HHS Public Access

Author manuscript

Transl Med Aging. Author manuscript; available in PMC 2019 September 11.

Published in final edited form as:

Transl Med Aging. 2019 ; 3: 52–56. doi:10.1016/j.tma.2019.05.001.

An inexpensive microscopy system for microfluidic studies in budding yeast

Kenneth L. Chen[a,b,c], **Toby N. Ven**[a], **Matthew M. Crane**[a], **Dexter E. Chen**[a], **Yen-Chi Feng**[a], **Nozomi Suzuki**[a], **Adam E. Russell**[a], **Diogo de Moraes**[a], **Matt Kaeberlein**[a,b,*]

[a]Department of Pathology, School of Medicine, University of Washington, Seattle, WA, USA

[b]Department of Genome Sciences, School of Medicine, University of Washington, Seattle, WA, USA

[c]Medical Scientist Training Program, School of Medicine, University of Washington, Seattle, WA, USA

Abstract

Recently, microfluidic technologies have been developed to allow higher throughput collection of yeast replicative lifespan data. Adoption of these devices has been limited, in part, due to the high cost of the motorized microscopy instrumentation from mainline manufacturers. Inspired by recent development of open source microscopy hardware and software, we developed minimal-cost hardware attachments to provide long-term focus stabilization for lower-cost microscopes and open source software to manage concurrent time-lapse image acquisition from multiple microscopes. We hope that these tools will help spur the wider adoption of microfluidic technologies for the study of aging in yeast.

Keywords

Yeast; Microfluidics; Microscopy; Replicative aging; Microdissection; Lifespan; 3D printing; Arduino

1. Introduction

The finite replicative lifespan of budding yeast cells was first reported by Mortimer and Johnson in 1959 [1]. *S. cerevisiae* cells divide asymmetrically, segregating age-accumulated damage and producing a smaller, rejuvenated daughter cell [2]. Mortimer and Johnson monitored individual mother cells grown on an agar pad. To maintain observation of the

*Corresponding author. Department of Pathology, School of Medicine, University of Washington, Seattle, WA, USA. kaeber@uw.edu (M. Kaeberlein).

Conflict of interest

The authors declare that they have no Conflict of Interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tma.2019.05.001.

original cells, they manually removed mature daughter cells using a micromanipulator, preventing the accumulation of an exponentially growing population.

Since then, countless researchers have exploited the scientific convenience and minimal husbandry costs of the budding yeast to discover multiple conserved longevity pathways [3–5]. The gold standard for the collection of yeast replicative lifespan data remains Mortimer and Johnson's microdissection technique. Unfortunately, this method is a time- and labor-intensive task. Consequently, insufficient sample sizes are common in replicative lifespan studies, and many labs avoid performing replicative aging experiments. There is only one genome-wide replicative lifespan study to date [6], which was only made feasible by utilizing a strategy that initially filtered the deletion collection using lifespan analysis of only 5 cells per deletion strain [7,8]. In some ways, the microdissection assay is a bottleneck that limits progress of aging research in the budding yeast.

In the last few years, several microfluidic devices have been developed to automate the microdissection process [9–16]. These devices generally feature structures that utilize the size difference between mother and daughter cells or the unipolar budding patterns of haploid yeast cells to mechanically trap mother cells. Use of these devices allows advantages over traditional microdissection—fluid flow automates the removal of progeny replacing hours of manual labor, the tiny size of the device traps allows for the concurrent monitoring of hundreds of cells, and the continuous nature of the progeny removal shortens the data collection time from several weeks to several days. These devices have already been used to make important discoveries about aging biology [17–22]. However, utilization has been limited to only a handful of laboratories, most of which are the developers of the original devices or close collaborators at the same institution.

One reason for the limited use of microfluidic devices among the yeast research community is the expertise and clean-room equipment needed to fabricate the molds used to produce these devices. Once molds have been fabricated, however, generation of devices from those molds is both facile and affordable [23]. Another reason is the cost of microscopy. Studies utilizing microfluidic devices for yeast aging have generally used expensive motorized inverted microscopes from the traditionally well-known manufacturers [9,17,20,24]. Costing tens of thousands of dollars, they are often outside the budget of many labs interested conducting regular yeast aging studies.

