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ARTICLE

A simplified design for the *C. elegans* lifespan machine

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Abbreviations used: CFM, cubic feet per minute; CITP, *Caenorhabditis* Intervention Testing Program; EMF, electromagnetic field; TPU, transparency unit

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ABSTRACT

Caenorhabditis elegans (*C. elegans*) lifespan assays constitute a broadly used approach for investigating the fundamental biology of longevity. Traditional *C. elegans* lifespan assays require labor-intensive microscopic monitoring of individual animals to evaluate life/death over a period of weeks, making large-scale high throughput studies impractical. The lifespan machine developed by Stroustrup *et al*. (2013) adapted flatbed scanner technologies to contribute a major technical advance in the efficiency of *C. elegans* survival assays. Introducing a platform in which large portions of a lifespan assay are automated enabled longevity studies of a scope not possible with previous exclusively manual assays and facilitated novel discovery. Still, as initially described, constructing and operating scanner-based lifespan machines requires considerable effort and expertise. Here we report on design modifications that simplify construction, decrease cost, eliminate certain mechanical failures, and decrease assay workload requirements. The modifications we document should make the lifespan machine more accessible to interested laboratories.

Keywords: *Caenorhabditis elegans*, lifespan machine, automation, aging, longevity, anti-aging interventions

INTRODUCTION

The short lifespan (~3 weeks), high fecundity, and low maintenance costs for whole-life studies of the 959-celled nematode *Caenorhabditis elegans* (*C. elegans*) constitute unique advantages for the investigation of genetic, environmental, pharmacological, epigenetic, and stochastic factors that influence longevity. In the lab, *C. elegans* are most commonly grown on solid agar culture plates where they subsist on a diet of abundant *E. coli*. A manually performed lifespan experiment typically involves determining animal viability in this plate environment at regular time intervals by using a light dissecting microscope to visualize movement, either unsolicited or induced by gentle prodding [1]. Animals must be individually transferred to fresh plates routinely to prevent starvation and in some cases to separate the aging population from their younger progeny. Throughput is thus limited by the considerable manual labor required to maintain and track worms.

Alternatives to the manual lifespan assay include microfluidic platforms [2], culture of individuals on agar plugs [3], and the lifespan machine [4,5]. In the lifespan machine, life/death discrimination is accomplished using a commercially available flatbed scanner instrument to scan *C. elegans* plate cultures that are placed onto the scanner bed. Images of plates are captured at regular intervals over the lifespan of the population, and a custom software package compares successive images to detect animal movement *via* changes in animal position. The time of death is called once an animal fails to exhibit movement for a few consecutive images. The lifespan machine is exceptionally scalable, potentially capturing the lifespans of tens of thousands of animals or more at a time, depending on the number of scanners used for an experiment [4-6]. Once an assay is set up, the animals require no further monitoring or maintenance until the end of the study is reached. The experimental design markedly reduces assay workload, making high throughput, large scale assays possible with *C. elegans*.

C. elegans lifespans are exquisitely sensitive to temperature [5] so a controlled temperature environment must be maintained on the scanners

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to obtain reliable data. The original instrument plan used Epson V700 flatbed scanners that were modified substantially to address potential heat buildup inside the plates [4]. More specifically, holes are cut in multiple places in the scanner body and desktop computer fans of various sizes are hung on the scanners using hot glue, twine and holes drilled into the scanner lid ([4] and see **Fig. 1A** and **1B**). These fans are connected to a computer power supply and must be run continuously for the entirety of each experiment to exhaust the heat generated by each scanner.

Figure 1. Lifespan machine designs. A. The original lifespan machine design, with pc fans connected to power supplies. **B.** The revised V700 lifespan machine design, with two cutouts (arrows) in the left side (i) and right side (ii). **C.** The V700 lifespan machine standard array. Computer power supplies (S) are wired to scanner fans. Some scanners (1) require additional fans to control temperature compared to others (2). **D.** New V700 lifespan machine array. Scanners are arranged on vertical racks with drawer slides for easy loading. Each box fan (B) covers two scanners and has an attached filter (F) to remove particulates from the airflow.

To image the nematodes, petri plates are inverted onto a glass slide with a rubber mat used to separate the plates from each other. The glass slide is then placed on the scanner bed. Because the glass slide and the inverted plate elevate the animals out of the focal plane of the scanner, the initial experimental set up requires full disassembly of the scanner to access and manually focus the lens. This step is accomplished by adjusting the lens by fractions of a millimeter, reassembling the scanner, and inspecting the focal plane *via* trial and error (details for this modification are well described in the original Life Machine paper) [4]. Modified scanners are then placed in an incubator to ensure static environmental temperature.

A point to note is that the glass and rubber mat initially described for plate assembly did not have precisely set positions on the scanner. Because each installation was a bit different, a custom image "mask" had to be generated for each scanner in each experiment using imaging software to identify the locations of the plates in the scanner bed. This image mask was used by the lifespan machine software to scan each plate domain at set intervals. Mask generation was not overly time consuming (~5 min) but time investment required for runs on multiple scanners could add up to hinder scalability.

