Journal of Visualized Experiments

Evaluation of motor impairment in C. elegans models of amyotrophic lateral sclerosis (ALS)

--Manuscript Draft--

Article Type:	Invited Methods Collection - JoVE Produced Video
Manuscript Number:	JoVE62699R2
Full Title:	Evaluation of motor impairment in C. elegans models of amyotrophic lateral sclerosis (ALS)
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Additional Information:	

Question	Response
Please specify the section of the submitted manuscript.	Neuroscience
Please indicate whether this article will be Standard Access or Open Access.	Open Access ($3900)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Seattle, Washington, United States of America
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1   **TITLE:**
2   Evaluation of Motor Impairment in *C. elegans* Models of Amyotrophic Lateral Sclerosis
3
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19 **SUMMARY:**
20 This protocol describes two sensitive assays for discriminating among mild, moderate, and severe
21 motor impairment in *C. elegans* models of amyotrophic lateral sclerosis, with general utility for
22 *C. elegans* strains, with altered motility.
23
24 **ABSTRACT:**
25 The neurodegenerative disease amyotrophic lateral sclerosis (ALS) features progressive loss of
26 motor neurons accompanied by muscle weakness and motor impairment that worsens with time.
27 While considerable advances have been made in determining genetic drivers of ALS for a subset
28 of patients, the majority of cases have an unknown etiology. Further, the mechanisms underlying
29 motor neuron dysfunction and degeneration are not well understood; therefore, there is an
30 ongoing need to develop and characterize representative models to study these processes.
31 *Caenorhabditis elegans* can adapt their movement to the physical constraints of their
32 surroundings, with two primary movement paradigms studied in a laboratory environment-
33 crawling on a solid surface and swimming in liquid. These represent a complex interplay between
34 sensation, motor neurons, and muscles. *C. elegans* models of ALS can exhibit impairment in one
35 or both of these movement paradigms. This protocol describes two sensitive assays for
36 evaluating motility in *C. elegans*: an optimized radial locomotion assay measuring crawling on a
37 solid surface and an automated method for tracking and analyzing swimming in liquid (thrashing).
38 In addition to the characterization of baseline motor impairment of ALS models, these assays can
39 detect suppression or enhancement of the phenotypes from genetic or small molecule
40 interventions. Thus, these methods have utility for studying ALS models and any *C. elegans* strain
41 that exhibits altered motility.
42
43 **INTRODUCTION:**
44 Amyotrophic Lateral Sclerosis (ALS) is a debilitating, aging-related neurodegenerative disease

45 with a particular impact on motor neurons. The disease features loss of motor neurons in the
46 brain and spinal cord and progressive motor impairment. This results in major functional
47 disability and premature death, typically within 3-5 years after diagnosis[1]. Mutations in at least
48 38 genes can cause ALS; however, most patients with ALS accumulate ubiquitinated inclusions of
49 the protein TDP-43 as their primary pathology in neurons and glial cells[2-4]. A number of animal
50 models have been developed to study the underlying mechanisms causing or contributing to ALS
51 *in vivo* (reviewed in [5]). In *C. elegans,* these models include genetic loss-of-function mutations in
52 homologs of ALS-causing genes or transgenic expression of human ALS genes. There are
53 numerous advantages to modeling ALS in *C. elegans*. *C. elegans* are a tractable simple animal
54 with a differentiated nervous system, well-characterized behavioral paradigms, and considerable
55 genetic homology to humans[6,7]. Many tools exist for working with *C. elegans*, including robust
56 genome editing capabilities, *in vivo* fluorescent reporters of neurodegeneration, RNAi screening
57 paradigms, tractable genetics, and established behavioral and phenotypic assays. *C. elegans*
58 models of ALS recapitulate aspects of human disease, including accumulation of insoluble
59 protein, neurodegeneration, and early death[8,9]. Further, motor dysfunction featuring
60 disturbance of both crawling and swimming behaviors is present in many *C. elegans* ALS models.
61
62 This protocol describes two methods to characterize *C. elegans* motor phenotypes: the radial
63 locomotion assay for evaluating crawling on a solid surface and the assessment of swimming in
64 liquid (thrashing) using the WormLab automated tracking and analysis. These sensitive methods
65 for characterizing motor deficits allow comparisons of severity and offer tools for measuring
66 suppression and enhancements of motor phenotypes. The radial locomotion assay quantifies
67 differences in crawling motility (sinusoidal movement on a solid surface) among populations of
68 worms. This assay takes advantage of *C. elegans* natural unstimulated exploration behavior by
69 placing worms in a single location on a plate and marking their final location after a given period
70 of time[10]. Alternatively, swimming in liquid (thrashing) assays count body bends of individual
71 worms over a set period of time. The manual counting of body bends by the human eye is time-
72 intensive and typically exhibits considerable variability between experimenters. The use of
73 computer-assisted automated tracking and analysis can eliminate much of that variability. In
74 addition to the characterization of baseline motor impairment of ALS models, both radial
75 locomotion and swimming assays can detect modulation of distinct locomotor phenotypes from
76 genetic or small molecule interventions. These methods have utility for studying ALS models and
77 any *C. elegans* strain that exhibits altered motility.
78
79 **PROTOCOL:**
80
81 **1.   Radial locomotion assay**
82
83 1.1.   Prepare 100 mm or 150 mm diameter Petri dishes about half full of nematode growth
84 media (NGM) and seeded with a uniform lawn of OP50 bacteria to cover the entire surface of
85 the plate.
86
87 1.2.   Flip the NGM assay plates upside-down and label the bottom with an identifier for the
88 *C. elegans* strains to be assayed, 2 plates per strain (**Figure 1A**).

89

1.3.   Make a small dot with the marker in the center of the upside-down plate (**Figure 1A**).

90

91

92   1.4.   While working with a dissecting microscope, transfer 15-20 staged matched[11] worms to
93   the center of the assay plate directly over the center dot (visible through the NGM) using a
94   platinum-wire pick (**Figure 1B**).

95

96   NOTE: Try to transfer all the worms at the same time or as quickly as possible.

97

98   1.4.1. Set a timer for 30 min after worms are placed in the center of the plate. Put the lid back
99   on the plate and set it aside (**Figure 1C**).

100

101   1.5.   Continue transferring worms until all strains are on the designated assay plates, making
102   a note of approximately how long it takes to transfer between plates (aim for ~ 1 min between
103   plates). Keep all plates in the same order that they were set up.

104

105   1.6.   After 30 min, begin scoring the first plate.

106

107   1.6.1. Remove the lid and place the plate face down under the dissecting microscope, such
108   that the labeled back of the plate is facing up, and the NGM agar is between the line of sight
109   and the worms. The plate will be upside-down from the normal usage.

110

111   1.6.2. Adjust the microscope focus until worms are visible through the agar.

112

113   1.6.3. Using a different colored felt tip pen from the center point, put a small dot at the
114   location of each worm - follow the worm tracks through the bacterial lawn to find the most
115   distal animals.

116

117   1.6.4. Check the edge of the plate as some worms may end up there. Also, count and record
118   how many worms did not move from the center point (**Figure 1D**).

119

120   1.7.   Continue marking all assay plates in order, such that all plates have 30 min of activity
121   time, being careful to account for the time it took to set up the plate. Then, perform manual or
122   digital measurements as described in steps 1.8 or 1.9.

123

124   1.8.   Perform manual measurements using a ruler.

125

126   1.8.1. Use a ruler to measure in mm, the distance from the center point to the final location
127   markings for each worm and record the distance. To help keep track of progress during scoring,
128   label the first scored point with a small marking line.

129

130   1.8.2. Record length data for each dot consecutively by rotating the plate in a clockwise
131   fashion (**Figure 1E**).

132

133 1.9. Perform digital measurement using a scanner and ImageJ.
134
135 1.9.1. Using a flatbed scanner, scan the back of the assay plate(s) next to a ruler (numbers are
136 facing the scanning surface) (**Figure 2A**).
137
138 1.9.2. Open the scanned plate image in ImageJ or a similar program.
139
140 NOTE: ImageJ is a free Java-based image processing software hosted by the NIH.
141
142 1.9.3. Click on the **Straight-Line Tool** and then draw a line connecting the 1 cm and 2 cm
143 points on the scanned image of the ruler (**Figure 2B**).
144
145 NOTE: Holding the **Shift** computer key will force the line to the nearest 45˚ angle.
146
147 1.9.4. Navigate to the **Analyze** menu and use it to set the scale (**Analyze | Set Scale**) (**Figure
148 2C**).
149
150 1.9.5. Enter the **Known Distance** (10) and **Units of Length** (mm) in the appropriate boxes, do
151 not adjust the distance in pixels number. Check **Global** to apply the same scale to all images
152 being analyzed in each ImageJ session; otherwise, set the scale for each image upon opening
153 (**Figure 2D**). Click **Ok**.
154
155 NOTE: Steps 1.9.2—1.9.5 are performed to set the measurement scale. Measurements for this
156 image will now automatically relate the length of lines to the defined pixel: length relationship.
157 Images scanned at 300 dpi will have an approximate relationship of 11.8 pixels per mm.
158
159 1.9.6. Use the **Paintbrush Tool** to mark just above the first point to be scored.
160
161 NOTE: This is a visual indicator for the first worm scored.
162
163 1.9.7. Use the **Straight-Line Tool** to draw a line from the center point to the first worm data
164 point.
165
166 1.9.8. Click the **M** key on the keyboard to measure the distance. Alternatively, navigate to the
167 **Analyze** menu and choose **Measure** (**Analyze | Measure**). In both cases, the measurement will
168 appear in a new window; this window will collect all measurements per image.
169
170 1.9.9. Keeping the center point constant, move the end of the line touching the first worm
171 point to the next worm point, moving clockwise, and click the **M** key to measure.
172
173 1.9.10. Continue measuring each point in a clockwise fashion until all points are scored.
174
175 1.9.11. **Copy** and **Paste** length measurement data into a spreadsheet to save it. Then, repeat
176 the process for each scanned plate image.

177

178   NOTE: Steps 1.9.6 – 1.9.11 measure the worm displacement values (**Figure 2E-F**).

179

180   1.10.   Perform statistical analysis.

181

182   1.10.1. Combine replicates within a single experiment so that a total number scored is between
183   30-40 worms.

184

185   1.10.2. Perform independent experimental replicates on different days and with independent
186   populations of worms to end up with 3 independent experimental replicates.

187

188   1.10.3. Analyze data using appropriate statistical analyses such as Student's t-test for 2 strains
189   or 1-way ANOVA for three or more strains.

190

191   **2.   Computer-analyzed swimming assay**

192

193   NOTE: This protocol contains detailed instructions for the commercially available WormLab
194   hardware and software system (see **Table of Materials**). However, the workflow can be applied
195   to other computer-analyzed swimming assay systems.

196

197   2.1.   Setting up and recording videos.

198

199   2.2.   Raise the shielding on the imaging hardware (**Figure 3**) and verify that the camera height
200   is set to the expected location.

201

202   NOTE: The optimal height for capturing worms on a 35 mm plate is such that the third from the
203   bottom threaded screw bore is visible below the adjustable camera mounting bracket, but no
204   additional mounting hole is seen above the screw bore (approximately 30.5 cm above the
205   stage) (**Figure 3C**). This will result in a capture ratio of 11.43 µm/pixel. Consider adding a
206   "correct height" indicator so that this is easier to confirm prior to beginning the assay. See
207   protocol step 2.18 to account for height adjustments.

208

209   2.3.   Open the associated software (**Supplementary Figure 1A**).

210

211   2.4.   Click the **Video Capture** icon (film with a right-pointing triangle in the middle) to open a
212   new **Video Capture** window. The camera will be in the **Live View,** but the display will be black
213   (**Supplementary Figure 1B**).

214

215   2.5.   Press down on the dimmer knob and turn it clockwise to adjust the light.

216

217   NOTE: The dimmer knob is on the stage of the imaging system near the bright field light source.
218   The **Video Capture** live view display will turn from black to illuminated.

219

220   2.6.   Back in the **Video Capture** window, click on the **Settings** tab, adjust as follows: **Video**
221   **mode**: 2456 x 2052_Mono8, **Frame rate**: 14, **Output**: monochrome, **Exposure**: 0.00300 s, **Gain**:
222   1 dB**, Gamma**: 1, **Rotate 180** (check on).

