

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[3] based on Palaniswamy article [5] 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

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, from segmentation to registration of each image, considered as scene, to one chosen as a good representant of the mandible and named the model. The figure 2 explains the different steps of the workflow. In this section, we

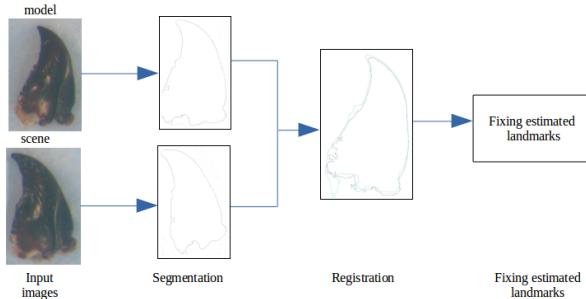


Figure 2: Overview of the proposed method

will describe three steps in our method: segmentation, registration and setting of estimated landmarks. It is worst to note that a protocol to get each image has been defined. All have been taken with in the same conditions, same camera and same resolution.

2.1 Image segmentation

Segmentation is often the first and the bottleneck of image processing chains. The main well-known algorithms are often classified as contours or regions based segmentation. We have chosen a contours one, the Canny algorithm[1], which allows determining the list of edges around the shape in the image. To use this method, two threshold values have been set. As it is after mentioned, fix the right values for these thresholds is often a drawback of Canny algorithm[2]. The mandatory *threshold value* used by Canny algorithm has been

determined by analyzing the image histogram (see [3] for detail and we assume (after experiments) that the upper threshold equals 3 times the value of the lower one. 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. From the histogram, the mean(*meanHist*) and median(*medianHist*) of the intensity value in the image can be extracted. The lower threshold is set following this rule: Firstly, the minimum and maximum value between the mean and median value are calculated.

$$\begin{aligned} minValue &= \min(meanHist, medianHist), \\ maxValue &= \max(meanHist, medianHist) \end{aligned} \quad (1)$$

Secondly, we calculate the peaks and the valley in the histogram. The first peak in the histogram is the bin that has the maximum value from the beginning of the histogram to the *minValue* value. Then, the valley is determined which is the lowest value from the location of the first peak to the *maxValue*. The second peak in the histogram is the location that has the maximum value from the location of the valley to the end of the histogram. Finally, the average values between two peaks and the valley are calculated. The final threshold value is mean of two average values minus to 5% of it.

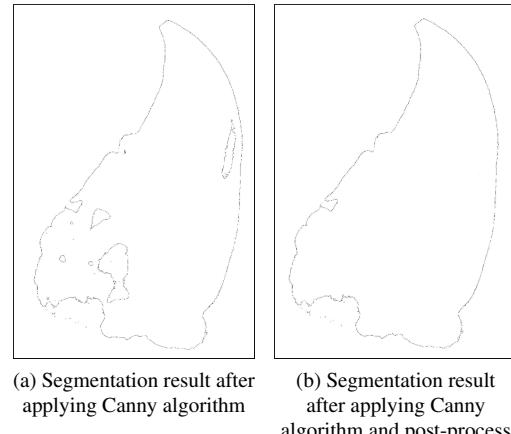


Figure 3: The segmentation results of the image

The curves from Canny algorithm will be post-processed to remove the unnecessary curves. As it is shown in figure 3, the final result of Canny generate 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 shape.

2.2 Image registration

As we have mentioned, all images have been captured following the same protocol. But it remains differences in size of the mandibles (cause of size of the animals), or orientation, position the mandible under the camera.

At this step, we will try to align the segmentations of two images before estimating the landmarks. Besides, the translation and rotation between the images are determined by Principal Component Analysis Iteration (PCAI). PCAI is the method that is improved from PCA method[6]. The work flows of PCAI are illustrated in figure 4

