

Automatic morphology: Application on biological images

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Abstract

Phenotype of species are characterized by several informations like, age, sex, morphological measures and environment parameters. Biologists are familiar to manually getting morphological measures. In the case of analysis at the macro level (tissues, small parts of animal ...) that can be done directly by measuring the element geometry: length, width, diameter, angles Another way is to take pictures of these elements and to run image processing algorithms. From a collection of beetle pictures, we have initiated this work to test the feasability of automatically positionning landmarks on biological images. The set of images contains 293 beetles and for each, an image of the left and right mandibles. For each mandible, a set of 16 and 18 landmarks (resp. left and right) have been manually set. In a previous work[4] based on Palaniswamy article [6] we have shown that if we consider the centroid measure of the mandible as the parameter to obtain, the probabilistic Hough Transform (PHT) can provide very interesting results. But if the goal is to consider more precisely the position or the geometry of landmarks area, the results are not enough accurate to consider estimated landmarks instead of manual landmarks. In this next turn, we have kept the same method to segment and to register scene images with a model but we have changed the way to set the model landmarks on the scene. This new procedure uses a SIFT algorithm as the last operation of our process. SIFT algorithm is not applied as usual on the full image but only on areas selected by the previous steps. We have compared all the obtained results with the previous ones and concluded that this new workflow to label the mandible images can be delivered to biologist to continue their analysis. This workflow, MAElab is available as library function on a github website.

Keywords

Keywords Automatic morphology, landmarks identification, image registration.

1 INTRODUCTION

In biology, morphology analysis is widely used to keep the changing information of the organism or detecting the difference information between the organisms. From the result of morphology analysis, we can conclude the evolution of an organism family, or we may classify the organisms. Especially in agriculture, morphology is one of best ways to learn about the variations of the insect on crops. The morphology methods may be divided into the groups by the features which are used by the methods such as shape, structure, color, pattern or size of the object. In the aim to study the potential links between these variations and agricultural ecosystems, a set of 291 beetles has been

collected with all the information about the sex, place where they are found and agricultural practices in each field were recorded. For each beetle, the morphometric landmarks has been defined on each part (each insect includes five parts: head, pronotum, body, left and right mandible) of the insect by the biologies. In this context, we try to indicate the landmarks on two parts of beetle (left and right mandible (see figure 1)). Morphometric landmarks are points that can be defined in all specimens and located precisely. Landmarks are widely used in many biological studies and they are currently included into the classification procedures.



(a) Left mandible (b) Right mandible

Figure 1: The mandibles of beetle

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In this paper, we focus on a method that can automatic identification of landmarks on 2D images of beetle, specify the mandibles of beetle. Through whole the article, we use two images to automatic extraction the landmarks: model image and scene image. The method mainly includes three stages: firstly, we extract the features of the object in the image; secondly, principal component analysis iteration is used to register two images, the translation and rotation between two images are also determined; finally, a refinement of the estimated landmarks is done by SIFT method.

In section 2, the steps of our methods will be presented. All experiments and evaluation are described in section 3.

2 METHOD

The problem to solve is to suppress the manual operation of setting landmarks on each image. To do that we propose a chain of operations, a workflow, including segmentation step of each image and a registration with a model image. The model image is more or less chosen randomly, we first verify by hand that it is not an image of some broken mandibles. The figure 2 shows the different steps of the workflow.

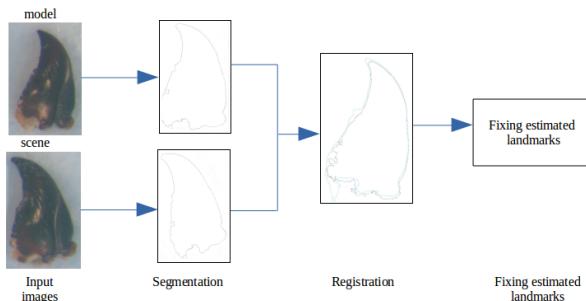


Figure 2: Overview of the proposed method

In this section, we will describe the different algorithms that we have used. It is worth to note that a protocol to take pictures of each mandible has been defined. All have been taken in the same conditions, the same camera with the same resolution.

