Pollen Grains Contour Analysis on Verification Approach

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Abstract. Earth's biodiversity has been suffering the effects of human contamination, and as a result there are many species of plants and animals that are dying. Automatic recognition of pollen species by means of computer vision helps to locate specific species and through this identification, study all the diseases and predators which affect this specie, so biologist can improve methods to preserve this species. This work focuses on analysis and classification stages. A classification approach using binarization of pollen grain images, contour and feature extraction to locate the pollen grain objects within the images is being proposed. A Hidden Markov Model classifier was used to classify 17 genders and species from 11 different families of tropical honey bee's plants achieving a mean of 98.77% of success.

Keywords: Pollen grains, Pollen classification, contour features, HMM, contour extraction, noise elimination.

1 Introduction

The recognition of pollen grains is an issue that has gained the attention on tropical countries, on both the theoretical and practical life. This topic has many applications, for example, species recognition, population estimation of species according to its biodiversity, knowledge of the extinction's patterns or region's reproduction. Within the field of pattern recognition, the pollen grains recognition is one of the areas that has embodied to this type of classification, not only by its shape or contour but also by its color, taking this to a higher level of complexity and variety when classifying patterns.

In the field of automatic pollen species classification, there have been a high number of research proposals, gaining a lot of progress in the species classification field. One of the first works in which texture features and neural networks were used for pollen grains identification task is shown in [1]. Another approach is presented by

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France et al. in [2], which addresses the problem based on improving the quality of the image processing; involving the processes of detection and classification of pollen grains. There have been many studies which model the features of pollen grains, in order to obtain a better classification process, which works as basis to understand the improvements made so far in the field. In [3], the shape and ornamentation of the grains were analyzed, using simple geometric measures. Measurement of textures using concurrence matrices were used by P.Li et al. in [4], using this measurements for automated identification process with optical images of pollen grains. Some others researchers have combined both techniques described above in their experiments as in [5] and [6], which used an approach of a Multi-Layer Perceptron neural network. In [7] brightness and shape information were used as vector features to improve its classification system. Besides these approaches, there are a vast number of methods used nowadays. An exciting approach is found in [8], which consists in a combination of statistical reasoning, feature learning and expertise knowledge from the application. Feature extraction was applied alternating 2D and 3D representations. Iterative refinement of hypotheses was used during the classification process. Another interesting method describes a full 3D volume recorded database of pollen grains using a confocal laser scanning microscope as found in [9]; this method extracted 14 invariant gray-scale features based on integration over the 3D Euclidian transformation used for classification. A more recent work with an ambitious approach is shown in [10]. It describes an automatic optical recognition and classification of pollen grains system. This method is capable to locate pollen grains on slides, focus and capture them in pictures, and finally identify the species applying a trained neural network.

There is no doubt the field of computer vision in biology has been made a lot of progress; nevertheless, more knowledge is needed combining different types of features so the number of species that systems can recognize and classify increments considerably. This work introduces a hybrid classification approach using Hidden Markov Models to classify successfully the contour of pollen grains images due to the results of previous works in different scenarios that has demonstrated that HMM is successful in the image contour classification area. The hybrid proposed system is based on image signal processing and decisions support systems, taking the advantages of both techniques to create a useful bioinformatics system to classify species. Moreover, this work introduces a contour analysis of pollen grains using a verification approach where the images are analyzed and classified by a HMM classifier. Two approaches have been used, identification for a first stage and verification for a second stage. The first stage consists of a learning process where the systems let us identify and classify species; towards that classification, a verification process is done to confirm the correct recognition of tropical honey bee's species, in comparison to previous work where identification was the core approach. This approach has given a 72.92% of success rate in the identification stage, but its success rate has considerable increased to 98.77% thanks to the novelty use of a verification process in the recognition system, being the first work in the area to use a verification stage. We consider that the verification stage we have incorporated to the system makes it unique and accurate due to the reliability in species recognition. This stage allows the system to recognize and classify successfully species of tropical honey bee's plants that are present in the database used. Compared to previous works, if a pollen grain is not documented in the database, those systems tend to fail in the identification stage.

