COMBINATION OF MULTIPLE PIXEL CLASSIFIERS FOR MICROSCOPIC IMAGE SEGMENTATION

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Abstract: The combination of classifiers has been proposed as a method allowing to improve the quality of recognition systems as compared to a single classifier. This paper describes a segmentation scheme based on a combination of pixel classifications. The aim of this paper is to show the influence of the neighborhood information and of the number of classifiers used in several combination processes. In the first part, we detail the ground of our study for a microscopic application. Then, we name the different steps of the new segmentation scheme. In the third and fourth part, we detail the different rules that can be used to combine classifiers and the classifications results obtained on colour microscopic images. Finally, we draw a conclusion on the improvement of the quality of the segmentation at the end of treatment.
Keywords: classifier combination, segmentation, pixel classification, colour, microscopy.

1 Introduction

Image analysis in the field of lung cancer is a diagnosis tool for cytopathology. The quantitative analysis of colour and texture of nuclei coming from microscopic colour images brings to the pathologist valuable information for diagnosis assistance. This analysis can only be performed from perfectly segmented objects. The segmentation of the bronchial cells is a difficult task because the mucus present in the background has the same aspect as some cells (cytoplasm, nucleus) in the setting of the international coloration of Papanicolaou. Our last works [1, 2] showed that an unsupervised or supervised pixel classification brings satisfactory results but that a combination of pixel classifications might improve our segmentation. Several studies [3, 4, 5, 6, 7] shown that this technique has became more and more used to improve the quality of recognition systems in several applications and notably in medical systems [8]. The difficulty to affirm the superiority of a classifier in relation to another brings us to couple several classifiers simultaneously. It enables to use their complementarity and to increase the quality of recognition of our segmentation system.To this aim, we propose an automatic segmentation scheme based on combination of pixel classifications. It is given in six steps: a simplification step to reduce the noise, pixel classifications to obtain three classes (background, cytoplasm and nucleus) in all images, a combination of pixel classifications, a marker extraction by using an operation of mathematical morphology and a colour watershed growing to correctly segment the objects. The paper is organized as follows: in section 2, we describe the colour segmentation scheme. In section 3, we detail the combination of pixel classifications step. In section 4, we give experimental results on the combination of pixel classifications schemes with an evaluation method adapted to microscopic images. Finally we draw a conclusion on the quality of the segmentation.

2 The segmentation scheme

The segmentation scheme is given in six steps [1, 2]:

❶ Image simplification: the simplification step consists in a pre-treatment phase with the aim of smoothing the initial image to reduce the importance of noise. The produced image is used to compute the gradient needed in the colour watershed step. The growing quality depends greatly on the gradient image. This smoothed image is also used as input to the pixel classification step in order to reduce the classifier sensitivity to the presence of noise (see in [9] for more details).

❷ Pixel classification: the classification step consists in determining for each pixel of the image, a class among background, cytoplasm or nucleus. To realize this classification, we have used several unsupervised classifiers using a Hierarchical Ascendant Classification (based on K-means or Fisher) [1] and supervised classifiers (Bayes, kNN, SVM, MLP) using a learning data base that was built from four images segmented by an expert in cytopathology [2].

❸ Combination of pixel classifications: this step permits to increase the recognition rate of objects. To this aim, we use the complementarity which can exists between several classifiers. We combine by different methods the pixel classifications produced in the previous step. In this paper, we give a detailed description of this step by presenting several combination schemes.

❹ Marker extraction: with the image produced in the previous step, a pixel subset is recognized as belonging to the cytoplasm or the nucleus, this subset corresponds to true markers. The marker extraction is based on mathematical morphology operations which consists in a variable number of erosions on the level of the boundaries according to the marker type.

❺ Colour watershed: from the markers previously extracted and the smoothed image, a watershed performs a growing using image colour information. The obtained regions correspond to the cytoplasms and nuclei [10].

