The automatic counting of head thrashing and omega turn behavior in *C. elegans* can map the function of motor neurons

*Zhan Wenyue* 展文乐

Cyberspace Security Institute

**1 Abstract**

Model organisms have played a significant role in developing biological sciences and have broad applications in various fields. As a model organism, the nematode *Caenorhabditis elegans* has become instrumental in studying neurodegenerative diseases. Amyotrophic lateral sclerosis is a classic neurodegenerative disease caused by defects in motor neurons. Due to the homology between nematode and human genes, the nematode is a crucial model for investigating the pathogenesis of Amyotrophic lateral sclerosis. Therefore, quantitative analysis of the nematode's locomotion behavior is essential. Among the various movement behaviors of nematodes, head thrashing and omega turn behaviors have been selected as indicators of motor neuron functionality. Then, utilizing a model for head and tail position localization in nematodes, the head and tail positions of the nematode are identified, enabling automated counting of head thrashing and body omega turn behaviors. Comparing the head thrashing and body omega turn behaviors between normal nematodes and those with apparent motor neuron defects confirmed that the frequency of these behaviors was significantly lower in nematodes with motor neuron defects than in those with intact motor neurons. Demonstrating the automated counting results of head thrashing and omega turn behavior in nematodes can serve as indicators to determine the integrity of motor neurons.

模式生物在生物科学的发展过程中发挥了巨大的作用，并且在各个领域都有广泛的应用。线虫作为一种模式生物，早已成为研究神经退行性疾病的重要模型。肌萎缩侧索硬化症是一种典型的由于运动神经元缺陷导致的神经退行性疾病。由于线虫与人类基因的同源性，线虫是研究肌萎缩侧索硬化症发病机制的一种重要模型。因此对线虫运动行为的定量研究至关重要。在线虫的多种运动行为中，选择线虫的头部摆动和omega转弯行为作为运动神经元功能的指示器，利用线虫头尾位置定位模型找到线虫的头尾位置，对线虫的头部摆动和身体Omega转弯行为进行自动计数。比较正常线虫与运动神经有明显缺陷线虫的头部摆动和身体Omega转弯行为，证明了运动神经有缺陷线虫的头部摆动和omega转弯行为的发生频率明显低于运动神经没有缺陷的线虫。说明对线虫头部摆动和omega转弯行为的自动计数结果可以作为判定运动神经元是否完整的指标。

Keywords**:** *C. elegans*, Head thrashing, Omega turn, Automatic count

**2 Introduction**

Model organisms have frequently graced the podium of the most prestigious award in the scientific community, the Nobel Prize, making them indispensable and significant stars in biology. Biologists have explored universal principles underlying various biological phenomena through scientific research conducted on model organisms. For example, the drosophila melanogaster[1], which has won scientists the highest honor in scientific research multiple times, is an important model organism for studying genetics and development. The model microorganism Escherichia coli[2] has propelled significant advancements in biotechnology, microbiology, molecular biology, and bioinformatics. It can be said that model organisms have played a significant and indelible role in the development of biological science. The nematode, *Caenorhabditis elegans*(*C. elegans*), is also a model organism and has graced the Nobel Prize stage due to its landmark role in developmental biology[3]. Currently, nematodes are widely employed in multiple fields including aging[4], development[5], neuroscience[6], behavior[7], genetics[8], drug screening[9], and toxicology research[10].

There are numerous advantages to using the nematode *C. elegans* as a unique model organism. Firstly, its small size and ease of cultivation reduce space requirements. Secondly, its short developmental cycle minimizes experimental duration. Additionally, its transparent body enables observation of internal structures and processes such as cell division and death during growth. These are unique advantages that set *C. elegans* apart from other model organisms. Furthermore, due to its minimal genome, consisting of only 6 chromosomes and 1 mitochondrial genome, *C. elegans* became the first multicellular organism to have its entire genome sequenced[11]. It is also the only organism in which every cell in the body can be traced back to its origin[12]. Furthermore, the homologous genes of *C. elegans* account for 60-80% of human genes[13], with many homologs of human disease-causing genes present in *C. elegans*[14]. In the genome sequence of *C. elegans*, there are 533 genes homologous to genes related to human diseases, and among the identified signaling pathways, there are 12 homologous to those in humans[15]. Because of these characteristics, nematodes are used as model organisms for studying the pathological mechanisms and screening candidate drugs for neurodegenerative diseases[16]. Common neurodegenerative diseases include epilepsy[17], Alzheimer's disease (AD)[18], Parkinson's disease (PD)[19], and others. Amyotrophic lateral sclerosis (ALS)[20] is also a type of neurodegenerative disease. Amyotrophic lateral sclerosis, commonly known as "Lou Gehrig's disease," is a condition caused by defects in motor neurons. Since locomotion behavior can reflect the functional state of motor neurons, quantitative analysis of nematode locomotion behavior can assess its motor ability and the functionality of motor neurons. Furthermore, due to the homology between nematodes and human genes, nematodes have become an important model for studying the pathogenesis of ALS[21]. Therefore, quantitative studies of worm locomotion behavior are crucial. Due to the sensitivity of head thrashing behavior[22] and the ease of observing omega turn behavior in nematodes among various locomotion behaviors, head thrashing and omega turn behaviors have been selected as the studied locomotion behaviors. The schematic diagram of head thrashing and omega turn in *C. elegans* is depicted in Figure 1(a) and Figure 1(b).