2.1 Image segmentation

Segmentation is often the first task and the bottleneck of image processing chain. The most well-known algorithms are mainly classified as contours or regions based segmentation. We have chosen a contours one, the Canny algorithm[2], which allows to determine the list of edges belonging to the shape of the image. To use this method, two threshold values have to be set. As

it is often mentioned, fixing the right values for these thresholds could be difficult[3]. The mandatory *threshold value* used by Canny algorithm has been determined by analyzing the image histogram (see [4] for detail). Most often authors define from this threshold, a lower and an upper one. The usual ratio of these two threshold is $T_{lower} = (1/2) * T_{upper}$. In order to consider a larger range of values, we have prefer to set T_{lower} to $1/3$ of T_{upper} . For optimisation purpose of the computing time, during the computing of Canny algorithm the gradient direction of each pixel which belongs to the curves is kept for the next steps of the method.

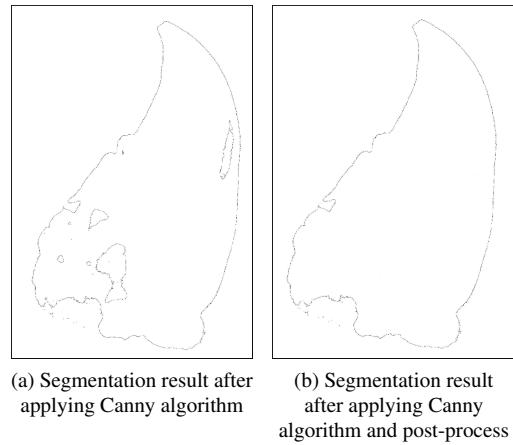


Figure 3: The segmentation results of the image

To achieve the segmentaion step, the obtained contours are post-processed to remove unnecessary ones. As it is shown in figure 3, the final result of Canny generates some contours which do not belong to the shape of the mandible. With a simple algorithm, we browse the image and suppress the edges inside the main shape.

2.2 Image registration

As we have mentioned, all images have been captured following the same protocol and at the same scale. But it remains differences in size between the mandibles (cause of size of the beetles), or orientation and position because of the mandible position under the camera. The next step concerns registration of model and scene before estimating the landmarks. Principal Component Analysis (PCA) is a well-known method to find the rotation and translation parameter values between two images. In our workflow, we have used a classical PCA computing[7], [8].

As input values, we use the lists of points which has been defined by the segmentation step. Firstly, the centroid point and principal axis of each image are defined: the centroid point is the point which has the coordinate equal to the mean coordinate of all boundary points; the principal axis is a connected line

from the centroid point to a point in the list of contours points which is determined as followed(algorithm 1):

Algorithm 1: The algorithm for finding the principal axis of a list of contour points

Input : Centroid point, list points of contours
Output: The principal axis

```

1 for point i in the contour points do
2   Draw line l between i and centroid;
3   for point j in the contour points do
4     if i ≠ j then
5       Compute the perpendicular distance
      between line l and point j;
6     end
7   end
8   Compute the average perpendicular distance
      between line l and other point j ≠ i;
9   Choose the i that has the minimal average
      perpendicular distance;
10 end
11 The principal axis is the line between centroid and
      point i which is chosen.
```

for all point i in the contours, draw a line l between i and centroid; for all points j in the contours ($i \neq j$), compute the perpendicular distance between line l and point j ; compute the average perpendicular distance of all points j to l . When the computing is finished, the principal axis is the line that has the minimum mean perpendicular distance to all points of the contours. The translation is indicated by the coordinate difference between the centroid points of the scene and the model. The rotation angle is the angle between the principal axes of these two images. As the rotation direction is determined by checking coordinate of the endpoints on the principal axes of the model and the scene. Then, the scene is moved to matching with the position of the model. However, in some case, the translation and rotation between two images are not enough right because the result of the segmentation may be not perfect. To improve registration, we have enhanced the PCA by an iteration state (PCAI). We have considered some specificity of our images and observed that the tip part of the mandible is less noisy than the base. So, we sort the points according to their y-value. We build a subset of points which contains half part of points, these ones which belong to the upper part of the image. PCA is again completed for this subset to refine the rotation and translation values. This operation is iterated until the new computing angle is less than 1.5 degree (see figure 4).

