

# **Nemo: A computational tool for analyzing nematode locomotion**

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## **AF1. Description of the steps ensued for quantitative analysis and features of the algorithm and the GUI**

### **Image analysis and object extraction**

The first step in image analysis is the removal of noise introduced during acquisition of the images. A mean low-pas filter is applied to smooth images (Fig.1A), which replaces every pixel with the average of its 3x3 neighbourhood. The free ImageJ software (<http://rsb.info.nih.gov/ij/>) is used to perform filtering operations on all images. Every image is subsequently segmented by subdividing it into its constituent objects. Segmentation is performed by means of a threshold value  $T$  defined by the user and binary images are generated (Fig.1B). Black pixels correspond to background while white pixels correspond to the animal.

In order to extract useful quantitative information about the individual animals in the frame, a number of morphological operations are applied on the binary images. Dilation

is one of the basic operators used to gradually enlarge the boundaries of regions of foreground pixels. Thus, areas of foreground pixels grow in size while holes within those regions become smaller. This is an important morphological operation, helping to clean small spots inside the animal body. For labelling connected components, every image is scanned and pixels are grouped into components based on pixel connectivity. Large objects are assigned to worms in the image while smaller objects outside the perimeter of the animals are removed. Using built-in functions provided by the Image Analysis Toolbox of Matlab, the perimeter of each worm can be easily obtained (Fig.1C). The ‘spine’ (or ‘skeleton’) of the animal is obtained in a similar fashion (Fig.2). An algorithm has been developed to remove small ‘branches’ on the skeleton (Fig.3). Final skeleton correction is achieved by removing redundant branches manually. The image processing procedure is summarised in Fig.4.

The distinction between the head and the tail is based on relative brightness (the head is less dark than the tail). This can also be accomplished with minimum intervention by the user. The user defines in the first frame of the sequence the head and the tail (points A and B in Fig. 5A). In the second frame, the skeleton has again two endpoints A’ and B’ (Fig.5). We assign ‘Head’ to be the endpoint corresponding to  $\min(\text{distance}(A,A'), \text{distance}(A,B'))$ . Similarly, ‘Tail’ is determined by  $\min(\text{distance}(B,A'), \text{distance}(B,B'))$ . The same procedure is repeated for the rest of the images in the video sequence comparing distances in successive frames. This approach is effective provided that the distance between the head and the tail is relatively large and the animal does not form a closed loop.

## Development of the algorithm

The algorithm we developed aims to calculate a number of parameters that can be used to analyse worm movement. After the initial extraction of the area occupied by the animal, attention is restricted only to small boxes containing the animal (Fig.6). The advantage of reducing the region of interest analysis is that it reduces time consuming computations.

The total number of white pixels representing the mask in the binary counterpart of the raw image yields the area of the animal in pixels. The area occupied by the animal in physical units can be computed. The perimeter points of all nematodes in the video sequence are determined by means of built-in functions from the Matlab Image Analysis Toolbox.

The skeleton of the animal helps to compute the length of the animal adding the sum of the distances between adjacent pixels along the spine of the animal. Knowing the approximate equivalence of the physical units to pixel size, the actual size of the worm spine can be calculated.

To analyse the movement of the animal, the coordinates of points along the spine must be computed. To this end, the body of the worm is divided into a number of segments  $N$  (in this experiment  $N=7$  as seen in Fig.7). The position of the centre of mass of every part (Fig.8A) as well as the centroid of the whole animal are recorded (Fig.8B). The centre of mass of every segment is approximated by computing the centre of mass of the skeleton section in this segment. A more precise but time consuming procedure would require the calculation of the coordinates of the centre of mass considering all body

pixels within that particular segment. However, we have noted that the difference between the two estimations of the centre of mass is small.

The thickness of the animal is computed in all segments except from the front (head) and back (tail). In order to compute the thickness of each segment, we use the binary image of the worm with the perimeter, skeleton and centre of mass of the segment sketched in the picture (Fig.9A). We define a quantity  $W$  to be equal to half of the thickness of the segment. The user chooses a circle of a radius bigger than  $W$  in such a way that it will ensure intersection with the perimeter in four points. These intersections define two segments along the perimeter and the distance between each point and the centre of mass is computed. The average of the smallest distances determines the approximate  $W$  (Fig.9B) and thereby the thickness can be easily estimated. The user is required to intervene only in the first frame to define the circle radius. This value is passed to the rest of the frames and the automated procedure is fast and efficient.