However, in practical applications, quantitative studies of worm locomotion behavior often rely on manual counting methods[22]. However, manual counting requires prior training of the experimenters, and the counting process often consumes a significant amount of their time. Additionally, manual counting is not suitable for long-term, continuous experiments. Consequently, manual counting methods are becoming increasingly inadequate for large-scale counting tasks. With the advancement of computer technology in the field of imaging, automated methods for counting worm locomotion behavior have emerged, greatly reducing manpower resources and saving time.

For instance, Swierczek et al. proposed a real-time computer vision system called the Multi-Worm Tracker (MWT)[23], which can quantify the behavior of dozens of worms on a petri dish at the same rate as video. That means the MWT system can rapidly quantify behavior with minimal human intervention. Correspondingly, when the MWT system processes too many worms, there is a possibility of dropped frames due to the system being overloaded. Moreover, as the number of worms increases, so does the frequency of collisions between them. When two or more worms collide, their sizes are summed up and counted as a single entity. These combined animals are ignored by the MWT system until they separate and are once again identified as individual worms by the search algorithm[24]. This also leads to the loss of worm features, affecting experimental results. In addition, the MWT system is unable to distinguish between the head and tail of the nematode[23], so it cannot analyze the movement of the head.

Zhang et al. proposed a method for automatically identifying and counting the head’s thrashing behavior of individual worms from experimental videos, achieving automatic recognition and analysis of the head’s thrashing behavior in nematodes[25]. The method employs two criteria to distinguish between the head and tail of a worm in the first frame of the video. The first criterion is that the head of the worm is typically rounder than the tail, so the sharpest point on the worm contour is defined as the tail. The second criterion is that the tail is usually darker than the head, so the brightness of the two endpoints in the binary image is compared, and the endpoint with lower brightness is defined as the tail. However, manual determination of the head and tail of the nematode is still required when there are inconsistent results between the two criteria.

****

**Fig.1** **Movement behaviors, bending angles, and a schematic diagram of the general process (a) Schematic representation of the head thrashing behavior of nematode. (b) Schematic diagram illustrating a complete omega turn behavior in the worm. (c) Schematic of bending angle in WormLab. (d) The general outline of the proposed method.**

WormLab[24] is a commercially available worm-tracking software developed by MicroBrightField. It allows for the tracking of the centroid, head, or tail markers of selected worms, as well as the analysis of various parameters such as speed, area, direction, wavelength, trajectory length, omega turns, and reversals. WormLab defines the supplement of the angle between the head, midbody, and tail of a worm as the bending angle[26] and considers worms with bending angles exceeding 90 degrees as undergoing omega turns. However, the typical definition of an omega turn is when the worm's head almost reaches its tail, or when there is a 135° reorientation during a single head bending[27]. That is, when the worm bends at an angle greater than >90°, the head and tail of the worm may not be close to each other, or redirection of the head may not occur, as shown in Figure 1(c).

In summary, although there are already some automated analysis tools available for *C. elegans'* locomotion behavior, some are commercial while others are free. However, existing automated methods may either overlook finer aspects of worm locomotion, such as head thrashing and omega turn, require manual intervention with room for improvement, or deviate somewhat from traditional behavior definitions. Therefore, this study considers the various disadvantages of manual counting and the shortcomings of existing automated counting methods. It constructs a model for locating the head and tail positions of nematodes and proposes an automatic identification method for the head thrashing and omega turn behaviors of individual nematodes. This approach successfully distinguishes between the head and tail of the nematode, analyzes both the head thrashing and body omega turn behaviors simultaneously, preserves the characteristics of nematode movement, and fully conforms to the traditional definition of nematode movement behaviors without the need for manual intervention. Based on this, the study investigates the problem of using head thrashing and omega turn behaviors of nematodes as indicators to map the functional states of their motion neurons.

**3 Method**

To facilitate researchers from other fields in directly utilizing the automatic analysis results of nematode head thrashing and omega turn behaviors as one of the evaluation methods for their studies, a method for automatically analyzing these behaviors has been proposed. This method includes processing videos and images, implementing a nematode head-tail localization model, and counting the head thrashing and omega turn behaviors. The flowchart of the method is shown in Figure 1(d).

**3.1 Video processing method**

To automate the counting of head thrashing and omega turn behaviors in *C. elegans*, the videos that include *C. elegans* need to be processed. The videos used in the experiments are obtained from the *C. elegans* behavioral phenotype database[28]. Firstly, it is necessary to extract image frames from the nematode experiment video for convenient subsequent experimental processing. However, in practice, the duration and frame rate of the videos obtained directly from the database are not the same. To ensure the scientific integrity of the experiment, we selected videos with a frame rate of 30fps and only extracted a one-minute segment from each video. Then, to capture more details of the nematode's movement during the process, a single image was extracted from each frame of the video. Therefore, from each video, we obtained 1800 frame images. The original frame images are then read as grayscale images.

**3.2 Nematodes head and tail localization method**

To achieve automatic counting of head thrashing and omega turns in *C. elegans*, it is essential to determine the positions of the worm's head and tail. The most crucial step in determining the positions of the head and tail of the worm is distinguishing between the head and tail of the worm. Fortunately, the head and tail of the nematode possess distinct features. For instance, the head appears brighter than the tail due to differences in fat distribution. The head also exhibits a higher frequency of thrashing compared to the tail during foraging behavior. Furthermore, the head is wider than the tail, and the relative positions of the head and tail concerning the body's center point remain relatively stable[29], etc. Because of these different features the head and tail of the nematode can be distinguished.