Recently, several open-source designs have been published for microscopy hardware and software. These resources include designs for highly sophisticated light-sheet [25] and two-photon [26] microscopes, motorized accessories [27], cell-phone extension [28–30], and a design with production costs below $1 [31]. Inspired by these advances, we developed simple hardware attachments and open-source software that facilitates the facile and reliable collection of brightfield images for yeast replicative lifespan data on consumer-grade microscopes with a total system cost of less than $1000 (Fig. 1).

2. System design

Our goal was to develop a low-cost, light microscopy system to reliably measure yeast replicative lifespan using a microfluidic device. In order to measure lifespan in this way, it is necessary to acquire multi-day time-lapses comprised of high-resolution brightfield images of yeast mother cells (roughly 3–10 um in diameter) aging in a microfluidic device. Replicative lifespan can then be quantified by counting the number of daughter cells that are produced by each trapped mother cell throughout the course of the experiment.

A central challenge in long term microscopy imaging experiments is maintaining the sample in focus against vertical stage drift caused by thermal fluctuations, mechanical slack in the focus system, or other sources of tension. Automated compensation is possible if the distance between the objective and sample can be adjusted via motorized microscope components. To this end we had a wide array of microscopy hardware solutions to choose from. In choosing what design strategy to pursue, we wanted to balance several potentially conflicting priorities: cost, convenience, and performance. On one end of the spectrum, popular motorized microscopes from the traditional microscope manufacturers offered an expensive but highly convenient solution. These microscopes and the accompanying software featured both image-based computational autofocus as well as hardware attachments that continually measure the distance between objective and sample. With strong technical support and a well-documented history of high experimental performance, these solutions represented the high performance, high cost end of the spectrum. On the other end, instructions for completely built-in-house motorized microscopes could also be found on the internet and in multiple peer-reviewed scientific journals [26,27,32,33]. Many of these were low cost and varied in complexity and performance. To create an economical solution that could be easily adopted by a wide variety of laboratories with minimal engineering expertise, we chose to balance the convenience, cost, and performance of these extremes by starting with budget-friendly upright compound microscope. We chose a stand and optical components that provided high quality images and designed the necessary components to motorize the z-axis of the stage. On the software side, we created a Graphical User Interface-based software to control the motorized components, drive a robust image-analysis based autofocusing system, and manage the concurrent collection of time-lapse images for multiple microscopes.

2.1. Hardware

To motorize the z-axis of the microscope, we connected the fine focus knob of the microscope to a widely available stepper motor. We designed and 3D printed a scaffold to be bolted onto the body of the microscope, providing a stable frame for the motor. A 3D printed coupler was used to join the stepper motor and fine focus shafts (Fig. 2). The motor is controlled by our software through an Arduino and microcontroller card (Fig. 3). All components of the motorization, including the 3D printer and necessary hardware tools can be purchased through online retailers. Total cost of materials is less than $200 for the first motorized microscope. Motorization of additional microscopes is less than $30 per microscope, as initial materials including Arduino, 3D printing plastic, solder, etc. can used for multiple microscopes. Components can be assembled in less than 1 h. A bill of materials

as well as directions for assembly are included in the supplementary files available for download on our website (Table 1).

2.2. Software

To integrate control of our newly motorized microscope with time-lapse image collection, we created an intuitive GUI-based software. The open-source program offers simple mouse-based control of camera settings including exposure, frame rate, and resolution and provides live, zoom-able on-screen viewing of the sample image. It can also simultaneously manage the image data streams for multiple connected microscope cameras for concurrent time-lapse image collection. We have connected nine microscopes without issue but have not attempted more. To maintain image quality over a long-term, multi-day experiment, the software implements a simple but robust image-based autofocusing program. Briefly, we determine the sharpness of the image using the variance of the Laplacian. To find the direction of adjustment necessary, initial increasingly large steps are taken until the sharpness increases beyond a predefined minimum threshold. Both directions are sampled if necessary. Then the motor takes small steps in the correct direction until the sharpness passes a local maximum. While the method cannot correct arbitrarily large deviations from the optimal focal plane, it has shown itself to be sufficient for our purpose (Fig. 4). A video of the autofocus in action is provided in the downloadable files (Table 1).

3. Results and methods

To assess the utility of this low-budget microfluidic lifespan system, we captured whole-lifespan time-lapses of wild-type mother cells (downloadable, Table 1) using a standard haploid laboratory strain BY4741 [34]. After scoring daughter cell production for each mother by visually assessing the time-lapse images, we compare the results to data collected on the same strain via manual microdissection and find comparable results (Fig. 5).