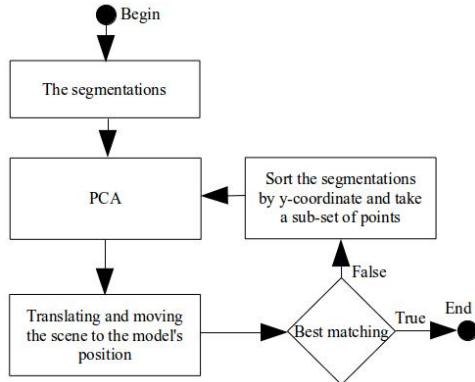


Figure 4: The flows in PCAI

PCAI received the result from segmentation step as the input. Firstly, the centroid point and principal axis of each image are defined: the centroid point is the point which has the coordinate by getting 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 curve points. The second endpoint on curve points are determined as described: For each point in the curve, we assume that it is the second endpoint of the principal axis. Then, the mean of the perpendicular distance from the remaining points to the axis is calculated. This work is finished for all points on the curve, and the principal axis is the line that has the minimum mean perpendicular distance to other points. The translation is indicated by the difference coordinate between the centroid points. The rotation angle is the angle between the principal axes. As well as, the rotation direction is determined by checking on each direction. 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 wrong indicated because the result of the segmentation may be not perfect. To make sure that we have obtained the best match between the images. We extract a sub-set of segmentation points to re-calculate the PCA. Before extracting, the points have been sorted by y-coordinate to suppress the unnecessary points. The iteration is continual until we get the best register of the segmentation.

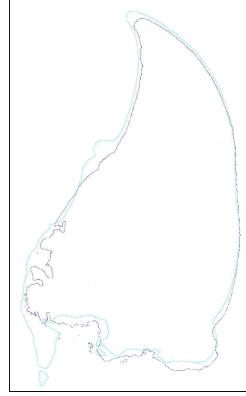


Figure 5: A registration result between two images.

Figure 5 shows an example of PCAI. In this figure, the aquamarine curve and blue curve are presented for the segmentation of the model and the scene image, respectively.

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.

As the result of PCAI, we have fitted between the model and the scene. So, by using the landmarks of the model image, we can also estimate the location of the scene image. Hence, before estimating their location, the translation and rotation are applied on scene image to match the pose between the scene and the model.

2.3 Fixing the estimated landmarks

The last step provides the best matching between manual landmarks and estimated landmarks. Now we are focus soon the determination of the landmarks position on the scene. The first information is given by the manual landmarks of the model which manually set by the biologist. This has to be done by using SIFT[4] method. Firstly, the regions around each manual and corresponding automatic landmark are created. Then, the orientation and gradient magnitude of each pixel in the region are calculated and presented into a descriptor. At the end, the comparing between the descriptors is done by using L_2 distance which is given by the equation (1).

$$L(x, y) = \sum_{x_i, y_i} \sqrt{(x_i - y_i)^2} \quad (2)$$

Where x_i, y_i are the corresponding location in the descriptors.

The figure 6 is the illustration of this process. For each manual landmark on the model and corresponding estimated landmark on the scene, the small patches I , T are created with the size s_1 , s_2 , respectively ($s_1 < s_2$). Next, for each pixel in the patch T , a sub-patch T' is extracted with the same size of the patch I . Then, their descriptor and measure distance are determined(between I and T'). This process is finished when all the pixels on the patch T are considered.

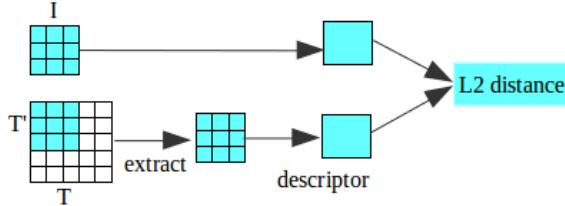


Figure 6: Illustrate the steps of descriptors comparing.

During the descriptor creation (I and T'), the orientation and gradient magnitude are calculated for each their pixel by the equation:

$$\begin{aligned} m(x,y) &= \sqrt{(L(x+1,y) - L(x-1,y))^2 + (L(x,y+1) - L(x,y-1))^2} \\ \theta(x,y) &= \tan^{-1}((L(x,y+1) - L(x,y-1)) / (L(x+1,y) - L(x-1,y))) \end{aligned} \quad (3)$$

Where:

- $m(x,y)$ is the gradient magnitude of the pixel at position (x,y)
- $\theta(x,y)$ is the orientation of the pixel at position (x,y)
- $L(x,y)$ is the value at position (x,y) in the image

Then, the descriptor is created for the patch. 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.

