**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 behavior 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 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 increases 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 organisms.

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.

The nematode is a unique model organism with numerous advantages for studying neurodegenerative diseases. 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. C. elegans' genetic features also enhance its usefulness as a model organism. For instance, 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, 60–80% of human genes are similar in C. elegans[18], and C. elegans has numerous homologs of human disease-causing genes[19]. Twelve of the identified signaling pathways are identical to humans, and 533 genes found in the nematode's genome sequence are homologous to those linked to human disorders[20]. Therefore, nematodes are a valuable tool for researching the causes of human diseases and vetting possible treatments. Nematodes provide scientists with a useful research platform because of their distinctive biological properties and genetic resemblance to humans, making them an essential model organism. Nematodes are very valuable in the investigation of neurodegenerative illnesses because of these characteristics[21].



Fig.1 General outline of the proposed method

Given that defects in motor neurons are frequently the source of neurodegenerative illnesses like amyotrophic lateral sclerosis, motor behavior can be utilized to assess the functional health of motor neurons[22]. Therefore, a better understanding of the pathophysiology of neurodegenerative illnesses depends on researching the connection between worm motor neurons and their motor behavior. The motor activity of worms must be quantitatively studied to accomplish this goal. Nematodes have demonstrated various motor behaviors in earlier research, including head thrash, body bend, and forward and backward movement, that can be utilized in quantitative investigations. These two locomotor behaviors were selected for study because of the sensitivity of nematode head thrash behavior and the simplicity of omega turn behavior observation. The motor capacity of nematodes can be more precisely determined by quantitatively analyzing these particular motor activities, which in turn reflects the motor neurons' functional state. However, in actual operations, manual labor is typically used to conduct the quantitative analysis of nematode motor behaviors[23] it demands that experimenters devote time to counting and requires prior training. The limitations of manual counting also include its inability to be used for ongoing, long-term research and its difficulty in meeting the demands of large-scale counting assignments. Thanks to the development of automatic counting techniques brought about by continual advances in computer technology in the field of image process, the study of nematode motor behavior now has new tools.

For instance, Swierczek et al. proposed a real-time computer vision system called the Multi-Worm Tracker (MWT)[24], which can measure the behavior of many dozen C. elegans worms in a petri dish at a speed equal to that of video. This indicates that the MWT system requires little human intervention to swiftly and effectively quantify the worms' behavior. On the one hand, if the MWT system is processing excessive worms, it may cause excessive system load and frame drops. On the other hand, the number of worm collisions rises with the worm population size. The system will add up the sizes of any colliding worms and treat them as a single unit until the worms are apart and the search algorithm recognizes them as distinct worms[25]. Consequently, worm traits may be lost in this scenario, which could impact the experiment result. 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.

Another commercial worm-tracking program that tracks a worm's head, tail, or center markings is called WormLab[25]. It can also examine the chosen worms' velocity, location, area, direction, wavelength, trajectory length, omega turns, and reversals. The bend angle is the additional angle measured by WormLab between the worm's head, midsection, and tail. Worms with bend angles greater than 90° are deemed to have completed an omega turn[26]. Nonetheless, an omega turn is commonly defined as a worm's head being nearly in contact with its tail or reorienting 135 degrees during a single head bend[27]. It follows that the worm's head and tail may not always be near each other or that the head may not have been reoriented when the bend angle of the worm is greater than 90°.

Other scholars have also put forth various automatic counting methods. For instance, Zhang et al. described a method for automatically recognizing and quantifying individual thrash motions of the worm head from experimental videos[28]. This method distinguishes between the worm's head and tail using two criteria. The first requirement states that the worm's head must be rounder than its tail; hence, the tail is designated as the point on the worm's contour with the sharpest. Since the worm's tail is darker than its head according to the second criterion, the tail is identified as the endpoint in the binary picture with the lower brightness. The head and tail of the worm must be determined by hand when the outcomes of these two criteria are contradictory.

