WormBot, an open-source robotics platform for survival and behavior analysis in *C. elegans*

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Abstract *Caenorhabditis elegans* is a popular organism for aging research owing to its highly conserved molecular pathways, short lifespan, small size, and extensive genetic and reverse genetic resources. Here we describe the WormBot, an open-source robotic image capture platform capable of conducting 144 parallel *C. elegans* survival and behavioral phenotyping experiments. The WormBot uses standard 12-well tissue culture plates suitable for solid agar media and is built from commercially available robotics hardware. The WormBot is controlled by a web-based interface allowing control and monitoring of experiments from any internet connected device. The standard WormBot hardware features the ability to take both time-lapse bright field images and real-time video micrographs, allowing investigators to measure lifespan, as well as healthspan metrics as worms age. The open-source nature of the hardware and software will allow for users to extend the platform and implement new software and hardware features. This extensibility, coupled with the low cost and simplicity of the system, allows the auto-

mation of *C. elegans* survival analysis even in small laboratory settings with modest budgets.

Keywords *C. elegans* · Automated · Survival · Healthspan · Open-source

Introduction

Over the last 30 years, the roundworm *Caenorhabditis elegans* has provided key insights into the genetic control of longevity. Many of the genetic pathways now known to control aging were first identified in the nematode and then later determined to play a role in mammalian systems (Kenyon 2011; Uno and Nishida 2016; Bitto et al. 2015). Studies of aging in the worm have capitalized on their small size, short lifespan, exquisitely defined cell lineage, and large genetic tool-set to identify specific genetic factors and the tissues in which they operate to influence the rate of aging. Nearly all of this progress was made using manual lifespan collection techniques, in which investigators examined animals grown on solid media in petri dishes daily to determine if worms had died since the previous time point by prodding the worms with a stick (platinum wire) to elicit movement.

More recently, several automated approaches to performing survival analyses have been developed (Xian et al. 2013; Stroustrup et al. 2013; Puckering et al. 2017; Churgin et al. 2017). These typically rely on photographic monitoring of spontaneous nematode movement; however, methods based on vital dyes or disruption of intestinal permeability have also been

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reported (Gill et al. 2003; Rera et al. 2018). Generally, the imaging-based approaches fall into two categories. First, several microfluidic devices have been developed that allow worms to be kept in liquid media and assayed by light microscopy-based systems (Xian et al. 2013; Banse et al. 2019a; Saberi-Bosari et al. 2018). Microfluidic systems have some benefits over traditional solid culture. Through the placement of properly sized sieves, progeny can be flushed out of the observation chamber allowing for automated survival experiments in the absence of 5-fluorodeoxyuridine (FUDR), which, while traditionally used in nematode survival experiments, has been reported to affect worm physiology and have genetic background specific effects (Burnaevskiy et al. 2018; Van Raamsdonk and Hekimi 2011; Wang et al. 2019). In addition, microfluidic systems allow for more precise control over the environmental factors to which the animals are exposed. Although microfluidic systems can be powerful for longitudinal imaging, they also suffer from drawbacks that impair their utility for studying aging in nematodes. First, they require culturing animals in an environment they typically do not experience in nature, as *C. elegans* is not an aquatic species but instead live in soil and in rotting fruit (Schulenburg and Felix 2017). Upon exposure to liquid environments, worms activate multiple stress response pathways and exhibit a fleeing behavior often described as “thrashing” that may impact aging (Laranjeiro et al. 2019). This also means that it may be difficult to reconcile data generated from animals aged in a microfluidic system with the vast majority of prior literature in the field, which has been obtained from animals cultured on solid agar media and fed *E. coli* OP50 (Brenner 1974). Finally, microfluidic systems generally require specialized equipment and expertise that most *C. elegans* laboratories are not equipped with, limiting utility among the broader community.