Due to the excellent performance of Convolutional Neural Networks (CNNs) [30] in the field of image processing, where they can automatically learn features within images and achieve impressive performance in tasks such as recognition, classification, segmentation, and generation, we consider applying CNNs to our image processing task. Given the outstanding performance of CNNs across various tasks, numerous architectures have emerged, such as VGGNet, and ResNet, among others, which have achieved significant results on different tasks and datasets. EfficientNet[31] is also a type of CNN that achieves better performance with the same computational resources. Therefore, we choose EfficientNet as our tool for locating the head and tail of nematodes. Additionally, since transfer learning[32] can utilize existing knowledge to expedite model training, improve performance, and address issues like data scarcity, we consider using pre-trained models for transfer learning to enhance the performance of our model. Finally, because the original grayscale nematode images retain more morphological features, we use grayscale images as our model's dataset.

The specific procedure is to randomly extract 2000 grayscale images containing nematodes from videos of nematodes of different strains in the nematode behavioral phenotyping database. Then, we used the open-source image annotation tool LabelMe[33] to label the head and tail coordinates of nematodes in these grayscale images. Next, to better align the dataset with the pre-trained model, the width and height of the grayscale images are uniformly resized to 224, and the annotated coordinates of the head and tail are also rescaled to match the size of the grayscale images. Then comes the construction of the head and tail localization model. Initially, we utilize the pre-trained model of EfficientNet as the base model. Here, we employ the EfficientNetB0 model, whose weights are trained on the ImageNet dataset. Then, onto the base model of EfficientNetB0, we add an attention[34] mechanism layer. Specifically, through two fully connected layers, the globally average-pooled feature vector is transformed into an attention-weight vector. L2 regularization is concurrently used to constrain the model's complexity. Subsequently, using a Multiply layer, the original feature vector, and the attention weight vector are element-wise multiplied to obtain a weighted feature vector, enhancing the model's focus on specific regions[35]. Finally, two fully connected layers are employed to respectively output the predicted positions of the head and tail. This completes the model construction.



**Fig.2** **The head and tail position localization model**

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. The implementation process of the worm's head and tail localization method is illustrated in Figure 2.

**3.3 Grayscale image binarization method**

To achieve automatic counting of nematode movement behaviors, the grayscale image needs to undergo further processing. The input grayscale image is shown in Figure 3(a). However, due to the spontaneous behaviors of *C. elegans* towards food[28], the grayscale images of the nematodes may contain negative interferences such as shadows. To eliminate these negative interferences and facilitate the subsequent experiments, clean binary images need to be generated next.



**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) Invert the color of images that have completed the XOR operation. (f) Mark the head, tail, and five equally spaced points on the body of the online worm. (g) To calculate the bending angle of the worm’s head using the first three marked points. (h) The distance between the head and tail of the worm.**

Since in grayscale images, the grayscale values of the shadows around the worm are significantly greater than the grayscale values of the worm itself and the background, the first step is to calculate the grayscale values of the shadows. The second step is to use the grayscale values of the shadows as the threshold and employ the global threshold algorithm[36] to obtain the initial binary image. The initial binary image is shown in Figure 3(b). The global threshold algorithm is defined as

In this equation, represents the pixel value at point in the grayscale image, and represents the pixel value at point in the initial binary image. The third step involves using an edge detection algorithm. Since edges refer to areas in the image where there is a sudden change in pixel values, and there is a clear distinction between the worm and its surrounding shadows against the background, an edge detection algorithm is applied to detect all contours in the image. The edge detection algorithm used here is the Sobel edge detection algorithm. The definition of the Sobel edge detection algorithm 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 point in the image. To reduce computational complexity and enable faster computation on computers, the above formula is simplified in practical applications as follows

Since the area occupied by the worm and its surrounding shadow is much larger than the area occupied by the interference and its surrounding shadow, the largest contour in terms of area is the contour containing the worm's body and its surrounding shadow, which is what we want to retain. The fourth step is to use the hole-filling algorithm to fill the largest contour by area. The hole-filling algorithm essentially involves using the scan-line algorithm to fill polygons. Simply put, the scan-line algorithm sorts the intersection points of the polygon's edges with a horizontal scan line and determines whether to fill based on the parity of these intersection points. The image after filling is shown in Figure 3(c). The fifth step involves performing an XOR operation on the initial binary image and the filled image to obtain a binary image. In the binary image, each pixel has a value of either 0 or 1, where 0 usually represents the background (black) and 1 represents the foreground (white). The rule for XOR operation is if the corresponding pixel values are the same (both 0 or both 1), then the resulting pixel value is 0. If the corresponding pixel values are different (one is 0 and the other is 1), then the resulting pixel value is 1. The image after performing the XOR operation is shown in Figure 3(d). The XOR operation is defined as

Here,represents the pixel value at point in the initial binary image, represents the pixel value at point in the image after filling, and represents the pixel value at point in the binary image obtained after performing 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. The image after the inversion process is shown in Figure 3(e). 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 both videos and images.

**3.4 Counting method**

Since binary image thinning can simplify images, highlight features, reduce computational complexity, and facilitate subsequent operations, to automatically count head thrashing and omega turns in *C. elegans*, the worm skeleton needs to be extracted from the binary images. Specifically, first, read the cleaned binary images, then sort them by the order of their filenames. Since the experimental video is segmented and named consecutively, the sorted binary images have a front-to-back order relationship. Next, perform thinning operations on the binary images to obtain the worm skeletons. The expression for thinning a binary image to obtain the worm skeleton is:

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.