224   2.7.   Move back to the **Capture** tab. Select the recording folder (**Supplementary Figure 1B**).

226   NOTE: This is where all recordings and project files will be stored. Create unique folders for
227   individual experiments to keep data organized.

229   2.8.   Assign a file name by entering it into the **File prefix** text box.

231   NOTE: The software will automatically append a progressive number to the end of the prefix in
232   the format "0001". It is advisable to have a consistent naming scheme for the files such as:
233   "YYYYMMDD_treatmentName_rep#_".

235   2.9.   Set other **Capture** settings as follows: **Buffer**: 128 frames (default), **Duration** (check on):
236   00 h:01min:00 s. Ignore or turn off other settings in this tab.

238   2.10.   Place the assay plate, lid up, on the device stage, and center it in the **Video Capture**
239   screen; remove the lid.

241   NOTE: Prepare assay plates ahead of time. Each plate consists of a 35 mm Petri dish
242   approximately half full of unseeded (no bacterial lawn) NGM.

244   2.11.   Use a micropipette to wash the stage synchronized[11] worms in 1 mL of M9 onto the
245   assay plate – aim for about 50 worms (**Figure 3D**). Gently swirl the plate to bring the animals to
246   the center or use a micropipette to add a few drops of M9 to separate animals (**Figure 3E**). Set a
247   timer for 60 s to allow worms to acclimate to swimming.

249   NOTE: The plate will not be 100% visible on the screen. Animals are not accurately tracked
250   while overlapping.

252   2.12.   While the timer counts down, manually adjust the camera focus by turning the focusing
253   ring on the lens body of the camera while watching the display (**Figure 3F**). It is possible to
254   zoom in on the worms using a scroll wheel mouse allowing for more precise focusing.

256   2.13.   After setting the focus, adjust the light knob such that the display is as bright as possible
257   without overexposure (shows up as red pixels on display).

259   2.14.   After 60 s, press the **Record** button.

261   NOTE: The video will go from the **Live View** to **Recording** and a timer will be visible. When the
262   capture is complete, the **Video Capture** display will return to the **Live View** (**Supplementary**
263   **Figure 1B**).

264

265   2.15.   Remove the plate after recording and set up the next plate. Rename the prefix as
266   needed. Wash worms into the assay plate, incubate for 60 s, and record each treatment.

267

268   2.16.   After completing all recordings for an experiment, close any open video tabs, and turn
269   off the light source by depressing the dimmer knob.

270

271   2.17.   Close the **Video Capture** window by clicking the red **x** button in the window's upper
272   right corner; alternatively, close the entire software package and reopen.

273

274   ==2.18.   Prepare videos for tracking.==

275

276   2.19.   ==From the **Workflow** sidebar menu, select the first button on this menu, **Import Image**==
277   ==**Sequence,** and find and double click on a video.== The video will open in the middle window
278   (**Supplementary Figure 2A**).

279

280   NOTE: The default display for the software is the **Workflow** sidebar menu; this menu can also
281   be accessed through the view top dropdown menu under **View** [**View | Workflow**]. The
282   **Workflow** menu has several view options; the **Track** view is appropriate at this point in the
283   protocol. If the **Set Sequence Info** button is not visible in the menu, then this is not the correct
284   view. First, look at the bottom of the **Menu** and check that the **Workflow** tab is active, next
285   check that the **Track** view is active by clicking on the **Track** icon. Where menus appear in the
286   window is dependent on monitor size and the location of other opened menus. Menus can be
287   moved by drag and drop, resized, and closed. Most of the menus can also be opened through
288   the **View** dropdown menu.

289

290   2.20.   ==From the Workflow menu, select **Set Sequence Info** (**Supplementary Figure 2B**). In the==
291   ==new menu window, check the naming scheme, add notes, and validate metadata.==

292

293   2.21.   Check that the scaling is set correctly; **Scale**: 11.43 µm/pixel and **Measure** will auto fill as
294   1000 µm is 87 pixels. If the camera height has been adjusted differently than prescribed here,
295   follow the instructions in this menu to update the scale. Click the **Save** button.

296

297   2.22.   In the Workflow menu, ==select **Adjust Image,** and a new popup menu called **Image**==
298   ==**Adjustments** will open== (**Supplementary Figure 2C**). Select **Dark worms on light background**
299   **(bright field)**.

300

301   ==2.23.   Adjust additional settings. Recommended **Image Processing Settings** are as follows:==
302   ==**Background smoothing**: 10, **Gaussian smoothing**: 5, **Fill holes**: 2, **Small object filter**: 0, and skip==
303   ==**Integral Derivative Segmentation**.==

304

305   2.24.   ==Adjust the **Threshold** level such that the animals are completely filled with green but==
306   ==still distinct from the background. Click **Apply**.==

307

308   NOTE: It is permissible to pick up some objects that are not worms.

309

310   2.25.   From the **Workflow** menu, select **Detect and Track** (**Supplementary Figure 2D**).

311

312   NOTE: A menu will appear to the side of the video with 3 tabs: **Detecting**, **Tracking**, and **Repair**.
313   The cursor will look like a pointing finger when over the video display. Alternatively, click the
314   **Select** icon (cursor arrow) in the icon menu in the upper left of the software.

315

316   2.25.1. Use the finger selector tool to select 3-7 worms and click the **Detect worms** button in
317   the **Detection** tab.

318

319   NOTE: This selection helps the software accurately identify worms for given conditions and
320   must be done for each video. After a few seconds, most of the worms will be highlighted in
321   green and have a number in yellow. Worms that are touching or curled up will likely not be
322   highlighted; however, the program will most likely find them during tracking. There is an option
323   to adjust the detection parameters; however, the defaults work well under most conditions. If
324   adjustments to the detection parameters are made, make sure to be consistent between
325   samples and replicates.

326

327   2.26.   Still in the **Detect and Track** window, move to the **Tracking** tab (**Supplementary Figure**
328   **2E**). In the **Tracking Parameters** check **Use backtracking**, uncheck **Track worms at the edge of**
329   **the image**.

330

331   2.27.   Set the **Max tracked hypothesis** to 5. Set the **Tracking mode** to **Swimming**.

332

333   NOTE: The Max tracked hypothesis setting adjusts how many potential worm-tracks a single
334   tracked worm is allowed to have and gives the program some leeway in how accurately to track
335   a single worm without losing track of it. 5 is a good setting for swimming.

336

337   2.28.   Move to the **Advanced Settings** section and set the following: **Frames worms can touch**
338   **boundary**: 50, **Frames worms can overlap**: 500, **Position tolerance**: 0.50, **Shape tolerance**:
339   0.50. Save these settings and deploy them for all videos in an experiment (and beyond) by
340   saving them as a configuration.

341

342   NOTE: **Track filtering** is not recommended and can easily be done post-processing, leave off.

343

344   2.29.   Navigate to the **Configuration Manager** (gear icon) in the upper-left icon menu. When
345   clicked, the **Configuration Manager** sidebar menu will open. Next, click the **Save (gear +) icon**,
346   give this configuration a name and description, then click the **OK** button.

347

348   2.29.1. To access this configuration in the future, open the **Configuration** menu, select the
349   desired configuration, and click the **Load** (gear up arrow) icon. Then add sequence information,
350   possibly fine-tune the threshold level, and select worms for detection. All other settings will be
351   the same.

352

353   2.30.   In the **Workflow** menu, click **Save Project**, verify the save location and add a project file
354   name (matching the project file name to the video name helps with continuity). Close the
355   video.

356

357   NOTE: These projects are saved with the .wpr filetype.

358

359   2.31.   Repeat for all videos: Import Image Sequence (2.18), Load saved configuration (2.28.1),
360   adjust the threshold (2.23), set sequence info (2.19), detect worms (2.24.1), save the project
361   (2.29), close video.

362

363   2.32.   Track videos

364

365   2.32.1. To track the project files, go to the **Workflow** menu and click the **Batch** (folder
366   containing worm pages) icon (**Supplementary Figure 2F**).

367

368   NOTE: The **Batch Processing** panel will be displayed (**Supplementary Figure 2G**). Sometimes it is
369   hidden but can be accessed at the very bottom of the **Batch Workflow** menu; the window may
370   need to be expanded to see it.

371

372   2.32.2. Click the **Add** button under the **File Selection** section of this **Batch Processing** menu.
373   Navigate to and select all the project files (.wpr) to be processed, click **Open**.

374

375   NOTE: The **Batch Processing** menu will fill with the selected file path, a status indicator and a
376   gray progress bar for each file.

377

378   2.32.3. Click the **Start** (play button icon). Note the green progress indicator on the first file.
379   Allow all the files to be processed. When all the files read as finished (green), the software can
380   be closed (**Supplementary Figure 2G**).

381

382   2.32.4. If a project file gets stuck, consider running the hung-up file by itself, this usually fixes
383   the problem. If this does not fix the problem, open the project file, delete the selected worms
384   (**Detect** and **Track** menu), and reselect from a new training set. Then reprocess the project file.

385

386   2.33.   Repairing tracks (recommended)

387

388   NOTE: Repairing tracks can increase the accuracy of the tracks. Common fixes are combining
389   tracks that clearly belong to the same worm as viewed by the human eye as, deleting tracks
390   that are not accurate or do not capture real worm data, and splitting tracks that are
391   misrepresented because worms are touching (remove the bad section and combine tracks from
392   before and after the poor section if practical).

393

394   2.33.1. Open the software and open a project file [**File | Open Project**] of interest. Click **Open**
395   and click **yes** to use this file. Wait for the temporal filter to load. The processed video will be on

396   the screen; worms will be highlighted in green and outlined in yellow. Use the video player to
397   scrub through or play the video.

398
399   2.33.2. From the **Workflow** menu, select **Detect and Track** (**Supplementary Figure 2D**). Switch
400   to the **Repair** tab. Activate the **Selector** cursor by clicking on the **Selector tool** icon (cursor
401   arrow). Scrub through the video, finding and selecting a worm track, and then use the repair
402   menu to create the desired change.

403
404   NOTE: To strategically fix most problems quickly, zoom in such that about 1/6th of the plate is
405   visible, start with the area surrounding track #1. Quickly scrub through the video checking for
406   worms that mysteriously appear out of nowhere, tracks where the numbers change, or worms
407   bumping into each other, editing at problems arise. Follow worms in numerical order from the
408   start position. Scrub back and forth to follow each worm as necessary until the whole plate is
409   repaired. Pay careful attention to the edges of the plate; often, fake worms appear in the
410   shadows of plate edges.

411
412   2.34.   Data analysis

413
414   2.34.1. From the **Workflow** menu, select **Analyze Data** (**Supplementary Figure 3A**).

415
416   NOTE: A new window named **Data Analysis and Plotting** will appear (**Supplementary Figure
417   3B**). This window has many ways to assess and view the movement data of a specific project
418   file's worm population. Most of these analyses allow for the selection of specific, multiple, or all
419   worms from a given video allowing for track information to be plotted in different ways.

420
421   2.34.2. To analyze turn count, navigate to the **Track Summary** [**Position & Speed | Track
422   Summary**] (**Supplementary Figure 3C**). Use the **Export** button in the bottom right to export
423   data in a spreadsheet readable format (.csv), choose a path to the desired location of the
424   spreadsheet, including renaming it to something memorable. The default settings are a good
425   choice (**Supplementary Figure 3D**).

426
427   2.34.3. Paste this data, as well as any other relevant project datasets into a consolidated
428   spreadsheet workbook (**Supplementary Figure 3E**).

429
430   2.34.4. Use the **Turn Count** and **Track Duration** to calculate turns per min for each track in using
431   spreadsheeting functions (=turns/track duration * 60) or use a different metric as desired
432   (**Supplementary Figure 3F**).

433
434   NOTE: Turns per minute captures very similar information as a manual thrashing assay which is a
435   widely used metric of activity in liquid, but there are many options, and it is quite possible that a
436   different metric may identify other interesting data for a given treatment or comparison.

