

# Automatic morphology: Application on biological images

LE Van Linh	BEURTON-AIMAR Marie	KRAHENBUHL Adrien	PARISEY Nicolas
LaBRI-CNRS 5800 ITDLU, Dalat Univ-V linhlv@dlu.edu.vn/ van-linh.le@labri.fr	LaBRI-CNRS 5800 Bordeaux University 33400 Talence-F beurton@labri.fr	LaBRI-CNRS 5800 Bordeaux University 33400 Talence-F adrien.krahenbuhl@labri.fr	IGEPP INRA 1349 35653 Le Rheu-F nparisey@rennes.inra.fr

## 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 feasibility of automatically positionning landmarks on biological images. The set of images contains 293 beetles and for all an image of the left and right mandibles. For each mandible, a set of 16 and 18 landmarks (resp. left and right) have been set manually. In a previous work[3] based on Palanaswami article [5] we have shown that if we consider the centroid measure of the mandible as the parameter to obtain, the probabilistic Hough Transform can provide very interesting results. But in the case of the main goal is to fix landmarks to get property about specific area (defined by biologists) the result is not as precised as we have expected. In this next turn, we have kept the same method to segment and to register scene images with model but we have change the way to set the model landmarks on the scene. This new procedure uses a SIFT algorithm as the last operation of our process. We will compare all the obtained results in order to define the new workflow to label the mandible images.

## 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 biologists. 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 four stages: firstly, we extract the features of the object in the image; secondly, generalizing Hough transform is used to detect the presence between two images; thirdly, the translation and rotation is identified by image registration; finally, a refinement of the estimated landmarks is done by comparing the descriptors between model landmarks and estimated landmarks.

In section 2, the steps of our methods will be discussed. All experiments and evaluation are described in section 3. Finally, we have some conclusion in section 4.

## 2 METHOD

For each image, a set of manual landmarks have been set by biologists corresponding to the morphological points of interest (18 landmarks for each right mandible and 16 landmarks for each left mandible). The automatic procedure to determine the landmarks is based on the segmentation of the image. The presence between two images are indicated by generalizing Hough transform, along with theirs translation and rotation are determine by applying registration. The last step is using descriptors measure (L2 distance) to verify the location of estimated landmarks.

### 2.1 Image segmentation

In the methods of image processing, feature extraction is always a important stage. Besides, depending on the nature of the method, the methods are applied before (pre-processing) or after (post-process) extracting the features. In our method, the original Canny algorith[m][2] is idea for detect the curves on the image. The ratio between lower threshold and upper threshold which are used in Canny algorithm is 1:3 (lower threshold approximated to 1 times *threshold value* and upper threshold set to 3 times threshold value). This value is evaluated by the experiments. The *threshold value* has been determined by analyzing the image histogram. During appling the Canny algorith[m] to detect the curves of object, the gradient direction of each pixel which belongs to the curves is kept for the next steps of the method.

In this study, the aim of segmentation stage is determined the outer border of the object which can be used to reconstruct the shape of the object as well as provide the best data for next step. The curves from Canny algorith[m] will be post-processed to remove the unnecessary curves i.e hole inside the border (see figure 2).

### 2.2 Generalizing Hough transform

The generalizing Hough transform (GHT)[1] is one of the keys of this study. With two input images,

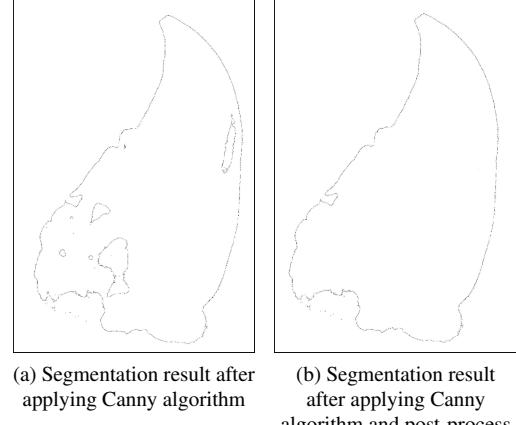


Figure 2: The segmentation results of the image

GHT is used to recognize the presence between two images. The GHT includes two phases: training and recognition.