❻ Evaluation: our evaluation method is based on an improved classification rate and is adapted to our study. The proposed method uses a reference manual segmentation provided by an expert and provides a recognition quality index of the cytoplasm ($IdCytoplasm$) and of the nucleus ($IdNucleus$) [2].

3 Combination of pixel classifications

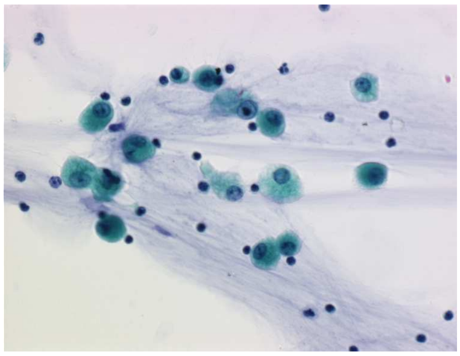
3.1 Definition of classifier

A classifier usually designates a recognition tool that provides class memberships information for a vector received in input. This tool can be described by a function e that with a feature vector x of the object to recognize, assign to x the class C_i among k possible ones:

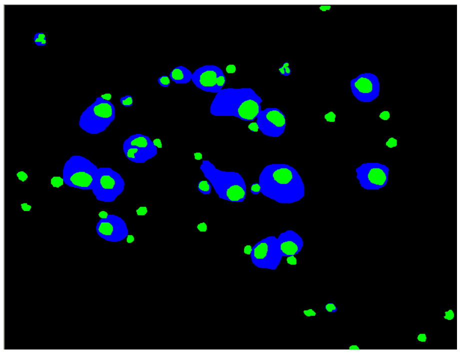
$$e : x \in R^n \rightarrow K \qquad \text{with } K \in \{C_1, ..., C_k\} \tag{1}$$

Moreover, the answers provided by the classifier can be classified in three categories [5]:

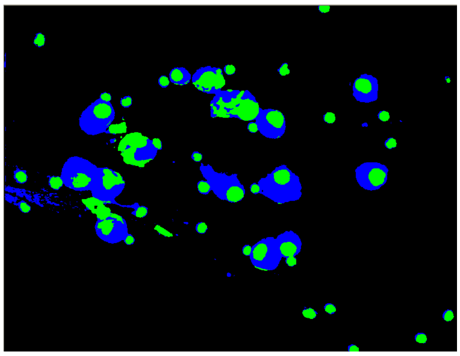
- Class type: $e(x) = C_i(i \in [\![1, k]\!])$, indicates that the classifier assigned the class C_i to x,
- Rank type: $e(x) = [\![r_1^j, ..., r_k^j]\!]$ where r_i^j is the assigned rank to the class i by the classifier,
- Measure type: $e(x) = [m_1^j, ..., m_k^j]$ where m_i^j is the measure assigned to the class i by the classifier.



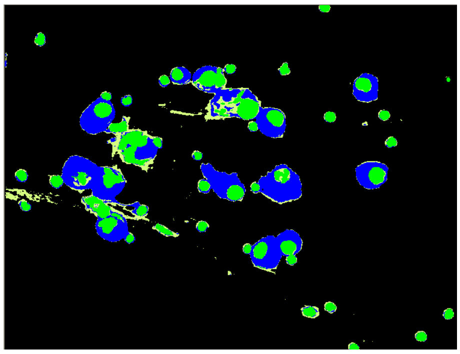
(a) Initial image.



(b) Manually segmented image.



(c) Classified image by SVM algorithm.



(d) incoherence zones between the different classifiers (yellow).

Figure 1: Pixel classification results.