437

438   2.34.5. If desired, screen for worm size, track duration, and/or some other chosen metric(s)
439   using conditional formatting tools to highlight tracks for removal if they are potentially
440   confounding. Maintain consistent data processing between treatments and replicates.
441
442   2.34.6. Analyze data using appropriate statistical analyses such as Student's t-test for 2 strains,
443   or 1-way ANOVA for 3 or more strains. Perform independent experimental replicates on
444   different days and with independent populations of worms for at least 3 independent
445   experimental replicates.
446
447   **REPRESENTATIVE RESULTS:**
448   Both radial locomotion and swimming assays offer sensitive detection of motility impairment
449   (**Figure 4** and **Figure 5**). To investigate the mechanisms underlying pathological TDP-43 in ALS, *C.*
450   *elegans* models have been developed that express wild-type or ALS-mutant human TDP-43 pan-
451   neuronally. These animals display molecular and cellular characteristics reminiscent of ALS,
452   including motor dysfunction[9]. Importantly, they exhibit moderate motility impairment with the
453   expression of wild-type human TDP-43 and more severe motility impairment in animals
454   expressing ALS-mutant TDP-43 using both radial locomotion and swimming assays. Some mutant
455   or transgenic animals will have greater impairment in crawling than swimming, or vice versa. By
456   using two different motility assays, a clearer picture of phenotypic differences between strains is
457   obtained.
458
459   **Radial locomotion**
460   When placed on a seeded agar plate, *C. elegans* explore their surroundings, including seeking out
461   the boundaries of their food source. Radial locomotion assays are one way to take advantage of
462   that behavior as a metric for physical fitness. By analyzing the locomotor behavior (crawling on a
463   solid surface) in a controlled and quantifiable way, the radial locomotor assay offers a simple and
464   effective tool for assessing the severity of motor deficits and other motor-related phenotypes.
465   Radial locomotion assays capture differences in motility in moderately or severely impaired ALS
466   model worms and offer a baseline to compare modulation of motility phenotypes or changes in
467   motility with age (**Figure 6**). This strategy can be applied to quantify the crawling behavior of any
468   strain that has altered movement from wild type (N2) or control worms. However, this method
469   may not be a good choice to assess animals unable to crawl normally, such as roller mutants or
470   animals that are paralyzed. Typically, wild-type worms will present an average displacement
471   between 200-300 μm/min when raised and tested at 20 ˚C. The example data presented in **Figure**
472   **4** show expected results comparing N2, two different transgenic strains expressing wild-type
473   human TDP-43 with a mild phenotype [TDP-43(WT-mild), CK402 (bkIs402[P*snb-1::TDP-43;* P*myo-*
474   *3::GFP*])] or stronger phenotype [TDP-43(WT-moderate), CK410 (bkIs402[P*snb-1::TDP-43;* P*myo-*
475   *2::GFP*])]), a transgenic strain expressing mutant human TDP-43 with a severe phenotype [TDP-
476   43(M337V), CK423 (bkIs423[P*snb-1::TDP-43;* P*myo-2::GFP*]), and a transgenic strain expressing
477   another neurodegenerative-disease associated protein, wild-type human tau [tau(WT), CK144
478   (bkIs144[P*aex-3::tau(4R1N)*; P*myo-2::GFP*])]. The two strains expressing wild-type TDP-43 have
479   different degrees of impairment as detected by radial locomotion. TDP-43(WT-low) is not
480   significantly different from N2, while TDP-43(WT-high) does exhibit clear differences in motility.
481   TDP-43(M337V) and tau (WT) strains have more severe impairments in motility.

482
483 **Swimming assay**
484 *C. elegans* engage in stereotypical swimming (thrashing) movement when immersed in liquid.
485 Immediately upon submersion, worms begin to characteristically bend head and tail towards
486 each other with a bend angle of approximately 45˚, with the angle vertex being the midpoint of
487 the worm. Worms alternate bending in the ventral and dorsal directions. One thrash, as
488 measured by the software, represents the movement of going from straight to a body bend angle
489 of 20˚ or greater, irrespective of directionality (angle thresholding can be adjusted post-
490 processing within the **Analysis** and **Plotting** window [**Workflow | Analyze Data | Body Shape |**
491 **Bending Angle | Mid-Point | Amplitude Threshold: # degrees**]. The swimming assay described
492 here uses automated computer-based tracking and analysis to provide unbiased scoring of
493 swimming activity. It is expected that wild-type (N2) worms will average between 150-200
494 thrashes per min when raised and recorded at 20 ˚C. The example data presented in **Figure 5**,
495 shows expected results comparing N2, two different transgenic strains expressing wild-type
496 human TDP-43 with a mild phenotype [TDP-43(WT-mild), CK402 (bkIs402[P*snb-1::TDP-43;* P*myo-*
497 *3::GFP*])] or stronger phenotype [TDP-43(WT-moderate), CK410 (bkIs402[P*snb-1::TDP-43;* P*myo-*
498 *2::GFP*])], and a transgenic strain expressing another neurodegenerative-disease associated
499 protein, wild-type human tau [tau(WT), CK144 (bkIs144[P*aex-3::tau(4R1N)*; P*myo-2::GFP*])]. The
500 transgenic strain expressing ALS-mutant TDP-43 [TDP-43(M337V)] does not thrash in liquid, and
501 so it is denoted on the graph as ND (no data). This assay can discriminate different phenotypes
502 than the radial locomotion assay shown in **Figure 4**. For example, in the radial locomotion assay
503 (**Figure 4**), TDP-43(WT-low) was not significantly different from N2. However, in the swimming
504 assay (**Figure 5**), both TDP-43(WT-low) and TDP-43(WT-high) are significantly different from N2,
505 as well as significantly different from one another. Also, despite both TDP-43(M337V) and
506 tau(WT) having severe crawling impairments by radial locomotion (**Figure 4**), only tau(WT) is able
507 to thrash enough to be tracked by the software (**Figure 5**). TDP-43(M337V) animals are unable to
508 thrash, and software-based analysis does not detect or track these worms accurately. Thus, data
509 on these worms were not collected (ND, no data).
510
511 **FIGURE AND TABLE LEGENDS:**
512
513 **Figure 1: Radial locomotion assay workflow.** Panels **A-E** show the generalized steps of the
514 radial locomotion assay. The steps are as follows: (**A**) NGM Plates, with OP50 seeded to the
515 edges, are prepared by labeling and marking central dot, (**B**) worms are placed in the center of
516 the agar as marked by the central dot, (**C**) worms are allowed to move freely for a set amount
517 of time, (**D**) plate is flipped bottom up and the final location of each worm is marked in a
518 different color than the central dot, (**E**) The distance from the center dot to each final worm
519 location in measured either by hand or digitally.
520
521 **Figure 2: Digital measurement of final location using ImageJ.** (**A**) Distance from center
522 measurements can be measured by hand or digitally, to measure digitally using ImageJ scan
523 backside of the plate with a ruler in the frame. (**B**) Draw a known length using the line tool. (**C**)
524 Use the known length drawn in (**B**) to set scale [**Analyze | Set Scale**…]. (**D**) Use the line tool to
525 draw a line from the center point to a final location mark, repeat for each mark. (**E**) A

526 paintbrush line marking the first mark measured aids in scoring (squiggly black line near the 1
527 mark). Final location marks are numbered in yellow to illustrate the directionality of scoring. (**F**)
528 Measurements are recorded in the Results window. Save results elsewhere for statistical
529 analysis.
530
531 **Figure 3: Materials and hardware adjustments for using the software.** (**A**) Materials used for
532 the software-assisted swimming assay. (**B**) General set up of the software and needed
533 materials, note that the housing is in the raised position. (**C**) The lens mounting bracket height
534 can be adjusted. This image shows the adjustable track and a tape indicator marking the
535 preferred height for performing swimming assays. It is necessary to adjust the brightfield light
536 and camera focus before recording. (**D**) A 35 mm assay plate is placed on the stage, animals in
537 M9 are added to the plate, and a 1 min timer is started. (**E**) It is sometimes advantageous to
538 gently swirl the plate to move worms closer to the center – observe location on the live capture
539 screen; alternatively, a few drops of M9 from a pipettor can be used to separate worms. (**F**)
540 Adjust the focus ring on the camera lens, observe worms on the screen to determine optimal
541 focus.
542
543 **Figure 4: Radial locomotion assays detect differences in crawling speed.** Unstimulated
544 dispersion of developmentally staged L4 larvae was measured using the radial locomotion assay
545 described above and graphed as µm/min traveled (**A-B**). The same data plotted in (**A**) and (**B**)
546 demonstrate two different possible graphical presentations. In (**A**), data are displayed as a bar
547 graph, making relative differences between strains clearer. In (**B**), the final displacement of each
548 worm is plotted within the graph, allowing the variation within the population to be better
549 visualized. To evaluate significance among strains tested, one-way analysis of variance (ANOVA)
550 with Tukey's multiple comparison test was used. **$p=0.0022$, ****$p<0.0001$, ns=not significant.
551 TDP-43(M337V) and tau(WT) are also significantly different from N2, $p<0.0001$. Error bars in (**A**)
552 are standard error of the mean (SEM) and in (**B**) are standard deviation.
553
554 **Figure 5: Swimming assays detect differences of thrashing in liquid.** Rates of swimming
555 (thrashing or undulation frequency in liquid) were measured using unbiased computer-assisted
556 scoring and analysis described above and graphed as thrashes/min (**A-B**). Same data plotted in
557 (**A**) and (**B**). In (**A**), data are graphed as a bar graph, which makes relative differences between
558 strains easier to see. In (**B**), data from each individual worm scored are plotted within the graph,
559 allowing the variation within the population to be better visualized. To evaluate significance
560 among strains tested, a one-way analysis of variance (ANOVA) with Tukey's multiple comparison
561 test was used. ****$p<0.0001$, ns=not significant. TDP-43(WT-moderate) and tau(WT) are also
562 significantly different from N2, $p<0.0001$.Error bars in (**A**) are standard error of the mean (SEM)
563 and in (**B**) are standard deviation.
564
565 **Figure 6: Mutant TDP-43 worms travel less than wild-type worms.** Plates showing a
566 representative difference in the final location of staged L4 N2 (wild-type) and TDP-43(M337V)
567 (CK423 (bkIs423[P*snb-1::TDP-43;* P*myo-2::GFP*]), a strain expressing mutant human TDP-43 with
568 severely impaired motor function after 1 hour of unstimulated crawling at room temperature.

569 Plates are marked with a red dot for the center point and blue dots for the final location of the
570 animals.
571
572 **Supplementary Figure 1: Recording swimming behavior using the imaging and tracking**
573 **software.** (**A**) The tracking workflow and the location of the video capture icon. (**B**) The **Video**
574 **Capture** window is shown in **Live View,** and the most important aspects are highlighted. The
575 green "Live view" words will change to a red "Recording" when the record button is activated.
576
577 **Supplementary Figure 2: Preparing and tracking swimming behavior**. This figure shows a series
578 of screenshots to aid in setting up a video image sequence for tracking. The general protocol for
579 preparing a video sequence is to open each workflow menu in this order: **Import Image**
580 **Sequence | Set Sequence Info | Adjust Image | Detect and Track | Save Project**. The **Analyze**
581 **Data** window can only be used after a project file is tracked. (**A**) shows an opened .avi (video)
582 file after importing the sequence into the software from within the **Track** workflow. (**B**) The **Set**
583 **Sequence Info** window provides a place for notes, set/change the scale, and to check the
584 metadata for a video sequence. Instructions for changing the scale are found in this window
585 and should be followed when the camera is lowered or raised (**B**). Image (**C**) shows the **Adjust**
586 **Image** window settings. (**D**) Screenshot showing the detection tab of the **Detect and Track**
587 window. Detecting worms is used to train the software and must be set for each video
588 sequence. Additional detection parameters can be set here if desired. (**E**) Screenshot of
589 **Tracking** tab of the **Detect and Track** window with the recommended settings for tracking
590 swimming behavior. This is the last step in setting up a video sequence. Save the sequence as a
591 project using the **Save Project** window. Repeat these steps for each video sequence. Settings
592 can be saved as a configuration to reduce workload and ensure that all sequences are treated
593 the same (not shown). To track project files for analysis, navigate to the **Batch** Workflow. (**F**)
594 shows the icon used to navigate to the **Batch** workflow, and (**G**) shows the batch processing
595 window, highlights the add and start buttons, and shows the expected appearance of a project
596 file that has been tracked.
597
598 **Supplementary Figure 3: Swimming behavior analysis.** After a project file has been tracked, it
599 can be opened using [**File | Open Project**]. Worms will appear in green as shown in (**A**).
600 Scrubbing through the video offers a quick check that the video processed as expect, green
601 highlighting will disappear while scrubbing. The Data Analysis and Plotting window is opened
602 from the **Analyze Data** workflow item. (**B**) shows the Data Analysis and Plotting window with
603 Position view opened (default), all tracks are highlighted. Below the datapoints is a plot
604 showing the recorded track for each highlighted worm. The track summary analysis is used to
605 calculated turns per minute. (**C-D**) show the track summary report and export details. (**E-F**)
606 show the track summary in a spreadsheet and how to calculate turns per minute, the output
607 measured in this assay.
608
609 **DISCUSSION:**
610
611 **Radial locomotion:**
612 The resolution of this assay is easily controlled by changing the time variable. Increasing the

