested reader can refer to [3].

TABLE I
MITOTIC RECOGNITION ACCURACY (CELL-WISE RESULTS).

		DATASET 1			DATASET 2		
	Features	**acc**	**gacc**	**bm**	**acc**	**gacc**	**bm**
Unsupervised	GLCM	0.842	0.724	0.225	0.925	0.756	0.180
	GABOR	0.788	0.548	0.353	0.904	0.582	0.303
	SURF	0.837	0.634	0.283	0.930	0.665	0.242
	LBP	0.796	0.618	0.306	0.904	0.655	0.253
	CoALBP	0.841	0.521	0.357	0.928	0.543	0.327
	RICLBP	0.791	0.579	0.333	0.904	0.615	0.281
Supervised [10]		0.851	0.610	0.295	n.a.		

In our experiments GLCM consistently obtained the best results (i.e. highest acc and $gacc$, lowest bm) among all the other tested features (see grey-colored row of Table I).

In order to assess the performance of our approach, in the last row of Table I we show for comparison the results obtained in the same dataset by the supervised technique proposed in [10]. For the latter approach, we report only the most performant configuration presented by the authors,

based on AdaBoost ensemble classifier with all parameters optimized on the validation set. Our unsupervised approach with GLCM-based features obtained comparable value of acc (0.842 against 0.851 of the supervised method) and better $gacc$ and bm values (respectively, 0.724 against 0.61 and 0.225 against 0.295).

As a final experiment on *dataset 1*, we measured the performance of our technique in the categorization of positive and negative mitoses (see *mitosis categorization* step in Section II-C). Since positive and negative mitoses have the same a priori probability, in this case we can apply classical evaluation measures for balanced binary classification problems. Hence, we computed accuracy (as in Equation 1), as well as precision and recall of the classification, as follows:

$$precision = \frac{TP}{TP + FP},\ recall = \frac{TP}{TP + FN} \quad (4)$$

where this time positive and negative mitoses were interpreted, respectively, as the positive and the negative class. The obtained values were 83% for recall, 91% for precision and 85% for accuracy, which demonstrates the good performance of our technique on a *cell-wise* classification basis.

As anticipated in Section I, one of the major applications of HEp-2 mitotic cell recognition is the identification of potentially faulted HEp-2 substrates, based on the absence of mitotic cells in the IIF image. Hence, in order to thoroughly assess the usability of our technique in the context of the ANA test, we need to measure its capability of discriminating not only between mitotic and non-mitotic cells, but also between images with and without mitotic cells. This type of evaluation (here referred to as *image-wise*) was not performed by previous literature.

In *dataset 1* images not containing any mitoses are only 3 out of 28, which makes the *image-wise* evaluation unfeasible. For this purpose, we integrated *dataset 1* with 20 additional images without mitoses, that were recently made available by the organizers of a contest at the conference ICPR [13]. The additional images have four different fluorescent patterns (homogeneous, speckled, nucleolar, centromere) and two intensity levels (intermediate and positive) and contain a total number of 2605 cells, all interphase. Hence, the resulting dataset (referred to as *dataset 2*) contains an almost equal number of images with and without mitoses (respectively, 25 and 23), which perfectly suits our validation purposes.

On this dataset, we evaluated the *image-wise* performance of our technique. This time, we interpreted positive and negative class as, respectively, images with at least one mitosis and images with no mitoses (the definitions of TP, TN, FP and FN were re-interpreted accordingly). Based on these assumptions, we measured our technique's performance on *dataset 2*, obtaining a 69% recall, a 100% precision, and an overall accuracy of 77%.

Cell-wise performance was also calculated on *dataset 2*, and was consistent with the results obtained for *dataset 1* (see right section of Table I).

IV. CONCLUSIONS AND FUTURE WORK

In this paper we tackled the problem of HEp-2 mitotic cell recognition in IIF images. Our technique automatically selects a limited set of candidate cells from the HEp-2 image and then recognizes mitotic ones by means of a clustering of textural features based on GLCM. Finally, the mitotic cells are categorized into positive or negative again based on the textural characteristics of different mitotic models.

Differently from previous works, we propose an unsupervised learning approach, which is less affected by class imbalance problem and intrinsically more robust to variations of the image characteristics, and hence more suitable to the demands of clinical practice. Experiments on public datasets of IIF images demonstrate the good performance of our technique compared to previous approaches.

As a future work, we plan to integrate our automated technique into a complete pipeline for the computer-aided analysis of IIF images, including automated techniques for cell segmentation [5] and for fluorescent pattern classification [3], and to test our tool in a real clinical setting.

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