3.2 Importance of the combination step

Since it is difficult to claim the superiority of classifiers one to another, a combination of classifier decisions seems necessary. The classifiers having not the same opinion of the class to be allotted to the same pixel, we were brought to carry out a combination of pixel classifications. The answer provided directly by the pixel classification is of class type. But this type of output being the one that brings the less information, we coupled it to a confidence index to perform the combination of pixel classifications. Fig. 1(a) presents an initial image to segment, Fig. 1(b) presents the ground truth segmentation and Fig. 1(c) gives the pixel classification result obtained by the SVM algorithm. Fig. 1(d) shows the superimposition of all the pixel classifications from several classifiers. On this figure, the background is presented in black, the cytoplasm in blue, the nucleus in green, and incoherence zones in yellow. These incoherence zones show the pixels where all the classifiers do not provide identical opinions on the class to be allotted to a same pixel.

3.3 Confidence index

A testing data base was built from four images containing objects with a wide variability and each image has been manually segmented (Fig. 1(b)) by an expert in cytopathology[1]. We evaluate every classifier on this testing data base (the testing data base is different of the learning data base). We obtain for every classifier, like describes it the following relation, a confidence index $index_j^i$. This confidence index represents the classification quality of the classifier j to the class i (with $i \in [\![1, k]\!]$). For a classifier $j \in [\![1, n]\!]$, we define:

$$index_j = \begin{pmatrix} index_j^0 \\ ... \\ index_j^k \end{pmatrix} \tag{2}$$

The confidence index is evaluated by a novel pixel classification quality index adapted to microscopic images (see in [2] for more details).

3.4 Combination schemes

A lot of different combination methods can be found in the literature [7, 3, 6, 5]. In this section, we propose to compare several of them to perform the combination of pixel classifications.

[1]The authors would like to thank Mr Michel Lecluse and the pathological anatomy and cytology department of the Louis Pasteur Hospital Center of Cherbourg for providing the ground truth reference images.

3.4.1 Untrained combination

Classical combination schemes usually combine several decisions coming from several classifiers, each classifier providing class memberships. In the case of pixel classification, this is directly applicable and one can combine the different outputs of the classifiers one to another. However, dealing with images, the spatial information involved in the pixel connectivity is not taken into account while combining several classifications for one pixel. It is therefore interesting to use not only one single value to describe the output of a classification method but several ones corresponding to all the classifications obtained for pixels neighbors to the central one considered. For a neighborhood of size i, the size of the feature vector associated to one classifier is of $(8i + 1)$ (with $i = 0$ one recovers the simplest case of only one classification per pixel). The combination methods [7] that we use are methods without training which can be described as follows:

If E the set of n classifiers used, we have $E = \{e_1, ..., e_n\}$. Every classifier associates a class C_i to an input vector x. We can thus define $E_{C_i}(x)$ as the set of classifiers which all associate to an input vector x the class C_i:

$$E_{C_i}(x) = \{e_j \in E | e_j(x) = C_i\} \tag{3}$$

We have clearly $\cup \{E_{C_i}(x)\} = E$ since a classifier takes only one decision of class type. With every $E_{C_i}(x)$ set with $i \in [\![1, k]\!]$, one can associate the set of confidence indexes for every classifier $e_j \in E_{C_i}(x)$. Each index corresponds to the confidence given to the classification carried out by the e_j classifier when it associates to x the class C_i. Let $I_{C_i}(x)$ denotes the set of these indexes:

$$I_{C_i}(x) = \{index_j^{C_i} | e_j \in E_{C_i}(x)\} \tag{4}$$

The set $I_{C_i}(x)$ corresponds to the respective confidence indexes of the classifiers who classify the input x as being of class C_i. From these sets, we can compute the membership probability of x to the class C_i by the following relation.

$$P(C_i|x) = g(I_{C_i}(x)) \tag{5}$$

where g is a combination rule among the followings: majority vote (MV), minimum (MIN), maximum (MAX), sum (SUM), average (AV), product (PDT).

We can then assign to the pixel p the class C_k such as:

$$P(C_k|x) = \underset{l}{argmax} \quad P(C_l|x) \tag{6}$$

This combination scheme therefore requires no training and is unsupervised.