613   length of time makes it easier to observe differences between animals with severe phenotypes,
614   thereby identifying subtle differences. However, because this assay measures displacement, if
615   the assay time is extended too long, animals with normal motility, such as N2, will travel to the
616   edges of the plate, and foraging behavior will lead to backtracking. This will artificially decrease
617   the measurement of distance traveled. Time periods that are too long may result in the
618   disappearance of differences between strains, particularly between animals with less severe
619   motor phenotypes, as animals become evenly dispersed across the plate. Shortening the time
620   variable will prevent more active worms from finding the edges of the plate. This method does
621   not track the total distance traveled for each worm but compresses the distance traveled for
622   each worm into a linear distance from the center of the plate. As such, it is inherently less robust
623   than a method that records the total track length of individual worms. However, the radial
624   locomotion assay requires very little researcher training, uses relatively affordable reagents that
625   are commonly available in most worm labs, and is sensitive enough to produce significant and
626   reproducible results. For labs that prefer automated video tracking, several methods have been
627   previously established to track and analyze crawling movements[12], or the software parameters
628   used for the swimming assay in this paper could be altered to permit crawling detection and
629   analysis.
630
631   This experiment is usually done in triplicate independent replicates, with a set of 30-40 worms
632   per replicate. Each replicate is split onto two different 100 mm or 150 mm plates, with 15-20
633   worms per plate. Using more worms than recommended per plate can make it hard to score
634   efficiently. A total number scored of 90+ is sufficiently powered to establish significance for mild,
635   moderate, or severe motility impairment. Being consistent with timing between strains scored is
636   essential for accuracy and reproducibility. 30 minutes is generally long enough to establish
637   differences between moderate to severe phenotypes such as transgenic strains expressing
638   human mutant TDP-43 compared to wild-type worms (**Figure 4**). If the time variable will be
639   extended, it is advisable to also increase the size of the plate from 100 mm to 150 mm.
640   Environmental factors such as temperature and humidity can affect this assay, which is typically
641   conducted at ambient room temperature, so it is important to always use a wild-type (N2) control
642   when comparing across replicates. In addition, this assay can measure the motility of some
643   strains that do not exhibit normal swimming behavior in liquid (thrashing), making it a useful
644   complement to the swimming assay.
645
646   **Swimming assay:**
647   The use of the imaging and acquisition system to automate tracking and analysis of worm
648   swimming allows for rigorous and unbiased data. However, there are several factors during initial
649   setup of the experiment that needs to be controlled between samples. These include the time to
650   acclimate to liquid before beginning recording, ambient conditions (i.e., temperature, humidity),
651   and consistent light and recording settings. On the recording stage, there are several features
652   that help reduce variability between plates. These include an integrated track-mounted camera
653   and bright-field stage that makes recording video consistent between plates, shielding around
654   the stage that prevents reflections, glare, and air movements while recording, and a robust
655   software package that reliably detects worms and allows for manual correction of tracks in video
656   post-processing. In this protocol, videos of a 35 mm plate with worms are recorded for 1 minute

657 and then processed using the software package. After processing, manual correction of tracks
658 ensures that worm behaviors are recorded accurately without confounding tracking errors. Turn
659 count and track duration data are used to determine turns per minute as a final readout. To
660 ensure reproducibility, data is collected over a minimum of 3 independent replicate experiments,
661 each with 40-50 animals scored, to achieve a combined final number of animals 120-150. This
662 number is sufficient to discriminate small differences in swimming behavior from control worms.
663 Some worms have motor deficits too severe to be captured by swimming assays. For example, if
664 the animals placed in a liquid medium curl instead of performing the expected thrashing
665 response, this assay will not record those movements accurately and another movement assay,
666 such as radial locomotion, may better capture those motility defects. The provided protocol uses
667 a commercially available imaging system (see **Table of Materials** for more details), but other
668 worm tracking systems may provide a similar utility, with some being open source[12]. Previously
669 published methods describe manual scoring of worm thrashing[13]. While the automated analysis
670 produces a number of metrics for each individual worm, detection of body bends which is
671 measured in thrashes per minute, provides consistent results between experiments and tracks
672 well with conventional scoring of worm thrashes by eye.

683 **DISCLOSURES:**
684 The authors declare that they have no disclosures.

686 **REFERENCES:**

688 1   Wijesekera, L. C., Leigh, P. N. Amyotrophic lateral sclerosis. *Orphanet Journal of Rare*
689 *Diseases.* **4**, 3 (2009).
690 2   Arai, T. et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in
691 frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochemical and*
692 *Biophysical Research Communication.* **351** (3), 602-611 (2006).
693 3   Neumann, M. et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and
694 amyotrophic lateral sclerosis. *Science.* **314** (5796), 130-133 (2006).
695 4   Mejzini, R. et al. ALS genetics, mechanisms, and therapeutics: Where are we now?
696 *Frontiers in Neuroscience.* **13**, 1310 (2019).
697 5   Tan, R. H., Ke, Y. D., Ittner, L. M., Halliday, G. M. ALS/FTLD: Experimental models and
698 reality. *Acta Neuropathologica.* **133** (2), 177-196 (2017).

699   6   Kim, W., Underwood, R. S., Greenwald, I., Shaye, D. D. OrthoList 2: A new comparative
700   genomic analysis of human and Caenorhabditis elegans genes. *Genetics.* **210** (2), 445-461
701   (2018).
702   7   White, J. G., Southgate, E., Thomson, J. N., Brenner, S. The structure of the nervous
703   system of the nematode Caenorhabditis elegans. *Philosophical Transaction of the Royal Society*
704   *of London B: Biological Sciences.* **314** (1165), 1-340 (1986).
705   8   Caldwell, K. A., Willicott, C. W., Caldwell, G. A. Modeling neurodegeneration in
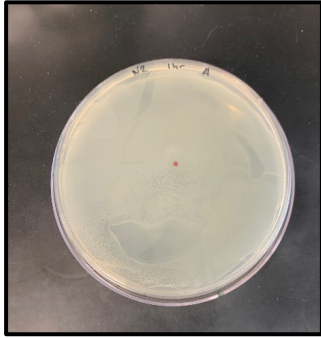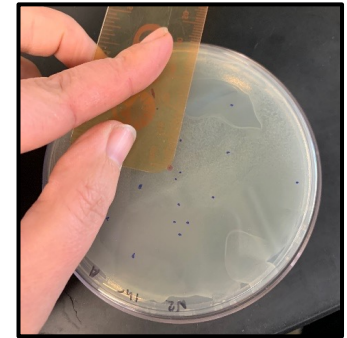706   Caenorhabditis elegans. *Disease Models and Mechanisms.* **13** (10), dmm.046110, (2020).
707   9   Liachko, N. F., Guthrie, C. R., Kraemer, B. C. Phosphorylation promotes neurotoxicity in a
708   Caenorhabditis elegans model of TDP-43 proteinopathy. *Journal of Neuroscience.* **30** (48),
709   16208-16219 (2010).
710   10   Robatzek, M., Thomas, J. H. Calcium/calmodulin-dependent protein kinase II regulates
711   Caenorhabditis elegans locomotion in concert with a G(o)/G(q) signaling network. *Genetics.* **156**
712   (3), 1069-1082 (2000).
713   11   Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A., Ceron, J. Basic Caenorhabditis
714   elegans methods: synchronization and observation. *Journal of Visualized Experiments.* (64),
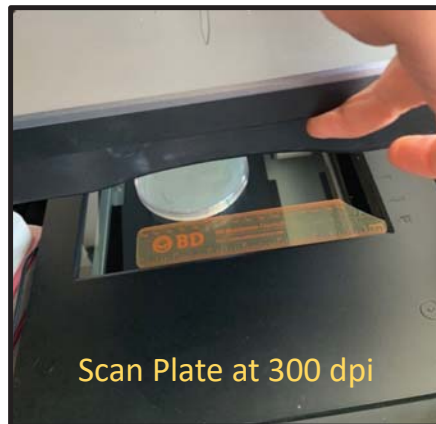715   e4019 (2012).
716   12   Husson, S. J., Costa, W. S., Schmitt, C., Gottschalk, A. Keeping track of worm trackers.
717   *WormBook.* 1-17 (2013).
718   13   Ringstad, N., Horvitz, B., and Koelle, M. *WormBook*. Editor: A. C. Hart, 1551-8507 (2006).
719

Figure 1

A

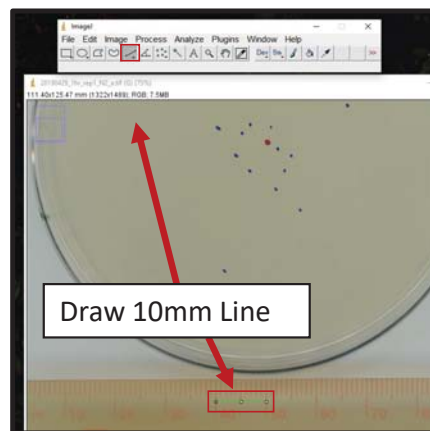Prepare Plate

B

Place Worms

C

Set Timer

D

Mark Final Location

E

Measure Distance

Figure 2

A

Scan Plate at 300 dpi

B

Draw 10mm Line

C

D

E

Move Line and
Click "M" to Measure

F

When Done, Paste Values
into a Spreadsheet for Analysis

Figure 3
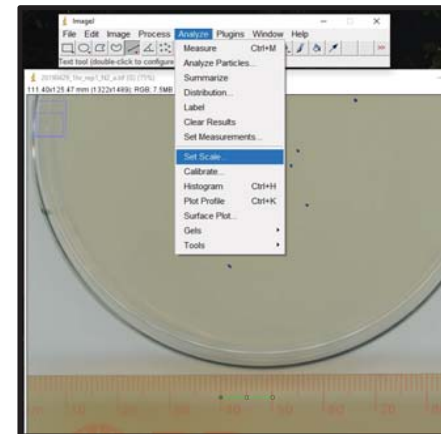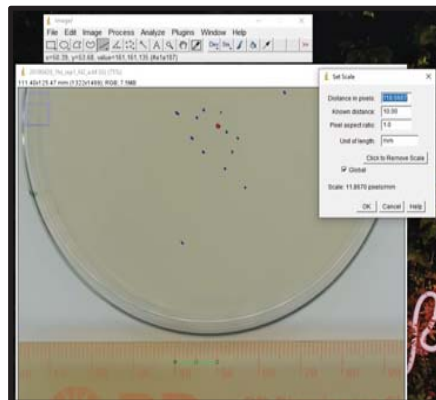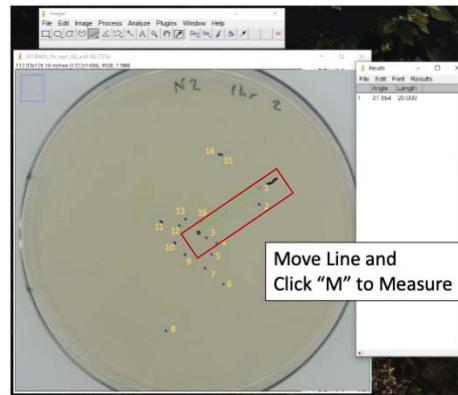
A

Materials

B

WormLab Setup

C

Camera Height Indicator

D

Washed Worms Added
to Assay Plate

E

Align Plate, Swirl Gently
to Center Worms (optional)

F

Adjust Focus Ring on
Camera Lens as Needed

Figure 4

A.