The second category of higher-throughput lifespan devices involves time-lapse microphotography to record images of the worms at fixed time intervals throughout the lifespan of the worm on solid media. The most developed of these systems, the Lifespan Machine (LM), uses an array of modified flatbed scanners to repeatedly scan low profile petri dishes sealed onto the glass bed of the scanner with a rubber gasket (Stroustrup et al. 2013). This system has been implemented by a small number of external labs, including the three sites of the National Institute on Aging *C. elegans* Intervention Testing Program (Banse et al. 2019b). However, our own

attempts to automate survival analysis using the LM identified some issues which ultimately led us to develop an alternative system. First, the system relies on modified flatbed transparency film scanners for imaging. The scanners used in the initial publication are no longer manufactured, and while the designers of the LM have issued updates to support newer scanners, we were concerned that rapid changes in the digital photography world could affect the long-term viability of sourcing components for the LM. Second, the temporal resolution of the scanned images is suboptimal. While transparency scanners have very high spatial resolution, in a LM at normal capacity, they are only capable of capturing an image of a single experimental plate once an hour. Additionally, LM protocols call for a modified version of nematode growth media (reduced calcium) that differs from media used in previous aging studies in the worm. Furthermore, under normal operation, an LM scanner’s light source travels the length of the scanner bed four times an hour, taking between 10 and 12 min per scan on the original model of scanner. This results in long exposures to intense light which has been reported to have effects upon lifespan in the LM (Banse et al. 2019b). Finally, the physically closed format of the scanner, which presented thermal management challenges to the system’s designers (Stroustrup et al. 2013), also limits extension of the device to experiments that require the worms to be placed in alternative gas environments. We have previously reported that hypoxia can increase lifespan (Mehta et al. 2009; Leiser et al. 2013) and the limited gas diffusion of plates sealed on a scanner, as well as the inability to modify the atmosphere of the plates sealed to a flatbed scanner was intractable for our experimental needs.

Another automated lifespan system that allows for solid media culture of worms is the WormMotel (Churgin et al. 2017), which uses a high-resolution camera to monitor single worms arrayed into individual wells in custom PDMS plate format. This system has been coupled with a plate handling robot, and since it relies on a camera, it is able to record the individually housed animal’s movements throughout life to generate longitudinal measures. While the WormMotel system is elegant and powerful, we felt the in-house manufactured custom plates and picking/seeding individual worms into single wells would make it difficult to use for high-throughput studies such as drug screens. Additionally, the necessity of the plate handling robot to increase throughput would limit its utility for many labs.

Here, we describe the WormBot, a new robotic system for semi-automated lifespan analysis in *C. elegans* which addresses some of the shortcomings of other systems. The WormBot is built from consumer robotics hardware that allows for solid media culture and use of standard 12-well tissue culture plates to provide high-throughput automated survival analysis of *C. elegans*.

Methods

Hardware construction

A MakeBlock XY plotter robotics kit (MakeBlock.com, Shenzhen, China) was purchased from amazon.com and assembled according to the manufacturer's instructions until page 21 of the build manual (<https://github.com/Makeblock-official/XY-Plotter-2.0/blob/master/XY%20Plotter%20V2.0%20Assembly%20Instruction.pdf>). Following this step, assembly diverged and a full list of additional parts is available at <http://wormbot.org/bom.xls> and in the Supporting Online Materials. Full video build tutorials are available at <http://wormbot.org> and on <https://www.youtube.com/channel/UCcvtj864r6CXAUZOVe4vCSg>, and in Supporting Online Materials. Optional, additional 3d-printed components were printed using 1.75 mm PLA filament on a Monoprice MakerUltimate 3D printer (Monoprice, Rancho Cucamonga, CA). STL files are available at wormbot.org, thingiverse.com, and in Supporting Online Materials. A 24 × 24 × 3/8 inch acrylic panel was purchased from TAP plastics (tapplastics.com, Oakland, CA) and machined by Front Panel Express (frontpanelexpress.com, Seattle, WA). CAD files for machining are available at <http://wormbot.org/wormbot.fpd> and in Supporting Online Materials.