Figure 5 shows an example obtained results from the different steps of PCAI. In this figure, the red contours is the model segmentation, the black contours is the scene segmentation after one iteration, and the blue

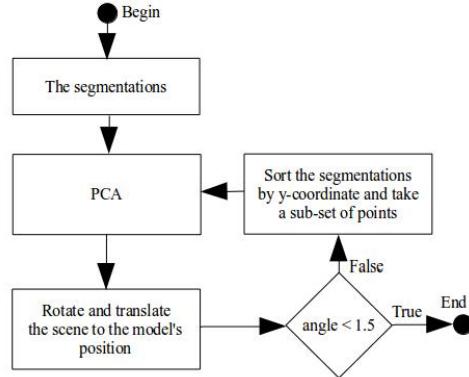


Figure 4: The flows in PCAI

contours is the last result after finished PCAI .

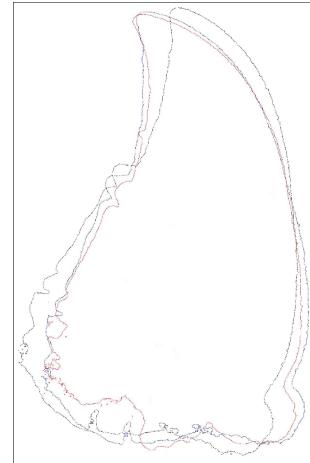


Figure 5: Differrent registration steps between two images

2.3 Fixing the estimated landmarks

The last task in the workflows concerns how to estimate landmarks of the scene from the manual ones of the model. This has to be done by using SIFT[5] method but as usually we do not consider all points of the image but only the area around the landmarks. Firstly, the region around each manual landmark (called patch) in the model is created and its corresponding position in the scene image is defined. Then, the SIFT descriptor is computed: the orientation and gradient magnitude are calculated for each their pixel by the equation:

$$m(x,y) = \sqrt{(P(x+1,y) - P(x-1,y))^2 + (P(x,y+1) - P(x,y-1))^2} \quad (1)$$

$$\theta(x,y) = \tan^{-1}((P(x,y+1) - P(x,y-1))/(P(x+1,y) - P(x-1,y)))$$

Where:

- $P(x,y)$ is the value at position (x,y) in the patch,
- $m(x,y)$ is the gradient magnitude of the pixel at position (x,y) ,

- $\theta(x,y)$ is the orientation of the pixel at position (x,y) .

Then, the descriptor is computed for each patch in the model and the scene. This is a histogram with eight direction for orientation, with the length of each bin is sum of the gradient magnitude of the pixels that corresponding with the orientation. The descriptor is formed from a vector containing the value of all the bins in the histogram. Finally, the vector feature is modified to reduce the effects of illumination change by normalization.

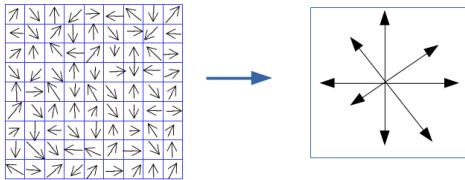


Figure 6: A landmarks descriptor is created by computing the orientation and gradient magnitude

For example, the figure 6 explains for our purpose, after experiments the best choice size to be 9×9 . The patch is create around the landmark. For each pixel in the region, the orientation and gradient magnitude are calculated (the orientation and length of the arrows). Then, the descriptor of this patch is computed by using 8-orientation histogram and the length of each arrow corresponding to the sum of the gradient magnitudes near that direction within the region.

The comparison between the SIFT descriptors is done by using L_2 distance which is given by the equation (1).

$$L(P, M) = \sum_{i=0}^n \sqrt{(P_i - M_i)^2} \quad (2)$$

Where:

- P, M are the descriptors with size of n ,
- P_i, M_i are the corresponding location i in the descriptors.