B.

Figure 5

A.

B.

Figure 6

WT
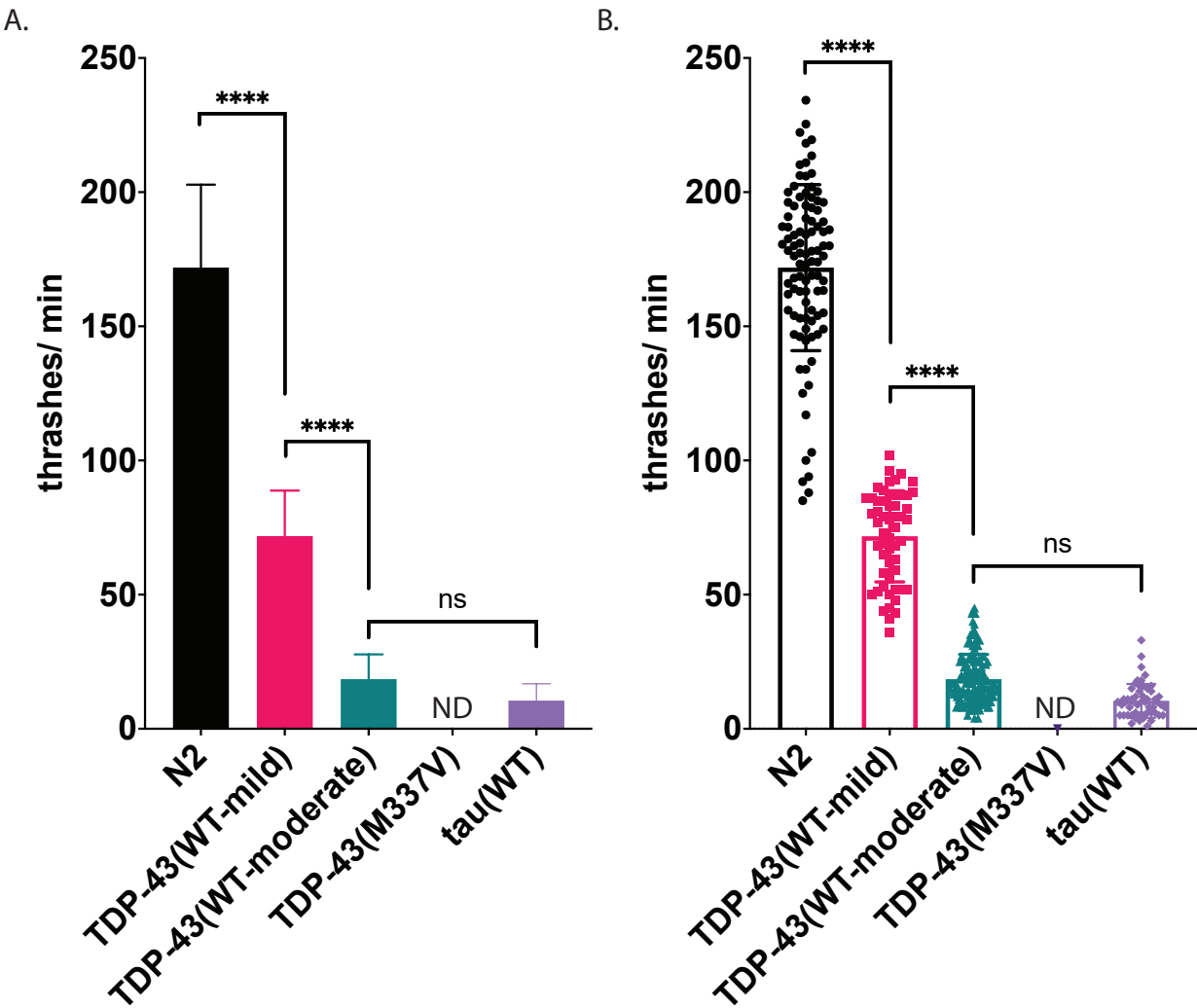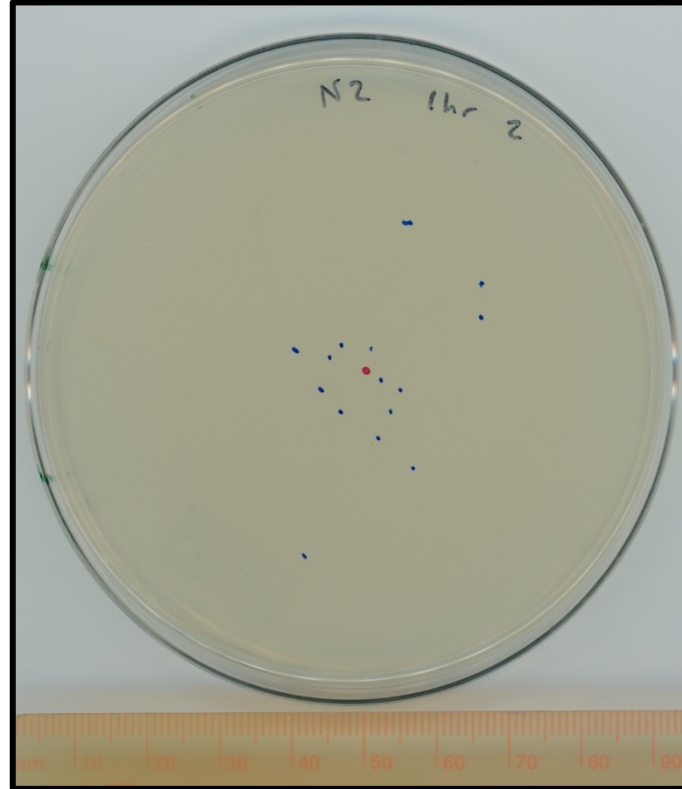
TDP-43(M337V)
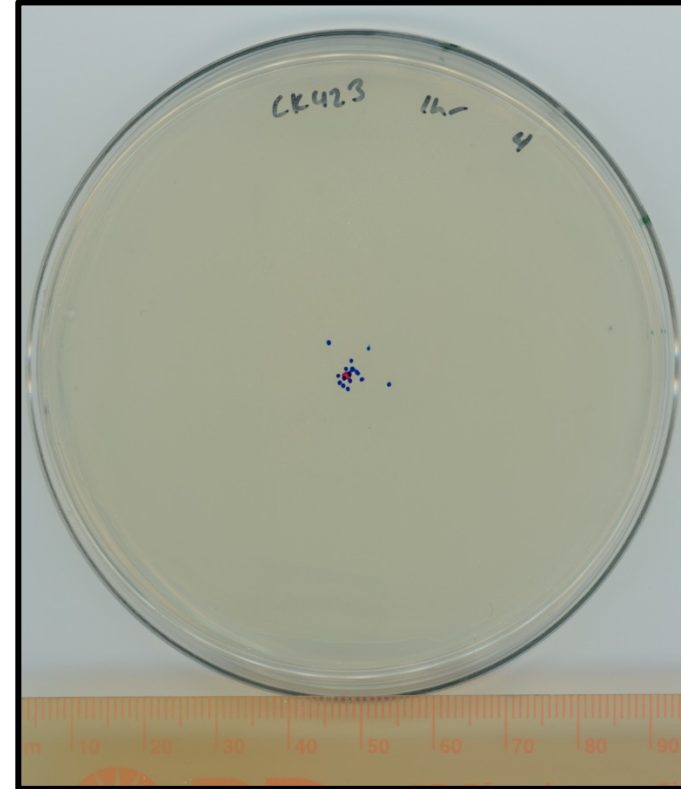
Table of Materials

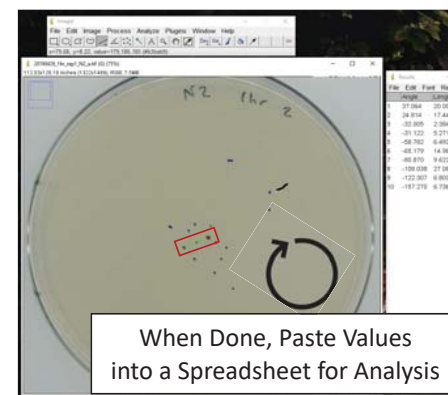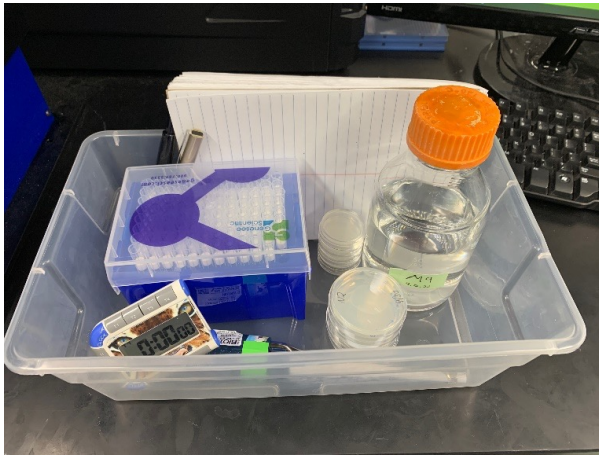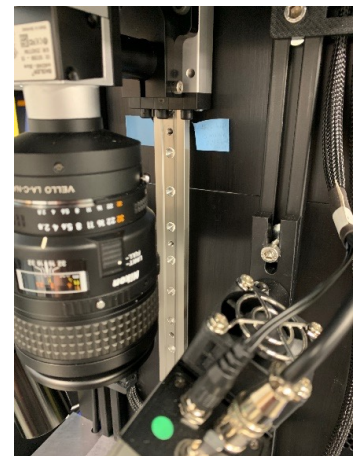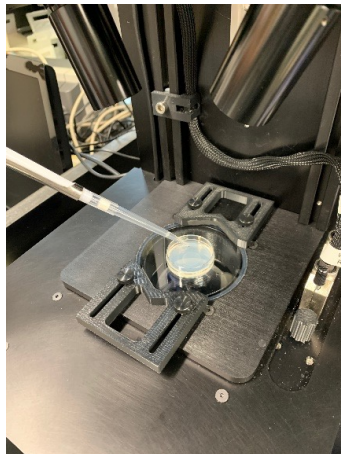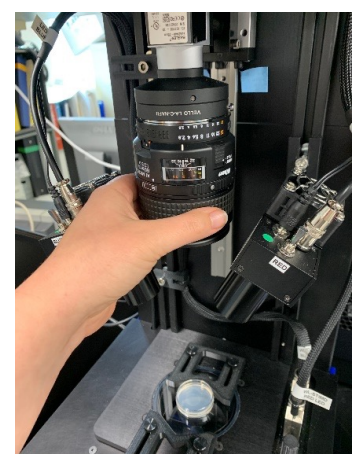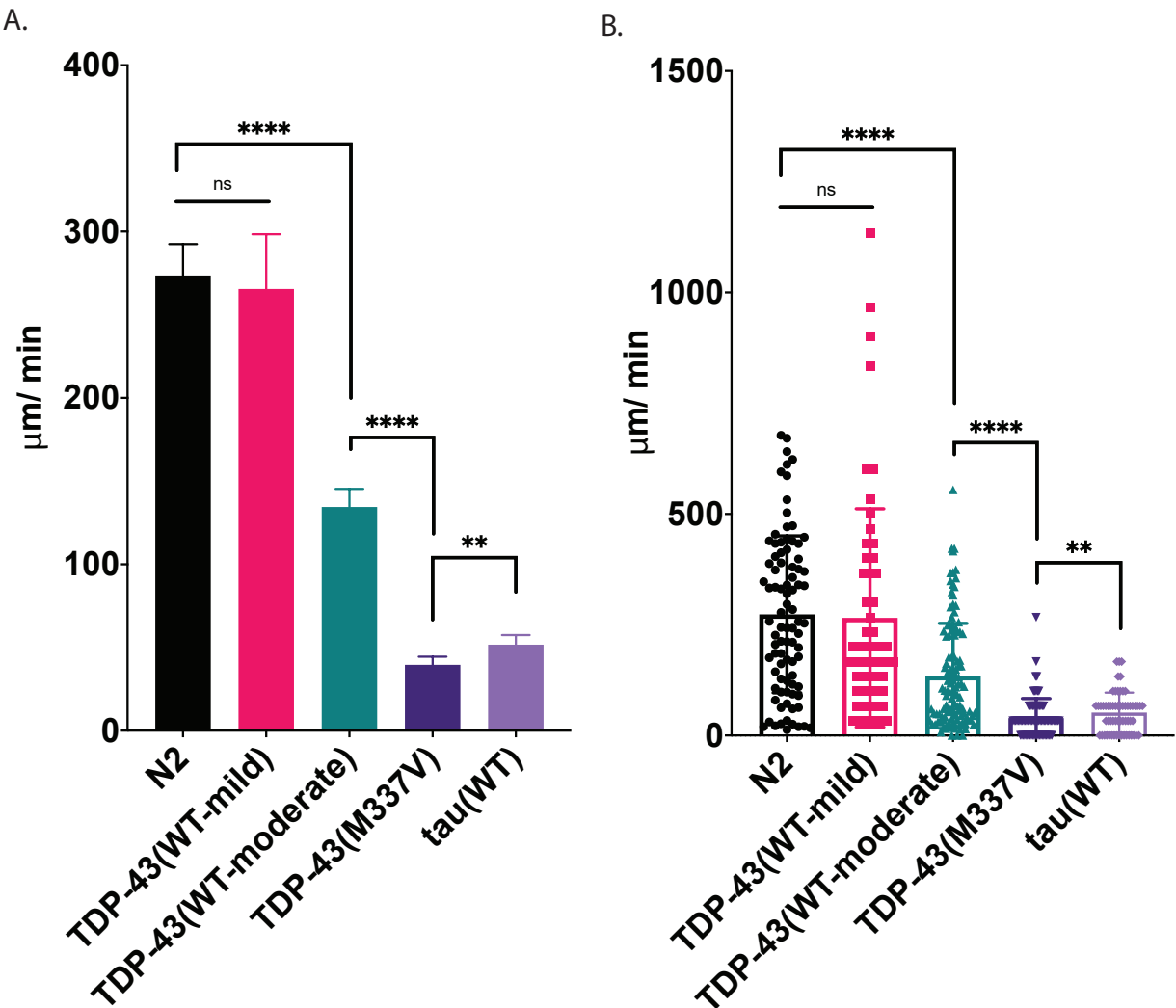| Name of Material/ Equipment	Company	Catalog Number	Comments/Description
C. elegans	Caenorhabditis Genetics Center (CGC)	-	Aquire your strains as desired, N2 is a
Disposable pasteur pipets, borosilicate glass	VWR	14673-010	Glass pipet used to create worm pick
Disposable petri dishes, 35x10mm	VWR	10799-192	Assay plates for WormLab Imaging Sys
Disposable petri dishes, 60x15mm	VWR	25384-090	Stock plates for worms
Disposable petri dishes, 100x15mm	VWR	25384-302	Standard radial locomotion assay plat
Disposable petri dishes, 150x15mm	VWR	25384-326	Longer time frame radial locomotion a
Dissecting microscope	Leica	M80	Scope for maintaining worms and sett
Fine-tipped markers	VWR	52877-810	Need at least 2 colors for radial locom
Flatbed Scanner	Amazon	Epson Perfection V850	Optional for radial locomotion assay.
ImageJ	NIH	-	Optional free software provided by th
M9 buffer	VWR	IC113037012	Medium used for swimming assay. Ca
NGM (Nematode Growth Medium)	VWR	76347-412	Medium used to cultivate C. elegans.
OP50 bacteria	Caenorhabditis Genetics Center (CGC)	OP50	Primary food source for C. elegans
p1000 pipettor	VWR	76207-552	Pipettor, used in swimming assay
p1000 tips	VWR	83007-384	Tips for pipettor, used in swimming as
Platinum wire, 0.2032mm diameter	VWR	BT136585-5M	Fine gauge platinum wire used to crea
Ruler	VWR	56510-001	Need to score radial locomotion assay
WormLab Imaging System	MBF Bioscience	WormLab	The Imaging System includes WormLa