Nevertheless, the incorporation of a verification process let the system successfully indicate that the pollen grain does not belong to a trained class; this way we can understand bee's behavior and if those bee's start to produce honey with different pollen grains. Furthermore, this work presents an angular parameterization which provides a contour invariant characterization compared to other approaches, removing the problems of scale, translation and rotation in images. Regarding the biological aspect, working with pollen grains contour supposed to have 2 relevant features, the variability and the similarity between its species. Likewise, the proposed method in this paper encompasses the classification using an HMM classifier due to the benefits it presents in pattern recognition as in temporal shape recognition, speech recognition, handwriting and gestures patterns, bioinformatics and others. The HMM statistical grounding gives the freedom to manipulate the training and verification processes as required by the problem, and give a mathematical and statistical analysis of the results and processes. Moreover, HMM classifiers provide a modular approach thanks to the modularity of its architecture, allowing the combination of HMMs into larger HMMs improving significantly the classification results. Furthermore, HMM allows incorporating prior knowledge into its architecture, letting initialize the model close to something believed to be correct and constraining the training process. The remainder of this paper is organized as follows. How the images were preprocessed is being described in Section 2. Section 3 describes the feature extraction process followed to obtaining the relevant information of the pollen grain contours. The HMM classification system used is described in Section 4. Then, Section 5 contains the experimental settings applied in this research; as are the database, the experiment methodology and the results obtained. Finally in Section 6, the conclusions of this work are shown.

2 Image Preprocessing

As can be seen in figure 1, it has been used a colorant to make the samples of each pollen grain more visible. Thus, the preprocessing was vital in order to obtain a good parameterization. The preprocessing steps are described above, those steps include: Binarization Process, Noise Elimination Process and Contour Extraction.

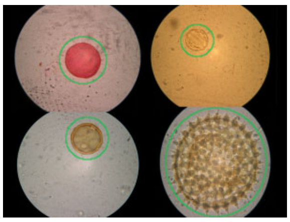

Fig. 1. Images to work with. Various kinds of pollen grains surrounded in green circles

Most pollen grain contour detection algorithms were proposed from binary images, which make it appropriate to transform a gray scale (or color) image to a binary one, allowing reducing data volume to process. One of the used methods to obtain a binary image is through its histogram, obtaining the number of pixels related to each level of gray which appears in the image. The binarization process must choose an adequate value in the range of gray levels (a threshold), all the gray levels inferior to that threshold would transformed to black and all the gray levels superior to that threshold would be transformed to white; being this process known as Otsu Method[11].

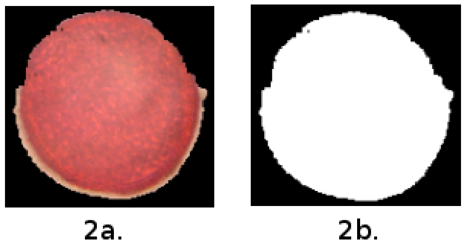

Fig. 2. Binarization Process. 2a) Pollen grain image, 2b) Binarized image

Once the images are binarized, the next step would correspond to eliminate the noise in the image, which would be removing any part of the image that does not belong to the pollen grain, like small bubbles created due to the liquid used to give color to pollen grains. The noise elimination process consists in using as reference the binarized image of the pollen grain. In this point, it is necessary to locate which part of the image corresponds to the pollen grain. To achieve this, we obtain the center pixel value of the whole image; if this value is equal 1 it means the pixel taken corresponds to the inner pixels of the pollen grain; if not, then we move to the right and take a new pixel value until we find the pollen grain. Once the pollen grain has been localized, it is used a selection algorithm which is useful to eliminate all the pixels that does not belong to the pollen grain. This algorithm consists in applying a mask using the binarized image of the pollen grain; which consist in taking the image and selecting the objects found in the image. Among those objects, the object which covers more than 90% of the image is the dominant, so the rest of them are excluded, obtaining a cleaner image and the pixels that correspond just to the pollen grain object. These results can be seen in figure 3.