In summary, despite the exceptional findings generated by Stroustrup and colleagues with the first generation scanner design [4,5], difficulties encountered with the original scanner set-up include: (1) complicated and time consuming construction; (2) a requirement for initialization of plate scan domains at run initiation; (3) fan failures during runs that could change experiment temperature; and (4) considerable space and expense requirements for the entire system.

Here we report on how we have streamlined scanner construction, addressed temperature regulation issues, decreased equipment costs, simplified the process of setting up and cleaning plates on the scanner bed, and improved reliability by eliminating some mechanical failure points. Our hope is that documenting these modifications to the scanner setup will increase the accessibility of the lifespan machine technology for the *C. elegans* community.

MATERIALS AND METHODS

Animal husbandry and scanner set up

We conducted all lifespan experiments using the *C. elegans* reference strain N2 [7] except those conducted at the University of Oregon, which used N2-PD1073. The N2-PD1073 strain is a clonal line derived from

the N2 strain VC2010, which was one of the strains used to generate a new *C. elegans* reference ("VC2010-1.0") genome [8]. We performed all animal husbandry at 20°C on NGM plates seeded with *E. coli* OP50-1 as a mono-cultured food source. We conducted survival assays as previously published for either manual or lifespan machine-assisted studies [6,9]. In brief, we grew synchronized cohorts of animals until day one of adulthood. We established replicate plates by picking 35–50 animals per plate, and transferred animals to fresh plates on days 1, 2, and 5 of adulthood. On day 5, we transferred animals to fresh scanner plates containing 100 mg/L Nystatin (to minimize fungal contamination), and positioned them in designated holes in the rubber mat for the rest of the lifetime. We initiated the experiment using the lifespan machine software [4]. All data are available as **File S1**.

Modifications to plate set up and scan initiation

To reproducibly position the experimental plates onto the scanner bed and limit time needed for initiation of scans, we made two significant adjustments. First, we designed a thin rubber mat into which we cut 16 plate-sized holes that reproducibly held plates in place for scanning. The mat is anchored to the scanner using the retention holes the scanner uses for film trays. This design ensures that the mat, and therefore the plates, maintains a static position in the scanner that is uniform from run to run. The benefit of this consistent positioning of plates is that the need for software mask generation that was previously required to start every scanner run is eliminated. Lifespan machine software can immediately recognize plate position in all runs after the first set up. After we established an optimal mat design, we arranged for their production by Xiamen Ruicheng Industrial Design Co, Ltd. (https://chinaruicheng.en.alibaba.com/) (part number RC001). Mats are reusable, we order one per scanner.

A second change from the original design is that we place air-tight closed plates directly onto the glass of the scanner bed itself, rather than placing "open" lidless plates onto holder glass slides. (Falcon Tight-Fit Lid Dishes, Corning Product number 351006; lids provide an airtight seal). The use of closed plates should eliminate the chance of contamination that might occur when sealing open plates to the glass slide, although other points of contamination, for example during the introduction of animals to the plate, are not different. Another advantage of this design is that the use of a glass sheet plate holder is eliminated, a modification that we found made efforts to focus scanners appropriately to plates significantly easier, and often no longer necessary.

Many plate models, including this one, contain a brand name imprinted on the base of the plate. This can be falsely detected by the worm detection software, and is resolved *via* storyboarding using the method outlined in the original Stroustrup paper to address this issue [4].

Lifespan machine scanner assembly

Most aspects of our lifespan machine design are identical to those previously described [4], with the structural modifications made to the image acquisition scanners and the scanner location in temperature-controlled rooms (as opposed to an incubator) as the major exceptions. We made no changes to any of the lifespan machine-associated software or to the computers used.

Lifespan machine construction

For the V700 redesign, we modified Epson V700 scanners by cutting four holes into the sides of the bottom main scanner unit and the top transparency unit (TPU) (**File S2**). First, we removed the screws attaching the scanner bed to the bottom unit, and placed gaffer's tape over the slit aperture of the scanning arm. We then gently pushed the scanning arm up to the back end of the scanner to protect it from shavings created by the cutting process. Contrary to the previous protocol [4], we found that a sufficiently powered Dremel tool (Dewalt DW 660) is able to cut through the sides of the scanners. Prior to cutting on the scanner itself, we first cut cardboard templates of the desired dimensions to ensure consistency of our construction among scanners.

We then cut holes into the main unit 2" in height and located about 0.75" below the top scanner bed surface. We cut holes into the TPU that were 1.5" in height and were located at the top of the unit. The locations of the holes are shown in **File S2** and are compared to the holes of the original design in **File S2**. After cutting, we removed the tape over the aperture slit, and vacuumed the plastic shavings out of the scanner interior before replacing the scanner bed. We then adjusted scanner cameras to ensure a proper focal plane for monitoring worms on plates as previously described [4].

