An inexpensive microscopy system for microfluidic studies in budding yeast

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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 \cite{Mortimer1959}. \textit{S. cerevisiae} cells divide asymmetrically, segregating age-accumulated damage and producing a smaller, rejuvenated daughter cell \cite{Johnson1959}. Mortimer and Johnson monitored individual mother cells grown on an agar pad. To maintain observation of the
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

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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.
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.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 |