Software

All of the WormBot's code is open-source and available at Github (<http://github.com/JasonNPitt/wormbot>). The WormBot codebase is primarily written in C++ and should function on any POSIX operating system; however, all testing has been done using Ubuntu 16.04 (Canonical LTD, London, UK). Wormbot software relies on the OpenCV library (Kaehler and Bradski 2016) (opencv.org), the FFMPEG project (ffmpeg.org), the Arduino platform (D'Ausilio 2012) (arduino.cc), and the

Apache web server (Laurie and Laurie 2003) (<http://apache.org>). Details of other code dependencies are available via Github. Wormbot software can be installed by cloning the git repository (> git clone <http://github.com/JasonNPitt/wormbot>) followed by running the install script (> sudo ~/wormbot/INSTALL). More detailed software installation instructions are available at wormbot.org and in the supporting online information.

Media preparation and RNAi experiments

Twelve-well tissue culture plates were purchased from Genesee Scientific (cat no. 25-101, genesee.com, El Cajon, CA). Each well contained 3 mL of sterilized Nematode Growth Medium (wormbook.org) supplemented with 100 units/mL Nystatin to prevent fungal growth and a final concentration of 50 µM FUDR to prevent progeny development. For RNA interference (RNAi) experiments, media also contained 100 µg/mL of ampicillin, 10 µg/mL tetracycline and 2mM IPTG. For all experiments, worms were synchronized by 10-min hypochlorite treatment (1% sodium hypochlorite, 250 mM potassium hydroxide and water) followed by four washes in M9 buffer to isolate eggs, followed by an overnight hatch off in 6 mL of M9 in a 60-mm petri dish sealed with parafilm in a 20 °C incubator (Torrey Pines Scientific, Torrey Pines, CA). RNAi clones were taken from the Vidal and Ahringer RNAi feeding libraries (Rual et al. 2004; Kamath et al. 2001), sequence verified and kept as – 80 °C frozen stocks prior to use. dsRNA induction was performed by growing RNAi feeding strains overnight in media containing antibiotics, but without IPTG. The following morning, stationary phase cultures were diluted fourfold with fresh media containing 2 mM IPTG and grown for 4 h at 37 °C on a shaker. Following induction, cells were pelleted and resuspended in the original overnight culture volume (4× concentrate) and seeded onto RNAi NGM media. Following 20 µL of seeding with RNAi bacteria, 12-well plates were allowed to dry for 20–60 min with their lids off in a laminar flow hood to allow all of the seeding solution to evaporate. RNAi experiments began by placing synchronized L1 worms on 10 cm RNAi plates until they reached the L4 larval stage when they were either hand picked or liquid transferred (in M9) to the seeded 12-well RNAi plates containing FUDR. Throughout the experiment, plate humidity was maintained by filling the interstitial spaces of the 12-well plate with double-distilled water and refilling as necessary during the course of the experiment.

Toxin exposure

Potassium cyanide (KCN) was purchased from Acros Organics (ThermoFisher, Waltham, MA) and resuspended at 100 mM in 100 mM sodium hydroxide. Hydrogen cyanide (HCN) exposure was performed as described (Gallagher and Manoil 2001), with modifications for the 12-well plate format. Briefly, in a fume hood, 12-well plate lids were ringed with petroleum jelly from a syringe and a 100- μ L drop of the 100 mM KCN solution was placed in the interstitial space of the 12-well dish near a 100 μ L drop of 200 mM hydrochloric acid. The lid was sealed and the plate tipped to mix the two drops liberating HCN gas into the sealed chamber and placed immediately onto the robot. Strains used in this study were provided by the Caenorhabditis Genetics Center (Madison, WI) which is supported by the NIH Office of Research Infrastructure Programs (P40 OD010440).