### ***Management of data and calculation of other parameters***

A Graphical User Interface (Fig.10) has been developed, aiming to provide the means to assist the researcher in analysing the collected data in a fast and efficient way. It operates as an information management tool and it allows the user to conduct further calculations. Graphs and histograms of measurable and computed parameters can be plotted and image figures can be produced by the GUI.

#### ***(a). Images:***

Image figures show raw frames with the worm, its perimeter, spine, segments, centre of mass of the entire animal, centre of mass of individual segments. The GUI offers an interactive tool to monitor all the above morphological quantities at any time point. The

total trajectory of the centre of mass ( $CM_{segment}$ ) of any segment of the worm skeleton can be also obtained, analysed and illustrated (Fig.11). X-coordinates and Y-coordinates of  $CM_{segment}$  as a function of time can be plotted (Fig.12).

(b). Computation of other parameters:

The knowledge of the location of particular points (centre of mass of the animal or centroids of segments) can facilitate the calculation of other parameters. In principle, the size of the animal allows a good and accurate estimation of the position of these points. It is, however, possible that a small deviation may generate meaningless patterns. For example, when the angle in the frame  $i$ ,

$$\phi_i = \arctan\left(\frac{y_{i+1} - y_i}{x_{i+1} - x_i}\right)$$

is calculated, where  $y$  and  $x$  are the position coordinates of the point in successive frames, the results demonstrate big fluctuations (Fig.13A). To eliminate this behaviour, position coordinates are averaged over a number of frames helping to obtain smoother results (Fig.13B). The GUI allows the researcher to choose the number of frames over which data will be averaged (i.e. in this experiment, the default value is 4).

### **1. Distance covered**

The distance covered by the worm is defined as the length of the total trajectory of the centre of mass (CM) of the animal. The distance between CM in successive frames is computed and then the sum represents the total length of the trajectory. In the current experiment, the distance covered equals 5.55mm.

## **2. *Animal thickness***

The average thickness of the animal as derived by considering all values produced by the automated algorithm (i.e. 0.092mm). In Fig.14A, a histogram of the values is plotted.

## **3. *Distance between head and tail***

The distance between the end-points does not equal the length of the animal. In Fig.14B, the histogram of values of this distance is sketched.

## **4. *Speed of CM or CM\_segment***

The displacement of the position of a point over time yields the speed of *CM* and *CM\_segment* and the plots are illustrated in the form of a time series or a histogram in Fig.15A and Fig.15B, respectively.

## **5. *Wavelength***

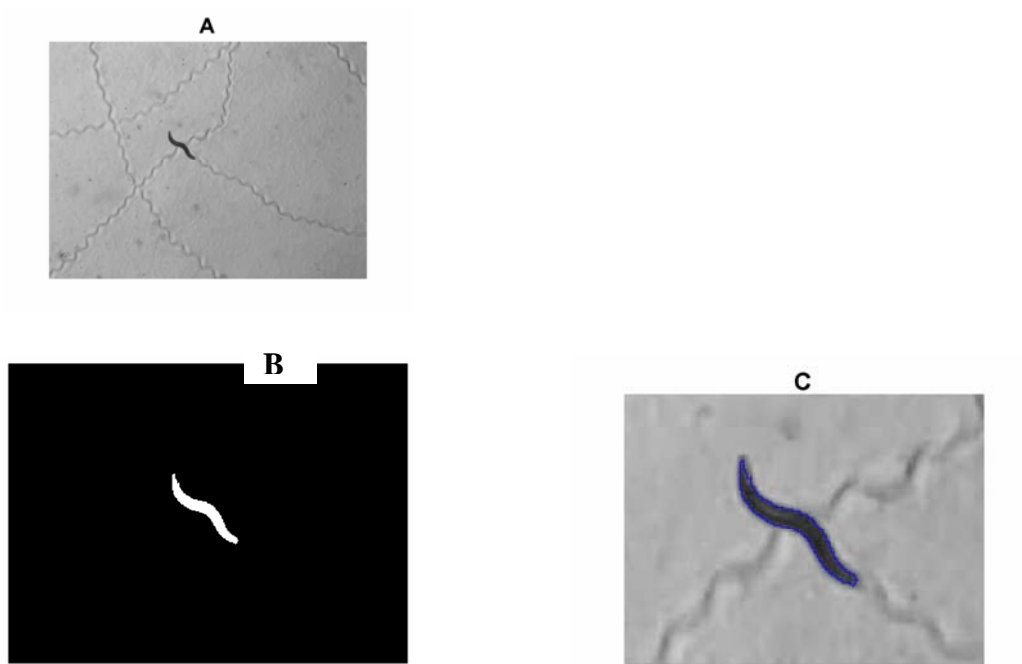
The calculation of the wavelength of the sinusoidal movement of the *C.elegans* is conducted by means of observing the change in the angle  $\phi_t$  (Fig.13B). The positions between two successive maxima (or minima) can be used to define the wavelength of the sinusoidal movement. In Fig.16A, the movement of the centre of mass of the second segment of the animal is illustrated. The line between two successive asterisks represents one wavelength (i.e. 0.37mm).