We did not make modifications of any kind to most of the Epson V800 scanners. We found that most V800s scanner can be removed from the shipping box and directly set up for scanning with the addition of a box fax. In this implementation, it is important to note that the Epson scanners have two lenses, a document lens and a slide lens. The slide lens is not adjustable, but the focal plane is slightly above the glass surface, enabling unadjusted usage. If focal adjustment is needed, usage of the document lens is necessary. If the slide lens is used, the time from opening the scanner box to scanning capability is on the order of one hour. The ease of V800 scanner set-up removes one impediment to lifespan machine use in the lab. Still, we caution that we found one lot of scanners for which focus adjustments were still required (instructions in Stroustrup *et al*. [4]).

A rack for stacking scanner units for ease of fan cooling and space conservation

To house multiple units in a relatively small space and to facilitate group fan cooling, we assembled shelving racks using slotted angle steel to form a frame, and mounted retractable MDF (Medium Density Fiberboard) shelves using drawer slides.

We found that 6 scanners can be housed on one rack with adequate room beneath the scanners to allow air circulation and between the scanners if we allowed a total height of 7'/scanner rack and 10" spacing between shelves. Cutout holes in the rack enable USB hubs, scanner power adapters, USB and power cables, and other necessary equipment to be easily ziptied to the shelves, reducing clutter and potential heat buildup from wiring. We attached box fans (Lasko model 3733) to the side of the shelves using zipties, which blew towards the scanners. We ziptied air filters (Honeywell MERV 11, 20" × 20" × 1") onto the intake side of the fans to keep dust from blowing onto the scanners.

Electromagnetic field measurements

We measured electromagnetic fields using a custom fabricated 1" × 1" × 0.063" aluminum antenna that fit inside a standard lifespan machine assay plate. The antenna was mounted on the agar surface of an NGM plate, which would normally house the nematodes during an experiment, which was located in the standard lifespan machine assay locations on the scanner. We initiated scans and monitored the signal using a Siglent SDS1202X-E oscilloscope. The electromagnetic field (EMF) waveform

followed during the scan. Using custom code (**File S3**), recording the max amplitude at a sample rate of 1 hz, we made measurements at all 16 plate positions in triplicate, for each of three scanners of each type (V700 and V800).

Light source measurements

We made light measurements with a USB4000 from Ocean Optics, Inc., using the manufacturer's supplied software, fiber optic cable, and cosine corrector. We made three measurements for each of three scanners of each type (V700 and V800; **File S1**). In the filtered light experiments we used 20" × 24" Roscolux Supergel R10 medium yellow filter sheets that were cut to size and affixed to the upper transparency unit which houses the light source for transillumination as previously published [6].

Temperature measurements

To make temperature measurements on the scanners we constructed custom 16-channel temperature recorders using OCR model 10k-5 temperature probes that operate within a measurement range of −20°C to 105°C. The probes have a 25 mm × 5 mm waterproof head that was mounted in the same petri dishes used for lifespan machine experiments. The schematics for a printable PCB board, noted with the necessary electrical components (*e.g.*, resistors and capacitors) and the necessary software for running the devices were previously published [10]. The thermistors were calibrated in-house using a general three-temperature reference point approach. The three temperatures used were approximately 0°C, 23°C, and 37°C. The temperatures used were selected to cover the spread of reasonable temperatures for *C. elegans* experimentation. For each temperature reference measurement, an insulated, ~2 gallon bucket of water was brought to the desired temperature. We chose to use an insulated bucket with at least 2 gallons of water to reduce thermal fluctuations in the water and to provide adequate thermal mass with the goal of maximizing the calibration accuracy. Each water bath was given enough time to equilibrate to the environment and reach the desired temperature before calibration was performed. For each temperature measurement, the thermistors, attached to the temperature recorder PCB and Raspberry Pi, were submersed in the prepared bucket of water, along with a certified reference digital thermometer probe that had been commercially calibrated. The digital thermometer was used to record the actual temperature of the water bath for reference in the thermistor calibrations. Once the thermistors had equilibrated with the temperature of the water bath, a Python script was run on the Raspberry Pi to sample the thermistor values 50 times per thermistor, at a sample rate of approximately 200 Hz. The average of the 50 samples was then used for the final resistance value for that thermistor at that temperature. The averaging was done to reduce the influence of random electrical noise and environmental EMF interference, and to attempt to maximize the measurement accuracy. Once the resistance value of each thermistor at each temperature was measured, we used the measured resistance-temperature pairs for each thermistor using an online calculator (https://www.thinksrs.com/downloads/programs/therm%20calc/ntccalibrator/ntccalculator.html) to calculate the Steinhart-Hart model coefficients for converting resistance measurements from each thermistor to temperature measurements. All probes for the temperature recorders were cross validated, both across the 16 probes, as well as between 6 different devices that were constructed simultaneously to make sure that measurement differences were less than 0.1 °C. After validation, the devices were delivered to the three CITP sites.

RESULTS

We are participating partners in the *Caenorhabditis* Intervention Testing Program (CITP), a collaborative effort in which labs at three distinct sites (University of Oregon, Rutgers University, and the Buck Institute on Aging Research) reproduce studies of compounds hypothesized to extend lifespan in a genetically diverse test set of *Caenorhabditis* species [6,11-14]. The CITP project emphasizes standardization of lifespan assays to generate reproducible survival analyses across research sites [15]. This project initially focused on manual lifespan analysis [11-13], but, in an effort to reduce the extensive labor needed for manual survival analyses by shifting more toward automation, the CITP labs set out to increase the throughput of the program by adopting the lifespan machine technology to automate lifespan analysis [6]. Here we describe our efforts to simplify assembly and use of the lifespan machine as originally published [4].