Because the distance between the head-to-head and tail-to-tail of the worm is closer between consecutive frames, the positions of the worm's head and tail can be determined based on this criterion in the frames following the first frame. To simplify the experiment and reduce the wastage of computational resources, only the worm head-tail positioning method needs to be used to predict the head and tail positions in the first frame of the worm image. Subsequent frames can determine the positions of the worm's head and tail based on distance. In concrete terms, in the first frame of the image, the positions of the worm's head and tail are obtained using the worm head and tail positioning method. The coordinate data is then read and saved separately in arrays for the worm's head and tail positions. Simultaneously, the positions of all the endpoints of the worm skeleton previously obtained are calculated. For example, in the second frame, the positions of the two endpoints are compared with the known head and tail positions from the first frame. The endpoint closer to the known worm head is considered the worm's head, and the endpoint closer to the known worm tail is considered the worm's tail. Then, the newly obtained positions of the worm's head and tail are saved in arrays for the worm's head and tail positions until the head and tail positions of all the worms in the images are obtained.

Next, calculate the curvature angle of the head of *C. elegans* to obtain the number of head thrashing of the nematode. To be more specific, the length of the head of *C. elegans* is approximately 1/6 of its total body length[37]. Therefore, the body of the nematode is divided into six equal parts using five equally spaced points. It is important to note that the body length refers to the total length of the nematode skeleton, not just the distance from the head to the tail. Subsequently, seven points are recorded in an array, including the head endpoint, tail endpoint, and five equally spaced points. The seven points are shown in Figure 3(f).

The bending angle of the nematode's head is calculated from the first three of these seven points, which will be labeled 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 . Then we will mark the supplement of as the bending angle of the nematode's head, as shown in Figure 3(g). 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. The bending angle of the head of *C. elegans* was calculated using the upper method. The angles of bending of the nematode's head are recorded in an array, and the bend angles of the nematode's head are calculated for each subsequent frame until the curvature angles of the heads of all nematodes in the images are calculated. Here, we consider thrashing the nematode's head from one side to the other and back as one complete head thrashing[38], so a change in the direction of the nematode's head bend twice is considered one head thrashing. Then, we need to calculate the number of changes in the direction of the nematode's head bend. Here, we consider the positive and negative changes in angle differences between bending frames as changes in the direction of the nematode's head bend, so we need to count the positive and negative changes in angle differences between consecutive frames. Finally, dividing the number of changes in the direction of the nematode's head bend by 2 gives the number of head thrashing of the nematode, thus obtaining the number of head thrashing of the nematode.

The next step is to calculate the distance between the head and tail of the *C. elegans* to determine the number of omega turns. Specifically, we will compute the distance between the head and tail coordinates of the worm. The schematic diagram of the distance between the head and tail of the nematode is shown in Figure 3(h). 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 . The calculated distance between the head and tail of the nematode will be recorded in an array of head-to-tail distance, and subsequently, the distance between the head and tail in the next frame will be computed, until distances for all nematodes in the images are calculated. Then, the farthest distance between the head and tail is determined and defined as the maximum head-to-tail distance. Here, if the distance between the head and tail of the nematode is less than half of the maximum head-to-tail distance, we consider an omega turn has occurred[39]. Therefore, when the head-to-tail distance is less than half of the maximum head-to-tail distance, the frame is marked as the start of an omega turn. Conversely, when the head-to-tail distance is greater than half of the maximum head-to-tail distance, it is marked as the end of an omega turn. A complete omega turn includes both the start and end of an omega turn. Finally, the number of worm’s omega turns in the nematode can be calculated.

In the above process, it is also possible to simultaneously detect abnormal behaviors of the nematodes. In concrete terms, if there is no change in the positions of the nematode’s head and tail for 100 consecutive frames, it is considered abnormal behavior, and a notification is sent to the experimenter. This enables automatic counting of head thrashing and omega turn of the nematode, as well as detection of abnormal behavior.

**4 Resul**

This section will conduct experimental validation to verify the effectiveness of the proposed algorithm. We will discuss three aspects: the results of head and tail identification in nematodes, the results of head thrashing and omega turn behaviors counting in nematodes, and a comparison of the locomotion behavior of nematodes with normal and defective motor neurons.



**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 at different epochs for the nematode head and tail localization model and the ResNet18 network model trained on the same dataset.**

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

Using the head and tail localization model of *C. elegans*, we obtained the positions of the worm's head and tail. To demonstrate the effectiveness of our method, it is necessary to compare the head and tail positions obtained from the localization model with manually annotated positions. The comparison between manually annotated and model-predicted head and tail positions is summarized in Table 1. The visual comparison results between manually annotated and model-predicted head and tail positions are shown in Figure 4(a). Here, we consider the difference between manually annotated head and tail positions and model-predicted head and tail positions as the distance threshold. To illustrate the efficiency of the head-tail localization model for nematodes, we compared the training results of the ResNet18 neural network model with the head-tail localization model on the same dataset at the same epoch. The comparison of different losses for the ResNet18 network model and the head-tail localization model at the same epoch is shown in Figure 4(b).

