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🌐 C. elegans worm size measurement

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| 1 *Works for me* | | ⤴ Share |

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ABSTRACT

Protocol to detect the size (length + width) of worms using a stereoscope and a camera.

The protocol was used in the publication:

Breimann L, Morao AK, Kim J, Sebastian Jimenez D, Maryn N, Bikkasani K, Carrozza MJ, Albritton SE, Kramer M, Street LA, Cerimi K, Schumann VF, Bahry E, Preibisch S, Woehler A, Ercan S (2022). The histone H4 lysine 20 demethylase DPY-21 regulates the dynamics of condensin DC binding.. Journal of cell science.
https://doi.org/pii:jcs258818.10.1242/jcs.258818

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Preparing worms for imaging

1   Select worm line(s) and prepare the worms for size measurement.

> To measure the sizes of worms, preparing the worms is critical. Typically you want to compare different worm lines or treatments at a specific stage. For this worms need to be synchronized and grown to the desired stage. Worms can be synchronized by picking, bleaching or hand selection of the desired stage directly.

2   For the synchronizing-by-picking method, pick (at least) 20 gravid Adult worms and place them on a fresh OP50 plate. Allow worms to lay eggs for 4 hours and then remove Adults from plates.   3h

> Try to keep the number of worms and time allowed to lay eggs constant between plates if the goal is to compare different lines.

3   Grow worms to the desired stage.   3d

4   Wash worms off the plate using M9. To remove all bacteria, let worms settle in a tube for 10 min and carefully replace the M9 with fresh M9. Repeat this step 3 times. After the last step, remove most M9 from the tube, leaving only 20 μl with the worms.   35m

5 To get a clear picture of the worms, lightly anesthetize the worms by adding 10 mM Levamisol 10m
to the tube. Wait 5 minutes and gently pipette worms onto an unseeded agar plate.

> Anesthetizing the worms stops their movement, making it easier to get unblurred images
> and capture worms only once while taking pictures.
> Try to keep the amount of liquid on the fresh plate minimal as it can interfere with
> analyzing the images later.

6 Separate the worms from each other using an eyelash pick. The goal is to have evenly spread
worms that are not touching and are not too close to the wall.

> It can be helpful to mark the plate on the bottom with thin marks to orient oneself while
> imaging

Imaging worms

7 To capture images of the worms, an eyepiece camera (for example the Dino-Eye camera) can
be used. It is also possible to capture images using a phone with an adapter. Phone adapters
can be 3D printed with many available designs online. Importantly, keep the zoom of the
camera and the microscope constant for one experiment. Try not to image worms twice. If the
camera allows it save raw file formats otherwise use the camera format that is available.

> AM4025X Dino-Eye Edge Eyepiece
> Camera
> Eyepiece Camera
>
> **Dino-Lite**   **AM4025X**   🔗

8 Use a microscope calibration ruler and take an image with the same settings. This will allow
you later to give the results a unit.
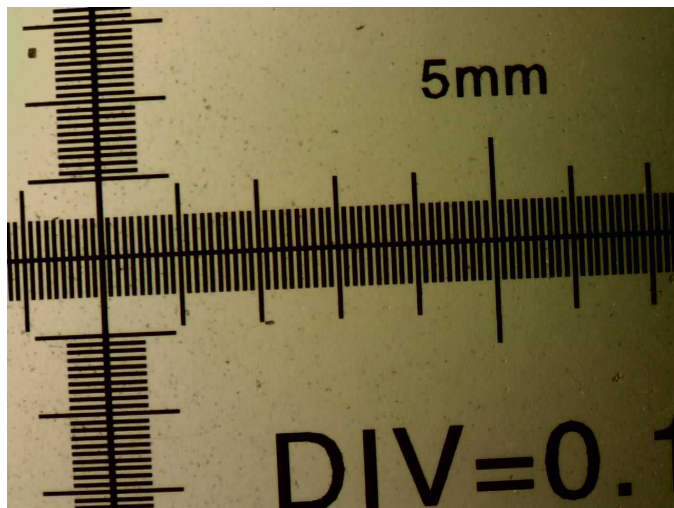
Microscope calibration rulers can be found [here.](#)

