**Automated recognition and analysis of head thrashes and omega turns behavior in C. elegans**

**1 Abstract**

Neurodegenerative illnesses have drawn a lot of public interest recently. These illnesses have a serious negative impact on patient's quality of life and may potentially endanger their lives. To better understand the pathophysiology of these illnesses and find viable treatments, scientists are focusing more and more on model organisms. Model organisms are essential to the progress of the biological sciences and are employed extensively in various fields. The nematode is an invaluable model organism in the research of neurodegenerative disorders. Understanding the relationship between nematode motor neurons and their motor activity is essential for comprehending the pathogenesis of neurodegenerative diseases. Among its several motor movements, the worm's head thrash and omega turn behaviors are chosen as indicators of the function of its motor neurons. It is possible to count head thrashes and omega turns automatically by using a nematode head and tail positioning model, which precisely recognizes the nematode's head and tail positions. The link between motor behavior and motor neurons can be illustrated by contrasting the variations in nematode strains' head thrashes and omega turns. This suggests that nematode behavior's automated counting findings can be a useful indicator of motor neuron integrity.

**2 Introduction**

Serious risks to human health and quality of life are posed by neurodegenerative disorders, which are chronic, widespread illnesses. The number of individuals afflicted by these diseases is rising year as aging worsens[1]. Among the common neurodegenerative disorders are epilepsy[2], Alzheimer's disease (AD)[3], and Parkinson's disease (PD)[4], among others. Furthermore, "Lou Gehrig's disease," or amyotrophic lateral sclerosis (ALS)[5], is a neurodegenerative illness similarly brought on by abnormalities in motor neurons. Gradually, to better understand the pathophysiology of these illnesses and find viable treatments, researchers have shifted their focus to model species.

Model organisms are essential resources that scientists use to look at particular biological processes. They are invaluable stars in biology and have repeatedly graced the Nobel Prize podium. For instance, the drosophila melanogaster is a crucial model organism for studies on genetics and development, and it has helped scientists earn numerous awards for their work in science[6]. Because it is a model microbe, Escherichia coli has contributed significantly to the advancement of molecular biology, biotechnology, microbiology, and bioinformatics[7]. In addition to drosophila melanogaster and Escherichia coli, the nematode, or Caenorhabditis elegans (C. elegans), is also an important model organism. It has played a landmark role in developmental biology and graced the Nobel Prize podium [8]. Nowadays, *C. elegans* is widely employed in many different disciplines of research, such as aging[9], development[10], neuroscience[11], behavior[12], genetics[13], drug screening[14], and toxicology[15], and so forth.

Nematodes are a special model organism that has several benefits for research on neurodegenerative illnesses. Firstly, they require less space to breed because of their small size and simplicity of cultivation. Second, shorter experiment durations are possible because of their brief developmental cycle. Furthermore, the transparent bodies of nematodes made it convenient for researchers to view interior structures and biological processes like cell division and death. Moreover, C. elegans' genetic features enhance its usefulness as a model organism. First, nematodes have a small genome—just six chromosomes and one mitochondrial genome. As a result, C. elegans became the first multicellular organism to have its whole genome sequenced[16], and it remains the only organism whose cells can all be traced individually[17]. Additionally, homologous genes in *C. elegans* account for 60-80% of human genes[18], and many homologs of human disease-causing genes are present in *C. elegans*[19]. Within the nematode's genome sequence, are 533 genes homologous to those associated with human diseases, and among the discovered signaling pathways, 12 are homologous to humans[20]. This makes nematodes an important tool for studying the mechanisms of human diseases and for screening potential drug candidates. Therefore, as an important model organism, nematodes provide scientists with a valuable research platform due to their unique biological characteristics and genetic similarity to humans. These features give nematodes significant value in the study of neurodegenerative diseases[21].