The statistical comparison data between manually annotated and model-predicted head and tail positions indicate that when the distance threshold is 5 pixels, the prediction accuracy for both the head and tail positions of the worm reaches 100%. When the distance threshold is above 4 pixels, the prediction accuracy for both the head and tail positions of the worm is above 90%. Even with a distance threshold of 3 pixels, the prediction accuracy for the tail position of the worm reaches 88%. However, when the distance threshold is two pixels or below, the accuracy of predicting the head and tail positions of the nematode is below 50%. The comparison between the head-tail localization model and the ResNet18 neural network model shows that although the loss rates decrease noticeably with increasing epochs for both models, the head-tail localization model exhibits significantly lower loss when at the same epoch.

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

|  |  |  |
| --- | --- | --- |
| **Distance threshold(pixel）** | **Head accuracy** | **Tail accuracy** |
| **1** | **2.60%** | **17.00%** |
| **2** | **13.80%** | **45.10%** |
| **3** | **66.10%** | **88.60%** |
| **4** | **94.60%** | **99.50%** |
| **5** | **99.70%** | **100.00%** |
| **6** | **100.00%** | **100.00%** |

The significant difference in accuracy corresponding to different distance thresholds between manually labeled and model-predicted positions of worm heads and tails is because both the manual labeling of worm head and tail positions and the image used for predicting the positions of worm heads and tails by the model are grayscale images. Specifically, on the one hand, due to various adverse factors like shadows in grayscale images surrounding the nematode, the predicted positions by the head-tail localization model can be influenced by these factors, leading to slight discrepancies within a very small pixel distance compared to manually annotated positions. On the other hand, because the comparison is made between the predictions of the head-tail localization model and manually annotated data, the latter inevitably contains errors. Therefore, variations in accuracy corresponding to different distance thresholds occur, which is an unavoidable drawback of manual annotation.

Certainly, this statistical data on the accuracy of manual annotation and model annotation also validates the effectiveness of the head and tail localization model for worms. Although the prediction accuracy for both the head and tail positions of the worm reaches 100% only when the distance threshold is 6 pixels, this data also indicates that the model consistently achieves 100% accuracy in distinguishing the head and tail. Therefore, in the subsequent counting experiment process, after obtaining the head and tail positions of the first frame using the head and tail position localization model, the principle that the distance between the head-to-head and tail-to-tail of the nematode is closer between adjacent frames can be used to obtain more accurate positions of the nematode’s head and tail. The comparison results between the head and tail localization model for worms and the ResNet18 neural network model indicate that the head and tail localization model for worms exhibits higher efficiency.

**4.2 *C. elegans* head bending and omega turning count results**

Before counting the thrashing of the worm’s head and omega turns, it is essential to calculate the bending angle of the worm’s head and the distance between the head and tail. Next, we will analyze the relationship between the head bend angle and the distance between the head and tail of the nematode during head thrashing., as well as the relationship between the head bend angle and the distance between the head and tail of the nematode during omega turns. Taking an experiment with nematodes of genotype Schafer Lab N2 as an example, the variations in head bending angle and the distance between the head and tail corresponding to different thrashing amplitudes are recorded in Figure 5. Similarly, the variations in head bend angle and the distance between the head and tail corresponding to different omega turn behaviors are recorded in Figure 6.



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



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

Due to the definition of a complete head thrashing as thrashing the nematode's head from one side to the other and back, a change in the direction of the nematode's head bending twice is considered one head thrashing. Therefore, the positive and negative changes in the angle difference between consecutive frames are considered as changes in the bending direction of the nematode's head, and the number of changes in the bending direction of the nematode's head is calculated. The number of changes in the bending direction of the nematode's head is divided by 2 to obtain the number of head thrashing of the nematode. Therefore, in the relationship chart between the head thrashing behavior of nematodes the angle of head bending, and the distance between the head and tail, each turning point may be considered as a reference point for the number of head trashing of the nematode (points marked in orange in the figure). Furthermore, the relationship chart between the head thrashing behavior of nematodes the angle of head bending and the distance between the head and tail shows that the greater the thrashing amplitude of the nematode's head (such as thrashing amplitude I), the greater the fluctuation in the angle of head bending, and the more pronounced the changes in the distance between the head and tail (as shown in region I); when the thrashing amplitude of the nematode's head is smaller (such as thrashing amplitude V), the fluctuation in the angle of head bending is smaller, and the changes in the distance between the head and tail are more gradual (as shown in region V).

The omega turn was considered to have occurred when the distance between the head and tail of the nematode was less than half of the maximum head-tail distance. Therefore, the relationship graph of *C. elegans* in omega turns and the angle of head bending and the distance between the head and tail indicates that when omega turn occurs, the distance between the head and tail of the worm significantly decreases, even to less than half of the maximum head-tail distance (as shown in regions II, IV, and VII). Furthermore, the situation where the distance between the head and tail of the worm is less than half of the maximum distance occurred three times, indicating that during this period, the worm underwent three omega turns.