useful control strain

- hold glass pipette in one hand and ~1" of platinum wire (held by pliers) in the other over a flame to join.

stem

e

assay plate
:ing up radial locomotion assays
iotion assays. Fine tips required for accuracy.

Protocol assumes a resolution of 300dpi, most scanners would work fine
e NIH - https://imagej.nih.gov/ij/
n be made from scratch, see WormBook: Maintenance of C. elegans

Can be made from scratch, see WormBook: Maintenance of C. elegans

ssay

ite worm pick - hold glass pipette in one hand and ~1" of platinum wire (held by pliers) in the other over a flame to join.
/s
b hardware (bright field stage, camera, and housing) and WormLab software. https://www.mbfbioscience.com/wormlab-imaging-system

Dear Editorial Staff,

Thank you for your edits of our article now titled "Evaluate motor impairment in *C. elegans* models of amyotrophic lateral sclerosis (ALS)". We appreciate the helpful comments and suggestions. As a result we have updated accompanying text according to the editor recommendations and moved several figures to the supplement.

best regards,
Nicki

Nicki Liachko, Ph.D.

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A
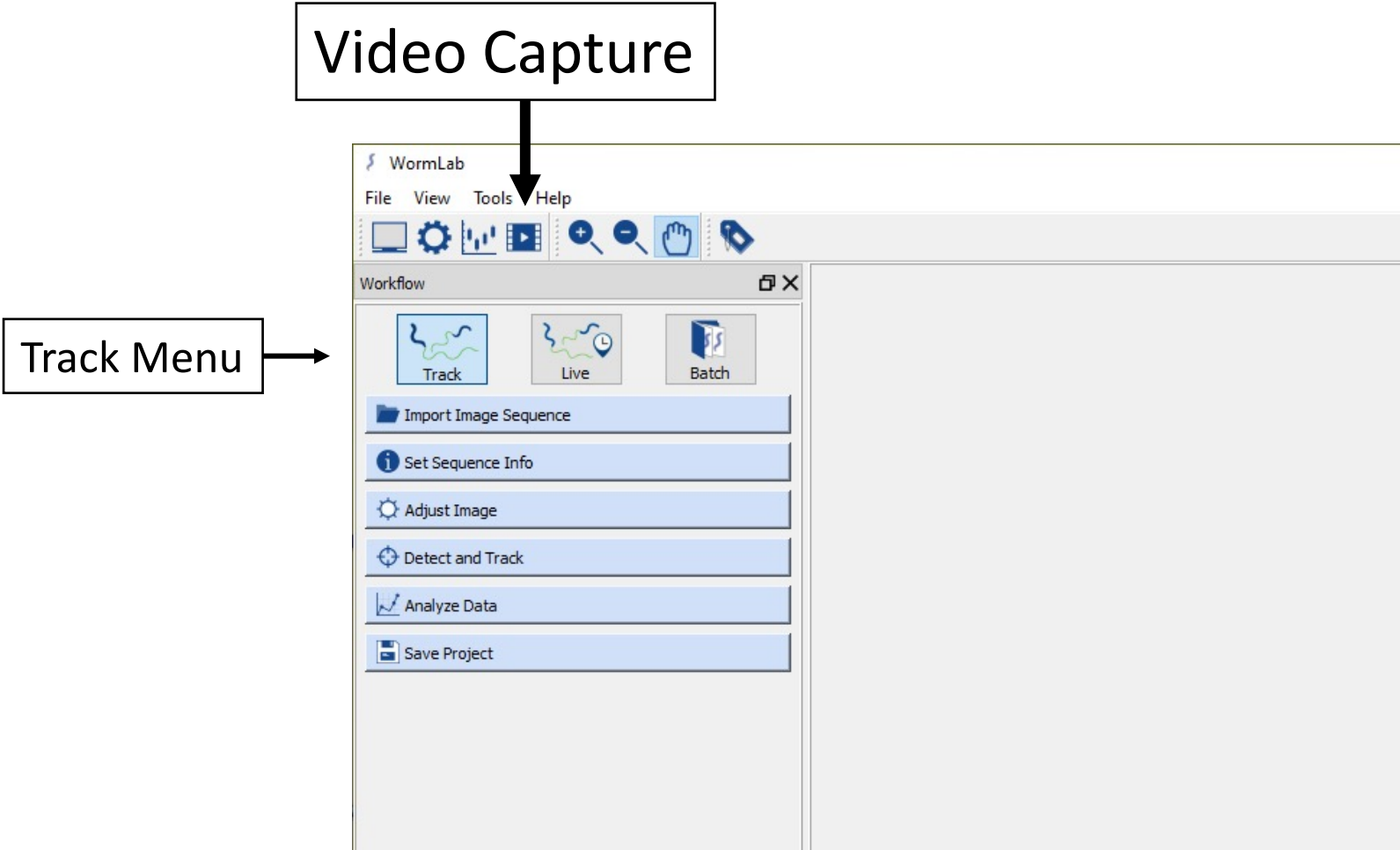
B

Video Capture

Camera

Live view

Basler - acA2440-35um
Time   00:09:05.629
Frame 7639
Rate   14.000
Buffer

[Display grid] [Display clipping]

Capture [Settings]

[Folder] racker/TDP-43 Worm Test

File prefix   20210503_CK1441;CK194:
Buffer   128   frames
☑ Duration   0 : 1 : 0
☐ Time-lapse   1.00   seconds
☑ Open video after recording
☐ Show Stimulus Controls

[Record] [Stop]
[Snapshot]

Live View – not recording

File location

File name

Duration (set to 1 min)