For microfluidics experiments, cells were serially diluted and grown overnight at 30 °C until log phase in synthetic complete (SC) 2% glucose medium. Microfluidic devices were filled with SC 2% glucose medium. Cells were loaded into a 5 mL syringe and injected into the device by hand. Media was flowed through the device via syringe pump at a rate of 2–10 μL per minute. Cells were imaged every 5 min for 120 h. The resulting time-lapse was scored for lifespans by eye. For manual microdissection, replicative lifespan was determined as previously described [35]. In brief, cells were lightly patched onto rich YPD and allowed to grow overnight prior to starting the experiment. Individual cells were arrayed by micromanipulation and daughters were removed and counted every approximately 90 min until all mother cells had stopped dividing.

4. Conclusions

A new family of microfluidic devices promises to spur the collection of vast amounts of yeast replicative lifespan data with significantly reduced time and effort. Currently, the most common instrumentation needed to use these devices presents a significant financial barrier to all but the most well-funded laboratories. To help democratize this new technology and increase its adoption by the broader scientific community, we have designed a simple

hardware and software solution to modify existing affordable microscopes. Our designs use only affordable and easy-to-find parts and require minimal technical experience. While our design goals were geared towards the greater adoption of microfluidic technologies for yeast replicative lifespan assays, our design will allow for the improved collection of any type of brightfield time- lapse images. Full instructions for use and assembly, CAD design.stl files for 3D printed parts, and executable software files as well as source code are available by download from the Kaeberlein Lab website at http://kaeberleinlab.org/budgetscopes/. See Table 1 for a complete list of individual files.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants P30AG013280 and R01AG056359 to MK. KLC was supported by NIH grant F30AG052225. MMC was supported by NIH grant T32AG000057.

References

[1]. Mortimer RK, Johnston JR, Life span of individual yeast cells, Nature 183 (4677) (1959) 1751–1752. [PubMed: 13666896]

[2]. Steinkraus KA, Kaeberlein M, Kennedy BK, Replicative aging in yeast: the means to the end, Annu. Rev. Cell Dev. Biol 24 (2008) 29–54. [PubMed: 18616424]

[3]. Denoth Lippuner A, Julou T, Barral Y, Budding yeast as a model organism to study the effects of age, FEMS Microbiol. Rev 38 (2) (2014) 300–325. [PubMed: 24484434]

[4]. Kaeberlein M, Lessons on longevity from budding yeast, Nature 464 (7288) (2010) 513–519. [PubMed: 20336133]

[5]. Wasko BM, Kaeberlein M, Yeast replicative aging: a paradigm for defining conserved longevity interventions, FEMS Yeast Res 14 (1) (2013) 148–159. [PubMed: 24119093]

[6]. McCormick MA, et al., A comprehensive analysis of replicative lifespan in 4,698 single-gene deletion strains uncovers conserved mechanisms of aging, Cell Metabol 22 (5) (2015) 895–906.

[7]. Kaeberlein M, et al., Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients, Science 310 (5751) (2005) 1193–1196. [PubMed: 16293764]

[8]. Kaeberlein M, Kennedy BK, Large-scale identification in yeast of conserved ageing genes, Mech. Ageing Dev 126 (1) (2005) 17–21. [PubMed: 15610758]

[9]. Chen KL, Crane MM, Kaeberlein M, Microfluidic technologies for yeast replicative lifespan studies, Mech. Ageing Dev 161 (2016) 262–269. [PubMed: 27015709]

[10]. Xie Z, et al., Molecular phenotyping of aging in single yeast cells using a novel microfluidic device, Aging Cell 11 (4) (2012) 599–606. [PubMed: 22498653]

[11]. Zhang Y, et al., Single cell analysis of yeast replicative aging using a new generation of microfluidic device, PLoS One 7 (11) (2012) e48275. [PubMed: 23144860]

[12]. Liu P, Young TZ, Acar M, Yeast replicator: a high-throughput multiplexed microfluidics platform for automated measurements of single-cell aging, Cell Rep 13 (3) (2015) 634–644. [PubMed: 26456818]

[13]. Fehrmann S, et al., Aging yeast cells undergo a sharp entry into senescence unrelated to the loss of mitochondrial membrane potential, Cell Rep 5 (6) (2013) 1589–1599. [PubMed: 24332850]

[14]. Jo MC, et al., High-throughput analysis of yeast replicative aging using a microfluidic system, Proc. Natl. Acad. Sci. U. S. A 112 (30) (2015) 9364–9369. [PubMed: 26170317]

[15]. Lee SS, et al., Whole lifespan microscopic observation of budding yeast aging through a microfluidic dissection platform, Proc. Natl. Acad. Sci. U. S. A 109 (13) (2012) 4916–4920. [PubMed: 22421136]

[16]. Crane MM, et al., A microfluidic system for studying ageing and dynamic single-cell responses in budding yeast, PLoS One 9 (6) (2014) el00042.