3.4.2 Trained combination: BKS

A trained combination scheme that can be used to combine several classifiers is the *Behaviour Knowledge Space* (BKS) method. The BKS rule estimates posterior probabilities from a training set by computing the frequency of each class corresponding to each combination of the classifiers decisions. For a k class problem, when $e_j(x)$ denotes the class decision provided by the jth classifier among the n used, the vector of all the classifiers decisions $(e_1(x), ..., e_n(x))$ defines a point in a k-dimensional discrete space which is called Behaviour Knowledge Space (BKS). Each point of the BKS can be considered as indexing a cell. The cell, which is the intersection of the classifiers' decisions, is called the focal point. For each cell, the value with the largest number of patterns is estimated from a training set. The BKS combination scheme assigns such a class to an input pattern x. The BKS can be regarded as a look-up table that maps the classifiers decision vector into a class: it associates the final classification to each combination of classifier outputs. The method requires the construction of a large look-up table which cross-references every possible combination of the classifiers decisions. With respect to the original formulation of this combination method, we weighted the decision according to their confidence index.

3.4.3 Dempster-shafer combination

The computation of the intersected image from all initial segmented ones is realized. A pixel is considered as well classified when the class is the same for all segmented images. A confusion of classification may remains when a same pixel belongs to different classes. Those kind of incoherent pixels are labelled to as misclassified pixels. In such a case, a combination of results obtained is realized to reach the final segmentation. To perform this step, the theory of evidence is used.

Basic principles of the Evidence Theory Let $\Omega = \{\omega_1, \ldots, \omega_N\}$ be the set of N possible classes for the input vector $\vec{x}$, called the *frame of discernment*. Instead of narrowing its measures to Ω (as the theory of probability does constrained by its axiom of additivity), the theory of evidence extends on the power set Ω, labeled as 2^Ω, the set of the 2^N subsets of Ω. An initial mass function m is then defined from 2^Ω to $[0, 1]$ and must satisfy:

$$\sum_{A \subseteq \Omega} m(A) = 1 \quad \text{et} \quad m(\varnothing) = 0 \tag{7}$$

where $\varnothing$ is the empty set. $m(A)$ quantifies the belief attached to the fact that the search class belongs to the subset $A \subseteq \Omega$ (and to none other subset of A). Subsets A such that $m(A) > 0$ are referred to as *focal elements*.

Two initial mass functions m_1 and m_2 representing respectively the information provided by two independent sources, can be combined according to Dempster's rule [11]:

$$m(A) = \frac{\sum_{B \cap C = A} m_1(B) m_2(C)}{1 - K}, \quad \begin{array}{l} \forall A \in 2^\Omega \\ A \neq \varnothing \end{array} \tag{8}$$

K is known as the *conflict factor* and represents the discrepancy between the two sources. It corresponds to the mass accorded to the empty set after combination, *i. e.* $K = \sum_{B \cap C = \varnothing} m_1(B) m_2(C)$. The Dempster's combination, also known as orthogonal sum is written as $m = m_1 \oplus m_2$. After performing the combination, the decision on the elements Ω need to be taken to assign the class to $\vec{x}$. Among the existing rules of decision, one commonly used is the maximum of the "pignistic" probability. This decision rule [12] uses the pignistic transformation that equally distributes the mass associated to a subset of Ω among each of its elements. The resulting pignistic probability is then:

$$\text{BetP}(\omega, m) = \sum_{\omega \in A \subseteq \Omega} \frac{m(A)}{|A|}, \forall \omega \in \Omega \tag{9}$$

where $|A|$ is the cardinality of A, and the decision on the best class to be assigned to $\vec{x}$ is:

$$\omega^* = \text{Arg} \left\{ \max_{\omega \in \Omega} [\text{BetP}(\omega, m)] \right\}. \tag{10}$$