In conclusion, even though free and commercial automatic analytic tools for worm locomotion behavior are currently accessible, these approaches still have certain drawbacks. For instance, certain techniques might not precisely record intricate movements in worms, like head thrashing and omega turns. Certain methods also define behaviors differently from traditional definitions. Others can be improved upon because they need human intervention. Therefore, a model for locating the head and tail positions of worms was built in this work, taking into the shortcomings of manual counting and the currently available automatic counting methods. It was considered to use an automatic recognition technique for distinct worm head thrashes and omega turns. This approach can evaluate head thrashes and omega turns at the same time and correctly distinguishes between the worm's head and tail. It is consistent with the conventional description of worm locomotion behaviors and maintains the features of worm locomotion. Furthermore, there is no need for manual intervention during the implementation process when using this strategy.

**3 Method**

An extensive description of the proposed method, including video and image processing, localization of the worm head and tail, and automatic counting techniques, will be given in this part. Figure 1 shows the method's workflow, with each step connected to the next to create an 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. A critical stage in this process is identifying the nematode's head from the tail. Thankfully, nematodes have distinct features for their head and tail. For instance, the nematode's head is brighter than its tail due to a difference in fat distribution; the head thrashes more frequently than the tail due to foraging behavior; the head is wider than the tail; and among other differences, the positions of the head and tail concerning the body center do not vary much[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

We have chosen to use Convolutional Neural Networks (CNNs) for locating nematodes' heads and tails due to their superior performance in image processing, CNNs have demonstrated exceptional performance in tasks including recognition, classification, segmentation, and generation. They can also automatically learn features from photos[30]. Many cutting-edge CNN designs, like VGGNet and ResNet, have surfaced in recent years and have demonstrated exceptional performance across workloads and datasets. Another kind of CNN appropriate for our purposes is EfficientNet[31], which performs well and demonstrates exceptional resource usage and efficiency. EfficientNet outperforms other models with the same computational resources we have selected it as our tool. We will use transfer learning strategies to enhance the model's performance even more. Furthermore, more morphological details are preserved in the original grayscale nematode photos. Therefore, the dataset for our model will consist of grayscale images. This will increase the accuracy of head and tail localization by assisting the model in better capturing the nuanced features of nematodes. To sum up, we will precisely localize the head and tail of nematodes by combining EfficientNet with transfer learning techniques, utilizing grayscale photos as the dataset.

We have performed the following actions to localize the nematode head and tail. Initially, we extracted 2000 grayscale pictures randomly from videos of various strains of nematodes included in the nematode behavior phenotypic database. Next, using Labelme[33], an open-source program for annotating images. 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 these 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. We layered an attention mechanism layer on top of EfficientNetB0 to improve the model's focus on particular regions[34]. To be more precise, we first reduced the dimensionality of the feature vectors using Global Average Pooling. Subsequently, the downscaled feature vectors were transformed into attention-weight vectors using two Dense (completely connected) layers. We used L2 regularization to limit the complexity of the model during this phase. We then obtained weighted feature vectors by performing element-wise multiplication between the original feature vectors and the attention weight vectors using a Multiply layer. Finally, we add two Dense layers, which produce the expected positions. We have so finished building the head-tail localization model. Figure 2 displays the model's schematic diagram. We precisely localize the head and tail of nematodes by following the above stages, guaranteeing the logical consistency of the data processing and model creation operations.

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 is 0.0001. The Adam optimizer is employed for parameter updates, and the model parameters are updated through backpropagation to optimize the prediction results. The evaluation metric is the mean absolute error (MAE), and the loss function is the mean squared error (MSE). 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**

Processing experimental recordings including the worms is necessary to count nematode movement behaviors automatically. The videos taken from the nematode behavioral phenotypes database[35] are not identical long. We screen the nematode videos from various strains and then extract one-minute video segments for each trial to ensure the scientific integrity of our work. This method ensures that every experiment has the same duration. Furthermore, we extract each frame from the video as an image to record additional features during worm motility. This allows us to record the entire nematode migration process. This procedure allows us to notice even the tiniest motion changes and ensures the consistency of the data utilized to analyze nematode activity.



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. (f) The distance between the head and tail of the worm. (g) Marking of nematodes. (h) To calculate the bending angle of the worm’s head using the first three marked points.