## **6. *Amplitude***

By applying a transform on the coordinates of the centroid trajectory of a particular segment, the waveform amplitude can be obtained. In Fig.16B, the sinusoidal movement waveform of the second segment is plotted yielding an approximate value for the amplitude equal to 0.03mm.

## **7. *Turning***

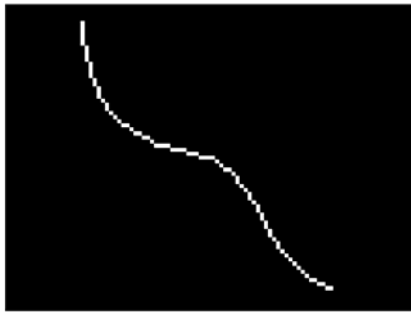
Deviations from a smooth sinusoidal movement (Fig.11) are quantified by computing the angle change between successive wavelengths (Fig.17).



**Figure 1**

Image processing. A. Raw Image. B. Mask after thresholding and morphological operations. C. Perimeter of the worm.





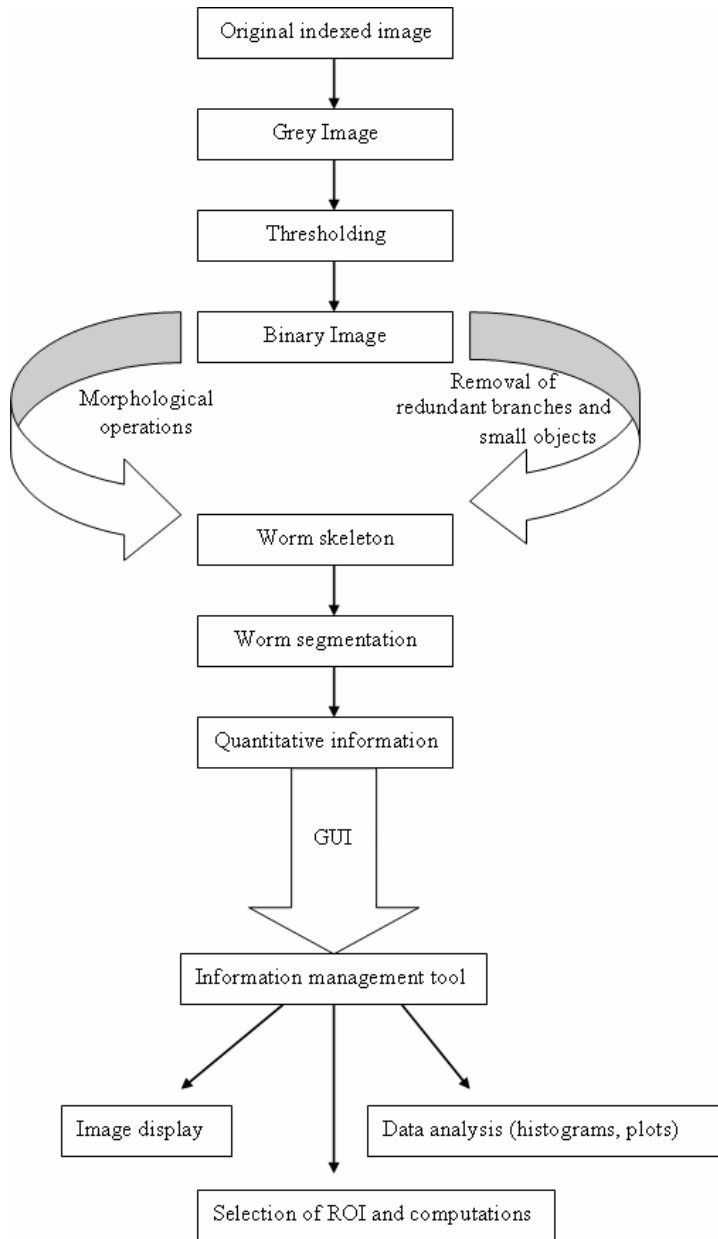
**Figure 2**

Image skeletonisation.



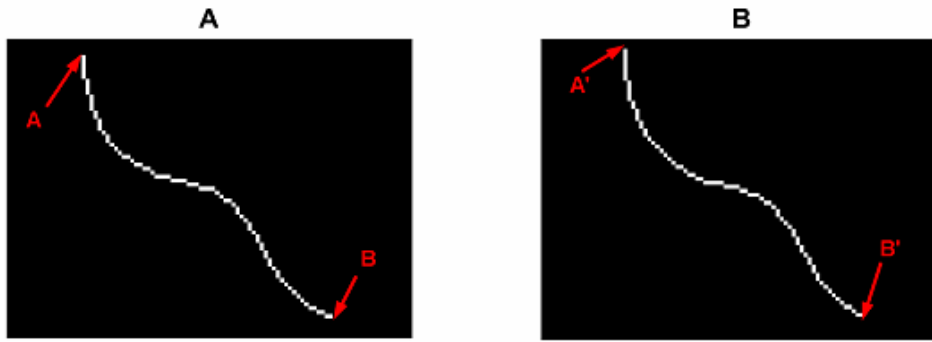
**Figure 3**

Branch removal. A. Skeleton with branches. B. Skeleton after removal of the branches.



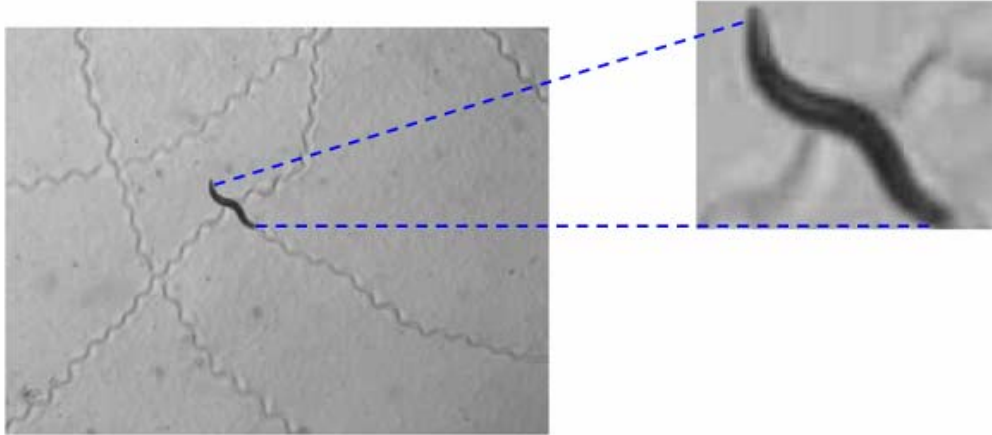
**Figure 4**

Image analysis algorithm. Steps of the image analysis procedure.