Health span/behavior measures

The WormBot can record brief 30 frame/s movies each day for each of the 144 wells in an experiment. To assay worm movement in Fig. 6c, one well from each of the 12 plates was randomly selected and the WormBot application *plateExplorer* was used to analyze 1250 frames from the selected wells for all 20 days of the experiment. The resulting 240 *plateExplorer* output images were loaded into the Gnu image manipulation program (<http://www.gimp.org>) with the center portion of the well containing the worms masked off and the total number of blue pixels measured, divided by the total number of worms (green objects) observed, divided by the number of seconds of video used to produce the image. This mean pixel/worm/s value was then converted to μm^2 by photographing a micrometer with the WormBot to determine the actual pixel size.

Results

Hardware overview

The WormBot system (<http://wormbot.org>) is a benchtop gantry robot that moves a USB microscope camera over a transparent acrylic optical table to image wells containing agar and nematodes in standard tissue culture plates (Fig. 1a). The camera is coupled to a

transillumination arm carrying an LED, optical tube, and reflector that provides off axis illumination and high contrast bright-field imaging (Figure S1). The system supports any camera that provides Video4Linux driver support, but the current software release and data presented here are based on a 1080p (1920 $\times$ 1080 pixel) USB 2.0 web camera with an f/2 4.8-mm S-mount micro video lens that yields images with a pixel size of 24.4 microns. While the system can work with higher-resolution cameras (we have successfully used USB 3.0 cameras with resolutions as high as 4208 $\times$ 3120, Figure S4), the extra storage required for the images generated and decreased light sensitivity have led us to favor standard HD cameras, which provide adequate resolution to resolve movements in adult worms. Additionally, these 1080p cameras are affordable and widely available.

The main hardware of the WormBot system consists of an XY-plotter extruded aluminum robotics kit made by the company MakeBlock (MakeBlock, makeblock.com, Shenzhen, China). The WormBot system simply replaces the drawing head of the XY plotter robot with a camera and attaches a transillumination arm to the camera head, much like a standard compound microscope with a very long and very curved arm. Due to the size and mass distribution of this arm, a 3-kg lead diving weight is placed on the camera head to serve as a counter-balance and reduce torsion on the linear bearings affording free movement of the camera head and transillumination arm. Movement of the camera head is MXL belt driven and powered by two NEMA 17 stepper motors running in an open loop control configuration by a clone of the Arduino UNO microcontroller board and stepper motor control boards that are provided in the MakeBlock kit. The WormBot's aluminum frame is attached to a 3/8-inch-thick acrylic panel that has been drilled to provide mounting holes for the robot chassis, four extruded aluminum or 3d printed legs, and an array of 1/8-inch steel dowel pins. These dowel pins serve to lock in 12 standard microtiter plates into position on the optical table. If using 12-well plates, this allows the system to analyze 144 individual wells. While this acrylic plate could be drilled by hand on a milling machine, it can also be ordered from an online machine shop (Front Panel Express, Seattle, WA, see Supporting Online Information for CAD files for ordering or drilling the panel). For complete video construction tutorials and assembly documentation, see <http://wormbot.org>.

Fig. 1 **a** Top down diagram of the WormBot system. **a** Plates are held in a 3-by-4 grid by steel dowel pins on a clear acrylic optical table. The camera head and transilluminator arm's motion is powered by a pair of stepper motors that are controlled by a microcontroller board that is connected a Linux workstation via USB. **b** Software components of the WormBot system. A pair of daemons handles image acquisition and image alignment. User interaction is handled through web server based tools that can be accessed by any web browser

The WormBot system is connected via two USB cables (one for the camera and one for the robot) to a single Linux workstation. It is advisable to install multiple large hard

drives into this workstation as the 1080p cameras generate approximately 50 GB per day at full capacity while 4kUHD cameras generate up to 300 GB of data per day.