To validate the effectiveness of our method, we chose to compare the results of manual counting with those of automatic counting. Firstly, we selected seven strains of *C. elegans* from the *C. elegans* Behavioral Database: Schafer Lab N2(N2), *ins-18*, *flp-13*, *nlp-2*, *unc-8*, *unc-44*, and *unc-116*. Among these, N2 is the wild-type strain, while the others are mutant strains. 30 one-minute videos were randomly chosen from each strain, totaling 210 one-minute videos, with 1800 frames per video. Head thrashings were manually and automatically counted for each video. Specifically, trained researchers counted head thrashing and omega turns for each worm in the videos, with three manual counts performed for each video to reduce errors. The manual counts reported in this paper represent the average of these three counts. Subsequently, an automatic counting algorithm was used to count head thrashing and omega turns for each video. The manual and automatic counts for head thrashing and omega turn, along with the statistics of abnormal behaviors, are summarized in Table 2. After calculation, the Pearson correlation coefficient between manual counting and automatic counting for head thrashing is 0.9953, with an average absolute error of 2.0429. Compared to the method proposed by Zhang et. al[25], the approach introduced in this paper avoids errors resulting from conflicts caused by using different methods for head and tail recognition. It enhances the accuracy of automatic counting of head thrashing behavior, offering greater stability and robustness. The Pearson correlation coefficient between manual counting and automatic counting for omega turns is 0.9856, with an average absolute error of 0.3343. This data demonstrates the effectiveness of the method proposed in this paper. The comparison of Pearson correlation coefficients and mean absolute errors for the two methods is shown in Figure 7(a) and Figure 7(b), respectively. The scatterplots of counting results for the head thrashing and omega turns of nematodes are presented in Figure 7(c) and Figure 7(d), respectively.

**Table 2 Data on head thrashing and omega turn and abnormal behavior in different genotypes of nematodes.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Head Thrash Count** | | **Omega Turn Count** | | **Abnormal Behavior** |
| **Automatic** | **Manual** | **Automatic** | **Manual** |
| ***ins-18*** | **83.8** | **82.4** | **5.8** | **5.4** | **False** |
| ***flp-13*** | **93.0** | **92.3** | **5.3** | **6.0** | **False** |
| ***nlp-2*** | **83.8** | **82.5** | **5.0** | **4.5** | **False** |
| **N2** | **90.8** | **90.8** | **4.0** | **3.7** | **False** |
| ***unc-8*** | **32.2** | **32.0** | **0.4** | **0.2** | **False** |
| ***unc-44*** | **27.6** | **26.8** | **1.4** | **1.1** | **False** |
| ***unc-116*** | **30.3** | **30.4** | **1.3** | **1.1** | **False** |

The purpose of automatically counting the head thrashing and omega turns of nematodes is to reduce the various costs of manpower and resources required for manual counting, and to avoid the drawbacks of manual counting, such as the inability to work continuously for long periods and the potential for errors. This allows the results of automatic counting of head thrashing and omega turns to be directly applicable to other different fields. Therefore, even if there is a slight fluctuation between the results of automatic counting and the validation data from manual counting, as long as this fluctuation is within a certain range, the goal of saving manpower and resources is still achieved.

****

**Fig.7** **Counting results of different counting methods and data processing plots (a) Comparative plots of Pearson's correlation coefficients for manual and automated counts. (b) The plot of comparison of mean absolute error between manual and automatic counts. (c) Scatter plots of the counting results of the two methods for nematode head thrashing behavior. (d) Scatter plots of counting results of nematode omega turn behavior by the two methods. (e) Comparison between manual and automatic counting of head thrashing behavior in *C. elegans*. (f) Comparison between manual and automatic counting of omega turn behavior in *C. elegans*.**

**4.3 Comparison of locomotion behavior between nematodes with normal motor neurons and those with motor neuron defects**

Because the movement behaviors of nematodes can reflect the integrity of their motor neurons, to further validate our inference — that the head thrashing and omega turn behaviors of nematodes can reflect the integrity of their motor neurons — we selected some nematodes with no obvious defects in their motor neurons and compared their head thrashing and omega turn behaviors with those of nematodes with obvious defects in their motor neurons. The gene types of the nematodes we selected with no obvious defects in their motor neurons are *ins-18*, *flp-13*, N2, *and nlp-2*, while the genetic types of worms with clear defects in their motor neurons are *unc-8*[40], *unc-44*[41], *unc-116*[42]. For each genotype of nematode, 30 one-minute experimental videos were randomly selected, and then the head thrashing and omega turn behaviors of the nematodes were manually and automatically counted separately, with the final counting results averaged. The data statistics of head thrashing and omega turn behaviors of nematodes with different genotypes are presented in Table 2. The average manual and automatic counts for head thrashing behavior are shown in Figure 7(e), and the average manual and automatic counts for omega turn behavior are also shown in Figure 7(f).

The statistical results indicate that the average counts of manually and automatically counted head thrashing and omega turn behaviors of nematodes are generally consistent, validating the accuracy and robustness of the automatic counting method. Furthermore, nematodes with no obvious defects in their motor neurons exhibit a significantly higher average number of head thrashing per minute compared to those with obvious defects in their motor neurons. Additionally, nematodes with no obvious defects in their motor neurons also show a higher average number of omega turns per minute compared to those with obvious defects in their motor neurons. This is because the motor neurons of the nematodes are damaged, whether it is GABAergic motor neurons or cholinergic motor neurons, it will affect the worm's movement behaviors, including head thrashing and omega turn behaviors. Therefore, there is a clear distinction in the quantification of head thrashing and omega turn behaviors between worms with no obvious defects in their motor neurons and those with clear defects. Specifically, worms with no obvious defects in their motor neurons exhibit higher quantification values for head thrashing and omega turn behaviors, while worms with clear defects in their motor neurons exhibit lower quantification values for head thrashing and omega turn behaviors.

The statistical data of head thrashing and omega turn behaviors in *C. elegans* further validate our goal of using these behaviors to map the integrity of the worm's motor neurons through automatic counting. The frequency of head thrashing and omega turn behaviors in worms with clear defects in their motor neurons is significantly lower than that in worms with no defects. It is important to note that even in nematodes with intact motor neurons, there may be low-frequency occurrences of head thrashing and omega turn behaviors, especially omega turns. This variability is due to individual differences among nematodes, which underscores the necessity for averaging statistical data.