Application to unclassified incoherent pixels The information provided by each classification process (*i.e.*, from each color plane RG, GB and RB) is represented by an initial mass function $(m_i)_{i \in \{RG, GB, RB\}}$ taking into account the uncertainty associated to each color plane. Thus, classes that are very close according to a particular plane are brought together into a single focal element.$\overline{A_i}$ is the complement of $A_i \subseteq \Omega$ with respect to Ω. From [13],

$$\begin{aligned} A_i = \{&\omega \in \Omega, \\ &\omega = \text{Classe}(\vec{x}) | d_i(\vec{x}, \vec{x}^*) \leq \varepsilon_i d_i(\vec{x_1}, \vec{x}^*), \forall \vec{x}\}, \end{aligned} \tag{11}$$

for $i \in \{RG, GB, RB\}$. $\vec{x}^*$ is the vector to be classified and $\vec{x_1}$ its nearest neighbor (according to a distance d_i). ε_i is a constant greater than 1, representing the chromatic specification of the plane i: the greater the sensitivity on this axis, the greater the value of ε_i. In particular, if $\varepsilon_i = 1$ then $A_i = \{\text{Class}(\vec{x_1})\}$ is a singleton corresponding to the nearest neighbor. The initial mass function for the axis i is based on A_i whose mass takes into account the distribution of the elements within the set A_i, represented by the mean distance between two of its elements. The initial mass function m_i is then defined as follows:

$$m_i(A_i) = \alpha_i e^{-\beta_i \overline{d}} \tag{12}$$

$$m_i(\overline{A_i}) = 1 - m_i(A_i) - m_i(\Omega) \tag{13}$$

$$m_i(\Omega) = 0.01 \tag{14}$$

where α_i is a constant and $\beta_i = 1/d_{\text{max}}$. d_{max} being the maximum distance between $\vec{x}^*$ and the elements of A_i within the RGB color space and $\overline{d}$ is the mean distance between elements of A_i within this space. Thus, the greater $\overline{d}$ (*i.e.*, the more the elements away the ones from one and all), the lower the mass A_i.

In order to avoid a total conflict between two sources (two planes), a constant mass is given to the frame of discernment. The remaining mass is assigned to $\overline{A_i}$.

Let m_{RG}, m_{GB} and m_{RB} be the three initial mass functions. The resulting mass function from the combination of the three functions is then $m = m_{RG} \oplus m_{GB} \oplus m_{RB}$ where $\oplus$ is the orthogonal sum [11] defined by equation (8). Then, the final class of $\vec{x}^*$ is selected from m based on the maximum of the pignistic probability (Eq. 9).

4 Experiments results

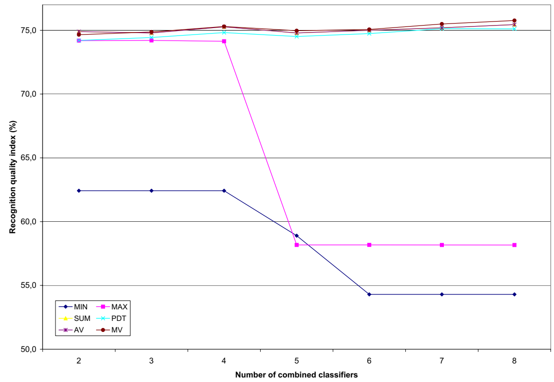
The images on which we work are microscopic cytology images of bronchial tumours acquired by a standardized platform. We provided the results of pixel classifications combination obtained on four cytological 24 bits colour images of size 752×574 pixels, each one containing hundreds of cells and all segmented manually by an expert in cytopathology to further assess the recognition quality.

In table 1, we present in order of merit the results of single pixel classifications obtained with the best colour space to further justify the importance of the combination step. The segmentation of nuclei bringing more information to the experts, we privilege the recognition quality index of the nucleus in relation to the cytoplasm. One can see that the best results are obtained with SVM supervised classification.