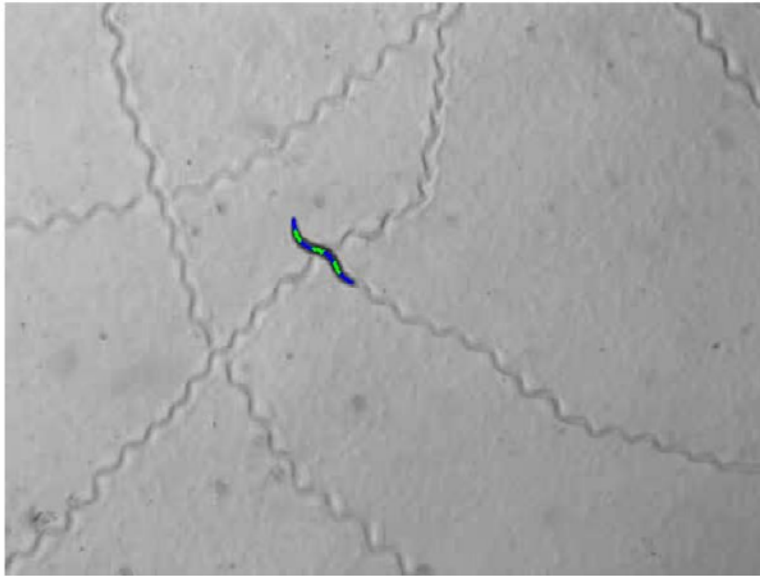
**Figure 5**

Head and tail of the animal in two successive images. A. The A and B within the panel represent the head and tail of the animal. B. A' and B' are the endpoints of the skeleton and minimisation of distances will determine whether they can be assigned as 'head' or 'tail'.



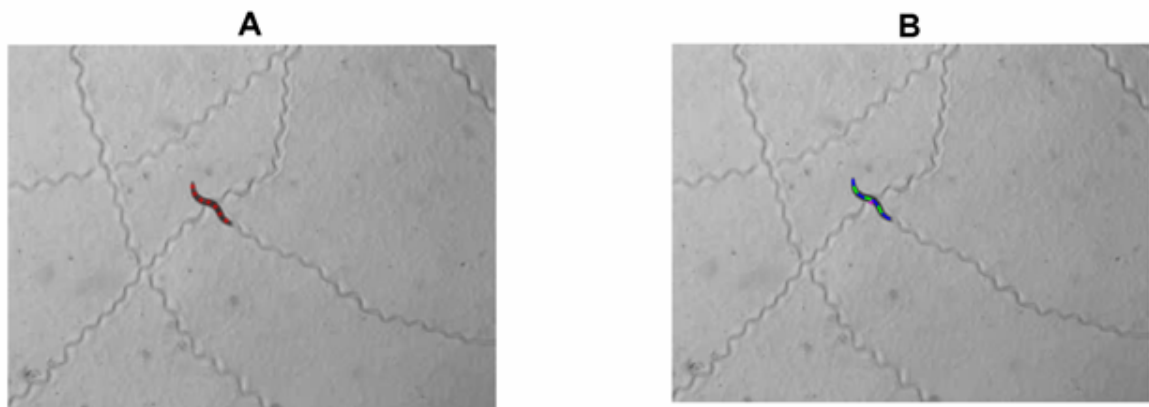
**Figure 6**

Reduction of area. Image analysis on a smaller region of interest (box bounding animal) increases calculation speed.



**Figure 7**

Worm segmentation. The skeleton is depicted.

**Figure 8**

Spine segments and centroid of animal. A. The skeleton of the animal and the Centroid of each segment (red stars). B. The skeleton of the animal and the centre of mass of the entire worm (magenta star).



**Figure 9**

Animal thickness. A. Perimeter of the animal and centroid of the first segment (red star). The yellow line represents the circle chosen by the user. B. Inner circle has a size that equals half the thickness of the segment.



**Nemo GUI**

**Data** ☐ Initial set  
☒ Saved set  
Set 1

**Whole animal Images**  
Only Image  
Frame = 274  
(-1) < > (+1)  
Area = 0.088707 mm<sup>2</sup>  
Thickness = 0.090643 mm  
Length = 0.99863 mm

**Spine and centroids**  
Spine  
Frame = 274  
(-1) < > (+1)

**Segment statistics**  
Number of segments 7  
Segment = 2  
Average over 4 frames  
lambda and amplitude

**Whole animal details in video sequence**  
Head-Tail distance  
Area (mean) = 0.091541 mm<sup>2</sup>  
Thickness (mean) = 0.092269 mm  
Length (mean) = 1 mm  
Distance covered by CM = 5.554 mm  
Head to tail distance (mean) = 0.78973 mm