[17]. Huberts DH, et al., Calorie restriction does not elicit a robust extension of replicative lifespan in Saccharomyces cerevisiae, Proc. Natl. Acad. Sci. U. S. A 111 (32) (2014) 11727–11731. [PubMed: 25071164]

[18]. Li Y, et al., Multigenerational silencing dynamics control cell aging, Proc. Natl. Acad. Sci. U. S. A 114 (42) (2017) 11253–11258. [PubMed: 29073021]

[19]. Janssens GE, et al., Protein biogenesis machinery is a driver of replicative aging in yeast, Elife 4 (2015) e08527. [PubMed: 26422514]

[20]. Xie Z, et al., Early telomerase inactivation accelerates aging independently of telomere length, Cell 160 (5) (2015) 928–939. [PubMed: 25723167]

[21]. Liu P, Acar M, The generational scalability of single-cell replicative aging, Sci. Adv 4 (1) (2018) eaao4666. [PubMed: 29399632]

[22]. Jin M, et al., Divergent aging of isogenic yeast cells revealed through singlecell phenotypic dynamics, Cell. Syst 8 (3) (2019) 242–253 e3. [PubMed: 30852250]

[23]. McDonald JC, et al., Fabrication of microfluidic systems in poly(-dimethylsiloxane), Electrophoresis 21 (1) (2000) 27–40. [PubMed: 10634468]

[24]. Saarikangas J, Barral Y, Protein Aggregates Are Associated with Replicative Aging without Compromising Protein Quality Control, vol. 4, Elife, 2015.

[25]. Pitrone PG, et al., OpenSPIM: an open-access light-sheet microscopy platform, Nat Methods 10 (7) (2013) 598–599. [PubMed: 23749304]

[26]. Rosenegger DG, et al., A high performance, cost-effective, open-source microscope for scanning two-photon microscopy that is modular and readily adaptable, PLoS One 9 (10) (2014) e110475. [PubMed: 25333934]

[27]. Campbell RA, Eifert RW, Turner GC, OpenStage: a low-cost motorized microscope stage with sub-micron positioning accuracy, PLoS One 9 (2) (2014) e88977. [PubMed: 24586468]

[28]. Jawale YK, Rapol U, Athale CA, Open Source 3D-printed focussing mechanism for cellphone-based cellular microscopy, J. Microsc 273 (2) (2019) 105–114. [PubMed: 30417401]

[29]. Switz NA, D'Ambrosio MV, Fletcher DA, Low-cost mobile phone microscopy with a reversed mobile phone camera lens, PLoS One 9 (5) (2014) e95330. [PubMed: 24854188]

[30]. Orth A, et al., A dual-mode mobile phone microscope using the onboard camera flash and ambient light, Sci. Rep 8 (1) (2018) 3298. [PubMed: 29459650]

[31]. Cybulski JS, Clements J, Prakash M, Foldscope: origami-based paper microscope, PLoS One 9 (6) (2014) e98781. [PubMed: 24940755]

[32]. Sharkey JP, et al., A one-piece 3D printed flexure translation stage for opensource microscopy, Rev. Sci. Instrum 87 (2) (2016) 025104. [PubMed: 26931888]

[33]. Schneidereit D, et al., Step-by-step guide to building an inexpensive 3D printed motorized positioning stage for automated high-content screening microscopy, Biosens. Bioelectron 92 (2017) 472–481. [PubMed: 27840039]

[34]. Kaeberlein M, et al., Genes determining yeast replicative life span in a longlived genetic background, Mech. Ageing Dev 126 (4) (2005) 491–504. [PubMed: 15722108]

[35]. Steffen KK, Kennedy BK, Kaeberlein M, Measuring replicative life span in the budding yeast, J. Vis. Exp (28) (2009).