The figure 7 is the illustration all the steps of this process. For each manual landmark on the model and corresponding estimated landmark on the scene, the patches P_m, P_s are created ($P_m < P_s$). For each pixel in the patch P_s , a sub-patch P'_s is extracted with the same size of P_m . Then, their descriptor and measure distance are calculated (between P_m and P'_s) by using equation 2. This process is finished when all the pixels on the patch P_s are considered. The coordinates of estimated landmark is the location in P_s that has the smallest measure distance value with P_m . Finally, the coordinates of the estimated landmarks is set to the original location of the scene image by applying the reverse operation of rotation and translation.

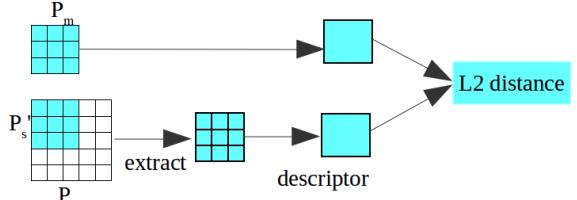


Figure 7: Illustrate the steps of descriptors comparison.

3 EXPERIMENTS AND RESULT

All the steps in our method are implemented in MAE-Lab¹. Two sets of beetle have been analyzed, right and left mandibles. After verifying the quality of the image, it remains 290 usable images for right mandible and 286 images for the left mandible. The removed images include the images that do not contain the mandible or the borken mandibles. Then, each dataset is divided into two sub-sets followed the size of the mandible. For each subset, an image is chosen as the model of the experiment process. In all valid images, a set of 18 manual landmarks of right mandible (16 landmarks for left mandible) are indicated by biologists. Along with choosing the centroid size to measure the mandible. This size is obtained by sum of all square distance from each landmarks to the centroid point (see [9]).

At the end of PCAI, a hypothesis is made to estimated the scale between the images. For each image, the bounding boxes are indicated by the coordinates of the points on the curve. The scale of x and y-direction are determined by the ratio between the corresponding sides of the bounding boxes. Then, the scene curve is scaled to fit the model curve.

Figure 8 and 9 show a complete result on one right mandible with the manual landmarks (red points) and estimated landmarks (yellow points). As we can see in the image, almost the estimated landmarks are quite near with the manual landmarks. This shows that our method is worked well for indicating the landmarks on the mandibles.

In next experiment, we want focus on two kinds of evaluation: centroid size of the image and the accuracy position of each landmark.

Firstly, our evaluation is done on comparing the centroid size of the estimated landmarks and manual landmarks. The experiment is done by choosing an arbitrary image in the dataset as the model. The automatic landmarks are estimated on remaining images with the method that we have described. Then, the

¹ MAELab is a free software in C++. It can be directly obtained by request the authors.

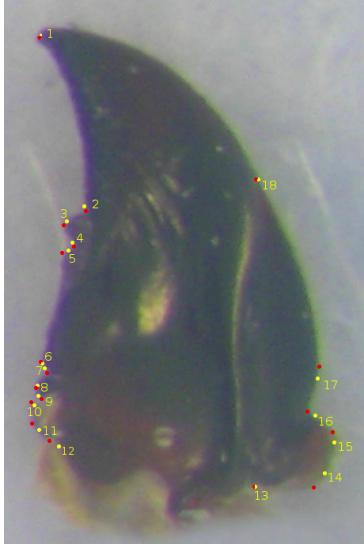


Figure 8: The manual and estimated landmarks on right mandible

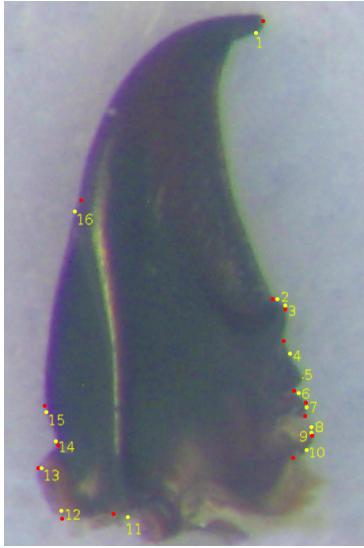


Figure 9: The manual and estimated landmarks on left mandible

centroid size of each image is calculated and evaluated. We can see in figure 10a and figure 10b for all images, the correct proportions of centroid size which based on the estimated landmarks are 93,45% for the right mandibles and 90,21% for the left mandibles with the standard deviation[1].