Preparing images for analysis

9   Save images for one condition in a folder.

10   **Use Fiji to open the images and remove background.**

FIJI (FIJI Is Just ImageJ) 🔗

1. Open image using Fiji
2. Set the image to 8-bit: Image > Type > 8-bit
3. Subtract background: Process -> Subtract Background. Set the preview checkbox to see how changing rolling ball radius changes the results.
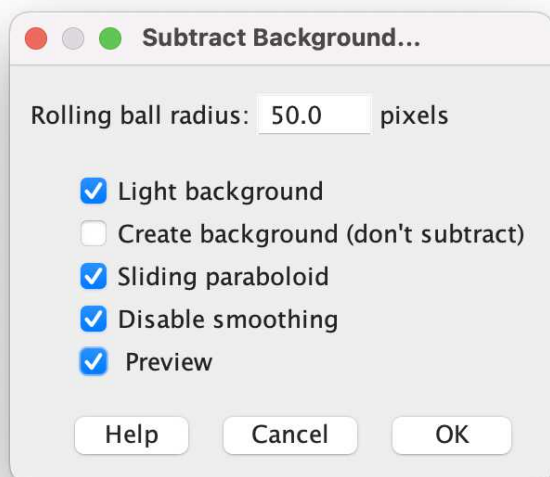
4. Threshold: Image -> Adjust -> Threshold; where it says "Default", change it to "Otsu". Move the sliding bars so that you get as much of the worms highlighted, and as little of the background. Then click "Apply".
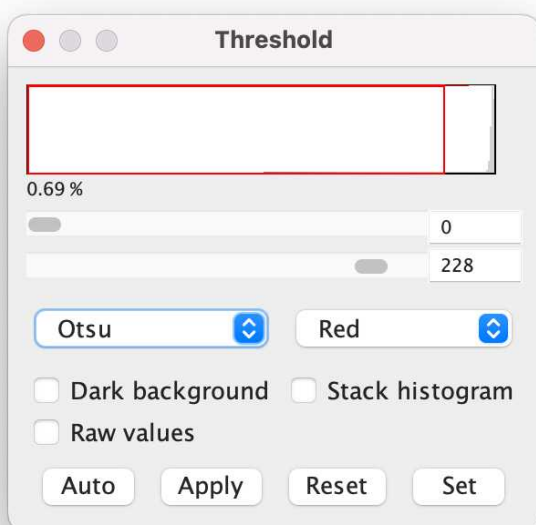
5. Save the image to a new folder for further analysis.

11   To run the above code on many images, you can use this Fiji macro. Make sure to adapt the

parameters in the script after finding them manually.

https://github.com/ercanlab/2021_Breimann_et_al/blob/main/WormSize_analysis/prep_worms_for_wormsizer.ijm

12   To get the **resolution** of your measurements, open the image of the calibration ruler.
- Use the line tool in Fiji

- Draw a line between two known points on your ruler (for instance a millimetre)
- Press command + M to open a results Window with the measurements to give you the length in pixels.

- For "Microns per Pixel", divide the number of microns your line is (In our case it was a mm, so it would be 1000 microns), by the length of the line in pixels from the step above.
- Write down this number for the next step

Alternatively, read here for more instructions.

Measuring worm size

13   Download the WormSizer plugin through the Fiji plugin menu. In Fiji navigate to "Help" > "Update" > "Manage Update Sites" and select "WormSizer" in the list of plugins and click "Close" and "Apply changes". After restarting Fiji, the plugin can be found in the Plugin drop-down menu.

Moore BT, Jordan JM, Baugh LR (2013). WormSizer: high-throughput analysis of nematode size and shape.. PloS one.
https://doi.org/10.1371/journal.pone.0057142

WormSizer: (ver. 1.2.5)

WormSizer is developed at the Baugh Lab.