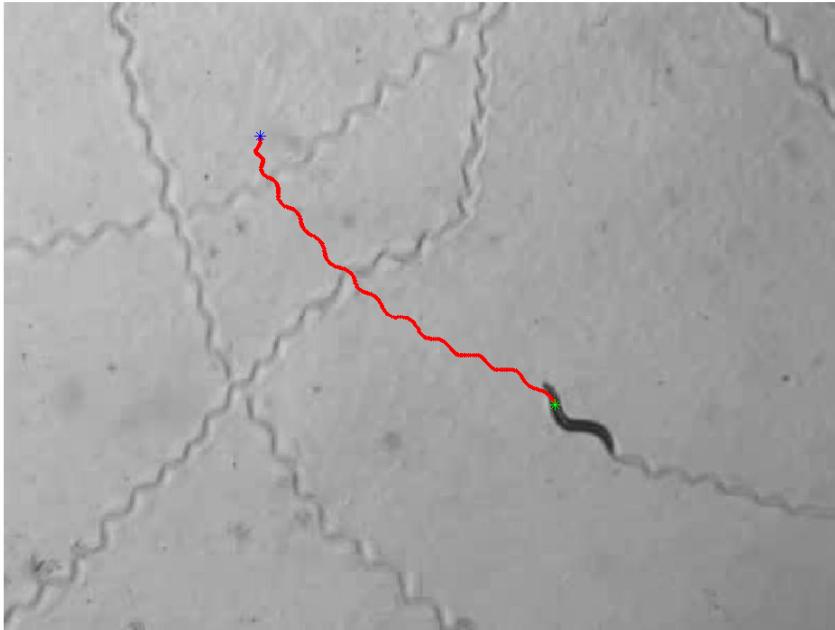
**Experiment details**  
Frame range = 95-431  
Frames/sec = 4  
Image dimensions (r,c) = 600x800  
Image dimensions (in mm<sup>2</sup>) = 6x8

**Close GUI**

**Figure 10**

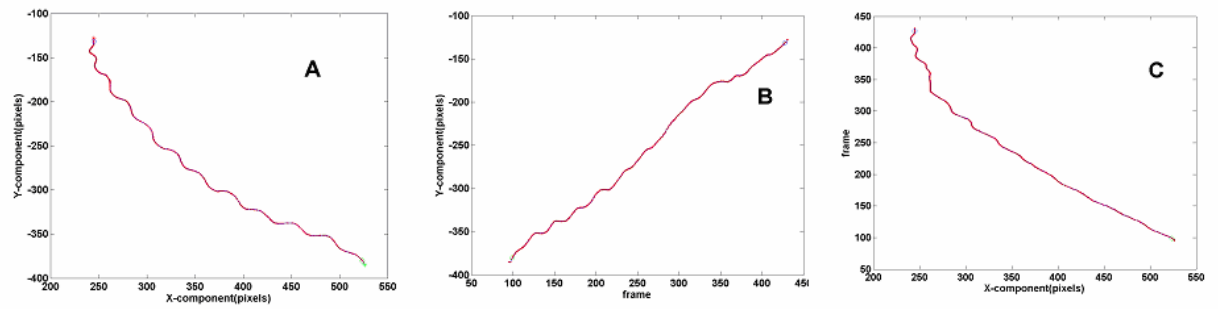
Graphical User Interface.