Fig.1 General outline of the proposed method

Neurodegenerative diseases are often caused by defects in motor neurons, such as amyotrophic lateral sclerosis, and motor behavior can be used to reflect the functional status of motor neurons[22]. Therefore, studying the relationship between the motor neurons of nematodes and their motor behavior is crucial for a deeper understanding of the pathogenesis of neurodegenerative diseases. To achieve this goal, it is essential to quantitatively study the motor behavior of nematodes. In previous studies, nematodes have exhibited many types of motor behaviors that can be quantitatively studied, such as head thrashes, body bending, forward and backward movement, etc. Considering the sensitivity of head thrashes behavior and the ease of observing omega turns in nematodes, these two types of motor behavior were chosen as the research objects. Through the quantitative analysis of these specific motor behaviors, the motor capacity of nematodes can be more accurately assessed, indirectly reflecting the functional status of their motor neurons. However, in practical operation, the quantitative study of nematode motor behavior is usually carried out manually[23], which requires experimenters to undergo prior training and invest a significant amount of time in counting. Nevertheless, the limitation of manual counting lies in its inapplicability to long-term continuous experiments and its difficulty in meeting the demands of large-scale counting tasks. With the continuous advancement of computer technology in the field of image processing, automatic counting methods have emerged, providing new solutions for the study of nematode motor behavior.

For instance, Swierczek et al. proposed a real-time computer vision system called the Multi-Worm Tracker (MWT)[24], which can quantify the behavior of dozens of *C. elegans* worms on a petri dish at the same rate as video. This means that the MWT system can quickly and efficiently quantify the behavior of the worms with minimal human effort. However, when the number of worms being processed by the MWT system becomes too large, the system load can become excessive, potentially leading to frame drops. Additionally, as the number of worms increases, the number of collisions between them also rises. When two or more worms collide, the system will sum their sizes and treat them as a single entity until they separate again and are recognized as individual worms by the search algorithm[25]. Therefore, this situation can lead to loss of worm characteristics, affecting experimental results. Moreover, the MWT system is fundamentally unable to distinguish the head and tail of the worms[24], making it impossible to analyze the head movements. These limitations can impact the accuracy of experimental results.

WormLab[25] is another commercial software for worm tracking that can track the center, head, or tail markings of selected worms. It can also analyze the speed, position, area, direction, wavelength, trajectory length, omega turns, and reversals of the selected worms. WormLab defines the supplement angle between the worm's head, midpoint, and tail as the bend angle, and considers worms with bend angles exceeding 90° as having performed an omega turn[26]. However, the typical definition of an omega turn is when a worm's head is almost in proximity to its tail, or when there is a 135° reorientation during a single head bending[27]. This means that when the bend angle of the worm exceeds 90°, the worm's head and tail may not necessarily be close together, or there may not have been a reorientation of the head.

Other researchers have also proposed different automatic counting methods. For example, Zhang et al. presented a method for automatically identifying and counting individual worm head thrash behaviors from experimental videos, achieving automatic recognition of worm head thrashes[28]. This method uses two criteria to distinguish the head and tail of the worm when identifying the worm's head. The first criterion is that the worm's head is rounder than its tail, so the sharpest point on the worm's outline is defined as the tail. The second criterion is that the worm's tail is darker than its head, so by comparing the brightness of the two endpoints in the binary image, the endpoint with a lower brightness is defined as the tail. When the results of these two criteria are inconsistent, manual judgment is required to determine the head and tail of the worm.

In summary, although there are existing automatic analysis tools available for worm locomotion behavior, including both commercial and free tools, these methods still have some limitations. For example, some methods may not accurately capture complex behaviors such as head thrashes and omega turns in worms. Some methods also differ in their definition of behaviors compared to traditional definitions, and others require manual intervention, leaving room for improvement. Therefore, in this study, considering the drawbacks of manual counting and existing automatic counting methods, a model for locating the head and tail positions of worms was constructed. An automatic recognition method for individual worm head thrashes and omega turns was proposed. This method successfully achieves accurate differentiation between the worm's head and tail and can simultaneously analyze head thrashes and omega turns. It not only preserves the characteristics of worm locomotion but also aligns with the traditional definition of worm locomotion behaviors. Moreover, this method does not require manual intervention during the implementation process. Based on this, we further analyzed the relationship between worm locomotion behavior and the functional state of its locomotion neurons, providing a more precise and automated tool for studying worm locomotion behavior.

**3 Method**

This section will provide a detailed overview of the proposed method, including worm head and tail localization, video and image processing, and automatic counting methods. The entire workflow of the method is illustrated in Figure 1, where each step is interconnected, forming a complete automated counting process.

**3.1 Nematodes head and tail localization method**

To automatically count the head thrashes and omega turns of nematodes, it is necessary to determine the positions of the nematode's head and tail. Distinguishing between the head and tail of the nematode is a crucial step in this process. Fortunately, the head and tail of the nematode have different characteristics. For example, due to the difference in the distribution of fat, the nematode's head is brighter than its tail; because of the nematode's foraging behavior, the head thrashes more frequently than the tail; the head is wider than the tail; and the positions of the head and tail relative to the center of the body do not change much, among other differences[29]. Because of these distinct characteristics, the head and tail of the nematode can be differentiated.