**5 Conclusion**

This paper utilized an automated counting method to investigate the use of head thrashing and omega turn behaviors in nematodes as indicators mapping the functional state of their motor neurons. Firstly, we achieved automatic localization of the head and tail positions in nematodes, with a localization accuracy of 100% within the allowable error range. Next, we automatically counted the head thrashing and omega turn behaviors in nematodes and compared the results with manual counting. The similarity between the two sets of results demonstrated the practicality of our proposed method. Finally, we performed counts on the head thrashing and omega turn behaviors of nematodes with normal motor neurons and those with obvious motor neuron defects. The results confirmed that nematodes with motor neuron defects exhibited a lower frequency of head thrashing and omega turn behaviors compared to nematodes with intact motor neurons. Therefore, the automatic counting results of head thrashing and omega turns in *C. elegans* can serve as indicators of the integrity of motor neurons.

**6 Reference**

1. Nüsslein-Volhard C (2022) The Toll gene in Drosophila pattern formation. Trends in Genetics 38:231–245. https://doi.org/10.1016/j.tig.2021.09.006

2. Janowski M, Andrzejewska A (2022) The legacy of mRNA engineering: A lineup of pioneers for the Nobel Prize. Molecular Therapy - Nucleic Acids 29:272–284. https://doi.org/10.1016/j.omtn.2022.07.003

3. Ishioka N, Higashibata A (2019) Space Experiments Using C. elegans as a Model Organism. In: Pathak Y, Araújo Dos Santos M, Zea L (eds) Handbook of Space Pharmaceuticals. Springer International Publishing, Cham, pp 1–32

4. Scharf A, Pohl F, Egan BM, et al (2021) Reproductive Aging in Caenorhabditis elegans: From Molecules to Ecology. Front Cell Dev Biol 9:718522. https://doi.org/10.3389/fcell.2021.718522

5. Cao X, Xie Y, Yang H, et al (2023) EAT-2 attenuates C. elegans development via metabolic remodeling in a chemically defined food environment. Cell Mol Life Sci 80:205. https://doi.org/10.1007/s00018-023-04849-x

6. Yemini E, Lin A, Nejatbakhsh A, et al (2021) NeuroPAL: A Multicolor Atlas for Whole-Brain Neuronal Identification in C. elegans. Cell 184:272-288.e11. https://doi.org/10.1016/j.cell.2020.12.012

7. Koopman M, Peter Q, Seinstra RI, et al (2020) Assessing motor-related phenotypes of Caenorhabditis elegans with the wide field-of-view nematode tracking platform. Nat Protoc 15:2071–2106. https://doi.org/10.1038/s41596-020-0321-9

8. Schwartz ML, Davis MW, Rich MS, Jorgensen EM (2021) High-efficiency CRISPR gene editing in C. elegans using Cas9 integrated into the genome. PLoS Genet 17:e1009755. https://doi.org/10.1371/journal.pgen.1009755

9. Sohrabi S, Mor DE, Kaletsky R, et al (2021) High-throughput behavioral screen in C. elegans reveals Parkinson’s disease drug candidates. Commun Biol 4:203. https://doi.org/10.1038/s42003-021-01731-z

10. Dou T, Chen J, Wang R, et al (2022) Complementary protective effects of autophagy and oxidative response against graphene oxide toxicity in Caenorhabditis elegans. Ecotoxicology and Environmental Safety 248:114289. https://doi.org/10.1016/j.ecoenv.2022.114289

11. Rapti G (2020) A perspective on *C. elegans* neurodevelopment: from early visionaries to a booming neuroscience research. Journal of Neurogenetics 34:259–272. https://doi.org/10.1080/01677063.2020.1837799

12. Sulston JE, Schierenberg E, White JG, Thomson JN (1983) The embryonic cell lineage of the nematode Caenorhabditis elegans. Developmental Biology 100:64–119. https://doi.org/10.1016/0012-1606(83)90201-4

13. O’Reilly LP, Luke CJ, Perlmutter DH, et al (2014) C. elegans in high-throughput drug discovery. Adv Drug Deliv Rev 69–70:247–253. https://doi.org/10.1016/j.addr.2013.12.001

14. Kaletta T, Hengartner MO (2006) Finding function in novel targets: C. elegans as a model organism. Nat Rev Drug Discov 5:387–398. https://doi.org/10.1038/nrd2031

15. C. elegans Sequencing Consortium (1998) Genome sequence of the nematode C. elegans: a platform for investigating biology. Science 282:2012–2018. https://doi.org/10.1126/science.282.5396.2012

16. Wilson DM, Cookson MR, Van Den Bosch L, et al (2023) Hallmarks of neurodegenerative diseases. Cell 186:693–714. https://doi.org/10.1016/j.cell.2022.12.032

17. Blank LJ, Acton EK, Thibault D, Willis AW (2021) Neurodegenerative disease is associated with increased incidence of epilepsy: a population based study of older adults. Age Ageing 50:205–212. https://doi.org/10.1093/ageing/afaa194

18. Nixon RA (2005) Endosome function and dysfunction in Alzheimer’s disease and other neurodegenerative diseases. Neurobiology of Aging 26:373–382. https://doi.org/10.1016/j.neurobiolaging.2004.09.018