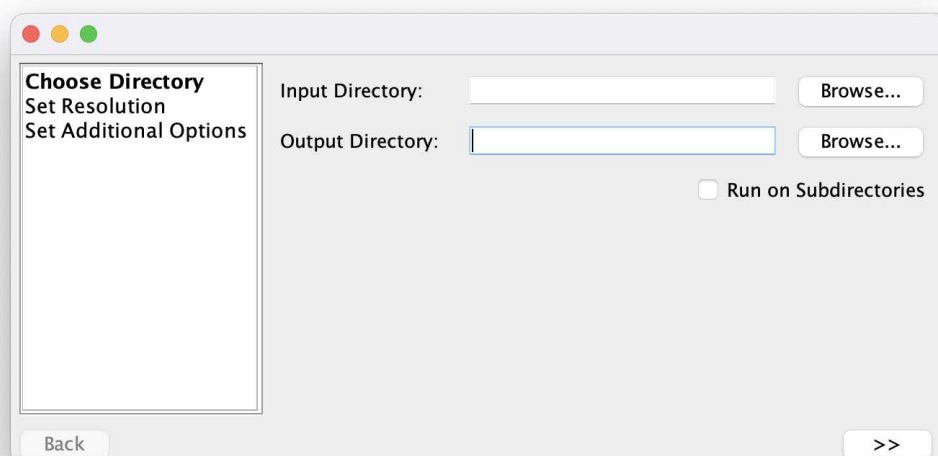Updates and instructions can be found here.

WormSizer is free to use and open source. If you use WormSizer results in your work, please cite us: Moore BT, Jordan JM, Baugh LR (2013) WormSizer: High-throughput Analysis of Nematode Size and Shape. PLoS ONE 8(2): e57142. doi:10.1371/journal.pone.0057142
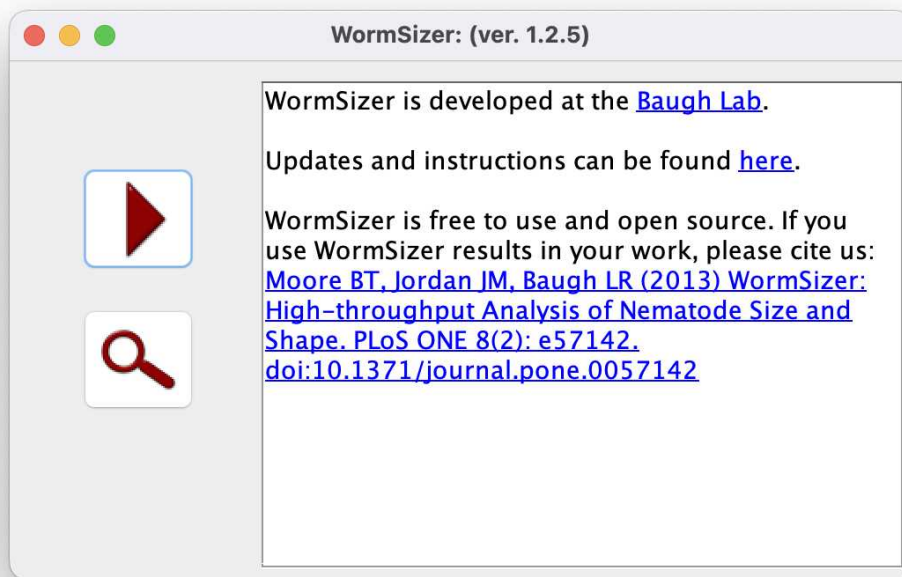
More info on how to use the plugin can be found here.

14   Select an input directory including your pictures and an output directory to save the results. Click the >> button to continue.

Choose Directory
Set Resolution
Set Additional Options

Input Directory: Browse...

Output Directory: Browse...

                                    Run on Subdirectories

Back   >>

15 Set the length in microns that were measured for the calibration ruler. Click the >> button to continue (this can take a moment depending on the number of images in the folder).

Choose Directory	Micrometer File: _____ Browse...
Set Resolution	
Set Additional Options	Objective: [⌄]
	Zoom: 1.0
	Microns per Pixel: 6.443
Back	>>

16 Next, select the settings to find the worm outlines. Depending on the images and the magnification they will be different. After the selection, pressing the >> button will start the detection in all images in the folder.

|	
Choose Directory	Rolling Ball Radius: 10.0
Set Resolution	Threshold Method: Otsu [⌄]
Set Additional Options	Close Radius: 5.0
	Worm Area Filter: 1000.0 to 99999.0
	Minimum Tubeness Filter: 0.0
	Sample Interval Width: 10
	☐ Review after Processing ☑ Preview
Back	>>

The processing complete image will signal when the plugin detected all worms and the review can start.

WormSizer Progress

Processing complete!