**segment = 2**



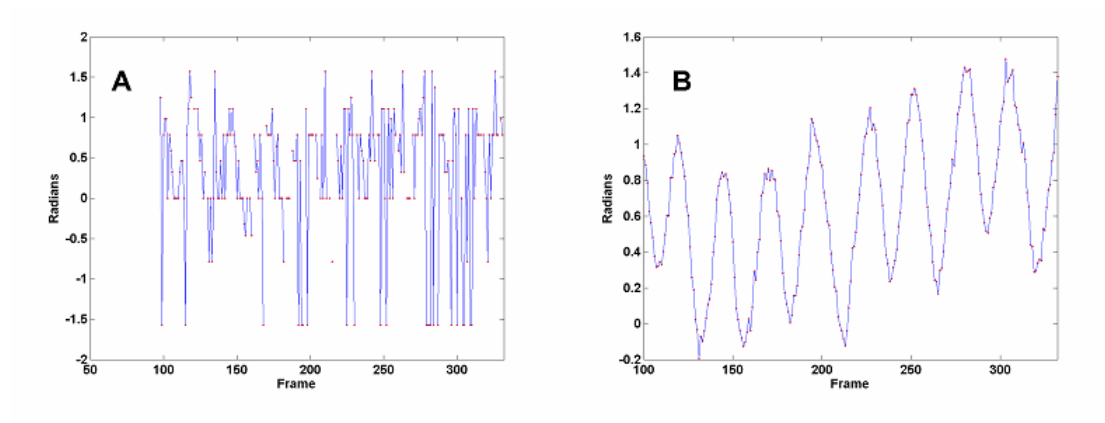
**Figure 11**

Trajectory of the centre of mass of a segment. Trajectory of centre of mass of segment 2 of the worm (green star: starting point, blue star: final point).



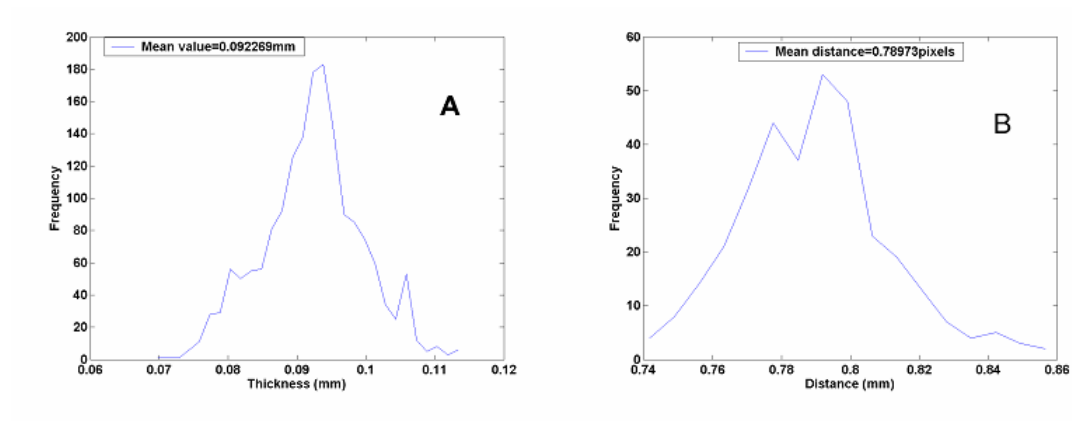
**Figure 12**

Graphical User Interface functions. A trajectory of centre of mass (CM\_segment) of segment 2. B. Y-coordinate of CM\_segment . C. X-coordinate of CM\_segment . (green star: starting point, blue star: final point).



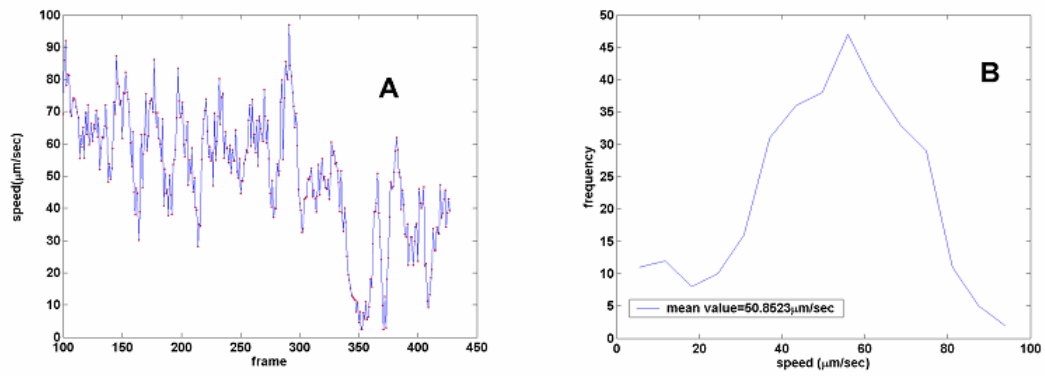
**Figure 13**

Angle change. A Angle evolution with respect to time before averaging. B Angle evolution with respect to time after averaging.