Software overview

The WormBot hardware communicates via USB to a set of software daemons on the workstation (Fig. 1b). The **controller** daemon coordinates movement of the robot hardware and captures the image data from the camera. The captured images are stored as PNG files and served to the **aligner** that uses an ECC image alignment algorithm (Evangelidis and Psarakis 2008) built into the OpenCV library (Kaehler and Bradski 2016) to align each time-point for a well to previous timepoints. This step is necessary as the spatial resolution of the robot kit stepper motors running in open loop configuration is limited; therefore, the images must be aligned to reduce frame to frame noise.

The WormBot is conceived to be used by an entire laboratory group; therefore, we designed the software's user interface to work inside of a web browser so that any internet connected device could be used to control the robot and analyze data on the system from any location. This also allows data to be shared easily between lab members and with collaborators. The workstation connected to the WormBot runs a standard Apache Web Server (The Apache Software Foundation, Wakefield, MA) that is installed and configured automatically when the WormBot software package is installed. Experiments are started and stopped on the WormBot using the **scheduler** application (Fig. 2a, /cgi-bin/scheduler). The **scheduler** page is broken into two parts. The upper red portion lists currently running experiments that can be individually stopped or clicked on to access the **marker** application (Fig. 2b). The lower green portion lists available plate slots on the WormBot and allows users to add new experiments to the robot's joblist. The WormBot performs two types of data collection that can be used simultaneously. *Time-lapse*: where the robot takes a single image of a well every 10 min, and *Daily-Monitor*: where once per day, the robot will position the camera over the well and take a 1–5-min video at 30 frames per second. We typically rely on time-lapse data for determining time of death and daily monitor videos for healthspan, behavior, or other movement metrics, but they could be used interchangeably. The **experimentbrowser** application (Fig. 2c, /cgi-bin/experimentbrowser) allows users to see all of the experiments that are currently stored on the server and to back them up or delete them.

In order to generate survival data, the WormBot system provides two different applications. **Marker** is

a cgi-based web application that can run on any internet connected device, like an iPad, smartphone, or web browser (Fig. 2d, cgi-bin/marker?loadedexpID=X). **Marker** allows the user to manually scroll through the stack of time-lapse images and click on a worm when it ceases all movements. Marking a death event produces a red circle which the **marker** application can also embed and export as a time-lapse movie of the entire experiment (see supporting online VideoS1). The application also lists basic experimental statistics and provides the data in an OASIS compatible format (Han et al. 2016) to be further analyzed. We have created another application, **retrograde** (Fig. 2e, /retrograde/retrograde.html), which attempts to automate the process of identifying the time of death, alleviating the need to manually annotate images. **Retrograde** is a JavaScript application that must be run on a web browser and controlled with a three-button mouse and provides all of the same basic manual scoring functionality as the **marker** application. For data to be fed into the **retrograde** prediction algorithm, the user goes to the end of the time-lapse image data and uses the mouse to drag boxes around each worm corpse. The user then initiates the application, and the software detects the point at which the objects in the boxes began to move and marks them as the death points. **Retrograde** relies on OpenCV's image thresholding and a Canny edge detection algorithm to detect worm-sized objects in the user-defined bounding boxes and determine when they have moved outside of the noise thresholds that are required due to time-point to time-point image variability.

In order to validate the ability of the WormBot to detect differences in *C. elegans* survival, we used RNAi to reduce expression of members of the well-described insulin/IGF signaling pathway (Kapahi et al. 2017; Finch and Ruvkun 2001) (Fig. 3). Specifically, we knocked down expression of the gene encoding the longevity promoting transcription factor DAF-16 or the gene encoding the insulin-like growth factor receptor DAF-2. Results from the WormBot were compared to similarly treated animals that were kept in identical 12-well plates stored next to the WormBot but were scored by hand using a worm pick and dissecting microscope. Both in empty vector controls and in worms treated *daf-16*(RNAi), results were not statistically different when scored by traditional methods or the WormBot **marker** application (Fig. 3). When scored by traditional methods or with the **marker** application, *daf-2*(RNAi) resulted in