Streamlining construction of scanner units

In the original lifespan machine design, open experimental culture plates are sealed to a glass plate using a rubber mat; this assembly is then placed on the scanner bed. To simplify the initial positioning of the experimental plates onto the scanner bed, we made two significant adjustments. First, we designed a thin rubber mat with 16 holes that could house plates, which we place directly onto the glass of the scanner bed itself (**Fig. S1A**). The mat is anchored to the scanner using the retention holes the scanner uses for film trays. This design ensures that the mat maintains a static position in the scanner that is uniform from run to run and eliminates the need for software mask generation that was previously required at the start of every scanner run. The elimination of the mask set up saved about 5 min per scanner experiment initiation, a time saving that adds up when using multiple scanners. Second, rather than place inverted "open" (lidless) agar plates onto glass plates, we placed sealed, air-tight petri-plates directly on the scanner bed into the mat holes we described above. Although we did not conduct a quantitative comparison, we note that closed plates seemed to be contaminated less frequently (possibly by decreasing the chance of contamination that might occur when sealing open plates to the glass slide), and better maintained plate hydration during survival assays.

Another advantage of our alternate plate setup (direct positioning of plates onto the scanner bed rather than using a glass plate) is that the scanner focal plane remains quite close to that intended by the manufacturer for scanning purposes. We find that for many of the V800 scanners we purchased, the focal plane of the scanner did not need to be adjusted to get sharp images of animals on the scanner (this was true for 3 of 4 V800 scanner lots that we purchased, so the focus still needs to be checked and possibly adjusted according to previously published instructions [4]). The direct positioning design greatly accelerates the time it takes to set up a scanner for *C. elegans* imaging, eliminating the disassembly of the scanner for trial-and-error refocusing of the camera that was previously required. Overall, the modified setup plan is fast and simple since time required for scanner disassembly and focus adjustment is eliminated.

An alternative cooling system

We sought to increase the ease of scanner construction while verifying that temperature control would be maintained. The original lifespan machine design features small fans mounted onto the scanner

chassis by cutting holes into the casing; power cords are needed for each fan ([4] and see **Fig. 1A-1C**). We found that the gluing of fans directly to the scanner required considerable manual labor and risked permanently damaging an expensive piece of equipment. In our Epson V700 redesign, we eliminated the fans embedded in the scanner, replacing the fans with a single large box fan positioned on the side of a scanner stack (**Fig. 1D**; we refer to this as V700 + box fan). We made four rectangular large cutouts in the chassis of the scanner through which fan-blown air could circulate (**Fig. 1B**, see also **File S2**). We added MERV 11 air filters on the box fan inlets to reduce airborne dust that might pass over the scanner optics, and to reduce potential dust accumulation inside the equipment. Note that in the original lifespan machine design, air is unfiltered.

The box fans have higher cubic feet per minute (2000 CFM) ratings compared to the smaller computer fans (< 150 CFM), so we anticipated that their use should offer improved airflow over the heat-generating components of the scanners. A key point is that no circular cuts need to be introduced in the chassis, and there is no fan gluing or scanner disassembly required with this alternate fan configuration. Another advantage of the alternative air flow design is that the need for separate power supplies is eliminated, decreasing assembly time, cost, and a bulky feature of the initial lifespan machine scanner set up. A final point is that in the original design, we found that the small fans were a common point of failure because they would break down or disconnect frequently, creating local environmental changes that necessitated dropping the data from that particular scanner. The larger box fans introduce a more reliable air circulation alternative.

To ensure that the redesigned lifespan machine maintained thermal characteristics of the original lifespan machine, we captured thermal images using a "FLIR One Pro" thermal infrared camera (**Fig. 2A**). We found that the redesigned V700 + box fan scanners had smaller thermal gradients as compared to the original V700 lifespan machine design, likely due to the higher airflow of the box fans used. Additionally, we detected minor temperature differences among scanners, likely due to either differences in scanner location or variation in the individual scanners themselves. We were able to fine-tune the temperature by adjusting the fan speed, adding additional filters, or taping over portions of the scanner cutouts. Using these approaches, we achieved variations among scanners of less than 1°C.