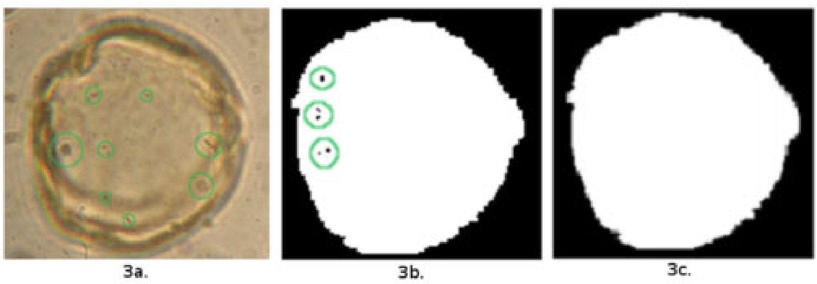

Fig. 3. 3a) Pollen grain image with colorant bubbles surrounded in green circles, 3b) Binarized image with some noise surrounded in green circles, 3c) Image without noise

Contour extraction is used in computer vision to recognize objects in 2 dimensions [6] [12], region segmentation and object's border extraction. To obtain the border of an image, there are a vast number of methods to achieve this goal like Sobel method, Prewitt method, Roberts's method, Canny Method, among others. In order to obtain the border of the pollen grain, it has been applied the Canny algorithm, finding out that it is not efficient enough in terms of how fast the border extraction is done.

Therefore, the method of extraction was changed to morphological operations. In this case, the image of the pollen grain and the interior pixels are removed, so the border can be obtained. To achieve this, the pollen grain pixels are taken an analyzed this way: If a pixel has all its 4-connected neighbors with a 1 value, then this pixel is set to 0. This helps us to process and leave only the boundary of the image with the pixel value in 1. This method is more efficient than Canny algorithm in terms of how fast the border extraction is done, and allows to obtain the contour of the image due to the image skeletonization; obtaining the results seen in figure 4.

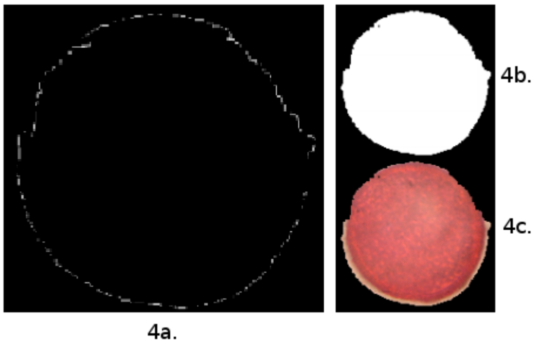

Fig. 4. 4a) Pollen grain contour, 4b) Binary noiseless image, 4c) Original Pollen grain image

3 Feature Extraction

In order to make a good feature extraction process, a set of steps has been proposed to locate a set of features of every image. Once the contour of the pollen grain has been obtained, the first thing that should be done is make the contour points selection as shown in figure 5b and obtain a set of points organized in a clockwise manner keeping the circular form of the contour.

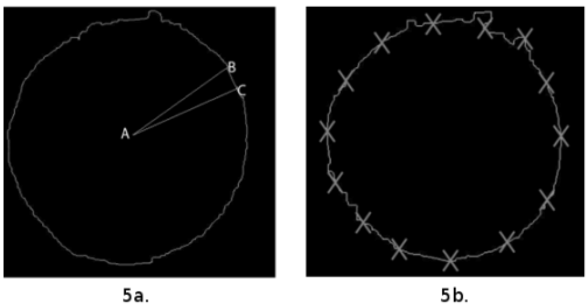

Fig. 5. 5a) Location of angles, 5b) Set of points

After the set of points has been calculated, the centroid of the object is obtained with formula (1), (2) and (3) where the *n* vertices are defined by *(xi,y_i)* and the centroid correspond to *(Cx, Cy)*. With this information, a triangle can be formed using the centroid of the pollen grain been this point the A vertex of the triangle, and the B and C vertex would be 2 neighbor points of the contour. Using the Pythagoras' theorem and Cosine' theorem, the inner angles of the triangle can be obtained.