Next
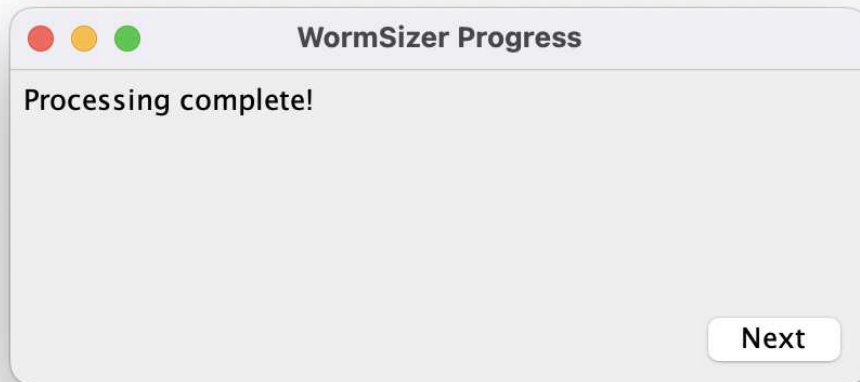
17   The plugin produced three files in the output folder:

  - settings .csv file
 - experiment .xml file
  - results csv file

The settings file saves the settings selected for detection. The XML file saves the outline information about each worm and can be used to review the outlines. The results file contains the worm measurements for each detected worm.

experimentId	imageFile	id	pass	volume	length	middleWidth	meanWidth	surfaceArea
WS_input	../WS_input/20210112_031358932_png.tif	0	NA	2722.5240181735344	568.6258954358349	92.92226747125792	73.77643603524287	178692.3561159262
WS_input	../WS_input/20210112_031358932_png.tif	1	NA	2088.3004949684114	710.3718954358349	64.42999999999999	58.560943636593706	149851.69570277448
WS_input	../WS_input/20210112_031358932_png.tif	2	NA	3954.198123320313	716.8148954358348	90.202	77.4141773646611	519903.1164935613

18   Reviewing the detections is done using the magnifying symbol. Navigate to the output folder and select the .xml file.
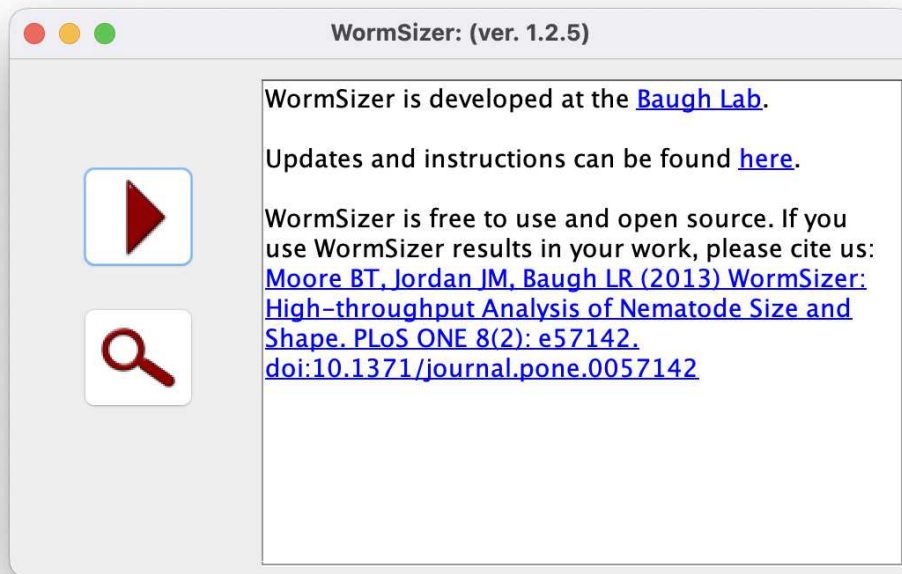
19   In the review part of the plugin, you can go through all detected worms in all images and select whether you want them to be included in the downstream analysis.
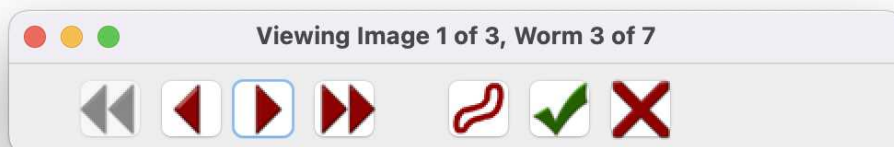
The results file is updated while reviewing and changes the value in the "pass" column to either TRUE or FALSE.

Plotting results

20   The results file can be used to plot the results. An example script using Python can be found in this GitHub repository.

Length of adult worms

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