Recording Controls

A
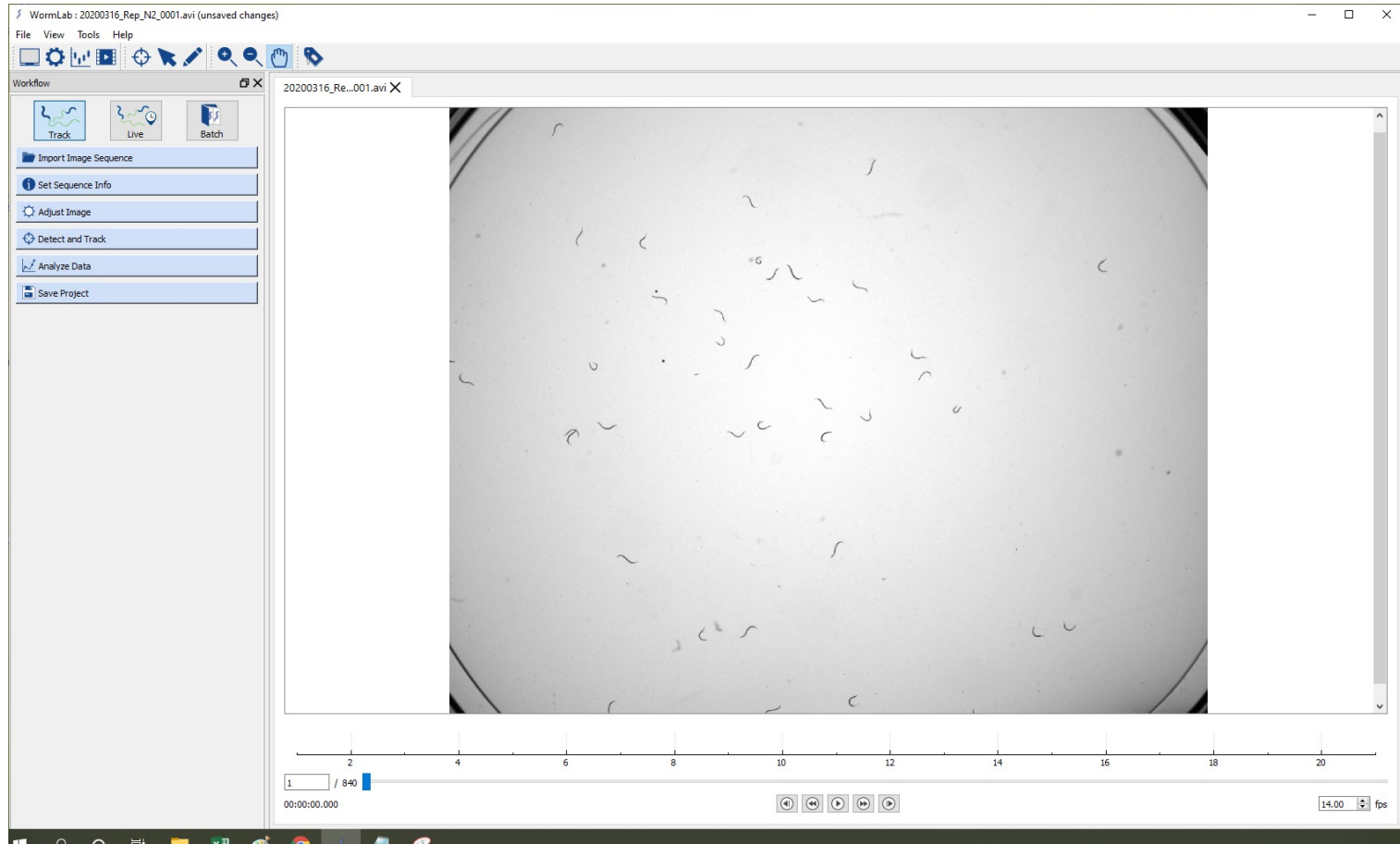
B
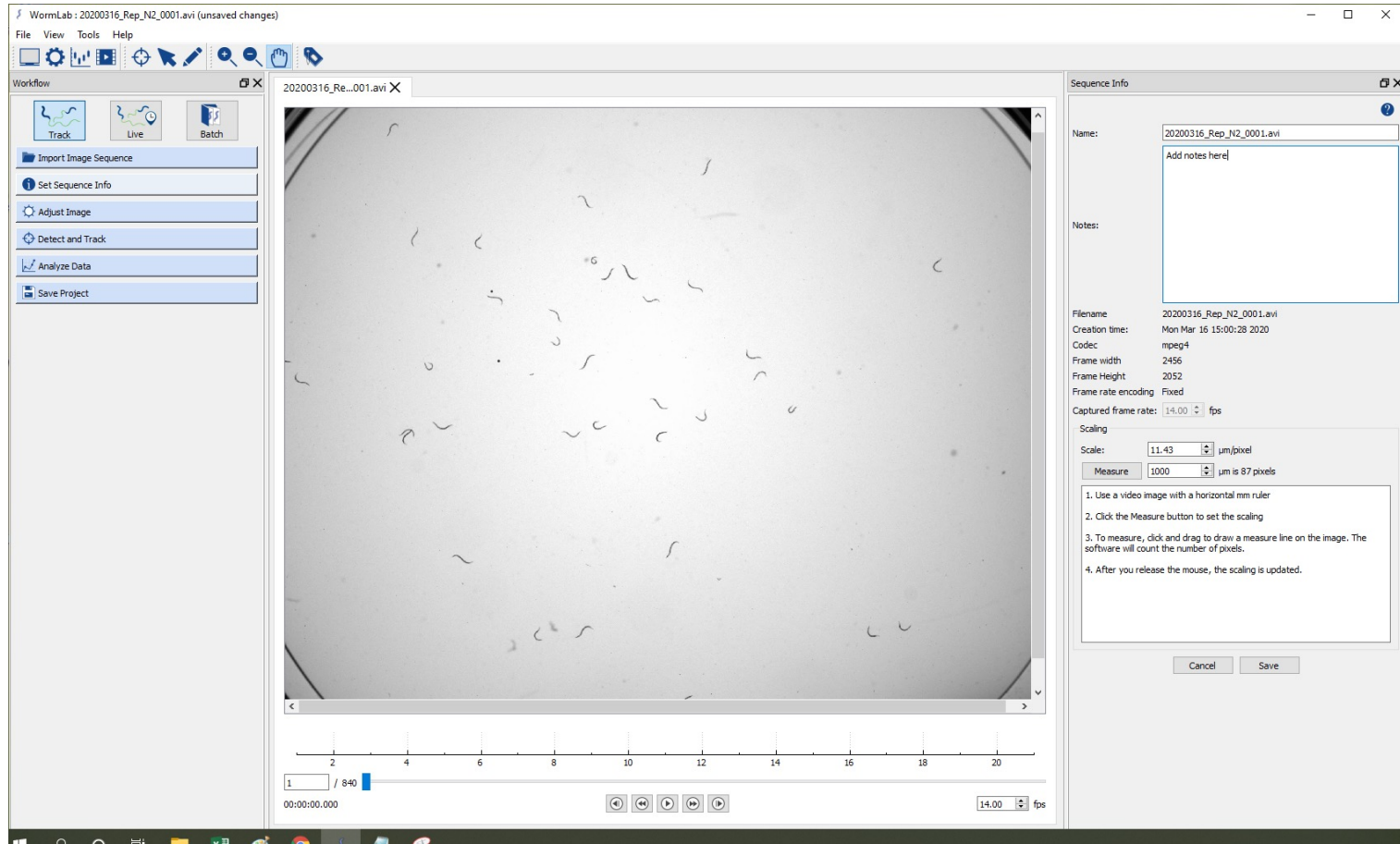
C
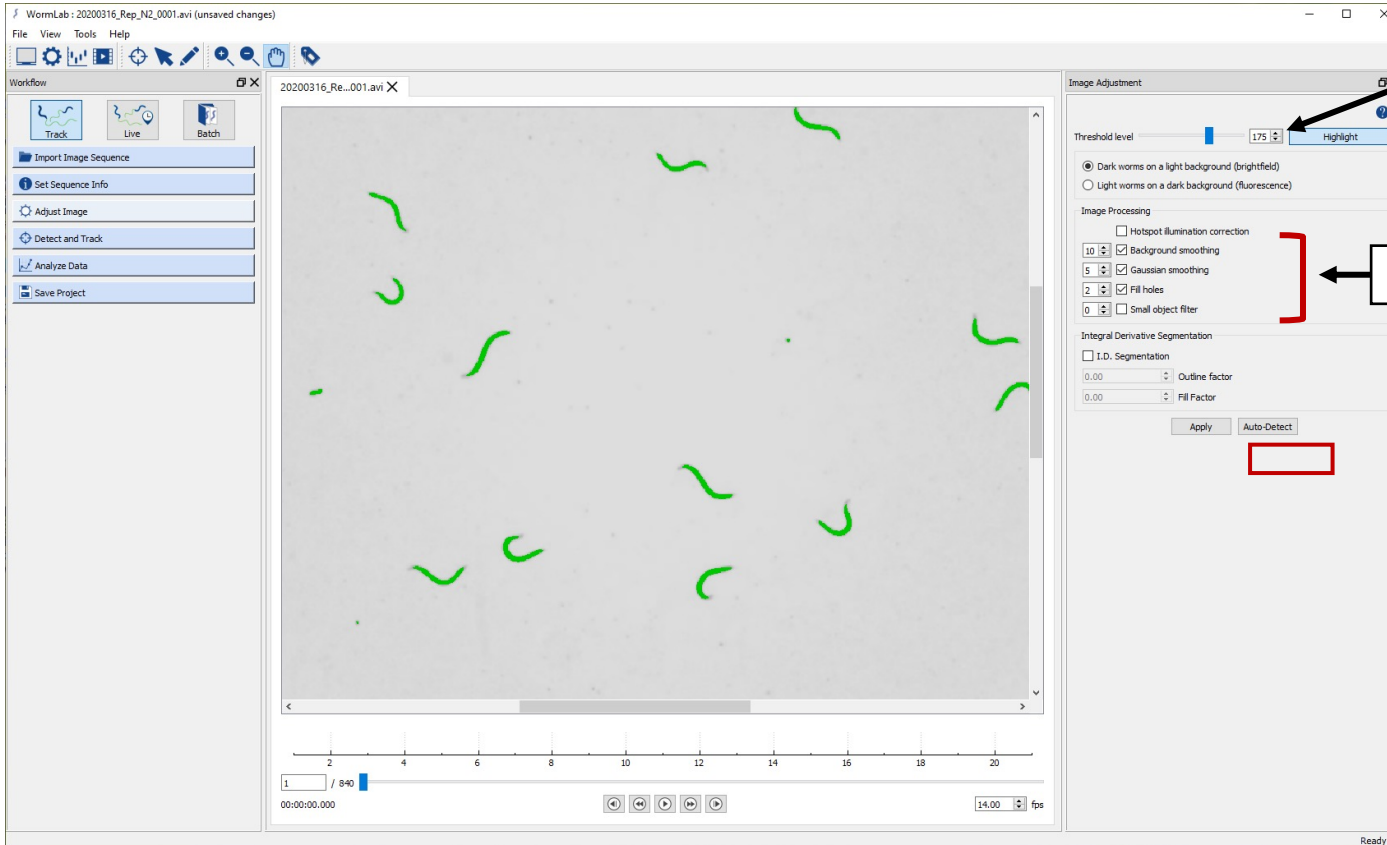
Set threshold such that worms are green

Image Processing Settings

D

1. Use cursor to select several worms
2. Click "Detect Worms"
3. Wait for most worms to be highlighted in green

E

Save as project when done!

Tracking Settings

F

WormLab

File   View   Tools   Help

Workflow

Track   Live   Batch

Move to the Batch Workflow

For each video:

1. Use the Track workflow to setup parameters. Do not click Start in the tracking tab!

2. In the workflow, click Save Project.

3. In the Batch Processing panel, click Add to add the project file (.wpr) to the list.

Once all the projects have been added, click the Start button to begin tracking all videos in the project list.

G

Add Project Files,
Click "Start" to Run

A

Open Analyze Data

Scrubber and media player controls

B

Go to Track Summary

C

Data Analysis and Plotting

Data | Report

Select an Analysis
- ∨ Position & Speed
  - Position (um)
  - Center Points (um)
  - Direction
  - Speed (um/s)
  - Moving Average Speed (um/s)
  - Smoothed Speed (um/s)
  - Reversal
  - Mobility
  - Validation
  - Position Speed Statistics
  - Pirouette
  - **Track Summary**
- › Body Shape
- › Histograms
- › Label Analysis
- › Chemotaxis
- › Swimming

Filtering option
- ☑ Kalman Smoothing

Export selected

Track Summary ⓘ

Refresh Data

Track Number

Metrics	1	2	3	4	5	6	7	8	9
Mean Worm Length (um)	674.26	686.52	732.80	720.40	718.74	788.12	862.28	722.57	743.37
Mean Width (um)	58.21	61.04	61.53	59.10	61.42	60.78	70.28	73.29	60.90
Mean Area (um^2)	39924.05	42604.36	45874.72	43307.35	44904.44	48731.65	61642.91	54192.86	46048.25
Track Length (um) [Center point trajectory]	23794.35	11736.97	26822.62	28227.75	32378.65	23988.81	15957.33	12211.07	7806.49
Center point Speed (um/s) [Center point trajectory/time]	397.05	195.85	447.58	471.02	540.29	465.16	521.97	348.18	481.46
Absolute Peristaltic Track Length (um) [forward + reversals]	14875.82	5508.27	17183.21	15938.67	21243.16	17197.74	10625.01	7215.72	5305.72
Peristaltic Speed (um/s) [Absolute peristaltic track length/tim	248.23	91.91	286.73	265.96	354.47	333.47	347.55	205.74	327.23
Peristaltic Track Length (um) [forward - reversals]	6257.98	-3624.59	-13734.06	-15767.12	-21048.29	-13297.44	-7427.13	3110.29	-5267.94
Peristaltic Speed (um/s) [peristaltic track length/time]	104.42	-60.48	-229.17	-263.10	-351.22	-257.85	-242.94	88.68	-324.90
Straight-line Distance (um)	2687.76	80.24	1822.21	2160.97	1346.03	6120.54	1371.07	1455.47	1116.95
Wavelength (um)	388.19	404.05	429.81	422.49	449.54	494.14	532.99	403.69	465.05
Mean Amplitude (um)	65.38	48.61	66.61	71.57	70.03	61.67	83.92	63.14	71.47
Max Amplitude (um)	131.10	117.79	153.45	132.01	131.96	140.60	182.18	162.88	129.18
Turn Count	183.00	69.00	207.00	196.00	183.00	154.00	90.00	78.00	60.00
Track duration (s)	59.93	59.93	59.93	59.93	59.93	51.57	30.57	35.07	16.21
Distance Traveled Forward(um)	10566.90	941.84	1724.58	85.77	97.44	1950.15	1598.94	5163.01	18.89
Distance Traveled in Reverse(um)	4308.92	4566.43	15458.64	15852.89	21145.73	15247.59	9026.07	2052.72	5286.83
Number of Reversals	7.00	17.00	2.00	1.00	1.00	3.00	1.00	5.00	1.00
Cumulative Reversal Time (s)	24.07	45.00	46.50	60.00	60.00	48.00	20.50	11.57	16.21
Cumulative Forward Time (s)	35.93	15.00	13.50	0.00	0.00	3.64	10.14	23.57	0.07
Number of Pirouette	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Pirouette Time (s)	0.86	0.00	0.00	0.00	0.00	0.00	0.00	1.57	0.00
Mobility reversal time (s)	25.50	47.29	46.21	60.00	60.00	47.93	20.93	13.07	16.29
Mobility idle time (s)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mobility forward time (s)	34.50	12.71	13.79	0.00	0.00	3.71	9.71	22.07	0.00

Copy Selected Data | Export...