$$C_x = \frac{1}{6A}\sum_{i=0}^{n-1}(x_i + x_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \tag{1}$$

$$C_y = \frac{1}{6A}\sum_{i=0}^{n-1}(y_i + y_{i+1})(x_i y_{i+1} - x_{i+1} y_i) \tag{2}$$

$$A = \frac{1}{2}\sum_{i=0}^{n-1}(x_i y_{i+1} - x_{i+1} y_i) \tag{3}$$

Once the angles are obtained, those values are stored and a new triangle is formed; keeping the centroid as the A vertex, the new B vertex value would be the same value of the C vertex of the last triangle, and the new C vertex value would be the next neighbor point of the B vertex. In other words, the B and C vertex are 2 neighbor points of the contour taking those points in a clockwise manner as shown in figure 5a.

The angles used in this feature extraction are angular rather than polar, taking the advantage of obtaining information of the contour independent of the size, translation and rotation of the pollen grains. Polar angles are not a good choice since polar angles gives information dependent of the contour size, being this a relevant factor due to the importance of a good classification in which the size does not predominate. This parameterization is used to treat discrete systems like the Hidden Markov Model (HMM) classifier.

4 Classification System

The classification system proposed to accomplish the recognition of the pollen grains from the features obtained is based in the Hidden Markov Model (HMM). A HMM is a string of states $\boldsymbol{q}$, jointed with a stochastic process which takes values in an alphabet S which depends of $\boldsymbol{q}$. These systems evolves in time passing randomly from one state to another and issuing in each moment a random symbol of the S alphabet.

When the system is in the state $\boldsymbol{q_{t-1} = i}$, it has a probability $\boldsymbol{a_{ij}}$ of moving to the state $\boldsymbol{q_t = j}$ in the next instant of time and the probability $\boldsymbol{b_j(k)}$ of issuing the symbol $\boldsymbol{o_t = vk}$ in time $\boldsymbol{t}$. Only the symbols issued by the state $\boldsymbol{q}$ are observable, nor the route or the sequence of states $\boldsymbol{q}$; that's why the HMM obtain the appellative of "Hidden" since the Markov process is not observable.

We have worked with an HMM called "left to right" or Bakis, which is particularly appropriate for sequences. These "left to right" HMM's turn out to be especially appropriate for hand edge because the transition through the states is produced in a single direction, and therefore, it always advances during the transition of its states. This provides for this type of model the ability to keep a certain order with respect to the observations produced where the temporary distance among the most representative changes.

5 Experimental Settings

In order to implement the classification system based on HMM classifier, a set of experimental settings has been configured. These settings includes the Database used in all the experiment scenarios, the methodology followed to achieve a good classification result, and the analysis of the results obtained.

5.1 Database

The database used has been obtained directly from the Research Center of Tropical Bees, CINAT (Centro de Investigación de Abejas Tropicales from its Spanish acronym), of the National University of Costa Rica (UNA); located in Heredia, Costa Rica. This database contains a total of 426 pollen grain sub-images corresponding to 17 genders and species from 11 different families of tropical honey plants from Costa Rica. This database has been used in previous research work done as in [14].

5.2 Experiment Methodology

The experiment methodology has been structured using a supervised classification technique which consists in using a set of pre-established knowledge to determine the best classification scenario. Therefore, this experiment is divided in two stages. The first stage describes the learning process based in HMM whereas the principal objective is to obtain the best model, which is the one capable of classify the contour of the pollen grain in every herbage. And the second stage represents the process of verification of the pollen grain contour used by the models generated in the learning stage, as shown in figure 6.

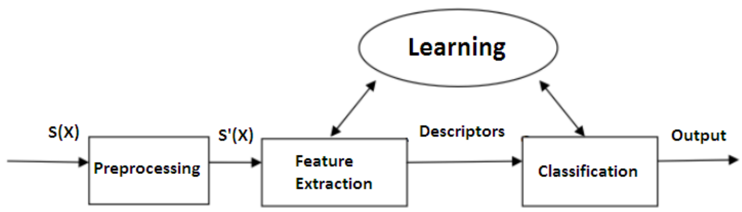

Fig. 6. Classifier procedure

Learning Process: It has been trained 41 models (one for each species), so the training was done with 17 different pollen grain contours coming from each different species. This training was done with the training function to which it sends as a parameter the observation information obtained in the feature extraction process. This function defines a state transition matrix and an initial observation symbols matrix.