Fig.2 The head and tail position localization model

Considering the excellent performance of Convolutional Neural Networks (CNNs) in the field of image processing, we have decided to apply them to the task of locating the head and tail of nematodes. CNNs are capable of automatically learning features in images and have achieved outstanding performance in tasks such as recognition, classification, segmentation, and generation[30]. In recent years, many state-of-the-art CNN architectures have emerged, such as VGGNet and ResNet, which have achieved remarkable results on various tasks and datasets. EfficientNet[31] is also a type of CNN that not only exhibits excellent performance but also excels in resource utilization and efficiency, making it highly suitable for our needs. Therefore, we have chosen EfficientNet as our tool because it performs better than other models with the same computational resources. To further improve the model's performance, we will employ transfer learning techniques[32]. Transfer learning leverages the existing knowledge of pre-trained models, accelerating model training, enhancing performance, and effectively addressing the issue of scarce data. This allows us to obtain better results in a shorter amount of time.

Additionally, the original grayscale images of nematodes preserve more morphological features. Therefore, we will use grayscale images as the dataset for our model. This will help the model capture the subtle characteristics of nematodes more accurately, thereby improving the accuracy of head and tail localization. To summarize, we will utilize EfficientNet combined with transfer learning techniques, using grayscale images as the dataset, to achieve precise localization of the head and tail of nematodes.

To achieve the localization of the head and tail of nematodes, we have taken the following steps. First, we randomly extracted 2000 grayscale images from videos of different nematode strains from the nematode behavior phenotype database. Then, using the open-source image annotation tool Labelme[33], we annotated these grayscale images, recording the coordinates of the nematode's head and tail. To make the dataset more compatible with the pre-trained model, we resized all grayscale images to a width and height of 224 pixels and correspondingly scaled the annotated coordinates of the head and tail to match the resized grayscale image dimensions. Next, we built the model. We use EfficientNetB0 as the base model, which was trained on the ImageNet dataset, providing good pre-training results. On top of EfficientNetB0, we added an attention mechanism layer to enhance the model's focus on specific regions[34]. Specifically, we first used Global Average Pooling to reduce the dimensionality of the feature vectors. Then, we used two Dense (fully connected) layers to convert the downscaled feature vectors into attention-weight vectors. During this process, we employed L2 regularization to constrain the model's complexity. Next, we used a Multiply layer to perform element-wise multiplication between the original feature vectors and the attention weight vectors, thereby obtaining weighted feature vectors. Finally, we added two Dense layers to the base model of EfficientNetB0, which respectively output the predicted positions of the head and tail. In this way, we completed the construction of the head-tail localization model. The schematic diagram of the model is shown in Figure 2. Through the above steps, we ensured the logical consistency of the data processing and model construction processes, ultimately achieving precise localization of the head and tail of nematodes.

It should be noted that during the model training process, 80% of the images in the dataset are used for the training dataset, while 20% are used for the validation dataset. The number of training epochs is set to 300, with a batch size of 64, and a learning rate of 0.0001. The Adam optimizer is employed for parameter updates, and the model parameters are updated through backpropagation to optimize the prediction results. The Mean Absolute Error (MAE) serves as the evaluation metric, while the Mean Squared Error (MSE) functions as the loss function. MAE measures the model's predictive performance, while MSE quantifies the disparity between the model's predictions and the actual values. The calculation method for MAE is as follows

The mean squared error is defined as

The can be defined as the number of samples, and represent the true head and tail positions, and the predicted head and tail positions, respectively.

**3.2 Video and image processing method**

To achieve automatic counting of nematode locomotion behaviors, we need to process experimental videos containing the nematodes. These videos are sourced from a nematode behavioral phenotypes database[35] but do not have consistent durations. To ensure the scientific integrity of our experiments, we extract one-minute video segments for each experiment after filtering the nematode videos from different strains. This approach guarantees that each experiment has the same duration for analysis purposes. Furthermore, to capture more details during nematode locomotion, we extract each frame of the video as an image. This enables us to capture the complete process of nematode movement. This workflow ensures consistent data sources for analyzing nematode behavior and allows us to observe all subtle movement changes.