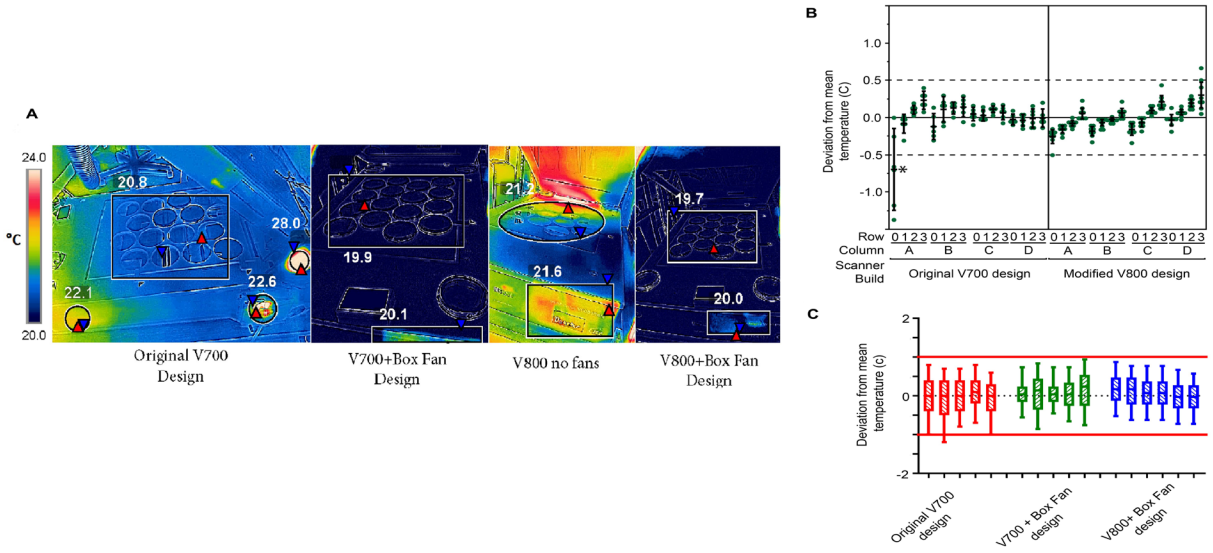

Figure 2. Thermal comparisons of lifespan machine installations. A. Thermal profile of lifespan machine designs used at Rutgers using a FLIR One Pro thermal camera. The original lifespan machine design, the revised V700 lifespan machine design, an unmodified V800 without cooling, and an uncut V800 lifespan machine with box fan are shown. Images were captured after scanners had been running continuously for 2 d at 15 min intervals. Temperature increases from the baseline blue color to green, then yellow, and finally red. Triangles indicate the warmest (red) and coldest (blue) points in each outlined area. Numbers indicate the average temperature for the outlined area. The red hotspots in the original design are due to the fans embedded in the scanner chassis, and the scanner bar is the cause of the heat visible on the left side of this image. The target area is the rough location of plates on the scanner or a hotspot. All three scanner designs met our target criteria of 20 ± 1°C. Ambient room temperature is ~19.5°C. The unmodified V800 scanner without a fan has a thermal variance of ± 1.5°C across the scanner bed and does not meet our criteria. **B.** A comparison of the temperature variation across the scanner bed from the lifespan machine designs used at the Oregon site. Recordings were made using a previously published 16-channel temperature recorder [10], which had been modified so that the temperature probes were embedded in mock plates mounted in the same positions as a typical lifespan machine experiment. The 16 positions show little deviation from the scanner average except position A0, marked with an *, on the original V700-based design. Those deviations proved to be artifactual due to electrical interference (see the result section). **C.** Temperature deviations among different scanner designs. Five to six temperature probes were spread across the scanner area for each of the 3 different scanner designs. The probes were set to record temperature at one-minute intervals over three days. Each scanner was set to scan continuously every fifteen minutes in a 20°C environment. The original V700 design probes are listed in red. The redesigned V700 probes are green. The V800 measurements are in blue.

Thermal stability over time is critical for accurate lifespan results. To test whether the temperature on the scanners is stable over time, we placed temperature probes on each scanner design and monitored the temperature in one-minute intervals. To detect potential local variations on the scanners (for example, plates closest to the light source resting spot between scans) we distributed probes evenly throughout the scanner bed. The target goal was to maintain temperature deviations on average less than 1°C across the scanner bed. On average, all lifespan machine designs easily achieved the target temperature stability (**Fig. 2B** and **2C**) and were slightly more stable on average compared to the original lifespan machine design.

V800 scanners feature LED lighting that offers advantages over the initial lifespan machine design

During the period in which we were implementing the scanner technologies into our lab, Epson redesigned scanners as the V800 model, a major feature of which is the use of an LED light source. We therefore set out to characterize the potential differences between the V700 and V800 scanner types, and to determine the optimal design requirements for the newer V800 scanners.

Heat generation is less evident in the V800s

The first question we addressed was the nature of the relative heat produced by the V700 *vs*. V800 scanners. We find that the V800 LED light source generates a more uniform heat distribution than the V700 + box fan fluorescent light source when used with our box fan racks (**Fig. 2A-2C**). We therefore used two alternative simplified lifespan machine designs. At Rutgers, it was determined that no cutouts or disassembly are necessary for the V800 model scanners (for full assembly instructions see **File S4**). A single box fan was sufficient to keep the scanners near ambient temperature. At Oregon box fans would not fit our lab space. We used a simplified design in which the number of cutouts and fans were reduced by half (for full assembly instructions see **File S5**).

