Journal of Laboratory Automation
2015, Vol. 20(1) 51–55

DOI: 10.1177/2211068214555414
jala.sagepub.com
SAGE

Miniaturized Plate Readers for Low-Cost, High-Throughput Phenotypic Screening

Paul A. Jensen[1,2], Bonnie V. Dougherty[1], Thomas J. Moutinho, Jr.[1,3], and Jason A. Papin[1]

Abstract

We present a miniaturized plate reader for measuring optical density in 96-well plates. Our standalone reader fits in most incubators, environmental chambers, or biological containment suites, allowing users to leverage their existing laboratory infrastructure. The device contains no moving parts, allowing an entire 96-well plate to be read several times per second. We demonstrate how the fast sampling rate allows our reader to detect small changes in optical density, even when the device is placed in a shaking incubator. A wireless communication module allows remote monitoring of multiple devices in real time. These features allow easy assembly of multiple readers to create a scalable, accurate solution for high-throughput phenotypic screening.

Keywords

phenotypic screening, plate reader, growth curve, multiwell plate assays

Introduction

High-throughput phenotypic screening maps the fitness of microorganisms to a set of chemical, genetic, or environmental perturbations. The simplest phenotypic assay monitors the optical density (OD) of a suspended cell culture throughout time.[1] The rate of change of OD corresponds to an organism's growth rate and correlates with its fitness. Growth rate assays are used in a wide range of microbiology and bioengineering research areas, including antibiotic testing,[2] laboratory evolution,[3] and process optimization.[4]

Most OD assays use a spectrophotometer to pass a fixed wavelength of light through a cell culture and measure the relative transmission. Historically, large batch cell cultures were sampled periodically and read in a separate cuvette or spectrophotometer device. Although this procedure is straightforward, it requires periodic human intervention, carries a risk of contamination during sampling, and has low throughput. For high-throughput screening, cells are cultured in multiwell plates and read continuously in specialized plate readers with built-in incubators and shakers. A robotic system can move plates in and out of the incubator and position each well beneath the spectrophotometer. These moving parts (and other sensors in the reader—fluorescence, etc.) drastically increase the size and cost of plate readers compared to low-throughput devices.

Incubating plate readers provide only a limited set of environments. Because the incubator and shaker are contained within the device, modulating the experimental conditions requires specialized hardware modules (CO_2 regulators, humidity controllers, etc.). Simultaneously screening more than 1 multiwell plate requires plate stackers and robotics to move plates from external incubators into the reader. Carefully controlling the environment during large screens can require encapsulating the entire robotic system in a climate-controlled incubator. Such systems are cost- and space-prohibitive for many laboratories, and they are difficult to reconfigure for new environments.

We present a novel instrument for continuously monitoring OD in a 96-well plate. Our design focused on 2 goals: (1) Create a compact device that can be placed inside any incubator or chamber, eliminating the need for additional hardware for environmental control; and (2) use only simple, low-cost components to produce an inexpensive device. Assembling several standalone readers creates a high-throughput system without additional robotic infrastructure.

[1]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, USA
[2]Present Address: Biology Department, Boston College, Chestnut Hill, MA, USA
[3]Present Address: Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, USA

Received Jul 5, 2014.

Corresponding Author:
Jason A. Papin, Department of Biomedical Engineering, University of Virginia, Box 800759, Health System, Charlottesville, VA 22908, USA.
Email: papin@virginia.edu