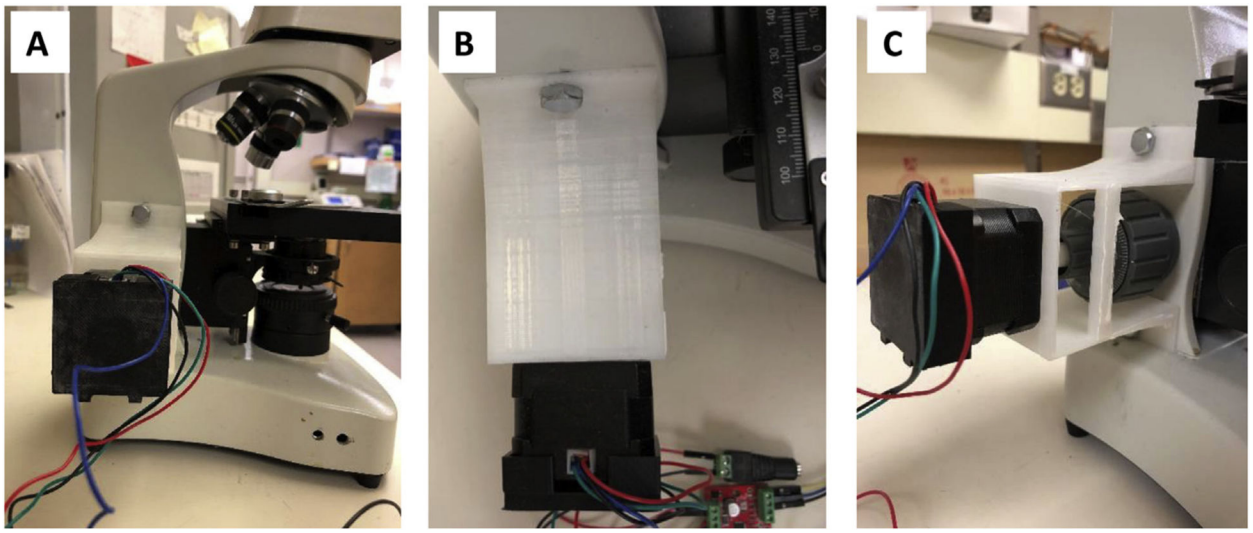

Fig. 1. 3D printed scaffold attaching stepper motor to microscope.
(**A**) side view (**B**) top view (**C**) oblique view.

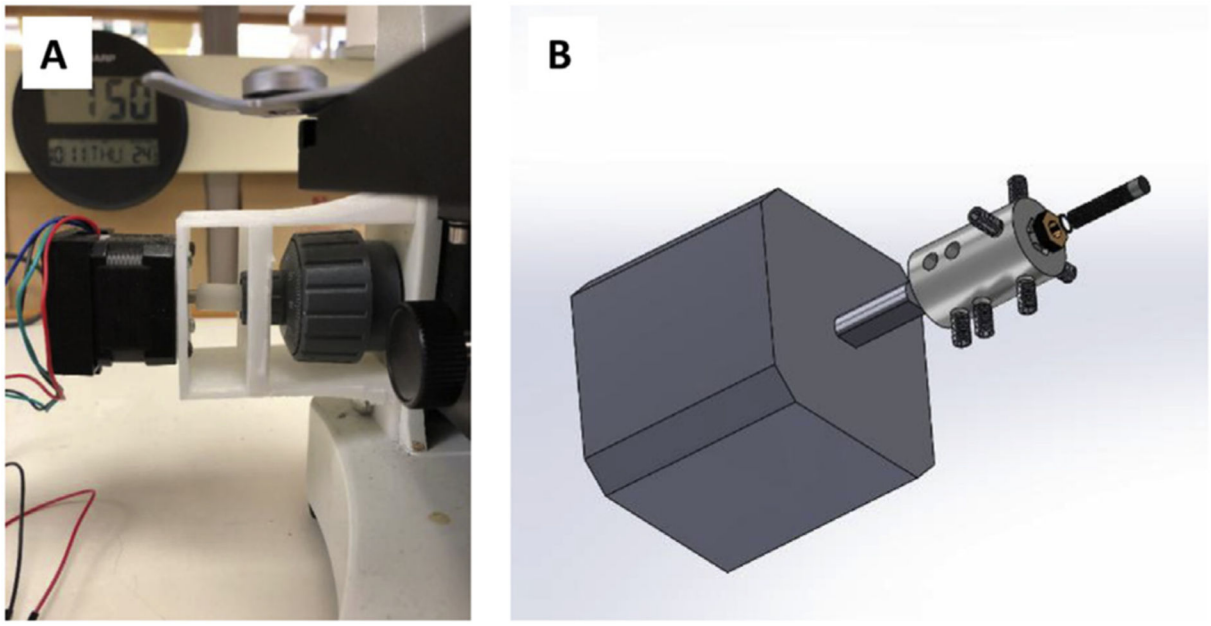

Fig. 2. 3D printed coupler to join the stepper motor and fine focus shafts.