The number of iterations in the HMM is 1681 using 17 types of pollen grain contours, this values corresponds to the number of observations obtained of each pollen grain contour. The behavior of the HMM classifier consists in comparing the input feature information vector corresponding to a determined class, with a set of pattern features vector. At least, there should be one pattern feature vector for each of the classes. The feature vectors of each class are introduced to a single HMM. For example; in class 1 there should be an input of the feature vectors corresponding to individual 1, in class 2 there should be an input of the feature vectors corresponding to individual 2, and so on. This way, a single HMM that contains the information of all the individuals is generated. Once the HMM is ready, the test mode can be pursued. In this mode, the system receives as input a feature vector and the system compares it with model generated in the learning stage. This comparison takes the maximum of the HMM with the output and the identified class. If the output exceeds this maximum, the input feature vector belongs to an individual in the database; otherwise the input feature vector belongs to an individual outside of the database.

Verification Process: The HMM classifier is treated as a bi-class classifier. It should be created as many models as there are classes, being each model composed by the feature vector of a class to which it is considered as positive and all the remaining feature vectors to which there are considered as negative. In the test mode of the verification process, the image to be classified is preprocessed according to the method described in Section 2, and then the features of this image are extracted according to the process described in section 3. The feature vector of the image to be verified is calculated according to the parameterization method chosen in each case. If the output exceeds a determined threshold, the pollen grain to be verified would be verified as from the class to which the individual is said to belong. Otherwise, the individual is rejected. The threshold obtaining process is based in the False Rejection Rate and the False Acceptance Rate. According to these concepts, the ROC curves are created, which indicates the system reliability. A high FRR indicates a decision threshold too strict provoking dissatisfaction from the system users due to the negation of authorized personal. Otherwise, a high FAR indicates a decision threshold too permissive, generating a big security problem. The important thing is to obtain an optimal threshold [13]. Representing the values of the rates in accordance to the decision thresholds, it can be obtained a TEER (Threshold of Equal Error Rate) and an EER (Equal Error Rate) of the system. The TEER refers to the threshold of EER, whereas the FAR and FRR are equal and the EER is the system error. This means that the TEER and EER are generated where the cutoff point between FAR and FRR is. Once the EER is calculated, the reliability of the system can be calculated through the following expression:

$$System\ Reliability\ (\%) = 100\ EER(\%) \tag{4}$$

6 Results and Analysis

In this section, it is presented serious different tests made with all the feature vectors and the classifier. Showing both the results of the identification process and the results of the verification process; providing an approach of which feature extraction methods and configurations of the classifiers offer a better Total Success Rate (TSR). Once all the results are obtained, it can be verified that the pollen grain contour system can identify and verify the individuals in a database, offering an acceptable success rate. The tests performed were made with a database of 43 classes with 17 samples of each class. In the tests made with the classifier, it has been used 10 samples for the system training and the remaining 7 samples for test purposes. The results of the tests are presented in tables were the column TSR shows the success rate in terms of mean and variance.

6.1 Results for the Identification Process

The experiments performed and its results obtained with each of the feature vectors calculated in the feature extraction process for the identification system using HMM are exposed in this section. The tests performed for the identification process were repeated a total of 16 times.

With this method, it was selected a determined number of contour points separated the same distance between them. The tests were performed for different number of contour points. The number of points selected for the tests comprises a range of 200 to 400 contour points, taking into account that the number of points is expressed in the form of which it takes 1 point and skips 16 points. The states of the HMM has been varied in a range of 100 to 200 states. This variation in the number of states of the HMM and the number of contour points selected has been used to create a high number of test scenarios, indicating that overpassing those boundaries, the results tends to decrease considerably making these values a good set to reach variation in the results during experimentation of the proposed system.