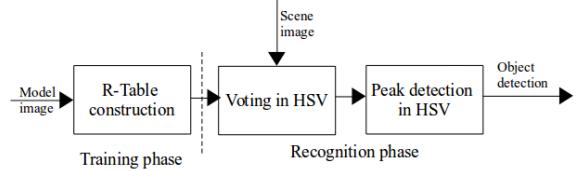


Figure 3: Block diagram of GHT

In the training phase of GHT, model image is used to construct R-table. This is table that contains the polar value of each point on model's curves (the result of segmentation). A polar coordinate system is initialized in the model image by fixing a reference point and using it as origin. The R-table records the polar coordinate values of all boundary points. The rows of R-table are indexed by the gradient directions of the points on the curves. It means that with a gradient direction can be exists many polar coordinate values.

Index	Polar values
gradient 1	(r1,l1), (r2,l2),(r3,l3)
gradient 2	(r2,l2),(r4,l4),(r1,l5),...
...	...
gradient n	(r1,l1),(r4,l5),(r5,l5),...

Table 1: An example of R-Table

During the recognition phase, a 2D accumulator is used, called Hough Space Voting (HSV). The axes of HSV express for the information of polar coordinate system. For each point on scene's curves, we try to find a record

in R-table that corresponding with the gradient direction of each scene point. The voting will be carried out at all location in HSV that found in R-table. At the end of voting process, if the scene image is identical to the model image, then the cell where have the highest number of votes corresponds to the reference point of the model in the scene image. Besides, the peak value would be equal to the number of curve points of the scene image when the model and scene image match perfectly.

### 2.3 Image registration

Our study is working on the 2D image, and the transformation between two images are inevitable. Though, GHT is just hired to detect the presence of the model image in the scene image. Translation and rotation are determined by applying an alignment method based on the set of boundary points of two images. In this case, we use Principal Component Analysis Iteration (PCAI) to finish this work. This method is improved from PCA method[6]. Firstly, the centroid point of each image is defined by getting the mean coordinate of all boundary points. Secondly, a principal axis of the images are indicated, this is a connected line from centroid point to a point in the list of curve points. For each point in the curve, we create a line from centroid point to them and assume it as an axis. The mean perpendicular distance from the remaining points to the axis are 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 translation between two centroid points. The rotation is the angle between the principal axis of two images. As well as, the rotation direction is determined by checking each direction. However, in some case, the translation and rotation between two images are wrong indicated. To make sure that we have obtained the best match between the images. We applied an iteration to get the best register of them. And instead consider all the points in the curve, we just need to consider a subset of it. For that, the curve points are sorted by y-coordinate first. Then a half points are extracted to check. The PCAI is applied on two new subsets until obtaining best matching of two images. Figure 4 shows two curves after registration by PCAI. The aquamarine curve and blue curve are segmentation of the model and the scene image, respectively. At the end of PCAI, a hypothesis is made to judge 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.

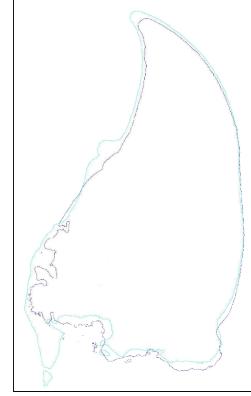


Figure 4: A registration result between two images.

At the result of PCAI, we have the fitting between model and scene. The landmarks of the scene image are estimated by the manual landmarks of model image. Hence, before estimating the landmarks, a translation and rotation are applied on scene image to match the pose between the scene and the model.

### 2.4 Refinement the estimated landmarks

The GHT and registration provide to us the best presence between the model and the scene. The automatic landmarks can be estimated for the scene by the manual landmarks of the model. However, the estimated landmarks can be located at the incorrect location. So, we need a method to verify each position of the estimated landmarks. For this reason, in the last step of the method, we apply a step in the SIFT[4] method to verify the location of automatic landmarks. Firstly, the region around each manual and corresponding automatic landmark are created. Then the orientation and gradient magnitude of each pixel in the region is calculated and presented into a descriptor. The comparing between the descriptors is done by using L2 distance which is given by the equation (1).

$$L(x, y) = \sum_{x_i, y_i} \sqrt{(x_i - y_i)^2} \quad (1)$$

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

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 ( $s_1 < s_2$ ). Then, 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 step is finish when all the pixels on the patch  $T$  are considered. The figure ?? is the illustration of this process.

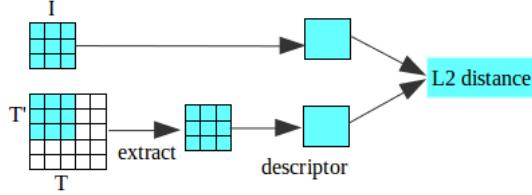


Figure 5: Illustrate the steps of descriptors comparing.

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

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

Where:

- $m(x,y)$  is the gradient magnitude
- $\theta(x,y)$  is the orientation
- $L(x,y)$  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 corresponding to the magnitude of that histogram (sum of the gradient magnitude for corresponding 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.