Fig.3 Schematic diagram of the image processing process and calculating head bending angle and head-to-tail distance (a) Frames were extracted from the experimental video and read in as greyscale images. (b) Processing greyscale images into binary images. (c) Fill the contour with the largest area in the original binary images. (d) Performs an XOR operation on the original binary images and the filled images. (e) The clean binary image n. (g) The distance between the head and tail of the worm. (h) To calculate the bending angle of the worm’s head using the first three marked points.

Next, we will read the extracted images in grayscale format. The grayscale image is shown in Fig.3a. However, due to the spontaneous behavior of *C. elegans* around food[35], the grayscale image may contain interferences such as food and shadows. We need to generate clean binary images to eliminate these negative interferences and ensure the smooth progress of subsequent experiments. Binary images can effectively remove background noise and shadows, retaining only the contours and movement trajectories of the nematodes. This step is crucial for obtaining accurate analysis results. When processing grayscale images, we use a global thresholding algorithm first, where the grayscale value of the shadows around the nematodes is used as the threshold to obtain the initial binary image. Therefore, determining the grayscale value of the shadows is a crucial step. Since the grayscale values of the shadows around the nematodes are significantly higher than the grayscale values of the nematodes themselves and the background, we can obtain the grayscale value of the shadows by calculating the maximum grayscale value in the grayscale image, thereby obtaining the initial binary image. The initial binary image is shown in Fig.3b. The definition of the global thresholding algorithm is as follows:

Where is the grayscale value at point in the grayscale image, and is the grayscale value at point in the initial binary image. Eliminating background noise and shadows to obtain a clean binary image, we will use an edge detection algorithm to find the contours. In image processing, edges refer to places in the image where the pixel values undergo sudden changes. Since the nematodes and their surrounding shadows are significantly distinguishable from the background, using an edge detection algorithm can effectively detect all the contours in the image. Here, we will use the Sobel edge detection operator to achieve this goal. The Sobel operator is a commonly used edge detection operator that can find edges by calculating the gradient of the image in the x and y directions. Specifically, the definition of the Sobel operator is as follows:

Where is the pixel gradient matrix in the horizontal direction of the image, is the pixel gradient matrix in the vertical direction of the image, and is the gradient value of each pixel in the image. To reduce the computational load and enable faster computation by the computer, the above formula is simplified in practical applications as follows:

Since the area of the nematode and its surrounding shadow is much larger than the area of interference, we can determine the region where the nematode body is located by selecting the largest area contour. In other words, the contour with the largest area is the one that we want to retain, which contains the nematode. Next, we will use the hole-filling algorithm to fill the largest contour. The essence of the hole-filling algorithm is to use the scan line algorithm to fill the polygon. Specifically, calculate the intersection points of the polygon edges with the horizontal scan line first; then, sort these intersection points; and finally, determine whether to fill based on the parity of the intersection points. On each scan line, the area between two odd intersection points will be filled. By scanning and filling the polygon line by line, we can effectively fill the largest contour and obtain the final result. The filled image is shown in Fig.3c. Next, it is necessary to perform an XOR operation on the initial binary image and the filled image. In a binary image, each pixel has a value of either 0 or 1, where 0 typically represents the background (black) and 1 represents the foreground (white). The rule for the XOR operation is: if the two corresponding pixel values are the same (both 0 or both 1), the resulting pixel value is 0; if the two corresponding pixel values are different (one is 0 and the other is 1), the resulting pixel value is 1. Therefore, through the XOR operation, we can obtain a binary image that contains only the nematode. The XORed image is shown in Fig.3d. The XOR operation is defined as follows:

Where is the pixel value at point in the initial binary image, is the pixel value at point in the filled image, and is the pixel value at point in the binary image obtained after the XOR operation. The final step is to perform an inverted color[36] on the binary image, which will result in a clean binary image. An example of a clean binary image is shown in Fig.3e. The inverted color is defined as

Here, represents the pixel value at point in the binary image, and represents the pixel value at point in the image after inverse processing. The above steps result in clean binary images, completing the preprocessing of all videos and images.