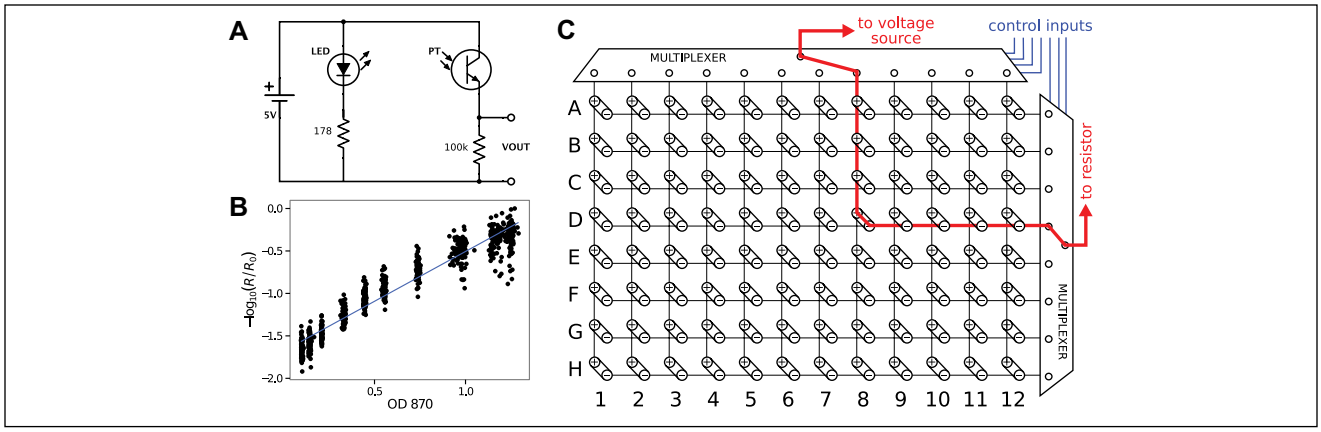

Figure 1. Design of a miniaturized multiwell optical density (OD) reader. (**A**) Schematic for OD sensor. An infrared light-emitting diode (LED) emits light at 870 nm, which is received by a phototransistor (PT). The light results in a proportional drop in voltage across the 100k ohm resistor, which is read at VOUT (output voltage). (**B**) Sensor output varies linearly throughout a range of OD. Readings for 96 wells on a single plate shown at 9 dilutions of a bacterial suspension. (**C**) A multiplexed grid for reading individual wells. Each of the 96 LEDs can be connected to the circuit independently by switching 2 analog multiplexers. One multiplexer connects all anodes in a single column, and the second connects all cathodes in a single row. The red line indicates an active connection through the LED corresponding to well D8. The same board layout can be used to connect the PTs above the multiwell plate.

Materials and Methods

Electrical Design

Measuring OD requires a relatively simple electronic circuit.[5] A light-emitting diode (LED) produces light focused on a peak emission wavelength. The light passes through the sample, where it is partly scattered by the suspended cells. The transmitted light is translated to a proportional voltage by a phototransistor (PT). The absorbed light is calculated as the difference between the emitted and transmitted light.

A single circuit for measuring OD is shown in **Figure 1A**. An LED produces a consistent light source at a fixed wavelength. The light travels vertically through the well of a microtiter plate, and transmitted light falls on a PT. We observed a linear response throughout a physiologically reasonable OD range (**Fig. 1B**).

We chose LED–PT pairs with peak emissions at 870 nm. This infrared wavelength is unaffected by the pigments produced by some bacteria. Pigments can absorb lower-wavelength light (e.g., 600 nm) and distort OD measurements. LED–PT pairs at 870 nm are used in many consumer electronics and are consequently inexpensive. LED–PT pairs are available for other wavelengths if desired.

Our device contains an 8×12 array of 96 LEDs spaced to match the wells of a multiwell plate (**Fig. 1C**). The anodes of each LED in a column are connected, as are the cathodes in each row. Selecting 1 column and row creates a complete circuit through only 1 LED, as shown in **Figure 1C**. Connecting analog multiplexers to the column and row wires allows the device to independently connect each LED to the emitter circuitry. A similar approach is used to connect each PT to the receiver circuitry.