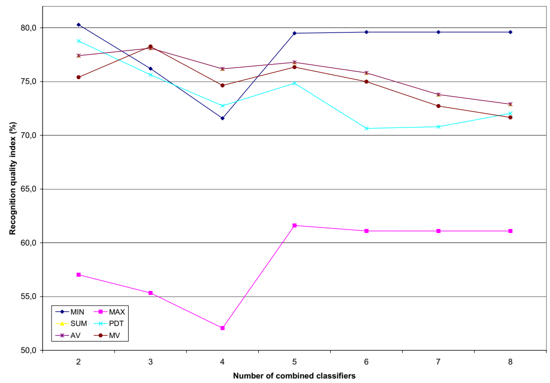
Table 1: Pixel classifications results with the best colour space before the combination step.

Classifier	Space	IdCytoplasm	IdNucleus
SVM	YCh_1Ch_2	77.4 %	74.2 %
Bayes	YCh_1Ch_2	72.4 %	74.6 %
k-means	YCh_1Ch_2	69.5 %	74.4 %
MLP	YC_bC_r	56.9 %	73 %
Fisher 1	RGB	50.8 %	72.3 %
kNN	HSL	79.9 %	70 %
Fisher 0	$I_1I_2I_3$	57.3 %	71.9 %
Fisher 2	HSL	59.9 %	69.8 %

Fig. 2(a) and 2(b) present the different untrained combination rules according to the number of combined classifiers. The classifiers being ordered as candidates for combination according to their confidence indexes. The majority vote (MV), and sum (SUM) are the methods which gave the best results for the whole cell (cytoplasm and nucleus). In the following, we comment our combination results only with these two methods. For the nucleus recognition, the indexes slightly increase with the growth of the number combined classifiers. For the cytoplasm recognition, a maxima of the indexes is obtained for 3 combined classifiers. We conclude that the best recognition of the whole cell is obtained with 3 combined classifiers.



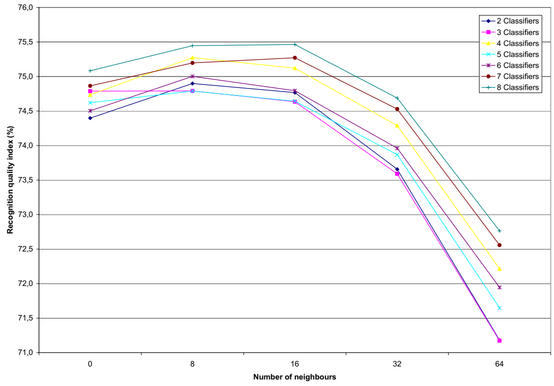
(a) Recognition quality index of the nucleus.



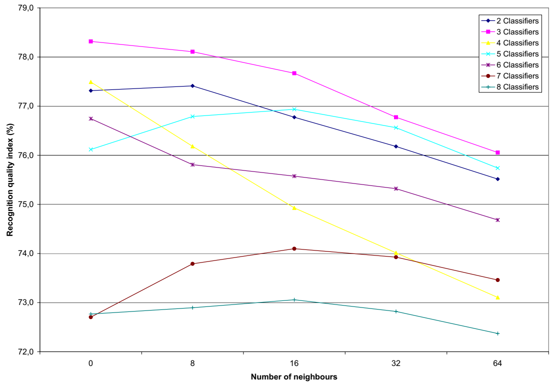
(b) Recognition quality index of the cytoplasm.

Figure 2: Influence of combination rules according to the number of merged classifiers.

Fig. 3(a) and 3(b) present the neighborhood influence in the combination process according to the number of classifiers with the SUM combination scheme. On can state that the nucleus recognition is increased by using 8 or 16 neighbors. The cytoplasm recognition is increased or decreased with 8 neighbors according to the combination rule used. Beyond this, the nucleus and cytoplasm recognition decreases. We can conclude that the best recognition of the whole cell is obtained for 8 neighbors with the 3 best classifiers for the SUM untrained combination rule.
For the BKS trained combination rule, the recognition decreases while the neighborhood increases. This can be explained since when the number of classifiers and/or the num-



(a) Recognition quality index of the nucleus.