The extracted images will be read in grayscale format. The grayscale picture is displayed in Figure 3a. However, the grayscale image could include interferences like food because of the spontaneous behavior of *C. elegans* around food[35], and the grayscale images 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. As a result, figuring out the shadows' grayscale value is an important step. We can determine the grayscale value of the shadows by calculating the maximum grayscale value in the grayscale image, which will yield the initial binary image because the grayscale values of the shadows surrounding the nematodes are significantly higher than the grayscale values of the nematodes and the background. 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. After removing shadows and background noise to create a clear binary image, we'll utilize an edge detection technique to identify the contours. Edges in image processing are regions of the image where abrupt changes in the pixel values. An edge detection method can successfully identify every contour in the image since the nematodes and the shadows surrounding them are easily distinguished from the background. To do this, we'll employ the Sobel edge detection operator. One popular edge detection operator that can locate edges is the Sobel operator, which computes the image's gradient in both the x and y dimensions. 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. The space between two odd junction sites on each scan line will be filled. We can efficiently fill the largest contour and get the desired outcome by scanning and filling the polygon line by line. In Figure 3c, the filled image is displayed. 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. As a result, we may create a binary image that only contains the nematode using the XOR method. 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 results 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**

The skeletons of the nematodes must be extracted from binary photos to accomplish automatic counting of their movement activity. This is because a binary picture that has been thinned can make the image simpler, draw attention to its best features, lower computational complexity, and make future operations easier. To be more precise, we read the cleaned binary pictures after processing. The binary images are then arranged according to their file names. The sorted binary images have a sequential order since the segmented images from the experimental videos are sequential. Next, we successively apply the thin operation to these sorted binary pictures. The nematodes' skeleton can be successfully extracted through thin operation, which leaves them in the image as basic line structures. We eventually achieve the nematode skeleton representation by thinning the binary pictures. The following expression can be used to extract the nematode skeleton and thin the binary picture.:

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.

The property that the distance between the nematode's head and head is closer in successive frames can be used to localize the nematode's head and tail positions. Specifically, the nematode head and tail localization approach allows us to forecast the head and tail positions in the first frame, and store the positional information in arrays for the nematode head and tail, respectively. We may then retrieve the nematode's skeleton and determine the endpoint locations in the second and following frames. Then, we compare the head and tail positions stored in the first frame with the two endpoints in the second frame. The endpoint is the closest to the known head position that is considered to be the head, and another endpoint is the tail. The head and tail positions that were just determined are then stored in the appropriate arrays. This method is repeated until all nematodes' head and tail positions are obtained in the pictures. This method eliminates the need to perform the head and tail localization process on each frame by gradually identifying the locations of each nematode's head and tail inside the images. This approach reduces experimental complexity without wasting computing resources.

The nematode's head-to-tail length and the number of omega turns are correlated. 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 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 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 in the nematode.

We must calculate the head bending angle to ascertain the number of head thrashings of a nematode because there is a correlation between the two. According to its body's length, we split the nematode into six equal pieces. It is crucial to understand that the term body's length describes the nematode skeleton's length, not the separation between its head and tail ends. Next, we recorded seven points in an array from head to tail: the head endpoint, the five equally divided points, and the tail endpoint. Fig. 3g depicts these seven sites. 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. We determined the C. elegans' head bending angle in each frame by the above method and stored these bending angles in an array. The nematode's head bending angle is then successively determined for every frame until the head bending angles in every image are determined. We define a complete head thrash as the nematode's head moving from side to side and back again to count the number of head thrashes. Consequently, two variations in the nematode's head's bending direction are regarded as one complete head thrash. The number of direction changes in the nematode's head bending must then be determined. In particular, we interpret a change in bending direction as the sign change in the difference of head bending angles between two successive frames. As a result, we tally the quantity of sign shifts in the angle disparities across succeeding frames. Lastly, to determine the number of head thrashes, we divide the total number of changes in the head bending direction by two. With the use of this technique, we were able to count the nematode's head thrashes.

By using the above procedure, we may concurrently identify any aberrant behavior displayed by the worm in addition to automatically counting its head thrashes and omega turns. specifically, if we don't see any changes in these positions throughout 100 consecutive frames, we label the nematode's behavior as aberrant and alert the experimenters. As a result, this thorough analytical technique makes it easier to count head thrashes and omega turns automatically and makes it possible to promptly identify and alert the experimenters to any unusual behavior the nematode may exhibit.