Mechanical Design

The LED–PT arrays were attached to an aluminum frame (**Fig. 2A–B**). The frames are stackable, and multiple devices fit into a bench top incubator (**Fig. 2C**). The bottom surface of the frame includes adjustable spacers to secure a multiwell plate. The spacing between the aluminum frames is adjustable to accommodate deep well plates or plates with thick lids.

OD Calculations

A 24-bit analog-to-digital converter (ADC) converts the voltage drop across the PT to a numeric value in proportion to the transmitted light. The ADC in our design (the LMP 90100; Texas Instruments, Dallas, TX) performs real-time temperature correction to remove artifacts when the reader is incubated. The ADC output is converted to OD by

$$\mathrm{OD} = c \log_{10}\left(R / R_0\right)$$

where R is the reading from the ADC, R_0 is the ADC reading from a blank plate, and c is a proportionality constant. The value of c was calculated by comparing the device output when measuring cultures of known OD. For the circuit

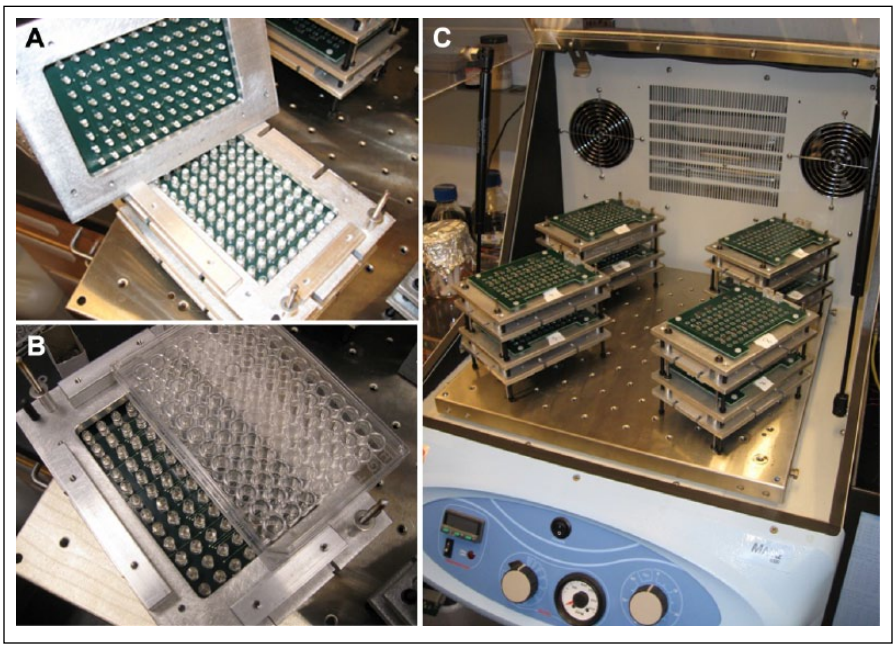

Figure 2. (**A**) Close-up of the phototransistor (PT) (top) and light-emitting diode (LED) (bottom) arrays. (**B**) The overall dimensions of the miniaturized reader are slightly larger than those of a standard 96-well plate. (**C**) Eight miniaturized plate readers stacked in a standard bench top incubator.

configuration shown in **Figure 1A** (870 nm LED, 5V, 178 ohm), we observed a linear response corresponding to an OD600 range between 0.0 and 2.0, with continued responsivity through OD600 3.0.

Experimental Procedures

Growth experiments in 96-well plates were performed similarly for both *Escherichia coli* strain K12 and *Pseudomonas aeruginosa* strain PA14. Overnight cultures were diluted to an OD600 of 0.05 in fresh media and loaded into the center 24 wells of a 96-well plate. Plates were covered and placed in either the miniaturized plate reader or a commercial Tecan Infinite Pro 200 instrument (Tecan, Männedorf, Switzerland). Except when noted, plates were grown for 20–24 h without agitation. OD readings were taken at 870 nm (miniaturized plate reader) or 600 nm (Tecan plate reader) every 120–360 s. Growth rates were calculated by a linear fit to a 2 h period during the exponential phase.[6] A sliding 2 h window was used to automatically identify the maximum growth rate, followed by visual confirmation that the corresponding period appeared during the exponential phase.