19. Franco-Iborra S, Vila M, Perier C (2018) Mitochondrial Quality Control in Neurodegenerative Diseases: Focus on Parkinson’s Disease and Huntington’s Disease. Front Neurosci 12:342. https://doi.org/10.3389/fnins.2018.00342

20. Masrori P, Van Damme P (2020) Amyotrophic lateral sclerosis: a clinical review. Euro J of Neurology 27:1918–1929. https://doi.org/10.1111/ene.14393

21. Chen L, Zhang S, Liu S, Gao S (2023) Caenorhabditis elegans Models Count. Biology and Life Sciences

22. Zhang X, Zhong H-Q, Chu Z-W, et al (2020) Arsenic induces transgenerational behavior disorders in Caenorhabditis elegans and its underlying mechanisms. Chemosphere 252:126510. https://doi.org/10.1016/j.chemosphere.2020.126510

23. Swierczek NA, Giles AC, Rankin CH, Kerr RA (2011) High-Throughput Behavioral Analysis in C. elegans. Nat Methods 8:592–598. https://doi.org/10.1038/nmeth.1625

24. Husson SJ, Costa WS, Schmitt C, Gottschalk A (2018) Keeping track of worm trackers. In: WormBook: The Online Review of C. elegans Biology [Internet]. WormBook

25. Zhang H, Gao S, Chen W (2022) Automated recognition and analysis of head thrashes behavior in C. elegans. BMC Bioinformatics 23:87. https://doi.org/10.1186/s12859-022-04622-0

26. Angstman NB, Frank H-G, Schmitz C (2016) Advanced Behavioral Analyses Show that the Presence of Food Causes Subtle Changes in C. elegans Movement. Front Behav Neurosci 10:60. https://doi.org/10.3389/fnbeh.2016.00060

27. Gray JM, Hill JJ, Bargmann CI (2005) A circuit for navigation in Caenorhabditis elegans. Proc Natl Acad Sci U S A 102:3184–3191. https://doi.org/10.1073/pnas.0409009101

28. Yemini E, Jucikas T, Grundy LJ, et al (2013) A database of Caenorhabditis elegans behavioral phenotypes. Nat Methods 10:877–879. https://doi.org/10.1038/nmeth.2560

29. Geng W, Cosman P, Berry CC, et al (2004) Automatic Tracking, Feature Extraction and Classification of C. elegans Phenotypes. IEEE Trans Biomed Eng 51:1811–1820. https://doi.org/10.1109/TBME.2004.831532

30. Jogin M, Mohana, Madhulika MS, et al (2018) Feature Extraction using Convolution Neural Networks (CNN) and Deep Learning. In: 2018 3rd IEEE International Conference on Recent Trends in Electronics, Information & Communication Technology (RTEICT). IEEE, Bangalore, India, pp 2319–2323

31. Tan M, Le Q (2019) EfficientNet: Rethinking Model Scaling for Convolutional Neural Networks. In: Proceedings of the 36th International Conference on Machine Learning. PMLR, pp 6105–6114

32. Zhuang F, Qi Z, Duan K, et al (2021) A Comprehensive Survey on Transfer Learning. Proc IEEE 109:43–76. https://doi.org/10.1109/JPROC.2020.3004555

33. Russell BC, Torralba A, Murphy KP, Freeman WT (2008) LabelMe: A Database and Web-Based Tool for Image Annotation. Int J Comput Vis 77:157–173. https://doi.org/10.1007/s11263-007-0090-8

34. Vaswani A, Shazeer NM, Parmar N, et al (2017) Attention is All you Need

35. Niu Z, Zhong G, Yu H (2021) A review on the attention mechanism of deep learning. Neurocomputing 452:48–62. https://doi.org/10.1016/j.neucom.2021.03.091

36. Gonzalez RC, Woods RE (2018) Digital image processing. Pearson, New York, NY

37. Cho JY, Choi T-W, Kim SH, et al (2021) Morphological Characterization of small, dumpy, and long Phenotypes in Caenorhabditis elegans. Molecules and Cells 44:160–167. https://doi.org/10.14348/molcells.2021.2236

38. Zheng F, Chen C, Aschner M (2022) Neurotoxicity Evaluation of Nanomaterials Using *C. elegans* : Survival, Locomotion Behaviors, and Oxidative Stress. Current Protocols 2:e496. https://doi.org/10.1002/cpz1.496

39. Ghosh R, Mohammadi A, Kruglyak L, Ryu WS (2012) Multiparameter behavioral profiling reveals distinct thermal response regimes in Caenorhabditis elegans. BMC Biol 10:85. https://doi.org/10.1186/1741-7007-10-85

40. Murray SM, Waddell BM, Wu C-W (2020) Neuron-specific toxicity of chronic acrylamide exposure in C. elegans. Neurotoxicology and Teratology 77:106848. https://doi.org/10.1016/j.ntt.2019.106848

41. Schmeisser K, Fardghassemi Y, Parker JA (2017) A rapid chemical-genetic screen utilizing impaired movement phenotypes in C. elegans: Input into genetics of neurodevelopmental disorders. Experimental Neurology 293:101–114. https://doi.org/10.1016/j.expneurol.2017.03.022

42. Soh MS, Cheng X, Vijayaraghavan T, et al (2020) Disruption of genes associated with Charcot-Marie-Tooth type 2 lead to common behavioural, cellular and molecular defects in Caenorhabditis elegans. PLoS ONE 15:e0231600. https://doi.org/10.1371/journal.pone.0231600