V700s produce electromagnetic fields

Our characterization of the temperature differences between the LED and fluorescent scanners revealed transient temperature drops recorded on the V700's that were not present during scans on the V800's (see position A0 noted with * in **Fig. 2B**). After instituting measures with alternative temperature probes to rule out probe-generated effects, we determined that the fluorescent bulb scanners emit electrical fields that interfered with our temperature probes, presumably *via* induction of current in the wires connecting the probes to the recorder. The induced current would give transient false measurements of resistance for the temperature probes. As such, we directly tested for the presence of electrical fields generated by the scanners. We find found that the inverter transformer that drives the fluorescent bulb generates a ~41 kHz EMF, with an observed signal strength as strong as ~9 volts when using a 1" × 1" × 0.063" aluminum antenna (**Fig. 3**). Because the inverter transformer is a point source mounted on the light bar carriage that moves with each scan (**Fig. 3A**), the observed field strength varies with position across the scan bed and with each scan (**Fig. 3B-3D**). In contrast, we were unable to detect any EMF above background in the V800 model scanner, suggesting another advantage of the V800 scanners.

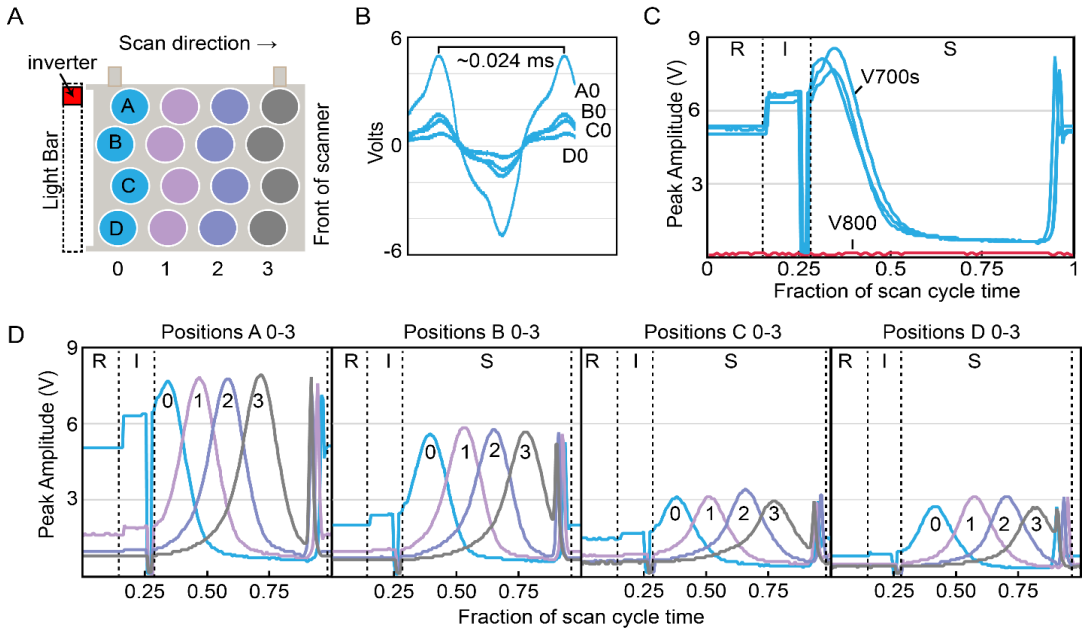

Figure 3. Worms grown on fluorescent bulb V700 based lifespan machines but not the LED V800 based lifespan machines experience a ~41 kHz electrical field. A. Plates in a standard lifespan machine are arrayed in 4 columns (from left to right on the scanner) and 4 rows (from back to front). The EMF signal is most strongly measured near the inverter transformer (arrow) which is housed on the edge of the scanner corresponding to column (A). **B.** Measurement of the EMF using an aluminum antenna (1" x 1" x 0.063") placed in the plane of the agar surface where the worms reside reveals a ~41 kHz EMF. **C.** Recordings of the EMF from the A0 position during three test scans from three scanners (V700s - blue, V800 -red) show a tightly reproducible measure between scan signal and strong EMF on the V700s, and virtually no detectible signal on the V800. **D.** Recordings of the EMF from each of the four rows (shown by color) in each of the columns during a scan run for a representative V700 scanner. The transitions between the scan phases (R, rest; I, initiation; S, scan) are shown by the vertical dashed lines.

Light output of the V700 and V800 is comparable

The CITP previously reported that the lifespans measured by the V700-based lifespan machines are shorter than comparable manual lifespans [6]. We speculated that the additional light exposure of animals grown on the scanners may underlie this difference, since visible light is known to shorten lifespan [16] and we previously found that filtering shorter wavelengths of light on the scanner can extend the lifespan of animals grown on the lifespan machine [6]. For this reason, we also characterized and compared the light output of the V700 and V800 scanner types, with a key question being how much potentially stressful UV light might be produced and controlled in the V800 design.

We find that the original fluorescent bulb V700 lifespan machine scanners have a broad emission in the visible spectrum, with two particularly strong peaks at ~440 nm and ~550 nm (**Fig. 4A**). Additionally, the spectral properties were relatively consistent across all tested scanners despite variable ages of the lights and the amount of cumulative usage. Notably, we did not observe any peaks in the UV region of the spectrum for the V700s (see **Fig. S2** and **File S1**).

