

Drosophila wings segmentation

Medical Image Analysis

Author: Vittorio Spadolini, Yinshuang Zhou

Background

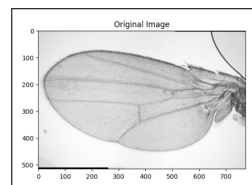
This report presents an automated system designed to segment *Drosophila* wings. *Drosophila* wings offer an exceptional subject for automated image analysis due to their straightforward structure and significance in genetic development, morphometrics, and evolutionary studies [1]. Despite the broad utilization of *Drosophila* wings in scientific inquiry, the current landscape of morphometric studies often relies on manual annotation of landmarks or key points, a labour-intensive process that has fueled interest in automation [2][3]. The project workflow began with a concise literature research to assess the state-of-the-art. Subsequently, a set of appropriate algorithms was chosen and developed in Python, and finally, the best segmentation method was utilized and evaluated on different test data.

Methods

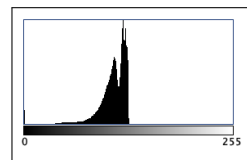
Various segmentation techniques were used to segment the wing boundaries and internal structures, nevertheless, a common procedure was used for each tested data.

1. Separate the wing from the background
2. Detect internal wing frontiers

We utilized the histogram to gain insights into the image's pixel intensity distribution, guiding the application of diverse segmentation techniques for enhanced analysis.



(a) Original *Drosophila* wing



(b) Histogram of pixel intensities

Figure 1: *Drosophila* wing and corresponding histogram

From the histogram the image is roughly divided into three modes of pixel intensity. Image background with higher intensity values, wing cell material and trailing edge from mid to high intensity values, wing vascular structure, leading edge and fly body with lower intensity values.

Thresholding

The first segmentation technique applied was global thresholding, used to separate objects from the regions of interest from the background in the grayscale image according to pixel intensity histogram. Resulting in a binary image where pixels representing the wings are set to black colour, and the background is set to white. In our case, a manual threshold value (threshold = 100) is set based on the analysis of the histogram.

Adaptive thresholding

As an improvement to the traditional global thresholding method, adaptive thresholding is used to deal with localised lighting variations. This method dynamically adjusts its threshold value according to the local neighbourhood characteristics of each pixel. The output of adaptive thresholding is a binary mask. In this binary mask, pixels are divided into two categories: pixels below the local adaptive threshold are considered part of the foreground, while pixels above the threshold are considered part of the background. This binary mask typically provides a fine division between foreground and background elements in the presence of local lighting variations.[4]

Watershed

The watershed segmentation method was used for partitioning the image into regions based on the topography of intensity gradients.

Skeletonization

Skeletonization was used to reduce the binary image to a "skeleton" or a thin representation of the wing structures while preserving the connectivity and topology.

CLAHE algorithm

In our project, we employed a multi-step image processing approach to enhance the visibility of intricate details in *Drosophila* wing images, it involved the following procedure:

1. The Contrast Limited Adaptive Histogram Equalization (CLAHE) algorithm was applied for contrast enhancement, emphasizing local variations and revealing finer structural details.

2. To further refine the segmentation process, background exclusion was implemented. This involved subtracting a locally averaged version of the contrast-enhanced image, effectively filtering out the background and highlighting features of interest.
3. The subsequent step involved binarization, where thresholding was applied to convert the background-excluded image into a binary format. This transformation facilitated the clear distinction between foreground and background elements.
4. In order to improve the segmentation's precision, we incorporated a step to remove smaller components from the binary image. This cleaning process was essential for eliminating noise and artifacts, resulting in a more accurate representation of the primary features.
5. To enhance the network of veins within the segmented image, morphological opening was employed. This involved morphological operations to smooth and refine the binary image, contributing to a more robust delineation of the intricate vein structures.

Sato algorithm

In our project, we applied the Sato filter which is based on the eigenvalues of the Hessian matrix to compute the similarity of an image region to tubes. The procedure involved the following steps:

1. Incorporating the Sato filter for edge enhancement to emphasize edges and linear structures within grayscale *Drosophila* wing images. Known for its effectiveness in highlighting features such as blood vessels, the Sato filter accentuated the structural details of interest.
2. Following the application of the Sato filter, we employed thresholding and binary mask creation to transform the filtered image into a binary mask. This process involved setting a threshold to distinguish potential edges from the background, where pixels above the threshold indicated potential edges, and those below represented the background.
3. To isolate and enhance the identified edges, an inversion step was introduced. Pixels in the binary mask falling below the threshold were inverted, effectively isolating potential edges and improving their visibility against the background.
4. Further refinement of the detected edges was achieved through the application of morphological operations. Specifically, morphological opening and closing operations were utilized to enhance the continuity of the detected structures and reduce noise. These operations contributed to a more precise delineation of edges and improved the overall quality of the enhanced image.