Export Data (all tracks)

D

E

Worm Data	Track Summary																					
Sequence	20200316_Rep_N2_0001.avi																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Mean Worm Length (um)	674.26	686.52	732.8	720.4	718.74	788.12	862.28	722.57	743.37	703.52	716.65	664.35	627.96	679.29	781.25	641.31	686.53	753.79	765.03	644.05	731.55	789.04
Mean Width (um)	58.21	61.04	61.53	59.1	61.42	60.78	70.28	73.29	60.9	64.31	59.97	58.42	55.76	65.7	64	61.01	59.89	58.56	62.05	62.74	60.46	65
Mean Area (um^2)	39924.05	42604.36	45874.72	43307.35	44904.44	48731.65	61642.91	54192.86	46048.25	45902.93	43715.97	39474.1	35630.87	45201.95	50858.77	39645.85	41818.33	44901.64	48286.08	41056.7	44965.99	52171.41
Track Length (um) [Center point trajectory]	23794.05	11736.97	26822.62	28227.75	32378.65	23988.81	15957.33	12211.07	7806.49	26370.85	29161.31	9494.03	11274.16	27159.58	29200.59	12764.09	5027.64	1158.44	22862.38	4290.85	20636.43	20624.62
Center point Speed (um/s) [Center point trajectory/time]	397.05	195.85	447.58	471.02	540.29	465.16	521.97	348.18	481.46	440.04	486.6	406.47	482.69	453.2	487.26	412.7	502.76	523.17	540.66	349.26	488.02	449.76
Absolute Peristaltic Track Length (um) [forward + reversals]	14875.82	5508.27	17183.21	15938.67	21243.16	17197.74	10625.01	7215.72	5305.72	17114.4	18793.41	6043.12	7578.37	17615.92	17503.27	8893.55	3118.43	770.73	15592.45	2338.46	13501.54	13008.22
Peristaltic Speed (um/s) [Absolute peristaltic track length/time]	248.23	91.91	286.73	265.96	354.47	333.47	347.55	205.74	327.23	285.58	313.6	258.73	324.46	293.95	292.07	287.55	311.84	348.07	368.74	190.34	319.29	283.67
Peristaltic Track Length (um) [forward - reversals]	6257.98	-3624.59	-13734.1	-15767.1	-21048.3	-13297.4	-7427.13	3110.29	-5267.94	7920.92	-11933.1	5882.6	-4039.66	8360.17	-8749.2	8089.91	1117.3	-766.9	-12441.3	1816.84	-7043.99	-6448.61
Peristaltic Speed (um/s) [peristaltic track length/time]	104.42	-60.48	-229.17	-263.1	-351.22	-257.85	-242.94	88.68	-324.9	132.17	-199.12	251.85	-172.95	139.5	-145.99	261.57	111.73	-346.34	-294.22	147.88	-166.58	-140.62
Straight-line Distance (um)	2687.76	80.24	1822.21	2160.97	1346.03	6120.54	1371.07	1455.47	1116.95	419.03	4530.41	1208.91	217.43	2064.61	3224.21	647.04	487.33	549.42	836.19	488.29	1712.89	1175.84
Wavelength (um)	388.19	404.05	429.81	422.49	449.54	494.14	532.99	403.69	465.05	399.77	434.94	411.48	361.89	386.15	479.97	376.61	378.01	514.33	476.36	316.62	460.29	492.08
Mean Amplitude (um)	65.38	48.61	66.61	71.57	70.03	61.67	83.92	63.14	71.47	73.87	68.13	61.3	62.68	60.38	68.34	43.43	65.78	67.85	74.86	60.9	68.82	72.55
Max Amplitude (um)	131.1	117.79	153.45	132.01	131.96	140.6	182.18	162.88	129.18	180.05	150.18	118.8	124.33	144.95	147.3	133.98	125.65	119.88	144.97	122.24	156.54	151.49
Turn Count	183	69	207	196	183	154	90	78	60	158	209	74	82	184	205	102	28	9	135	24	145	157
Track duration (s)	59.93	59.93	59.93	59.93	59.93	51.57	30.57	35.07	16.21	59.93	59.93	23.36	23.36	59.93	59.93	30.93	10	2.21	42.29	12.29	42.29	45.86
Distance Traveled Forward(um)	10566.9	941.84	1724.58	85.77	97.44	1950.15	1598.94	5163.01	18.89	12517.66	3430.14	5962.86	1769.36	12988.04	4377.03	8491.73	2117.87	1.92	1575.56	2077.65	3228.78	3279.81
Distance Traveled in Reverse(um)	4308.92	4566.43	15458.64	15852.89	21145.73	15247.59	9026.07	2052.72	5286.83	4596.74	15363.27	80.26	5809.01	4627.87	13126.23	401.82	1000.56	768.81	14016.89	260.81	10272.77	9728.42
Number of Reversals	7	17	2	1	1	3	1	5	1	4	4	0	1	5	2	0	1	1	2	0	3	3
Cumulative Reversal Time (s)	24.07	45	46.5	60	60	48	20.5	11.57	16.21	18.07	41.93	0	13.14	17.86	29.79	0	4.21	2.29	37.5	0	24.93	31.64
Cumulative Forward Time (s)	35.93	15	13.5	0	0	3.64	10.14	23.57	0.07	41.93	18.07	23.43	10.29	42.14	30.21	31	5.86	0	4.86	12.36	17.43	14.29
Number of Pirouette	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirouette Time (s)	0.86	0	0	0	0	0	1.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mobility reversal time (s)	25.5	47.29	46.21	60	60	47.93	20.93	13.07	16.29	19.07	42.07	0.79	13.21	18.21	29.5	2.21	4.29	2.29	37.36	0.43	25.14	32.14
Mobility idle time (s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mobility forward time (s)	34.5	12.71	13.79	0	0	3.71	9.71	22.07	0	40.93	17.93	22.64	10.21	41.79	30.5	28.79	5.79	0	5	11.93	17.21	13.79

TrackSummary

F

Turn Count / Duration * 60

=(B32/B33)*60

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Worm Data	Track Summary															
2	Sequence	20200316_Rep_N2_0001.avi															
3																	
4		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
5	Mean Worm Length (um)	674.26	686.52	732.8	720.4	718.74	788.12	862.28	722.57	743.37	703.52	716.65	664.35	627.96	679.29	781.25	641.31
6	Mean Width (um)	58.21	61.04	61.53	59.1	61.42	60.78	70.28	73.29	60.9	64.31	59.97	58.42	55.76	65.7	64	61.01
7	Mean Area (um^2)	39924.05	42604.36	45874.72	43307.35	44904.44	48731.65	61642.91	54192.86	46048.25	45902.93	43715.97	39474.1	35630.87	45201.95	50858.77	39645.85	41
8	Track Length (um) [Center point trajectory]	23794.35	11736.97	26822.62	28227.75	32378.65	23988.81	15957.33	12211.07	7806.49	26370.85	29161.31	9494.03	11274.16	27159.58	29200.59	12764.09	5
9	Center point Speed (um/s) [Center point trajectory/time]	397.05	195.85	447.58	471.02	540.29	465.16	521.97	348.18	481.46	440.04	486.6	406.47	482.69	453.2	487.26	412.7
10	Absolute Peristaltic Track Length (um) [forward + reversals]	14875.82	5508.27	17183.21	15938.67	21243.16	17197.74	10625.01	7215.72	5305.72	17114.4	18793.41	6043.12	7578.37	17615.92	17503.27	8893.55	3
11	Peristaltic Speed (um/s) [Absolute peristaltic track length/time]	248.23	91.91	286.73	265.96	354.47	333.47	347.55	205.74	327.23	285.58	313.6	258.73	324.46	293.95	292.07	287.55
12	Peristaltic Track Length (um) [forward - reversals]	6257.98	-3624.59	-13734.1	-15767.1	-21048.3	-13297.4	-7427.13	3110.29	-5267.94	7920.92	-11933.1	5882.6	-4039.66	8360.17	-8749.2	8089.91
13	Peristaltic Speed (um/s) [peristaltic track length/time]	104.42	-60.48	-229.17	-263.1	-351.22	-257.85	-242.94	88.68	-324.9	132.17	-199.12	251.85	-172.95	139.5	-145.99	261.57
14	Straight-line Distance (um)	2687.76	80.24	1822.21	2160.97	1346.03	6120.54	1371.07	1455.47	1116.95	419.03	4530.41	1208.91	217.43	2064.61	3224.61	647.04
15	Wavelength (um)	388.19	404.05	429.81	422.49	449.54	494.14	532.99	403.69	465.05	399.77	434.94	411.48	361.89	386.15	479.97	376.61
16	Mean Amplitude (um)	65.38	48.61	66.61	71.57	70.03	61.67	83.92	63.14	71.47	73.87	68.13	61.3	62.68	60.38	68.34	43.43
17	Max Amplitude (um)	131.1	117.79	153.45	132.01	131.96	140.6	182.18	162.88	129.18	180.05	150.18	118.8	124.33	144.95	147.3	133.98
18	Turn Count	183	69	207	196	183	154	90	78	60	158	209	74	82	184	205	102
19	Track duration (s)	59.93	59.93	59.93	59.93	59.93	51.57	30.57	35.07	16.21	59.93	59.93	23.36	23.36	59.93	59.93	30.93
20	Distance Traveled Forward(um)	10566.9	941.84	1724.58	85.77	97.44	1950.15	1598.94	5163.01	18.89	12517.66	3430.14	5962.86	1769.36	12988.04	4377.03	8491.73	2
21	Distance Traveled in Reverse(um)	4308.92	4566.43	15458.64	15852.89	21145.73	15247.59	9026.07	2052.72	5286.83	4596.74	15363.27	80.26	5809.01	4627.87	13126.23	401.82	1
22	Number of Reversals	7	17	2	1	1	3	1	5	1	4	4	0	1	5	2	0
23	Cumulative Reversal Time (s)	24.07	45	46.5	60	60	48	20.5	11.57	16.21	18.07	41.93	0	13.14	17.86	29.79	0
24	Cumulative Forward Time (s)	35.93	15	13.5	0	0	3.64	10.14	23.57	0.07	41.93	18.07	23.43	10.29	42.14	30.21	31
25	Number of Pirouette	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
26	Pirouette Time (s)	0.86	0	0	0	0	0	0	1.57	0	0	0	0	0	0	0	0
27	Mobility reversal time (s)	25.5	47.29	46.21	60	60	47.93	20.93	13.07	16.29	19.07	42.07	0.79	13.21	18.21	29.5	2.21
28	Mobility idle time (s)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	Mobility forward time (s)	34.5	12.71	13.79	0	0	3.71	9.71	22.07	0	40.93	17.93	22.64	10.21	41.79	30.5	28.79
30																	
31	Track #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
32	Turn Count	183	69	207	196	183	154	90	78	60	158	209	74	82	184	205	102
33	Track duration (s)	59.93	59.93	59.93	59.93	59.93	51.57	30.57	35.07	16.21	59.93	59.93	23.36	23.36	59.93	59.93	30.93
34	Turns Per Minute	=(B32/B33)*60															
35																	
36																	
37																	
38																	
39																	

TrackSummary

EDIT