Based on those experiments, it has been found that the best combination results are those who have an approximate relation of 2 contour points by each HMM state. It has been obtained a large number of results related to the variation in the number of states of the HMM and the number of selected contour points. From these results, it can be noticed that while the number of points chosen increase the results decrease, not being optimal results; as we can see in table 1. Among all the experiments done, it has been found that the combination of 280 selected contour points with 140 number of states for the HMM has given the best results in the identification process of the classes of the pollen grains as shown in table 1.

Table 1. Identification of pollen grains contour

Number of Points Chosen	Number of States HMM	TSR Mean%±std
350	180	54.17±5.89
300	160	70.83±5.90
280	140	72.92±2.95

In the classification process, it has been used a classifier based on HMM. Knowing that in most cases, by not specifying that all, the pollen grains have a circular form regardless of the termination of its class noting 2 different types of pollen shapes. This classification has given a good result which it helps to explain some issues: a)The number of points to be selected is a relevant factor since choosing a lower number of points in comparison to the optimal, the shape of the pollen grain could be lost converting it into a simple circle; and choosing a higher number of points would give us a lot of details in the pollen grains, causing worst results due to the memorization of the shape and not being able to recognize the shape pattern of pollen grains of the same species. This way, choosing the number of points to be selected is a crucial task so this number can create a balance between generalization and memorization allowing the system to correctly identify and classify the species. And, b) By introducing a higher number of points, the results would be impoverished due to the inclusion of noise to the classifier, giving bad results as seen in table 1.

6.2 Results for the Verification Process

The experiments for the verification process were performed only with the method were the best result has been obtained. Thanks to the identification system, the best result scenario has been obtained due to the combination of 280 selected contour points with 140 number of states for the HMM. Therefore, this result is used as a basis for the verification process. As a reminder, while in the identification process it was created just a single model with all the classes in it, for the verification process it is created a model for every class in the database where each of this models corresponds to a different class in the database. Each one of these models is evaluated, and the results are obtained varying the output threshold, obtaining the FAR and FRR for every value of the threshold. These rates are obtained analyzing the number of pollen grains images falsely accepted and the number of pollen grains images falsely rejected depending on the threshold. From the representation of FAR and FRR, the EER and the TEER can be obtained. As shown in figure 9 and table 2, the results of EER, TEER and the Reliability of the system for the performed tests for all the classes in the database, are calculated according to the equation given in section 5.2, it can be considered that a verification process can be done with a high level of credibility.

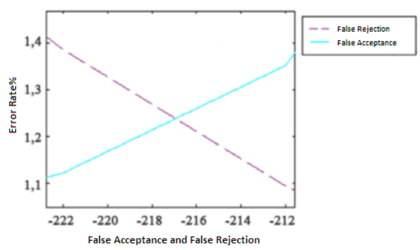

Fig. 7. Cutoff points for the verification

Table 2. EER, TEER and Reliability of the system obtained with HMM

EER	TEER	Reliability
1,23%	-217	98,77%

7 Conclusions

This work has introduced a classification system through the pollen grain contour, being capable of performing the recognition and verification of the pollen grain. The preprocessing stage has been a little bulky in the process of finding the appropriate threshold for the binarization of the image. The ideal method would be to calculate the threshold for each image, but this couldn't be done due to the obtainment of the images captures. The solution presented in this paper was performed by obtaining the mean of the thresholds establishing this mean as the binarizarion threshold. In the feature extraction stage, 2 methods of parameterization have been proposed; obtaining angular angles and polar angles. For this 2 methods, it has been obtained a better result using the angular angles because these values does not depend on the size of the pollen grain, gaining a better TSR result. The success rates in identification are around 72.95% with a variance of 2.95 and a trust interval of 0.5; having for the verification process a reliability of 98.77% working with our database. This shows that an approximate relation of 2 contour points by each HMM state, is the optimal choice to model the contour of pollen grains, letting the system identify and validate the different species of tropical honey bees. It is important to mention that this classification method proposed gives a better result rate than the methods presented previously by other authors like [4], [5], [6] and [7]. Moreover, this paper has contributed with a taxonomical quantitative model, showing that the pollen grain contour classification system can simplify the features in the moment of its classification obtaining a sufficient discriminatory result.

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