**4 Result**

To confirm the proposed algorithm's efficacy, we shall validate it from three angles in the section. The findings of the nematode's head and tail identification, the nematode's head thrashes and omega turns counted, and the comparison of the movement behaviors of nematodes with impaired motor neurons and normal worms are these three features. By verifying and contrasting these elements, we may evaluate the suggested algorithm's correctness and dependability completely, ascertaining its efficacy in recognizing and quantifying the movement behavior traits of nematodes.

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

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

Using the nematode head and tail localization method, we can determine the locations of their heads and tails. It is crucial to compare the positions of the nematode's head and tail that the localization model obtained with manually annotated positions to show how effective our method is. Here, the distance threshold is defined as the difference between the model's predicted and the manually annotated positions. The comparison results will be shown graphically in Fig. 4a and summarized in Table 1. We will also contrast the nematode head and tail localization model's performance with that of the ResNet18 neural network model, which was trained on the same dataset across an equal number of epochs. To illustrate the performance advantage of our suggested model, we will compare the losses for the ResNet18 network model with the nematode head and tail localization model at the same time in Fig. 4b.

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.

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



Since both the model and the manual annotation are based on grayscale images, these images show shadows surrounding the nematodes, which can impact both manual annotation and model prediction There is a substantial difference in accuracy associated with different distance thresholds when comparing the head and tail positions. As such, disparities may occur within small pixel distances between the manually annotated and model-predicted places. Additionally, as the comparison is based on manually annotated data, inevitable mistakes in manual annotation may result in discrepancies between the manually annotated and model-predicted positions, which could cause accuracy variations at various distance thresholds. This heterogeneity is one of the inevitable disadvantages of annotation by hand.

The efficiency of the nematode head and tail localization model was also confirmed through the comparative data between hand and model annotation. Although the distance criterion of 6 pixels caused the prediction accuracy for the nematode's head and tail positions to reach 100%, this also shows that the accuracy of this model continuously stays at 100% within a certain margin of error. The comparison results between the nematode head and tail localization model and the ResNet18 neural network model further illustrated the latter's superior efficiency.

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

It is crucial to determine the head bending angle and the distance between head and tail before delving into the analysis of the nematodes' head thrashes and omega turns. These computations are essential because they offer vital details regarding the nematode movement properties. We have recorded the changes in head bending angle and head-tail distance corresponding to different amplitudes of head thrashes using an experiment using N2 genotype nematodes as an example, as shown in Figure 5. Figure 5 shows the link between the head bending angle and the head-tail distance, as well as the amplitude of head thrashes. Similarly, Figure 6 shows the changes in head bending angle and head-tail distance that correlate to various omega turn behaviors. We may examine the relationship between omega turn behavior and the head bending angle and head-tail distance by analyzing Figure 6. In conclusion, we can determine the relationship between head thrashes behavior, omega turn behavior, and the head bending angle and head-tail distance of the nematodes by calculating their head bending angle and head-tail distance as well as by observing their head thrashes at various amplitudes and behaviors.



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

We must consider the definition of head thrashes and how to translate changes in the head bending direction into head thrash counts when examining the association between the nematode's head thrash behavior the head bending angle and the head-tail distance. Particularly, two variations in the head bending direction are regarded as a single head thrash since a full head thrash is defined as moving the nematode's head from one side to the other and back again[37]. As a result, we count the number of times the angle difference between consecutive frames changes, whether positive or negative, indicating a shift in the direction in which the head bends. To get the nematode's head thrashes count, we divide the total number of changes in the head bending direction by two. As a guide for head thrash counts, Figure 6's orange-marked inflection points may indicate locations when the nematode's head bending orientation changes. Figure 6 illustrates that the nematode's head thrashing amplitude (thrash amplitude I) is correlated with its head bending angle fluctuation and head-tail distance fluctuations (as indicated in area I). In contrast, the head bending angle fluctuates less and the head-tail distance varies more gradually when the head thrashes amplitude is less (such as oscillation amplitude V) (as indicated in area V). As a result, we can better comprehend the connection between the nematode's head thrashes behavior and the head bending angle thanks to this analytical method.

We must consider the definition of an omega turn and how to translate variations in the head-tail distance into omega turn counts when examining the relationship between the nematode's omega turns with the head bending angle and the head-tail distance[38]. To be more precise, we define an omega turn as taking place when the nematode's head-tail distance is less than half of its maximum. The distance between the nematode's head and tail thus drops dramatically during an omega turn in Figure 7, even going below half of the maximal head-tail distance (as demonstrated in areas II, IV, and VII). Given that the head-tail distance is less than half of the maximum head-tail distance that happened three times, the nematode made three omega turns during this time. The nematode's omega turn behavior may be recognized and counted using this analytical method, which also helps us to comprehend how it relates to variations in the head-tail distance.