**3.3 Automatic counting methods for motor behavior**

To achieve automatic counting of nematode locomotion behavior, we need to extract the skeleton of the nematodes from binary images. This is because a thinned binary image can simplify the image, highlight image features, reduce computational complexity, and facilitate subsequent operations. Specifically, we first read the processed clean binary images. Next, we sort the binary images based on their file names. Since the segmented images from the experimental videos are named sequentially, the sorted binary images have a sequential order. Then, we perform thinning operations on these sorted binary images one by one. The thinning operation can effectively extract the skeleton of the nematodes, representing them as simple line structures in the image. By thinning the binary images, we ultimately obtain the skeleton representation of the nematodes. The expression for thinning the binary image and obtaining the nematode skeleton is as follows:

Where denotes the skeleton of image , represents the nth skeleton of , is the last iteration number before the operation erodes into an empty set, i.e.,. stands for iteratively eroding with n times until no more pixels can be removed. Through this process, we can extract the nematode skeletons from the binary images, which is crucial for the subsequent automatic counting of nematode locomotion behavior.



Fig.4 Accuracy and loss rate plots for head and tail localization models (a) Prediction accuracy curves for nematode head and tail positioning at different distance thresholds. (b) The comparison of losses in different epochs for the nematode head and tail localization model and the ResNet18 network model trained on the same dataset.

To achieve automatic localization of the head and tail positions of nematodes, we can utilize the characteristic that in consecutive frames, the distance between the head and tails of the nematode is shorter. Based on this criterion, we can use the nematode head and tail localization method to predict the head and tail positions in the first frame and store the positional information in arrays for the nematode head and tail, respectively. Subsequently, in the second frame and subsequent frames, we can obtain the skeleton of the nematode and extract the endpoint positions. We then compare the two endpoints in the second frame with the known head and tail positions from the first frame. The endpoint closer to the known head position is identified as the nematode's head, while the endpoint closer to the known tail position is identified as the nematode's tail. The newly obtained head and tail positions are then saved in the respective arrays. This process is repeated until we obtain the head and tail positions for all nematodes in the images. By following this method, we can gradually obtain the head and tail positions of all nematodes in the images without needing to run the head and tail localization method for every frame. This approach reduces experimental complexity and minimizes the waste of computational resources.

The number of omega turns in the nematode is related to the distance between its head and tail. Therefore, we need to calculate the distance between the head and tail of the nematode to determine the number of omega turns. Specifically, we first calculate the distance between the head and tail coordinates of the nematode. The schematic diagram of the distance between the head and tail of the nematode is shown in Fig.3f. The calculation method for the distance between the head and tail of the nematode is defined as

Here, we'll label the head and tail of the *C. elegans* worm as points T and H, respectively. Therefore, the corresponding position coordinates for the head are , and for the tail are . Next, calculate the distance between the head and tail of the nematode in each frame of the images, and record these distances in an array. Then, sequentially calculate the distance between the head and tail in each frame until the distances for all images are computed. Subsequently, we will determine the maximum head-to-tail distance among all frames and define it as the maximum head-to-tail distance. During the analysis, if the distance between the head and tail of the nematode in a frame is less than half of the maximum head-to-tail distance, it will be considered as the start frame of an omega turn. When the head-to-tail distance exceeds half of the maximum head-to-tail distance again, it will be marked as the end frame of the omega turn. A complete omega turn consists of a start and end frame. Finally, analyzing the marked start and end frames, we can calculate the number of omega turns that occurred in the nematode.

Due to the relationship between the number of head thrashings of a nematode and its head bending angle, we need to calculate the head bending angle to determine the number of head thrashes. Specifically, we divide the nematode into six equal parts based on its body length. It is important to note that the body length of the nematode refers to the total length of the nematode's skeleton, not the distance between the head and tail ends. Then, we recorded seven points, including the head endpoint, the five equally divided points, and the tail endpoint, in an array in the order from head to tail. These seven points are shown in Fig.3g. Here, we stipulate that the head bending angle of the nematode is calculated using the first three points among these seven points, labeled as A, B, and C. The angle formed by points A, B, and C is calculated as follows:

We will mark the edge opposite point A as , the edge opposite point B as , the edge opposite point C as , and angle as α. Then we will mark the supplement of as the bending angle of the nematode's head, as shown in Fig.3h. The calculation method for the bending angle of the nematode's head is as follows:

Here, we will denote as the bending angle of the nematode's head. Using the above method, we calculated the head bending angle of the *C. elegans* in each frame of the images and recorded these bending angles in an array. Next, we sequentially calculate the nematode's head bending angle for each frame until the head bending angles in all images have been calculated. To determine the number of head thrashings, we define a full head thrash as the nematode's head moving from one side to the other and back again. Therefore, two changes in the bending direction of the nematode's head are considered one full head thrashes. Next, we need to calculate the number of direction changes in the nematode's head bending. Specifically, we treat the sign change in the difference of head bending angles between two consecutive frames as a change in bending direction. Hence, we count the number of sign changes in the angle differences between consecutive frames. Finally, we divide the total number of changes in the head bending direction by 2 to obtain the number of head thrashes. Using this method, we determined the number of head thrashes of the nematode.