Fig. 2 Web applications for the WormBot system. **a** The *scheduler* application allows users to start and stop experiments. **b** The *marker* application is designed for manual scoring of lifespan time-lapse data and generation of annotated time-lapse movie files. **c** *ExperimentBrowser* displays all of the experiments stored on the server and allows for data management. **d** The *retrograde* application allows for manual or automated lifespan scoring. Users mark the corpses (green rectangles) at the end of the time-lapse data, and the software automatically determines the death point (red circles) of the enclosed worm object

longer lifespan compared with EV controls, but *daf-2*(RNAi) animals were even longer lived when assayed on the WormBot (Fig. 3).

While the *marker* application yields results that are similar to traditional methods, it still requires a degree of manual user input in order to generate a survival curve.

The **retrograde** application attempts to remove much of this user effort by leveraging computer vision approaches to detect when worms cease movement. In all cases, the **retrograde** application was able to correctly detect the differences between the treatments (Fig. 3 bottom right); however, **retrograde** yielded absolute lifespan values that were significantly greater than those obtained by traditional hand scoring (Fig. 3, green curves).

Since the WormBot was able to detect changes in lifespan due to disruption of a canonical aging pathway, we next evaluated the variation in lifespan of wildtype animals across all of the wells of the robot in large number of individuals (Fig. 4a). Perhaps due to the open and symmetrical design of the WormBot, we find that when binning wells across all 12 plates, we detect no significant difference in mean lifespan in any of the 12 well positions (Fig. 4b, type I ANOVA, $F = 1.24$ $p = 0.27$). Average temperature variation between the WormBot wells and the surrounding environment was less than 0.04 °C with the system running 144 simultaneous experiments (Table S2).

One of the key features of robotic image acquisition is that it allows for finer temporal resolution survival data than is practical with human acquired datasets. This feature is useful when performing experiments with

short survival times on the order of hours, such as toxicity assays or stress resistance assays. From a practical drug or genetic screening standpoint, such assays can be powerful because the shorter timeframes afford the ability to greatly increase throughput. For example, resistance to high temperature stress has previously been shown to positively correlate with increased longevity, and exposure to 35 °C will kill wild-type worms within 10 h while the long lived *daf-2(e1370)* strain can survive up to 18 h (Gems et al. 1998).

In order to test the ability of the WormBot to perform short-term survival studies, we exposed *C. elegans* to the lethal toxin HCN. Previous work on the susceptibility of worms to the human pathogen *Pseudomonas aeruginosa* (PAO1) identified bacterial production of HCN as being responsible for the fast paralytic killing of the worms when exposed to the pathogen, and resistance screens identified the prolylhydroxylase EGL-9 as conferring resistance to lethal HCN exposure (Gallagher and Manoil 2001). Subsequent work showed this resistance was due to activation of the hypoxia-responsive transcription factor HIF-1 and its downstream targets (Budde and Roth 2010). We placed L4 larval stage worms, with loss of function mutations in either *egl-9* or *hif-1* into sealed 12-well plates in the presence of 270 µg of HCN and placed them onto the WormBot

Fig. 3 Variation in wild-type survival due to inhibition of insulin signaling by RNAi as analyzed by the Wormbot or by traditional methods. Top row and bottom row contain the same nine survival curves sorted by RNAi treatment (top) or by analysis type (bottom row). X axis is days post hatching. Wild-type worms were fed *E. coli* expressing double-stranded RNA for the insulin growth factor receptor DAF-2 or its downstream effector, the FOXO transcription factor DAF-16. All worms were cultured in 12-well

plates starting at the L4 larval stage. Plates were scored either by traditional hand scoring with a dissecting microscope (black curves on top row and bottom left panel), using the *marker* application from the WormBot system (magenta curves on top row and bottom middle panel), or with the automatic feature of the Retrograde application (green curves top row and bottom right panel). * indicates P value < 0.05 using Log-rank test. See Table S3