Four media were tested for each strain: lysogeny broth (LB); LB with 1 g/L glucose; LB with 1 g/L lactate; and synthetic cystic fibrosis media (SCFM), a defined solution of salts, amino acids, lactate, and glucose.[7] Each experiment was repeated 3 times on separate days.

For comparisons with batch cultures, 500 ml Erlenmeyer flasks were filled with 100 ml media and inoculated with overnight culture. Samples taken every 20 min were read at 600 nm using a cuvette-based spectrophotometer. Both the flasks and the miniaturized plate reader were shaken orbitally at 125 rpm. Four media were tested: LB, M9 with 4 g/L glucose, M9 with 4 g/L arginine, and M9 with 5 g/L glycerol.

Results and Discussion

Assembly and Configuration

With no moving parts, our device's sampling rate is limited only by the ADC and the switching time of the multiplexers. Our configuration allows a sampling rate of more than 100 Hz, allowing us to read the entire 96-well plate each second. Both the sampling rate and the reporting frequency can be adjusted by the user.

The OD sensors can be configured dynamically via software. The ADC gain, the emission current through the LED, and the well sampling order are all reprogrammable without hardware changes. Such adjustments can tune the device for reading very low- or high-OD cultures, and for plates with fewer than 96 wells.

Validation

We tested our miniaturized plate reader against a commercial plate reader using a variety of conditions. Cultures of the bacteria *E. coli* K12 and *P. aeruginosa* PA14 were grown in multiple media: a rich base (LB) with supplemental glucose or lactate; a minimal base (M9) with supplemental arginine, glucose, or glycerol; and SCFM, a media designed to recreate the in vivo metabolic environment of the cystic fibrotic lung.[7] We compared the exponential