(**A**) Front view of motor-microscope assembly showing coupler (white) joining stepper motor shaft to fine focus. (**B**) Exploded view of stepper motor shaft-coupler-fine focus shaft assembly (from left to right) including views of optional set screws, and internal nuts and washers.

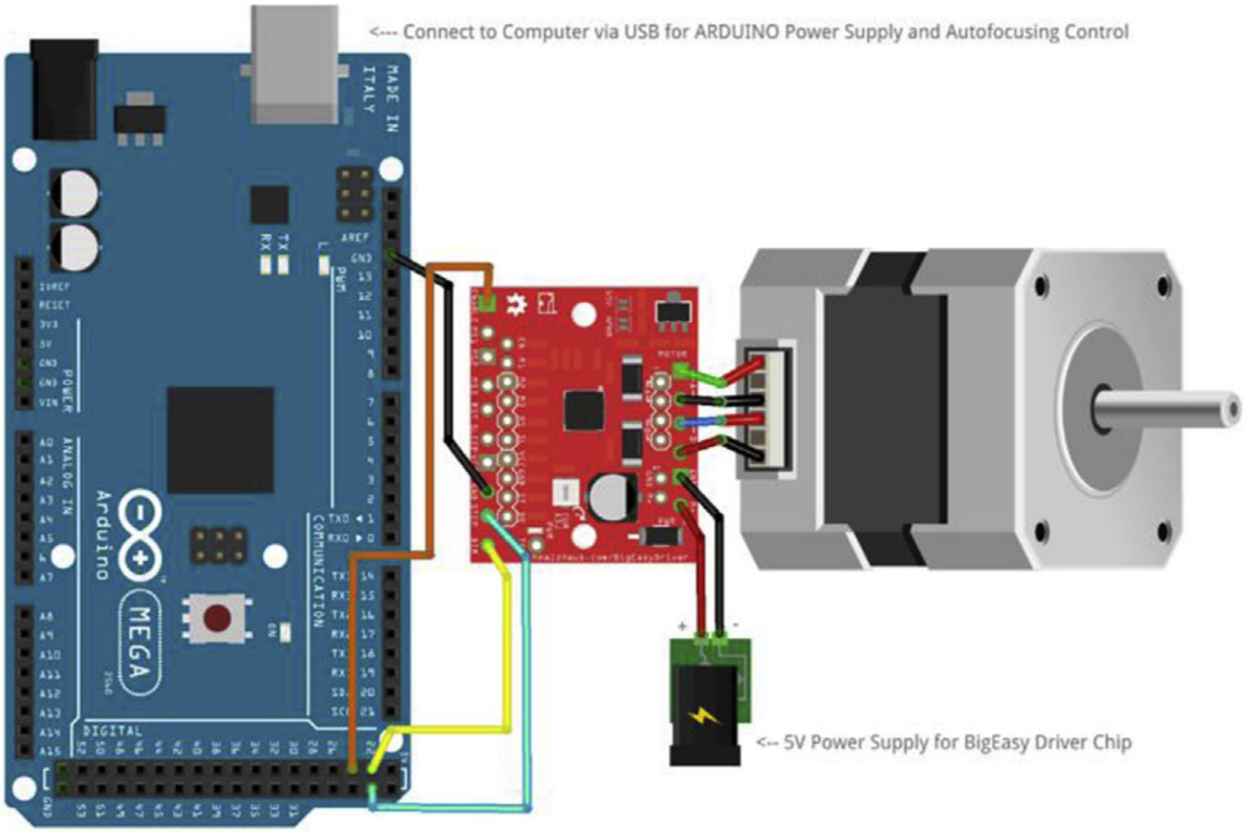

Fig. 3. Circuit diagram showing wiring of stepper motor through BigEasyDriver controller chip and Arduino.

Power supply is a 12 V DC adapter that can be plugged into a lab or household power outlet. Arduino is connected to computer via USB.