Fig. 4 Experimental variability in wild-type aging. **a** 142 wells of N2 grown on OP50 with curves from binned plates (Gray lines, see also Figure S2) shown along with mean of all wells (Black line). X axis is days post L4. Fractional day output was used and timepoints are separated by 10 min. See Table S1 for pairwise Log-

rank comparisons and Figure S3 for histogram. **b** Box and whisker plots of mean lifespan for individual well positions (red dots, A1, A2, etc.) were binned and mean lifespans for binned groups were found not to differ significantly (Table S3)

(Fig. 5). After 2000 min (200 time points), the data were analyzed. While only two *egl-9* animals died during the observed period, all of the *hif-1* animals were dead within 1300 min and 75% of wild-type animals were dead by 2000 min. These data show that the WormBot can provide high-resolution survival data for even extremely toxic and fast-acting compounds such as HCN.

While the maximum and mean lifespan are useful metrics to assess biological aging, the geroscience community has recently begun to favor the concept of healthspan as a useful metric to evaluate the effectiveness of anti-aging interventions (Fuellen et al. 2019). There is growing consensus that interventions that extend lifespan, but do not also reduce disease burden or

moribundity in animal models, are unlikely to be translationally relevant. While the health span concept presently lacks the same rigorous definition as lifespan, central to this concept is the ability to measure “health” metrics as the organism’s chronological age increases (Kaeberlein 2018). Health metrics that have been shown to decline during normative aging in *C. elegans* include crawling, feeding (pharynx pumping), reproduction, mechanosensation, and olfaction (Hahm et al. 2015; Russell et al. 2017; Bansal et al. 2015; Ewald et al. 2018; Pan et al. 2011; Leinwand et al. 2015; Rollins et al. 2017). When equipped with a high-resolution camera, the WormBot is able to detect laid embryos (Figure S4), but our standard low-resolution cameras

Fig. 5 High-resolution survival data in HCN. Wild-type (N2), *egl-9(sa307)*, and *hif-1(ia04)* worms were placed into a sealed 12-well plate with an acidified solution of KCN to liberate HCN gas. X axis is days post hatching. The WormBot's fractional day output was used to generate curves with a 10-min (0.007 day) temporal resolution starting from the time of HCN exposure at L4 (1-day-old animals). * indicates P value < 0.05 using Log-rank test

cannot resolve eggs unambiguously. However, spontaneous movement, as well as orientation to aversive or attractive odorants, could be quantified. In order to visualize the physical activity of animals, we used the *dailymonitor* feature of the WormBot software to acquire real-time video (30 frames/s) of the worms each day for the entire course of the experiment. These .avi video files can be used with any of the widely available worm tracking packages that have been previously described (Husson et al. 2013). Because implementing many of these trackers requires its own separate installation and batch processing procedures, we developed a simple command line application for the WormBot system called *plateExplorer* that processes all the *dailyMonitor* movies for an experiment and runs the same worm detection algorithms as the *retrograde* application to produce a graphical output of the paths worms take during the course of the recorded movies (Fig. 6a). These image files can then be mined with standard image processing applications (see “Materials and methods”) to quantify worm movement over the course of the lifespan experiment (Fig. 6b). As worms age, we find that their spontaneous movement peaks around days 2–3 and then declines (Fig. 6c), consistent with similar profiles observed in the WormMotel (Churgin et al. 2017).

Discussion

Here, we describe the WormBot, an open-source robotics system for high-throughput lifespan and behavioral phenotyping in *C. elegans*. We find that the automated lifespan scoring features of the WormBot software are able to differentiate between RNAi treatments known to

increase and decrease lifespan, and that human annotated WormBot data are comparable to those obtained from traditional manual lifespan methods, but at substantially reduced time and effort. In addition to quantifying mortality under standard conditions, we show that the WormBot can easily resolve survival differences under toxic conditions such as hydrogen cyanide exposure and can be used to quantify simple healthspan metrics such as motility.