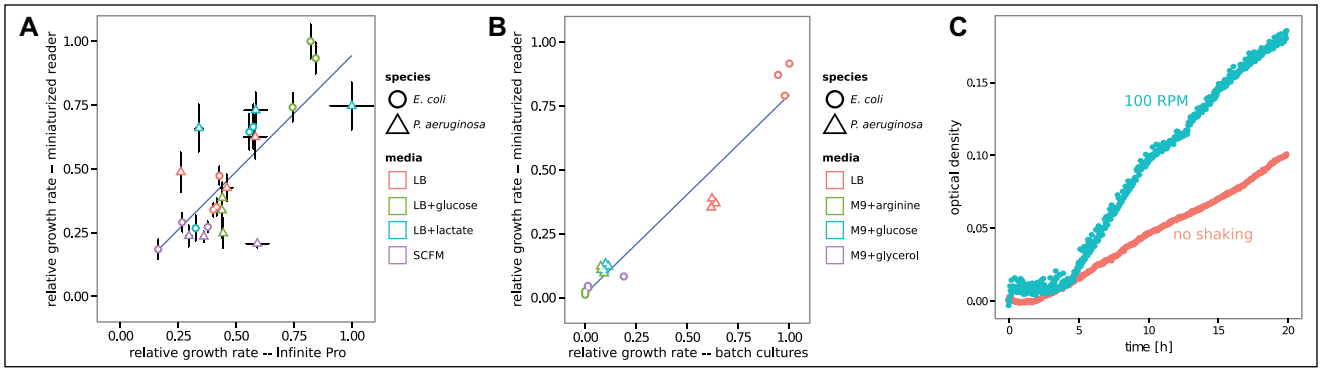

Figure 3. (**A**) Performance comparison between our miniaturized plate reader (vertical) and a commercial system, the Tecan Infinite Pro 200 (horizontal). The bacteria *Escherichia coli* K12 and *Pseudomonas aeruginosa* PA14 were grown in multiple conditions. Growth rates were normalized to *E. coli* grown in LB with supplemental glucose. Each point represents a biological replicate. Vertical and horizontal bars indicate the standard deviation from 24 technical replicates. (**B**) Comparison between miniaturized plate reader (vertical) and batch cultures grown in 500 ml flasks (horizontal). Growth rates were normalized to *E. coli* grown in LB. Each point represents a biological replicate. (**C**) Shaking does not affect device performance. Comparison between growth curve of *P. aeruginosa* in LB, with (blue) and without (red) shaking at 100 rpm. Readings were collected every 120 s.

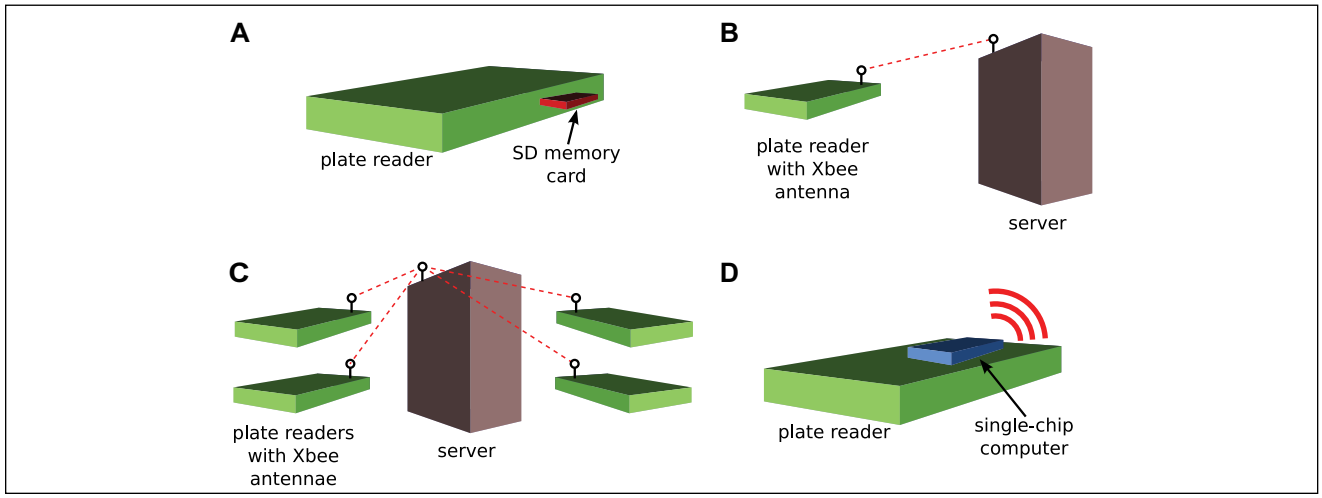

Figure 4. Multiple configurations for the miniaturized plate reader system. (**A**) Standalone reader with data stored on a Secure Digital (SD) memory card. (**B**) Reader transmitting data to a database server for storage and processing. (**C**) Multiple readers as part of a high-throughput screening system. (**D**) Reader with on-board computer. The computer provides a Wi-Fi hotspot to visualize data through the internet.

phase growth rates between our device and either a commercial incubating plate reader—the Tecan Infinite Pro 200 (**Fig. 3A**)—or batch cultures grown in 500 ml flasks (**Fig. 3B**). There was excellent agreement among the growth rates measured by each device.

Some cells require agitation for optimal growth or to prevent aggregation from highly filamentous or flocculating microbes. Our device does not include an internal shaking mechanism. Instead, the entire device can be placed on a shaking platform inside an incubator (**Fig. 2C**). We tested whether movement would introduce noise into the data collected from our device. As shown in **Figure 3C**, cultures of *P. aeruginosa* strain PA14 grow faster and reach a higher OD when shaken at 100 rpm.