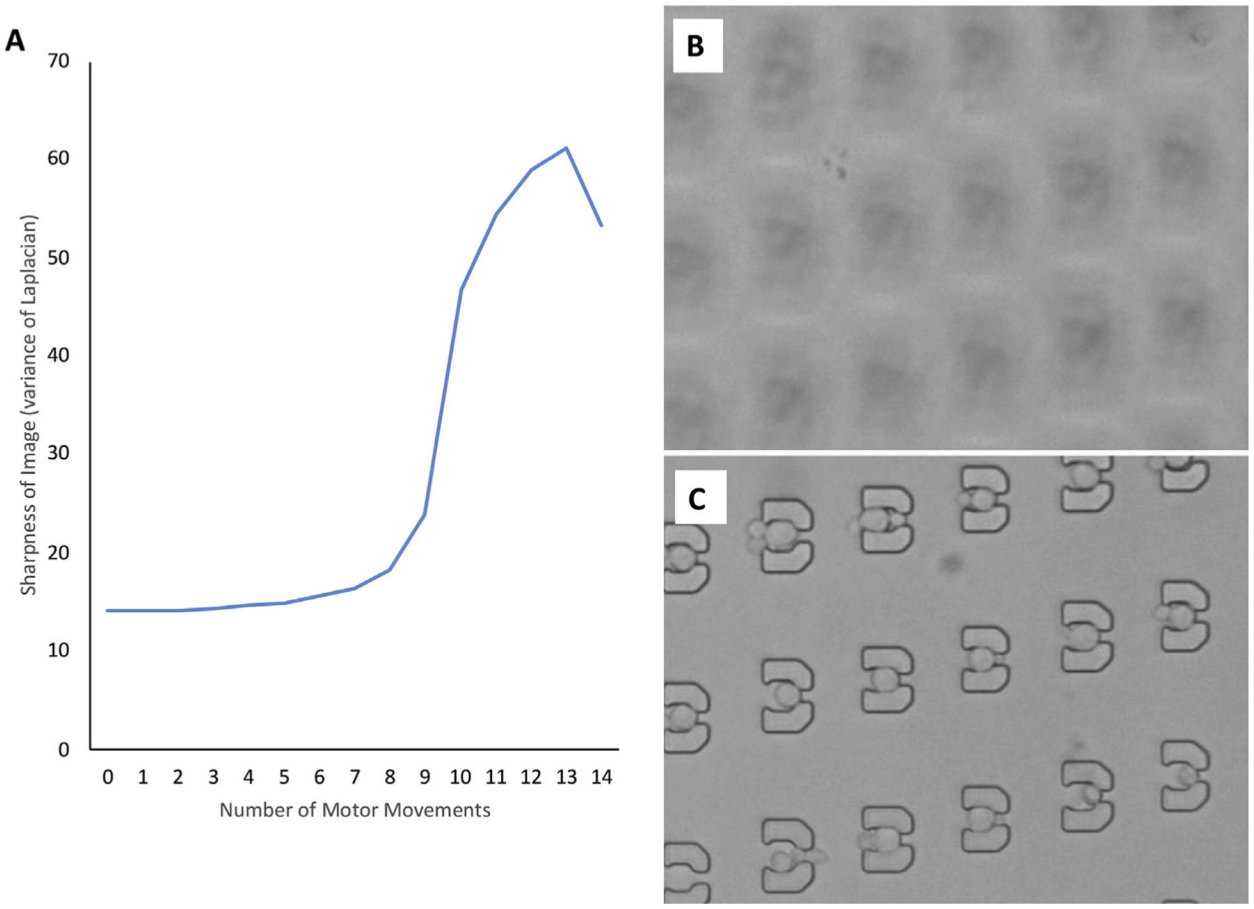

Fig. 4. Typical autofocus performance

(**A**) Trace of sharpness score of the live image as autofocus algorithm proceeds. (**B**) Cropped view of starting (top) and (**C**) ending (bottom) images through autofocus algorithm.