We also characterized light in the presence of agar plates that are used for the scanner experiments. We determined that neither the petri plates, nor the agar in the plates, selectively absorbed visible light of particular wavelengths (**Fig. 4B**).

We previously demonstrated that the shorter wavelengths of light emitted by the fluorescent scanners can profoundly alter the effects of some compounds on longevity and that adding short wavelength filters to the scanner can eliminate phototoxicity [6]. We therefore compared the effect of filtering the light on the V700 fluorescent bulb scanners *vs*. the V800 LED based scanners (**Fig. 4C** and **4D**). We find that filters are equally effective in reducing targeted wavelengths of light in both the V700 and V800 scanners.

In sum, our characterization of light exposure in the V700 and V800 designs reveals that the scanner types do not introduce much UV light, that distributions of wavelength exposures differ somewhat between the V700 and the V800, that scanner light properties tend to be maintained after heavy use, and that light filtering is efficacious for both models.

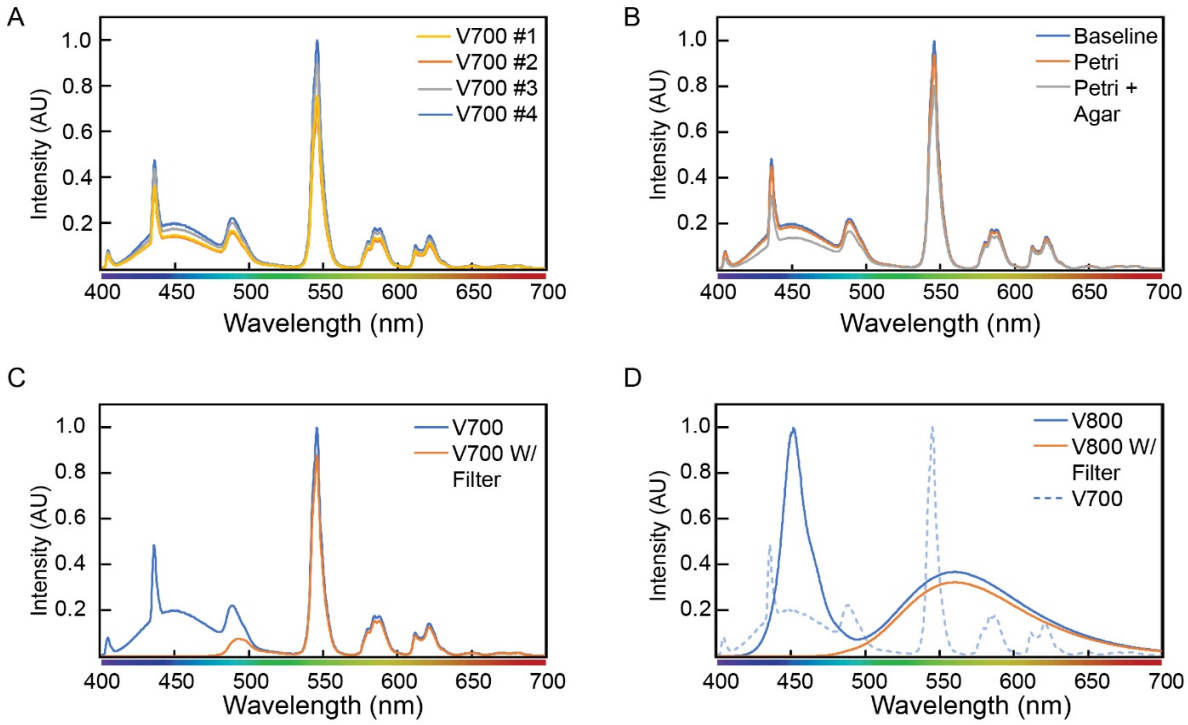

Figure 4. V700 *vs*. V800 light output. **A.** Comparison of four V700s of varying ages shows that while there may be variability in bulb intensity, the distribution across the spectrum remains consistent. **B.** The plastic plate and the agar absorb some of the light, but the absorption is not wavelength specific, and instead the light is dimmed across the entire spectrum. **C.** The filters previously used to filter the blue end of the visible spectrum worked on the V700s as we expected, with most of the blue light eliminated. **D.** The V800s have a strong peak on the blue end of the spectrum that is effectively removed by the filter.

Comparing lifespan outcomes in the V700 and V800 lifespan machines

Given minor differences we measured between V700 and V800 scanners, we compared *C. elegans* lifespans determined using the different scanner variations (**Fig. 5**). We found that lifespans measured on the redesigned V700 and V800 scanners exhibited a slight increase in survival curves as compared to the original design, a change that may be attributed to improved heat dissipation. The increase in lifespan in the new designs was one day or less (**Fig. 5**). We conclude that the changes in construction of lifespan machine units and in their light sources maintain the well documented capacity of the lifespan machines to generate longevity data [4,5], while greatly increasing the simplicity of lifespan machine technology set-up in the lab. In our hands, modest survival increases with modified units move outcomes more directly comparable to what CITP has determined for survival on traditional agar plates we reported [6,11].