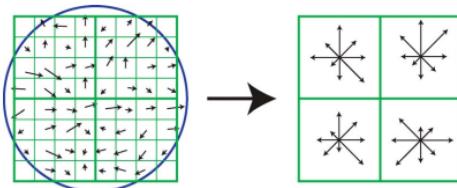
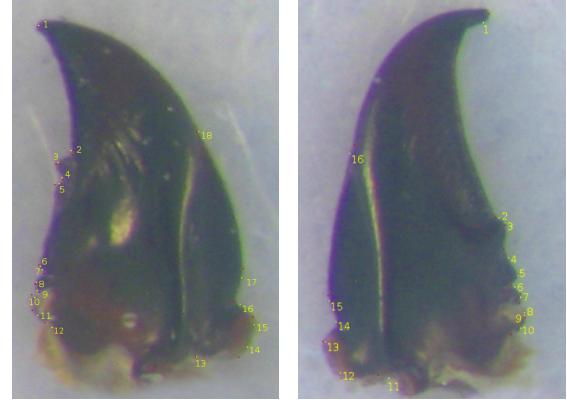


Figure 6: A landmarks descriptor is created by computing the orientation and gradient magnitude

In the example at figure 6, a patch with the size  $16 \times 16$  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  $4 \times 4$ . 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. 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 7: The automatic landmarks on mandibles

Figure 7 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-Lab<sup>1</sup>. Two sets of beetle have been analyzed, right and left mandible. After verifying the quality of the image, it remains 288 usable images for right mandible and 285 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]).

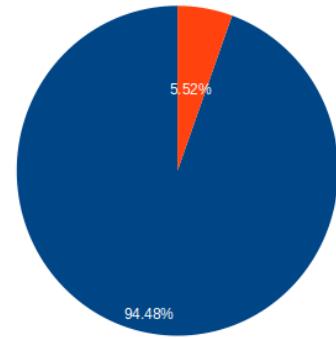


Figure 8: The percentage of correct proportions on right mandibles

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

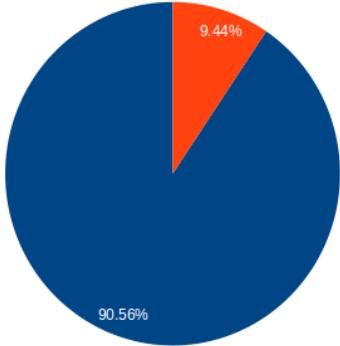


Figure 9: The percentage of correct proportions on left mandibles

By this way, we have compared the centroid size between manual landmarks and estimated landmarks. We can see in figure 8 and figure 9 for all images. The correct proportions of estimated landmarks are 94,48% for the right mandible and 90,56% for the left mandible. 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. And the results in figure 8 and 9 were a vindication of the propriety of the method.

Besides, using the centroid size to evaluate the method. We also consider the position of the estimated landmarks to verify the method. In this experiment way, we calculate the distance between each manual landmark and corresponding automatic landmark.

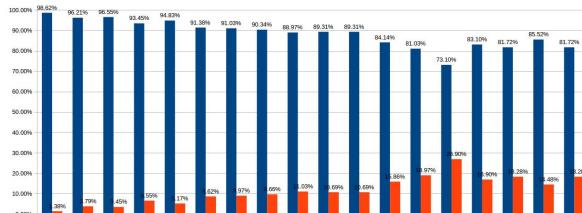


Figure 10: The correct proportions on each landmark of right mandibles

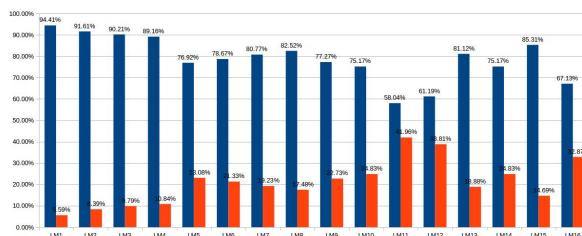


Figure 11: The correct proportions on each landmark of left mandibles

Figure 10 and 11 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 73,1% for fourteenth landmark. The remaining landmarks are also indicated with a high proportion (with the accuracy proportion greater than 80%). For left mandible, the highest and lowest success rate are 94,41% for the first landmark and 58,04% for the eleventh landmark. When we saw the datasets, the images in left mandible are having more type of size than on the right mandible. This explains why the success rate on right mandible is 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 GHT and image registration to 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.

## 5 REFERENCES

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