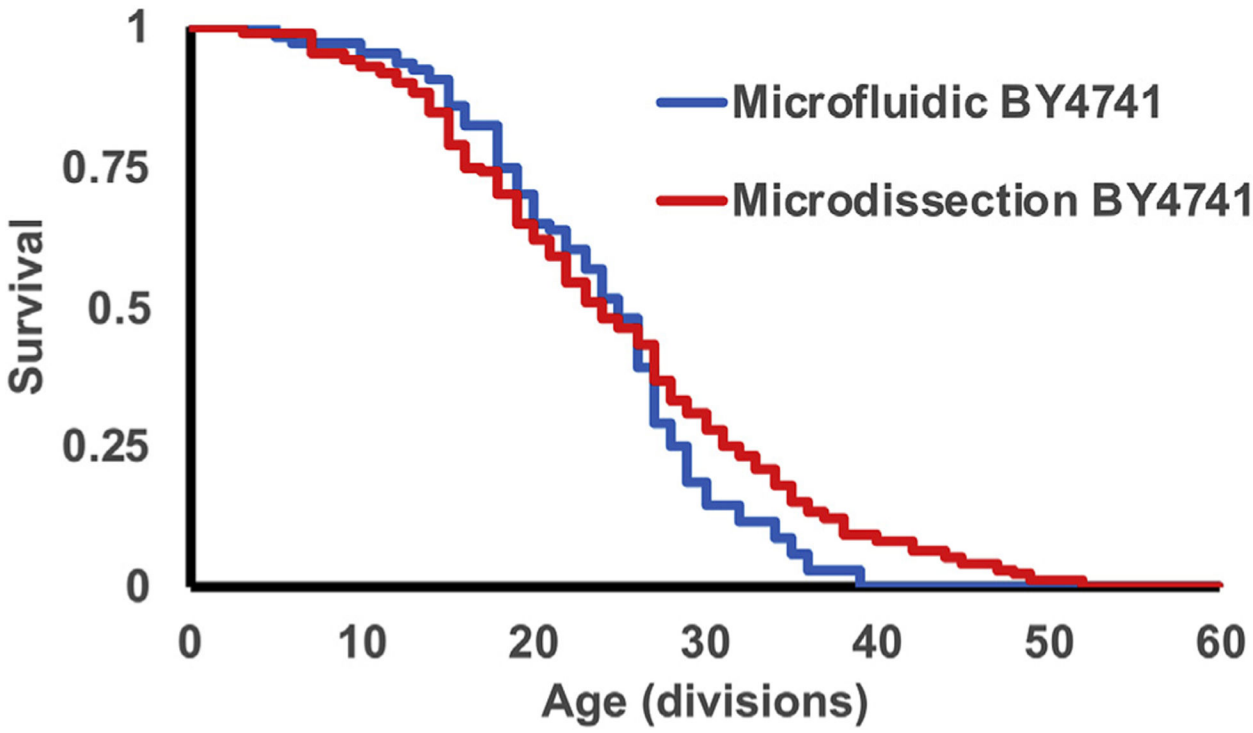

Fig. 5. Replicative lifespan measurement.

Lifespan for the standard laboratory haploid strain BY4741 was determined by microfluidics using the budget microscope system and compared to lifespan determined for the same strain by microdissection. N = 100 mother cells for microdissection and N = 67 mother cells for microfluidics.

Table 1

List of supporting files available for download.

Full instructions for use and assembly of the microscope system, CAD design.stl files for 3D printed parts, and executable software files as well as source code can be downloaded directly from the Kaeberlein Lab website at http://www. http://kaeberleinlab.org/budgetscopes/. Files can be downloaded as compressed.zip file or individually.

Folder	File	Purpose
Home	ChenSupplemental.zip	Compressed.zip file containing all of the available files for download
Home	Parts.xlsx	Excel file containing a detailed bill of parts
Engineered Parts	Microfluidic Device.dwg	CAD design of microfluidic device
Engineered Parts	Microfluidic Device Frame.STL	3D printed scaffold to hold device during experiments
Engineered Parts	Motor Backing.STL	3D printed heat sink for stepper motor, optional
Engineered Parts	Motor Scaffold.STL	3D printed scaffold to attach stepper motor to microscope
Engineered Parts	Shaft Coupler.STL	3D printed part to connect stepper motor shaft to fine focus shaft
Instructions	First run of program.pdf	Instructions to set up the software and run for the first time
Instructions	Instructions for wiring and driving the stepper motors.pdf	Instructions to set up the wiring of the stepper motor(s)
Instructions	Motor attachment Instruction.pdf	Instructions to connect stepper motor to microscope
Instructions	Tips for experimental setup.pdf	Tips for microfluidic experiments
Software	MotorArduinoCode.ino	Arduino code to drive stepper motor, see first run instructions
Software	MicrofluidicMicroscopeManager.zip	Zipped folder containing portable executable program and source code for software (MicrofluidicMicroscopeManager.py). See first run instructions.
Videos	Autofocus Demonstration 2x speed.mp4	Screenshare video showing live image being autofocused
Videos	Sample Experiment.avi	.avi video showing an example experiment at a rate of 2 h real time per second of video