We performed a direct comparison of data obtained on the WormBot to manual lifespan determination by traditional methods. In all cases, the WormBot data were comparable to manually obtained lifespan data and successfully discriminated short-lived (*daf-16*) and long-lived (*daf-2*) conditions from wild-type control. The fully automated *retrograde* survival analysis yielded survival curves with greater median and maximum values than manual annotation of the WormBot data or traditional hand scoring. We do not yet fully understand the reasons for this; however, because the relative differences in lifespan are maintained, we believe that the automated system is suitable for screening purposes. Because the WormBot data are stored as a series of image files, the data can always be re-analyzed using human annotation. The human annotated WormBot data yielded lifespan effects that were not significantly different from traditional manual methodology for both control and *daf-16(RNAi)* conditions, and slightly longer lifespan values for *daf-2(RNAi)*. We speculate that this may result from the damage caused by repeated manual prodding over the long lifespans of *daf-2* animals when the experiment is carried out using traditional methods. The WormBot design appears to be robust against position effects and thermal variation from well-to-well and plate-to-plate.

Fig. 6 Lifetime activity data. Plate exploration was scored using *dailyMonitor* movies from the N2 control data in Fig. 4. **a** Output of the WormBot application *plateExplorer* used to determine regions of the well worms explored during the recorded time frame. **b** Samples of zoomed in traces from days 1, 5, 10, and 15 of the same experimental well. Worm position at the start of the movie is marked in the green channel, the ending position in the red channel and all intervening frames (1250 in this analysis) summed into the blue channel. **c** Twelve wells were randomly sampled from each of the 12-plates (142 experiments) in Fig. 4 and each of their daily traces combined to generate the composite curve showing the average number of μm^2 explored by a worm per second as a function of age. See Supporting Online information for example *dailyMonitor* movies

A major unmet challenge of the current WormBot system, as well as other automated lifespan systems for *C. elegans*, is the inability to unambiguously identify the

precise time of death for each animal. While fully automated survival analysis is a goal we hope to achieve in the future, human curation of the time-lapse image data

currently provides survival curves that closely match traditional manual lifespan analysis. To do this, a person scores the time point immediately following the last observed spontaneous movement as a “death” point. While still requiring some human effort, we have found that this approach requires less than 20% of the time required for traditional manual lifespan experiments and can easily be performed by undergraduate students with significantly less training required.

While the current WormBot platform is geared largely toward survival analysis, it is a programmable robotic plate scanning microscope and as such could be used for any assay that could be scored with a low-power bright-field microscope. For example, a WormBot was recently built in order to study magnetotaxis in the worm (Andres Gadea-Vidal, pers commun) and, conceivably, any assay that involves determining the position of worms on a plate could be adapted to the WormBot. Obvious additional applications of the WormBot include pathogen sensitivity/resistance and chemosensory (avoidance/attractant) assays. The open-source nature of the WormBot software and the flexibility of the open-source hardware design will allow for future enhancements and upgrades of an already useful system. Thus, we anticipate that the WormBot has the potential to accelerate the pace of *C. elegans* research in a variety of contexts.

Finally, science is in the midst of a reproducibility crisis (Prinz et al. 2011; Baker 2016). The causes of this crisis are multifactorial, but range from simple statistical errors, like inadequate sample size, to outright data fabrication. Systems like the WormBot that automatically create archival time-stamped digital observations that can easily be blinded, shared, and reexamined are an important tool in addressing this crisis. In this aspect, automation itself is also beneficial, as it is designed to be carried out by machines and is therefore necessarily more highly controlled owing to the limitations of these mechanical systems. Automation also allows for larger sample sizes, decreased experimenter bias, and fewer human errors during data collection. All of these factors point to laboratory automation tools like the WormBot revolutionizing the scale, rigor, and scope of science in the future.

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Compliance with ethical standards

Conflict of interest JNP, BWB, and MK are shareholders of GeroTech, Inc., a company seeking to provide a commercial version of the WormBot.

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