Besides using the centroid size to evaluate the method. We are also interested in the accuracy on the position of the estimated landmarks. In this experiment way, we calculate the distance between each manual landmark and corresponding automatic landmark. Through, we want to examine replacing the manual landmarks by corresponding estimated landmarks.

Figure 11 and 12 show the correct proportion on

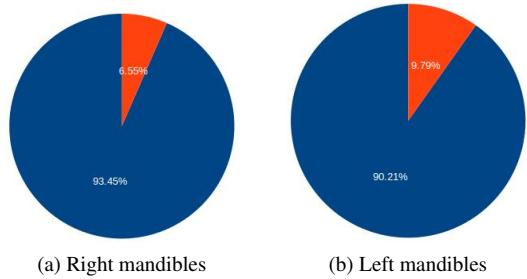


Figure 10: The correct proportions on centroid size of the mandibles

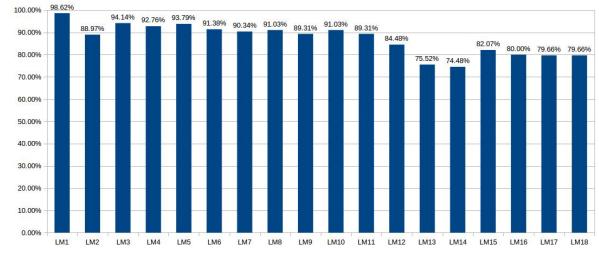


Figure 11: The correct proportions on each landmark of right mandibles

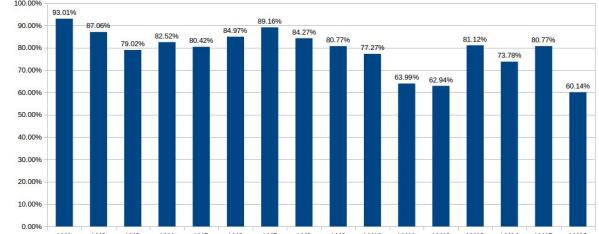


Figure 12: The correct proportions on each landmark of left mandibles

each landmark of mandible. With 18 landmarks of right mandible, the position of the first estimated landmarks is reasonably accurate with 98,62%, the lowest proportion is 74,48% for fourteenth landmark. The remaining landmarks are also indicated with a high proportion (with the accuracy proportion greater than 75%). For left mandible, the highest and lowest success rate are 93,01% for the first landmark and 60,14% for the sixteenth landmark. The statistic is done on each automatic landmarks of all the images with a standard deviation[1]. As we can see in figure 3, the noisy of the base in segmentation is higher than the tip. This explains why the correct proportion on 11th, 12th landmark of the left mandible or 13th, 14th landmark of the left mandible are less than other landmarks. Moreover, when we reconsider the datasets, the images in left mandible are having more type of size than on the right mandible (scale problem). This is shows the reason that the success rate on right mandible is always greater than left mandible in both of the experiments.

From two experiment ways, we can see that the method is success in indicating all landmarks for each image;

and the location of the landmarks is considered near with the manual landmarks in some aspect. In a different side, when comparing with our previous study (see in [4]). This method has more exactly about the position of automatic landmarks as well as the advantages for the implementation process. The memory to detecting the landmarks, along with the times to execute the process are decreased dramatically.

4 CONCLUSION

Morphometric analysis is a powerful tool in biology in classification the species. Automatic identification the characteristics biology of the organism is a difficult problem. In the content of this paper, we have begun to design a method to segment the beetle mandibles and to indicate automatically landmarks which have been determined by biologists. Each mandible is segmented by applying the Canny algorithm. Using PCAI to align the images and estimate the landmarks. Finally, a descriptor distance will be applied to refine the location of the estimated landmarks. The first version of this method has been implemented. From now, the next stage of our method is to add the features to have the position of landmarks more precisely, i.e diagnose on the scale of the image.

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