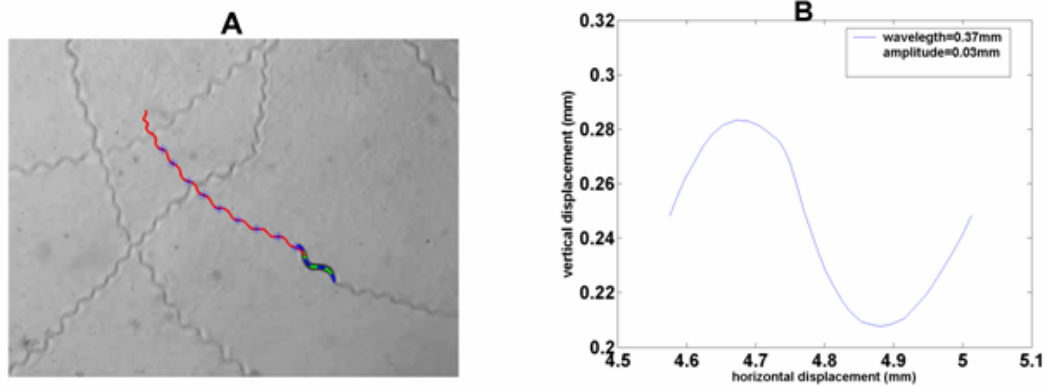


**Figure 14**

Thickness and distance between head and tail. A. Thickness histogram. B. Distance between head and tail.

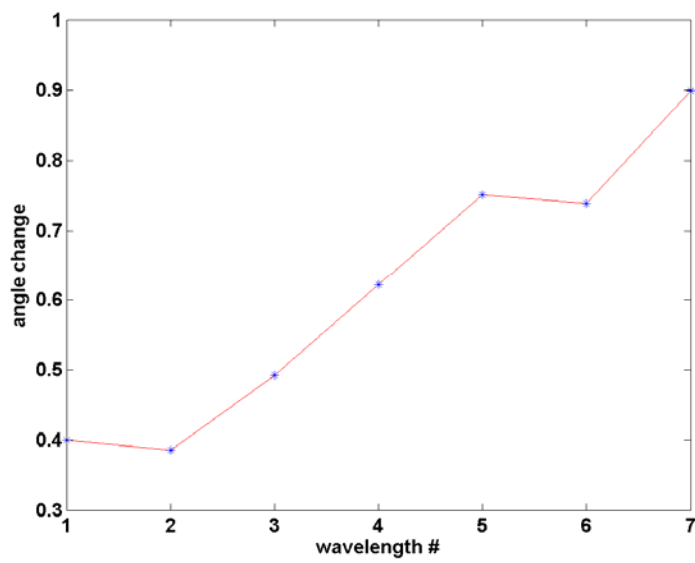
**Figure 15**

Speed. A. Speed series for segment 2. B. Speed histogram for segment 2.



**Figure 16**

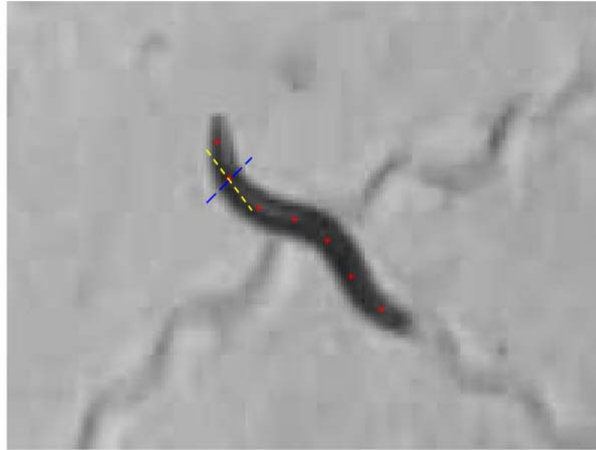
Features of sinusoidal movement. A. Trajectory of centre of mass of segment 2. The length between two successive blue asterisks equals one wavelength. B. Wavelength and amplitude of sinusoidal movement.



**Figure 17**

Angle change. Total angle change in each wavelength.





**Figure 18**

Computation of the thickness of a given segment. The yellow line goes through the centre of mass of the segment. Thickness is measured on the blue line, perpendicular to the yellow line.