Through the above process, we are not only able to automatically count the head thrashes and omega turns of the nematode but also simultaneously detect any abnormal behavior exhibited by the nematode. Specifically, we monitor the positions of the nematode's head and tail, and if there is no change observed in these positions for a consecutive 100 frames, we consider the nematode's behavior as abnormal and send a notification to the experimenters. Therefore, this comprehensive analytical approach not only facilitates the automated counting of head thrashes and omega turns of the nematode but also enables timely detection and notification of any abnormal behavior exhibited by the nematode to the experimenters.



Fig.5 Schematic representation of nematode head thrashes behavior about head bending angle and distance between head and tail.

**4 Result**

In the following section, we will validate the proposed algorithm from three aspects to verify its effectiveness. These three aspects are the identification results of the nematode's head and tail, the counting results of the nematode's head thrashes and omega turns, and the comparison of the movement behaviors between nematodes with defective motor neurons and normal nematodes. Through validation and comparison of these aspects, we can comprehensively assess the reliability and accuracy of the proposed algorithm, thereby determining its effectiveness in identifying and counting the movement behavior characteristics of nematodes.

**4.1 *C. elegans* head and tail identification results**

We have obtained the positions of their heads and tails through the head and tail localization method of nematodes. To demonstrate the effectiveness of our method, it is necessary to compare the positions of the nematode's head and tail obtained by the localization model with manually annotated positions. Here, we define the difference between the predicted positions by the model and the manually annotated positions as the distance threshold. The comparison results will be summarized in Table 1 and visualized in Fig.4a. Additionally, to evaluate the efficiency of the nematode head and tail localization model, we will compare it with the ResNet18 neural network model trained on the same dataset for the same number of epochs. We will present the comparison of losses between the ResNet18 network model and the nematode head and tail localization model at the same epoch in Fig.4b to demonstrate the performance advantage of our proposed model.

Table 1 Accuracy of the predicted position of head and tail over the different threshold



By comparing the manually annotated positions of nematode heads and tails with those predicted by the model, we found that when the distance threshold is 6 pixels, the prediction accuracy for both the head and tail positions of the nematodes reached 100%. When the distance threshold is above 4 pixels, the prediction accuracy for both the head and tail positions remained above 90%. Even with a distance threshold of 3 pixels, the prediction accuracy for the tail position reached 88%. However, when the distance threshold was 2 pixels or less, the prediction accuracy for both the head and tail positions dropped below 50%. Moreover, when comparing the nematode head and tail localization model with the ResNet18 neural network model, we found that as the number of epochs increased, both models showed a significant decrease in loss rate for the localization of nematode head and tail positions. However, at the same number of epochs, the nematode head and tail localization model exhibited noticeably lower loss and a faster convergence rate compared.

The significant variation in accuracy associated with different distance thresholds when comparing manually annotated and model-predicted nematode head and tail positions can be attributed to the fact that both the manual annotation and model prediction are based on grayscale images. Specifically, on the one hand, grayscale images exhibit shadows around the nematodes, which can affect both manual annotation and model prediction. Consequently, discrepancies between the manually annotated and model-predicted positions can arise within extremely small pixel distances. On the other hand, since the comparison is made with manually annotated data, inevitable errors in manual annotation can lead to differences between the model-predicted and manually annotated positions, resulting in fluctuations in accuracy at different distance thresholds. This variability is one of the unavoidable drawbacks of manual annotation.

Certainly, the comparison data between manual annotation and model annotation also validated the effectiveness of the nematode head and tail localization model. While the prediction accuracy for both the head and tail positions of the nematodes reached 100% when the distance threshold was 6 pixels, it also indicates that within a certain margin of error, the accuracy of this model consistently remains at 100%. Furthermore, the comparison results with the ResNet18 neural network model further demonstrated the higher efficiency of the nematode head and tail localization model.