Results and discussion

The resulting images reveal the shortcomings of various segmentation methods. Both threshold and watershed segmentation methods proved inadequate in addressing the segmentation challenges, leading to the inaccurate removal of intervein regions and accidental removal of portions of the leading edge. Additionally, skeletonization did not yield a satisfactory outcome, resulting in overly thin and disconnected structures that failed to capture the wing's topology effectively.

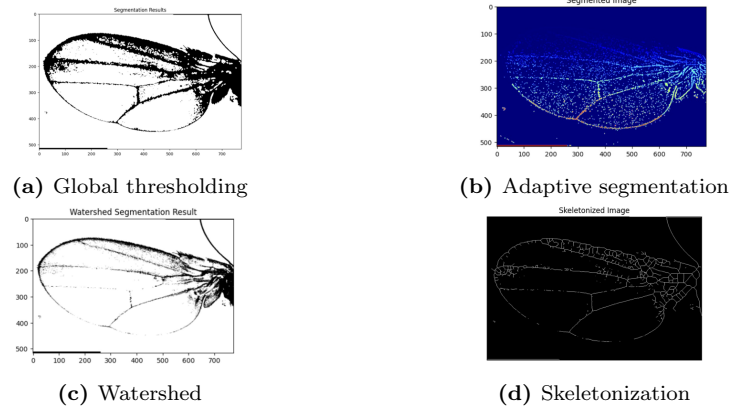


Figure 2: Results from global thresholding, adaptive thresholding, watershed segmentation, and skeletonization, collectively indicate the necessity for a more refined approach in addressing the challenges of Drosophila wing segmentation.

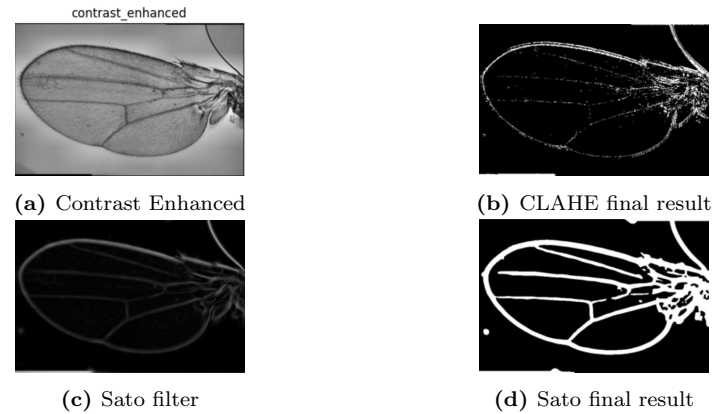


Figure 3: CLAHE and Sato provide much better results compared to the previous techniques

CLAHE segmentation is based on contrast enhancement with background exclusion, it leads to a successful wing segmentation. The algorithm effectively reduces intervein artifacts, however the overall wing structure can be more accurately segmented. On the other hand, Sato segmentation which is based on ridge operator leads to a successful wing segmentation. The algorithm effectively reduces most of the intervein artifacts, the overall wing structure seems accurately segmented.

Quantitative analysis of CLAHE and Sato algorithms

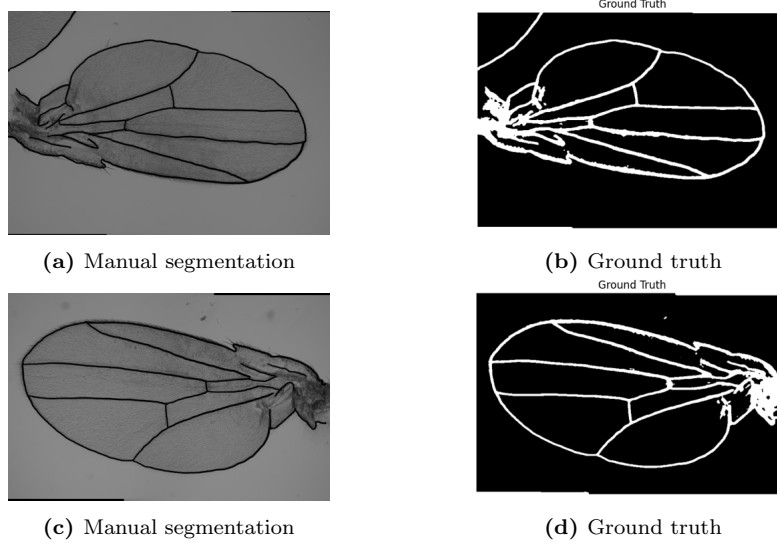


Figure 4: Manual segmentation and relative binary mask applied on the test dataset

Based on the outcomes, it is evident that both the CLAHE and Sato algorithms exhibit superior performance in wing segmentation. The next step involves assessing their efficacy on a distinct image dataset and comparing their results against a gold standard to determine the optimal algorithm. Manual segmentation was employed to generate a binary ground-truth mask, serving as a reference for the evaluation of segmentation results (Figure 4). The evaluation metrics we used include IoU, Dice Coefficient, Precision, Recall, F1 and AUC. According to the evaluation results Table 1, both CLAHE and Sato algorithms achieved high scores for Precision, which indicates good performance in positive predictions. What's more, the Sato segmentation method exhibits superior IoU, Dice coefficient compared to CLAHE, demonstrating better performance in overlapping with the ground truth. Overall, Sato consistently outperforms CLAHE across all metrics, indicating its superior segmentation performance on

the evaluated images.