We contrasted our method with other researchers' approaches to confirm its efficacy. Specifically, Zhao et al. proposed a method that takes into account changes in the bending direction of the middle area of the nematode's body as head thrashes[22], Zhang et al. proposed a way that takes into account changes in the bending direction of the nematode's head as head thrashes[28]. For the experiment, we used ten nematodes, and we manually counted the nematodes and compared our method with these two approaches. Fig. 7a displays the findings computed using the four approaches. Fig.7b shows the three automatic counting systems' average absolute mistakes. The Pearson correlation coefficients between the manual method and three automatic counting methods in Fig.7c. Upon doing a comparative analysis, we discovered that the outcomes of Zhao et al.'s method were noticeably higher than those of the other approaches. The results of manual counting were more closely aligned with Zhang et al. and the proposed method. Conflicts occurred during counting, though, with Zhang et al.'s approach. Our approach also resembled the findings of hand counting more closely and had a decreased average absolute error. As a result, the accuracy and dependability of our approach were greater. Still, there are certain restrictions with this algorithm. The movement behavior of individual C. elegans can only be identified and analyzed using it.

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

We looked into the nematodes' movement patterns in more detail. To compare the behavior of head thrashes and omega turns, we took a sample of some normal and significantly defective motor neuron nematodes. 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]. From the C. elegans movies of each strain, we took 30 one-minute video segments at random, making 210 one-minute videos in total. These 210 films were subjected to automatic and manual head thrashes and omega turns counting. These 210 videos were subjected to manual and automatic counting of head thrashes and omega turns. Every video was manually counted three times to minimize human counting errors. Figure 8a displays the findings of the experiment's manual and automated counting of nematode head thrashes, and Figure 8b displays the turns of omegas. Table 2 provides a summary of the average counting outcomes and deviation records. Figure 8c is the average manual and automatic head thrash counting results, and Fig.8d displays the average manual and automatic nematode omega turns.

The statistical findings demonstrate the accuracy and robustness of the automatic counting method by showing a general consistency between the average counts of head thrashes and omega rotations derived from manual counting and automatic counting. In addition, the average head thrash counts per minute of the normal worms are much higher than those of the nematodes with faulty motor neurons. Similarly, the average number of omega turns per minute is also higher in normal worms compared to those with abnormalities in their motor neurons. This is because nematode movement behavior, such as head thrashes and omega turns, is impacted by injury to their motor neurons, whether it be cholinergic or GABAergic motor neurons. As a result, there is a definite quantitative difference between the behaviors of normal nematodes and those with omega turns and head thrashes.

**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 |



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.

To be more precise, nematodes without motor neuron abnormalities tend to exhibit fewer head thrashes and omega turns than normal worms. The considerable variations in movement behaviors between worms with and without motor neuron abnormalities are shown by this analysis. It should be mentioned, nevertheless, that even in normal nematodes, there may be head thrashes and omega turns occur fewer, particularly in the case of omega turns. Averaging is required to acquire more reliable statistics because biological diversity among individuals may this. This explains why averages are used in statistical analysis to reduce the effect of individual variances on the outcomes.



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.

**5 Conclusion**

An automatic counting method was proposed to decrease the time and labor needed for manually counting nematodes' omega turns and head thrashes. This algorithm's correctness was confirmed by contrasting its output with results from hand counting. Moreover, comparative studies using alternative approaches proved its efficacy. It was shown that worms with motor neuron abnormalities had far fewer mobile behaviors than normal nematodes when their movement behaviors were compared to those of normal nematodes. This suggests that the automated counting of nematode omega turns and head thrashes can be useful markers for determining the health of motor neurons. In summary, utilizing the suggested automated counting technique, we can precisely and efficiently evaluate the head thrashes and omega turns in nematodes, thereby ascertaining the motor neurons' functioning state. In addition to saving time and resources for researchers, this offers a trustworthy evaluation metric and makes studying neurodegenerative illnesses easier.

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