Fig.6 Schematic representation of nematode omega turning behavior about head bending angle and distance between head and tail

**4.2 *C. elegans* head thrashing and omega turn count results**

Before analyzing the head thrashes and omega turn behaviors of the nematodes, it is essential to compute the head bending angle and the distance between the head and tail. These calculations are indispensable as they provide crucial information about the movement characteristics of the nematodes. Using an experiment with the N2 genotype nematodes as an example, we have recorded the variations in head bending angle and head-tail distance corresponding to different amplitudes of head thrashes, as shown in Figure 5. By observing Figure 5, we can analyze the relationship between the amplitude of head thrashes and the head bending angle, as well as the head-tail distance. Similarly, we have also recorded the variations in head bending angle and head-tail distance corresponding to different omega turn behaviors, and these are documented in Figure 6. Through analysis of Figure 6, we can investigate the relationship between omega turn behavior and the head bending angle, as well as the head-tail distance. In summary, by computing the head bending angle and head-tail distance of the nematodes, and observing the head thrashes at different amplitudes and different omega turn behaviors, we can establish the relationship between head thrashes behavior, omega turn behavior, and the head bending angle, as well as the head-tail distance.

When analyzing the relationship between the nematode's head thrash behavior and the head bending angle as well as the head-tail distance, we need to consider the definition of head thrashing and how to convert changes in the head bending direction into head thrash counts. Specifically, since a complete head thrash is defined as moving the nematode's head from one side to the other and back again, two changes in the head bending direction are considered one head thrash[37]. Therefore, we regard the positive or negative change in the angle difference between consecutive frames as a change in the head bending direction and count the number of these changes. Finally, we divide the number of changes in the head bending direction by 2 to obtain the head thrashes count of the nematode. In Figure 6, each inflection point, marked in orange, may be a point where the nematode's head bending direction changes and can be used as a reference for head thrash counts. Figure 6 shows that the larger the amplitude of the nematode's head thrashing (thrash amplitude I), the greater the fluctuation in the head bending angle, and the more significant the changes in the head-tail distance (as shown in region I). Conversely, when the head thrashes amplitude is smaller (such as oscillation amplitude V), the fluctuations in the head bending angle are smaller, and the changes in the head-tail distance are more gradual (as shown in region V). Therefore, through this analytical method, we can more clearly understand the relationship between the nematode's head thrashes behavior and the head bending angle.

When analyzing the relationship between the nematode's omega turns and the head bending angle as well as the head-tail distance, we need to consider the definition of an omega turn and how to convert changes in the head-tail distance into omega turn counts[38]. Specifically, we consider an omega turn to occur when the distance between the nematode's head and tail is less than half of the maximum head-tail distance. Thus, in Figure 7, during an omega turn, the distance between the nematode's head and tail significantly decreases, even falling below half of the maximum head-tail distance (as shown in regions II, IV, and VII). The distance between the head and tail is less than half of the maximum head-tail distance that occurred three times, indicating that the nematode performed three omega turns during this period.

Through this analytical method, we can identify and count the nematode's omega turn behavior and further understand its relationship with the changes in the head-tail distance.

To validate the effectiveness of our method, we compared it with methods proposed by other researchers. Specifically, Zhao et al. proposed a method that considers changes in the bending direction of the middle region of the nematode's body as head thrashes[22], while Zhang et al. proposed a method that considers changes in the head bending direction of the nematode as head thrashes[28]. We selected 10 nematodes for the experiment and compared our method with these two methods, along with manual counting. The results calculated by the four methods are shown in Fig.7a. The average absolute errors of the three automatic counting methods are shown in Fig.7b. The Pearson correlation coefficients between the three automatic counting methods and the manual counting method are also shown in Fig.7c. Through comparative analysis, we found that the results obtained by the method proposed by Zhao et al. were significantly higher than those of the other methods. In contrast, the method proposed by Zhang et al. and our method were closer to the results of manual counting. However, the method proposed by Zhang et al. exhibited conflicts during counting. Furthermore, our method had a smaller average absolute error and was more similar to manual counting results. Therefore, our method demonstrated superior performance in terms of accuracy and reliability. However, this algorithm also has some limitations. It can only be used to identify and analyze the movement behavior of individual *C. elegans*. In the future, we aim to automate the identification and analysis of the movement behavior of multiple nematodes in videos.



Fig.7 Graphs of nematode head thrashes statistics mean absolute error, and correlation index for different methods (a) Four different methods for counting nematode head thrashes. (b) Comparison of mean absolute errors of nematode head thrashes for three automatic counting methods. (c) Correlation of three automated counting methods with manual counting.