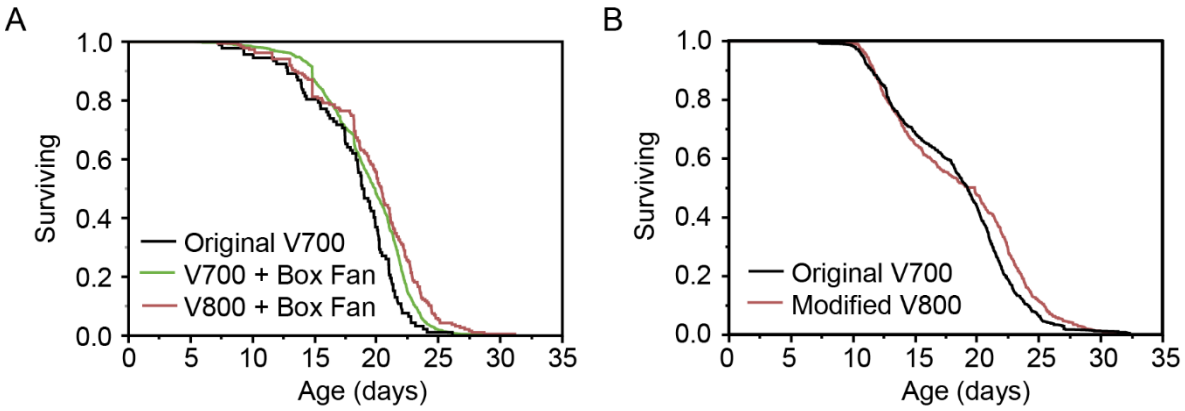

Figure 5. Automated lifespan measurements. A. Lifespan curves (shown in days) generated at the Rutgers site for *C. elegans* strain N2 using the original V700 lifespan machine (mean 18.2, *N* = 92), the V700 + Box Fan lifespan machine (mean 19.3, *N* = 1143), and V800 + Box Fan lifespan machine (mean 19.6, *N* = 187) designs. Log rank probability of difference among designs at Rutgers is 0.0293. **B.** Lifespan curves generated at the Oregon site for *C. elegans* strain N2-PD1073 using the original V700 lifespan machine (mean 18.3, *N* = 662) and Modified V800 lifespan machine (mean 18.6, *N* = 628) designs. Log rank probability of difference between designs at Oregon is 0.0038.

DISCUSSION

C. elegans is a premier model for longevity and aging studies as this animal lives for only a few weeks and is highly amenable to genetic, molecular, and pharmacological manipulation. A bottleneck in *C. elegans* longevity studies is the labor time devoted to manual survival assays conducted over the adult life of each individual in a population.

The lifespan machine made *C. elegans* high throughput lifespan assays conducted on standard agar plates feasible [7]. The machines eliminate a large amount of the tedium and personnel expense of one of the most important assays in longevity research. Indeed, we have adopted lifespan machine technology for coordinated efforts to identify compounds that robustly influence lifespan of genetically diverse *Caenorhabditis* strains [9]. However, the "start-up" investment for use of lifespan machines impressed us as a potential barrier to wide-spread deployment of the original lifespan machine designs. Lifespan machine construction (which included cutting holes and mounting fans on units) was particularly daunting when we considered setting up large numbers of units. The modifications we describe here make the instruments significantly easier to construct and to cool; in addition, the initial set up time for a lifespan experiment is shortened (an advantage most evident when multiple scanner runs are set up at the same time). In the case of the V800, the scanners can often be used as delivered by the manufacturer, with no construction adaptations required. The updated scanners also require less cooling equipment, decreasing incidents of experiment loss previously associated with fan failures. Our estimate for the cost of fans/unit is $18 per box fan/2 units *vs*. $67 per fan cooling equipment per scanner estimated in Stroustrup *et al*. [4] The plate holder adjustment improves throughput and set-up ease; and our impression is that the use of sealed culture plates lowers plate contamination incidence.

Importantly, the design modifications of the scanners and the availability of the next-generation V800 unit has made scanner technology easier to construct without impacting scanner functionality. We have documented differences in light exposure and electromagnetic field exposure between V700 and V800 designs, but because we find that longevity outcomes are similar with the units, the implementation of design shortcuts do not impair the functionality of the scanner units.

We do note that the V800 LED light sources feature light exposure that is somewhat different from the V700 fluorescent light sources, but survival data support this is not an impactful difference. We show that filters for low wavelength light can be added to the scanner bed to limit UV exposure, which can be important when controlling for photo-lability of added compounds or addressing light sensitivity of particular strains.

Overall, we hope that simplified construction and operation of the lifespan machine that we describe, as well as implementation of updated LED-based scanner technology, will enable wider adoption of a powerful tool for investigating the determinants of aging and longevity.

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Supplementary information

File S1. Data.

File S2. SOP for V700 + Box fan lifespan machine.

File S3. Code for electromagnetic field measurements.

File S4. SOP for V800 + Box fan lifespan machine.

File S5. SOP for modified V800 lifespan machine.

Figure S1. Schematic of custom designed plate holder, which anchors plates over the scanner bed.

Figure S2. V700 *vs*. V800 light output.

Supplementary information of this article can be found online at http://www.jbmethods.org/jbm/rt/suppFiles/332.