![JOSS - The Journal of Open Source Software]

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

Josep Llobet Lladó [1*], **Núria Garriga-Alonso** [1*], **Amanda Muñoz-Juan** [1,2], **and Anna Laromaine** [1¶]

1 Institut de Ciencia de Materials de Barcelona (ICMAB-CSIC), C/ dels Til.lers s/n, Campus UAB Bellaterra, Barcelona, Spain **2** Current: Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus, Denmark; and Danish Research Institute of Translational Neuroscience—DANDRITE, Nordic-EMBL Partnership for Molecular Medicine, Aarhus University, 8000 Aarhus, Denmark. ¶ Corresponding author * These authors contributed equally.

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. Typically, measurements are performed manually; however, software has been developed to automate this process. 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 ImageJ/FIJI, 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.

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

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

Brief Description of the Program Use and Features

WorMe is a software developed in *MATLAB version 9.11 (R2021b)* (Inc., 2021), using the *Image Processing Toolbox* (The MathWorks, 2021c), *Computer Vision Toolbox* (The MathWorks, 2021a), *Image Acquisition Toolbox* (The MathWorks, 2021b), and *Statistics and Machine Learning Toolbox* (The MathWorks, 2021d). The program is deployed as an executable using MATLAB Runtime, so it can be installed and run without a MATLAB license on the Windows Operating System. WorMe can also be used on Windows, Linux, or macOS, by running the code from the main script `WM_length_determination.m` from the MATLAB Desktop interface, version R2021b or greater.

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

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

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

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

Acknowledgements

References

Hakim, A., Mor, Y., Toker, I. A., Levine, A., Neuhof, M., Markovitz, Y., & Rechavi, O. (2018). WorMachine: Machine learning-based phenotypic analysis tool for worms. *BMC Biology*, *16*. https://doi.org/10.1186/s12915-017-0477-0

Inc., T. M. (2021). *MATLAB version 9.11 (R2021b)*.

Jung, S. K. (2021). AniLength: GUI-based automatic worm length measurement software using image processing and deep neural network. *SoftwareX*, *15*. https://doi.org/10.1016/j.softx.2021.100795

Jung, S. K., Aleman-Meza, B., Riepe, C., & Zhong, W. (2014). QuantWorm: A comprehensive software package for caenorhabditis elegans phenotypic assays. *PLoS ONE*, *9*. https://doi.org/10.1371/journal.pone.0084830

Jung, S. K., Qu, X., Aleman-Meza, B., Wang, T., Riepe, C., Liu, Z., Li, Q., & Zhong, W. (2015). Multi-endpoint, high-throughput study of nanomaterial toxicity in caenorhabditis elegans. *Environmental Science and Technology*, *49*, 2477–2485. https://doi.org/10.1021/ES5056462

Kaletta, T., & Hengartner, M. O. (2006). Finding function in novel targets: C. Elegans as a model organism. *Nature Reviews. Drug Discovery*, *5*, 387–399. https://doi.org/10.1038/NRD2031

Markaki, M., & Tavernarakis, N. (2020). Caenorhabditis elegans as a model system for human diseases. *Current Opinion in Biotechnology*, *63*, 118–125. https://doi.org/10.1016/j.copbio.2019.12.011

Moore, B. T., Jordan, J. M., & Baugh, L. R. (2013). WormSizer: High-throughput analysis of nematode size and shape. *PLoS ONE*, *8*. https://doi.org/10.1371/journal.pone.0057142

Muñoz-Juan, A., Assié, A., Esteve-Codina, A., Gut, M., Benseny-Cases, N., Samuel, B. S., Dalfó, E., & Laromaine, A. (2024). Caenorhabditis elegans endorse bacterial nanocellulose fibers as functional dietary fiber reducing lipid markers. *Carbohydrate Polymers*, *331*, 121815. https://doi.org/10.1016/J.CARBPOL.2024.121815

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. In *Nature Methods* (Vol. 9, pp. 676–682). https://doi.org/10.1038/nmeth.2019

Schröter, L., Jentsch, L., Maglioni, S., Muñoz-Juan, A., Wahle, T., Limke, A., Mikecz, A. V., Laromaine, A., Ventura, N., Schröter, L., Jentsch, L., Maglioni, S., Wahle, T., Limke, A., Mikecz, A. V., Ventura, N., Muñoz-Juan, A., & Laromaine, A. (2024). A multisystemic approach revealed aminated polystyrene nanoparticles-induced neurotoxicity. *Small*, *20*, 2302907. https://doi.org/10.1002/SMLL.202302907

So, S., Miyahara, K., & Ohshima, Y. (2011). Control of body size in c. Elegans dependent on food and insulin/IGF-1 signal. *Genes to Cells*, *16*, 639–651. https://doi.org/10.1111/J.1365-2443.2011.01514.X

Srinivasan, S. Y., Illera, P. A., Kukhtar, D., Benseny-Cases, N., Cerón, J., Álvarez, J., Fonteriz, R. I., Montero, M., & Laromaine, A. (2023). Arrhythmic effects evaluated on caenorhabditis elegans: The case of polypyrrole nanoparticles. *ACS Nano*, *17*, 17273–17284. https://doi.org/10.1021/ACSNANO.3C05245

Tain, L. S., Lozano, E., Sáez, A. G., & Leroi, A. M. (2008). Dietary regulation of hypodermal polyploidization in c. elegans. *BMC Developmental Biology*, *8*, 1–9. https://doi.org/10.1186/1471-213X-8-28

The MathWorks, Inc. (2021a). *Computer vision toolbox (R2021b)*. https://www.mathworks.com/help/vision/

The MathWorks, Inc. (2021b). *Image acquisition toolbox (R2021b)*. https://www.mathworks.com/help/imaq/

The MathWorks, Inc. (2021c). *Image processing toolbox (R2021b)*. https://www.mathworks.com/help/images/

The MathWorks, Inc. (2021d). *Statistics and machine learning toolbox (R2021b)*. https://www.mathworks.com/help/stats/

Wählby, C., Kamentsky, L., Liu, Z. H., Riklin-Raviv, T., Conery, A. L., O'Rourke, E. J., Sokolnicki, K. L., Visvikis, O., Ljosa, V., Irazoqui, J. E., Golland, P., Ruvkun, G., Ausubel, F. M., & Carpenter, A. E. (2012). An image analysis toolbox for high-throughput c. Elegans assays. *Nature Methods*, *9*, 714–716. https://doi.org/10.1038/nmeth.1984