




# WorMe: A user-oriented software for *Caenorhabditis elegans* length determination

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## Software

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## Summary

*Caenorhabditis elegans* is a widely used animal model for biomedical research. The length of this nematode is commonly used to assess its development and its health. Usually, measurements are performed manually, although software that automates this process has been developed. However, most of these tools are designed for stereomicroscope images, and none work reliably for compound microscopy images. Here we present WorMe, a user-friendly software that measures *C. elegans* length in compound microscopy images. The program is versatile in handling various image types, and can analyse multiple images collectively. Furthermore, the user can accept or discard the detected objects, separate joined worms, correct erroneous measurements, and manually add new worms. The length results are presented in a spreadsheet file, with each measurement linked to its image. Graphic data can also be exported.

## Statement of Need

*Caenorhabditis elegans* is a transparent nematode widely used as a robust early-stage research and toxicology studies model. Its small size (~1mm), short life cycle (~3days), and its proliferative cycle allow for cost-effective and high-throughput experiments ([Kaletta & Hengartner, 2006](#)). In addition, it is estimated that 60% of human genes have a homolog in *C. elegans* ([Markaki & Tavernarakis, 2020](#)).

Body size is a crucial endpoint used to assess the nematode's development that can be affected by dietary changes or altered temperatures ([Muñoz-Juan et al., 2024](#); [So et al., 2011](#); [Tain et al., 2008](#)). Furthermore, in toxicity studies, the nematode's body length is used to assess whether exposure inhibits growth ([Jung et al., 2015](#); [Schröter et al., 2024](#); [Srinivasan et al., 2023](#)).

The length of *C. elegans* is usually measured manually from microscopy images using FIJI-ImageJ, an image analysis software ([Schindelin et al., 2012](#)). This method presents several disadvantages as it is time-consuming and imprecise, since it involves manually tracing a line along the middle of each worm. Thus, the measured length would vary across attempts and experimenters.

Therefore, many software solutions have been developed to automate and improve this process, such as the WormSizer plugin for FIJI ([Moore et al., 2013](#)), WormToolBox from CellProfiler ([Wählby et al., 2012](#)), WormLength from QuantWorm ([Jung et al., 2014](#)), Anilength ([Jung, 2021](#)) or WorMachine ([Hakim et al., 2018](#)), among others. Most are designed to measure length from stereomicroscope images, as they capture a large number of worms in the same picture. However, the quality of these images is usually low.

41 For higher quality images, researchers use compound microscopy, where usually only one  
 42 or two worms are captured in the same image, ending with several files to analyse. This  
 43 microscopy technique can also be used for other measurements such as pharynx pumping rate  
 44 or, if the microscope allows, for fluorescence imaging. However, despite the wide range of  
 45 software available for the image analysis of *C. elegans*, no software has been found for length  
 46 determination from compound microscopy images.

47 In this work we present WorMe, a *Caenorhabditis elegans* length determination software.  
 48 WorMe is a MATLAB Runtime software that automates the nematode's length measurements  
 49 from compound microscopy images. It is open-source and user-friendly, since it works from a  
 50 graphical user interface. WorMe is also versatile, because it has a wide range of settings to  
 51 process many kinds of images, and it ensures data reliability since the user selects the worms  
 52 to be analysed. It is also fast, as the process is computationally optimized.

## 53 Brief Description of the Program Use and Features

54 WorMe is a software program implemented in MATLAB version 9.11 (R2021b) (Inc., 2021),  
 55 using the Image Processing Toolbox (The MathWorks, 2021c), Computer Vision Toolbox  
 56 (The MathWorks, 2021a), Image Acquisition Toolbox (The MathWorks, 2021b), and Statis-  
 57 tics and Machine Learning Toolbox (The MathWorks, 2021d). WorMe can be used on  
 58 Windows, macOS or Linux Operating Systems, running the code from the main script  
 59 WM\_length\_determination.m from the MATLAB Desktop interface. The program is also  
 60 deployed as an executable using MATLAB Runtime, so it can be installed and run without a  
 61 MATLAB license.

62 When the program is started, it prompts the user to provide the images for analysis, where one  
 63 or multiple images can be selected. The scale is then set by selecting the scale bar or writing  
 64 the scale value in pixels per unit.

65 Then, the images are processed to obtain the worms as binary objects. This step is done, as is  
 66 common, by converting the image to grayscale (MATLAB function `im2gray`), improving the  
 67 contrast (`imadjust`), binarizing the image (`imbinarize`), and removing noise and filling holes  
 68 (`bwareaopen`, `imopen`, `imclose`, `imfill`, `imclearborder`). The user can select from a list of  
 69 different sets of image modifications or apply their own if none display a workable result.

70 Afterwards, the program will skeletonize the worm object (using `bwmorph` among other MATLAB  
 71 functions), prune the branches, and elongate the main line, which is a longitudinal line along  
 72 the centre of the nematode. Then, the user can visualize and select which *C. elegans* to  
 73 measure, and exclude undesirable objects. The selection panel can be seen in Figure 1. This  
 74 process has been optimized to be fast and easy to use, for example, through the implementation  
 75 of keyboard shortcuts. Furthermore, the length value is shown in real time, which ensures the  
 76 measurements' reliability.

77 In this process, WorMe has some tools that can assist with worm selection. If two worms  
 78 are connected, their binary object can be split, and the program will reprocess the skeleton  
 79 for each new object. If the skeleton line does not span the entire worm, it can be extended.  
 80 Similarly, if it is partly erroneous, it can be cut and extended again. Lastly, a worm can be  
 81 added via manual analysis if it is not detected.



**Figure 1:** Image selection panel. In the left column we can see the Filters, which remove small objects, the Tools, which edit binary objects, and the Selection, where the user accepts or discards the selected object.

Finally, the results panel will return descriptive statistics of the length measurements, and these measurements can be exported to a spreadsheet. Since there is a measurement bias between manual and automatic measurements, as automatic lines contain more points and are always longer in curves, corrected results that skip some points when measuring are included to allow meaningful comparison. Furthermore, graphic data such as the binary images, indexed images, or PascalVOC data for other morphology measurements or AI model training can also be exported.

The user manual provides a more detailed explanation and examples of use. This document and the software binaries are provided in the [GitHub code repository](#).

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