(b) Recognition quality index of the cytoplasm.

Figure 3: Influence of neighborhood information.

ber of classes is large, this requires a huge training set to have a good chance of populating each element of the look-up table. Additionally the training set has to be a good representation of unseen data otherwise, as in the experiments of KUNCHEVA [14], BKS will perform well on training data while performing poorly on the testing data. This is what was stated again by our experiments. Good results are however obtained while performing KBS on a single pixel with the three best classifiers.
For the Dempster-Shafer combination rule, the classification rates are higher than the single classifiers. This combination rule does not use neither the confidence indexes of the single classifiers nor the neighborhood information, however the classification rates are very close to the others combination rules. This seems really promising: taking into account the previous criteria into the distance measure might further increase the recognition rate. Moreover this combination rule does not need any training set and is totally unsupervised.

Table 2 resumes the quality indexes of the pixel classification with the used combination rules. The results are very close but the untrained combination rule using the SUM seems more powerful than the others (this was already experimentally stated by KITTLER [7]. Table 3 presents the quality index of the segmentation obtained at the end of treatment. The combination step increases the segmentation quality of the whole compared to a segmentation with a single pixel classification taken alone (k-means or SVM algorithms). Fig. 4 presents the final segmentation with colored boundaries of the objects superimposed.

Table 2: Clasification combination rates.

	IdCytoplasm	IdNucleus
Untrained Comb. (SUM)	78.3 %	74.9 %
Untrained Comb. (VM)	78.1 %	74.8 %
Trained Comb.	78.5 %	74.8 %
Dempster-Shafer Comb.	76.3%	74.7%

Table 3: Final segmentation rate.

	IdCytoplasm	IdNucleus
k-means	72.8 %	76.2 %
SVM	73.2 %	75.8 %
Untrained Comb	76.5 %	76.4 %

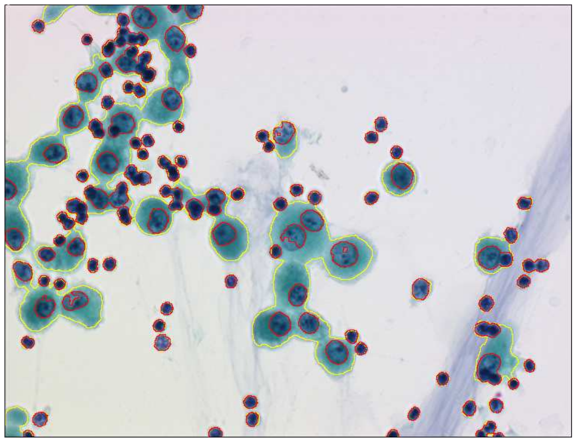


Figure 4: Segmented image.

5 Conclusion

When using multiple classifiers, combination problems arise since conflicting predictions between classifiers are possible and one has to arbiter among them. Combining multiple pixel classification (obtained from several inducers) can provide better results than a single pixel classification taken alone. This is why we propose a segmentation scheme of colour images based on a combination of pixel classification. This paper shows the improvement of the results by the use of a pixel classifications combination and of the neighborhood information. The best combination for our application in microscopic imagery consists in using a combination of the 3 best classifiers with the information of neighborhood (8 neighbors) for an untrained SUM combination rule. Future works will concern the amelioration of the Dempster-Shafer combination rule by the integration of the neighborhood information and quality indexes for each independant sources (the classifiers). Our method is suitable for the segmentation of colour images in a noisy environment and more particularly to the segmentation of cellular objects (Fig. 4 and table 2). We have finally improved the quality of our segmentation by the addition of this combination of multiple pixel classifiers step.

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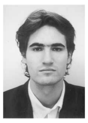

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