Metric	CLAHE	Sato
IoU	0.25	0.67
Dice coefficient	0.40	0.80
Precision	0.77	0.78
Recall	0.27	0.82
F1	0.40	0.80
AUC	0.62	0.89

Table 1: Comparison of segmentation metric results for the two algorithms

Validation of Sato algorithm

In order to assess the robustness of the Sato algorithm, a comprehensive validation has been carried out on different datasets (3 different images). The performance of the algorithm was systematically evaluated through the metrics mentioned before.

Metric	Image 1	Image 2	Image 3
IoU	0.67	0.51	0.50
Dice coefficient	0.80	0.68	0.67
Precision	0.78	0.54	0.52
Recall	0.82	0.90	0.93
F1	0.80	0.68	0.67
AUC	0.89	0.90	0.91

Table 2: Segmentation metric results for Sato algorithm

The implemented Sato algorithm demonstrated significant efficiency in segmenting the images, effectively reducing the intervein regions while preserving the overall structural integrity (Figure 5, only two results are presented, however, the statistical analysis was performed with an additional image not presented). The results were evaluated by quantitative metrics derived from the segmented images, they are presented in Table 2 and show that the algorithm performs "relatively well" with minimal deviations observed between the three different datasets. This consistency suggests a high level of reliability for the image sets evaluated.

However, the fine-grained analysis showed a slight decrease in segmentation scores for the second and third images compared to the first image. The reason for this difference can be addressed by different operators during the manual segmentation process. It is worth noting that different operators affect the segmentation of the ground truth, leading to subtle differences in algorithm performance.

This observation highlights the importance of understanding the impact of operator choices on algorithmic results and emphasises the need for careful consideration and standardisation when dividing ground truth between different datasets. As a final consideration, it is important to highlight the necessity for a diverse and larger set of images for testing. This is crucial to ensure algorithm robustness and generalization, promoting further development and uncovering limitations that drive iterative refinement.

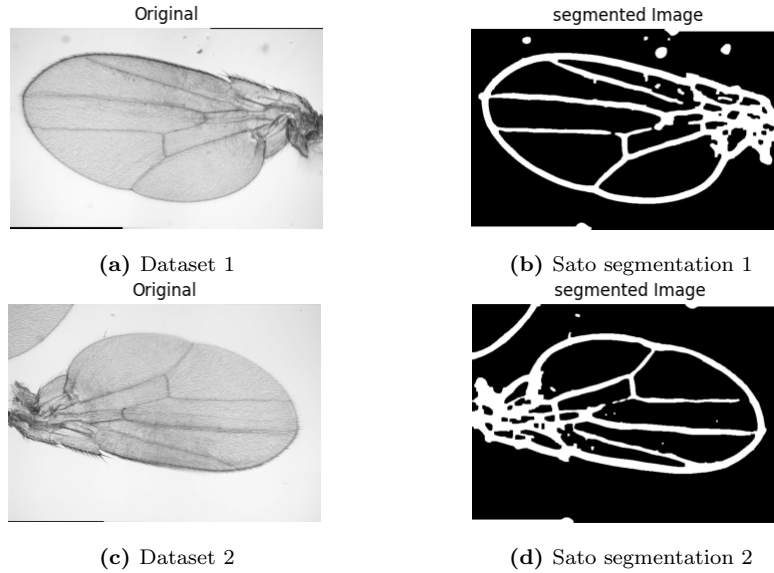


Figure 5: CLAHE algorithm on 2 different test images

References

- [1] Barbara H Jennings. “Drosophila—a versatile model in biology & medicine”. In: *Materials today* 14.5 (2011), pp. 190–195.
- [2] David Houle et al. “Automated measurement of Drosophila wings”. In: *BMC evolutionary biology* 3.1 (2003), pp. 1–13.
- [3] Sebastian Bas Kanã. *Automatic landmark identification in digital images of Drosophila wings for improved morphometric analysis*. 2019.
- [4] Gregory D Young. *Image segmentation and paired shapes asymmetry quantification: An application in a Drosophila wing image set*. California State University, Long Beach, 2015.