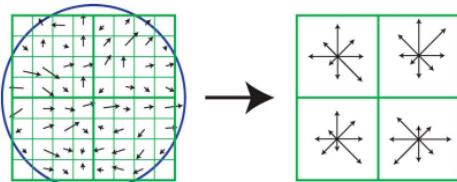
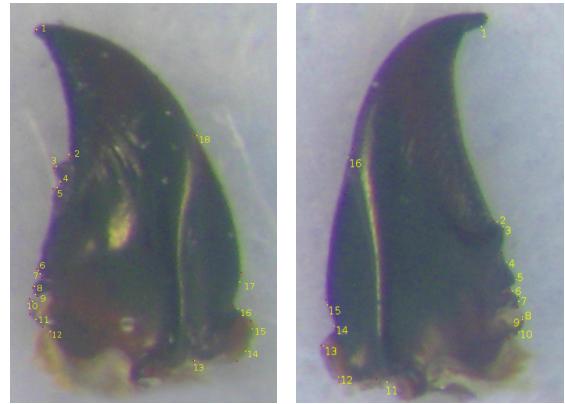


Figure 7: A landmarks descriptor is created by computing the orientation and gradient magnitude

In the example at figure 7, a patch with the size 16x16 is created 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 patch is divided into 4 sub-blocks 4x4. The

descriptor of each sub-block is determined 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.

As we mentioned before, the last location of estimated landmarks is the pixel in template T that has the smallest measure distance value (by $L2$ distance). Finally, the automatic landmarks are inverted to match with the real location in the scene image (by inverting the transformation and rotation).



(a) Result on right mandible (b) Result on left mandible
Figure 8: The automatic landmarks on mandibles

Figure 8 shows a complete result on one scene mandible with the manual landmarks (red points) and estimated landmarks (yellow points).

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 mandible. After verifying the quality of the image, it remains 290 usable images for right mandible and 286 images for left mandible. The removed images include the images that do not contain the mandible and the mandible is broken.

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 [7]).

Firstly, our evaluation is done on comparing the centroid size of the estimated landmarks and manual

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

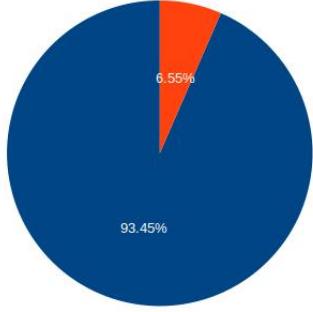


Figure 9: The percentage of correct proportions on right mandibles

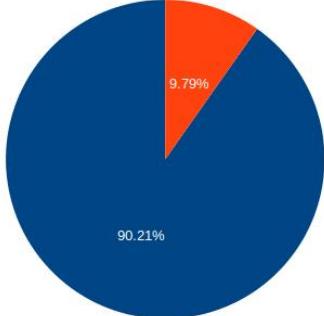


Figure 10: The percentage of correct proportions on left mandibles

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 centroid size of each image is calculated and evaluated. We can see in figure 9 and figure 10 for all images, the correct proportions of centroid size which based on the estimated landmarks are 93,45% for the right mandible and 90,21% for the left mandible. And the results in figure 9 and 10 were a vindication of the propriety of the method.

Besides, using the centroid size to evaluate the method. We are also interested in 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 automatic landmarks.

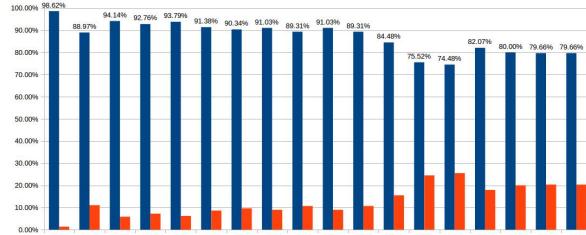


Figure 11: The correct proportions on each landmark of right mandibles

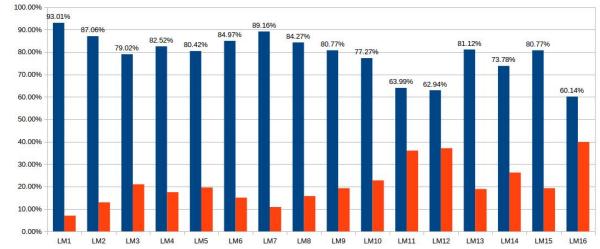


Figure 12: The correct proportions on each landmark of left mandibles

Figure 11 and 12 show the correct proportion on each landmark of mandible. The blue column is presented for the success rate. The orange column is expressed as the incorrect rate. With 18 landmarks of right mandible, the position of the first automatic 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.

In both of experiments, the success rate on right mandible is always greater than left mandible. So, when we reconsider the datasets, the images in left mandible are having more type of size than on the right mandible (scale problem). This explains why the success rate on right mandible is always higher than left mandible.

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 [3]). 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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