**4.3 Comparison of the motor behavior of worms with defective motor neurons with that of normal worms**

We conducted further analysis of the movement behavior of the nematodes. We selected some normal nematodes and nematodes with significant defects in motor neurons and compared their head thrashes and omega turns. The genetic types of the normal nematodes we chose were ins-18, flp-13, N2, and nlp-2, while the genetic types of the nematodes with significant defects in motor neurons included unc-8[39], unc-44[40], and unc-116[41]. We randomly selected 30 one-minute video segments from the *C. elegans* videos of each strain, resulting in a total of 210 one-minute videos. The automatic counting and manual counting of head thrashed and omega turns were performed on these 210 videos. Specifically, researchers first manually counted the head thrashes and omega turns of *C. elegans* in each video and recorded the results. To reduce human counting errors, each video was manually counted three times, and the average value was taken. Then, an automatic counting algorithm was used to calculate and record the head thrashes and omega turns of *C. elegans* in each video. In this experiment, the manual and automatic counting results of nematode head thrashes and omega turns are shown in Fig.8a and Fig.8b. The average counting results and records of abnormal behaviors are summarized in Table 2. The average manual and automatic counting results for head thrashes and omega turns of the nematodes are shown in Fig.8c and Fig.8d. **Table 2** Data on head thrashes and omega turns and abnormal behavior in different genotypes of nematodes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Head Thrash Count | | Omega Turn Count | | Abnormal Behavior |
| Automatic | Manual | Automatic | Manual |
| *ins-18* | 67.8 | 67.0 | 6.0 | 5.6 | False |
| *flp-13* | 68.8 | 67.7 | 4.1 | 3.8 | False |
| *nlp-2* | 72.1 | 70.8 | 4.0 | 3.6 | False |
| N2 | 83.1 | 80.2 | 4.0 | 3.7 | False |
| *unc-8* | 28.2 | 27.5 | 2.1 | 1.9 | False |
| *unc-44* | 19.4 | 19.7 | 1.5 | 1.3 | False |
| *unc-116* | 20.6 | 20.6 | 1.5 | 1.3 | False |

The statistical results indicate that the average counts of head thrashes and omega turns obtained from manual counting and automatic counting are generally consistent, verifying the accuracy and robustness of the automatic counting method. Additionally, the normal nematodes exhibit significantly higher average head thrash counts per minute compared to the nematodes with defective motor neurons. Similarly, the average omega turn counts per minute are also higher in the normal nematodes than in those with motor neuron defects. This is because damage to the motor neurons in nematodes, whether it involves GABAergic or cholinergic motor neurons, affects their movement behavior, including head thrashes and omega turns. Therefore, there is a clear quantitative distinction in head thrashes and omega turns behaviors between normal nematodes and those with motor neuron defects.

Specifically, normal nematodes typically display higher frequencies of head thrashes and omega turns, while nematodes with motor neuron defects tend to show lower frequencies of these behaviors. This analysis demonstrates the significant differences in movement behaviors between nematodes with and without motor neuron defects. However, it should be noted that even among normal nematodes, there can be instances where the frequency of head thrashes and omega turns is relatively low, especially for omega turns. This could be due to biological variability among individuals, thus averaging is necessary to obtain more reliable data. This explains the use of averages in statistical analysis to minimize the impact of individual differences on the results.

**5 Conclusion**

To reduce the time and manpower required for manual counting of nematode head thrashes and omega turns, an automatic counting method was proposed. The accuracy of this algorithm was validated by comparing it with manual counting results. Furthermore, its effectiveness was demonstrated through comparisons with other methods. When comparing the movement behaviors of normal nematodes and those with motor neuron defects, it was observed that the movement behaviors of nematodes with motor neuron defects were significantly lower than those of normal nematodes. This indicates that the automatic counting results of head thrashes and omega turns in nematodes can serve as important indicators for assessing the integrity of motor neurons. In conclusion, with the proposed automatic counting method, we can accurately and effectively assess the head thrashes and omega turns in nematodes, thereby determining the functional state of motor neurons. This saves researchers time and manpower, provides a reliable assessment metric, and facilitates the study of neurodegenerative diseases.



Fig.8 Results of automatic enumeration of different strains of nematodes (a) Automatic and manual counting results for head thrashes. (b) Automatic and manual counting results for omega turns. (c) Results of nematode head thrashes counts in different strains. (d) Results of nematode omega turn counts in different strains.

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