Agitating the device did not significantly change the variability between reads. The device's high sampling rate allows any small perturbations to be averaged into a smooth set of readings. In practice, the reader averages 25 samples for each reported OD measurement. (The number of samples can be adjusted by the user.) For 95% of the readings from a shaking experiment, the 25 individual samples fall between 0.0% and 1.5% of the reported average, with a median variation of less than 0.45%. We observed that the variability derives from the movement of the media and not

from the electronics; agitating a device containing an empty plate produces no variation in the readings.

Data Storage and Retrieval

The miniaturized reader can be operated in a "standalone" configuration (**Fig. 4A**). The device can be powered with a single 9 V battery for several days. We included a ZigBee wireless communication radio (ZigBee, San Ramon, CA, http://www.digi.com/xbee) to transmit data to a remote server for real-time data storage and visualization (**Fig. 4B**). The server can track and configure multiple devices via the internet (**Fig. 4C**). We have operated up to 16 devices simultaneously. Many more devices can be connected, with the total number limited only by the available wireless bandwidth. We have also integrated an on-board single-chip computer (the Raspberry Pi: Raspberry Pi Foundation, Caldecott, UK, http://www.raspberrypi.org) to provide data storage and visualization without an external server (**Fig. 4D**). The on-board computer allows use of the reader in remote field locations without access to additional computers or wireless networks.

Availability

Schematics, parts lists, assembly instructions, and software for the readers and database servers are available at http://github.com/csbl/platereader.

Conclusions

We have described a miniaturized plate reader system for high-throughput growth rate monitoring. The system is designed from low-cost electronic components and contains no robotic or moving parts, simplifying the assembly process. We assembled our prototype device for less than $500 USD per reader, yet it performs as well as other readers that cost 10 times more. The device is compact and can be placed in incubators, anaerobic chambers, and biohazard containment suites and on shakers.

The autonomous operation and networking capabilities allow multiple devices to be assembled into a multiplate high-throughput screening system. Importantly, the devices can easily be reconfigured and shared between labs, allowing customized phenotypic-profiling systems to be created "on demand." The web-based data analysis and monitoring software creates an easy-to-use screening system with throughput comparable to that of complex robotic platforms.

Acknowledgments

We thank Bruce Ferris (SPARK Engineering, Richmond, VA), Erik Hewlett (University of Virginia), and David Chen (University of Virginia) for their assistance with product design and testing; and Jennifer Bartell and Anna Blazier (University of Virginia) for help with the growth experiments.

Declaration of Conflicting Interests

JP and PJ have filed US Patent Application No. 61/710,961 on this technology. The other authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is supported by the National Institutes of Health (GM088244 to JP) and the Coulter Foundation at the University of Virginia. PJ was supported by a graduate fellowship from the National Science Foundation. TM was supported in part by the Summer Research Internship Program at the University of Virginia School of Medicine.

References

1. Dalgaard, P.; Ross, T.; Kamperman, L.; et al. Estimation of Bacterial Growth Rates from Turbidimetric and Viable Count Data. *Int. J. Food Microbiol*. **1994**, *23*(3–4), 391–404.
2. Imamovic, L.; Sommer, M. O. A. Use of Collateral Sensitivity Networks to Design Drug Cycling Protocols That Avoid Resistance Development. *Sci. Transl. Med*. **2013**, *5* (204), 204ra132.
3. Fong, S. S.; Palsson, B. Ø. Metabolic Gene-Deletion Strains of *Escherichia coli* Evolve to Computationally Predicted Growth Phenotypes. *Nat. Genet*. **2004**, *36* (10), 1056–1058.
4. Kim, B.; Du, J.; Eriksen, D. T.; et al. Combinatorial Design of a Highly Efficient Xylose-Utilizing Pathway in *Saccharomyces cerevisiae* for the Production of Cellulosic Biofuels. *Appl. Envir. Microbiol*. **2013**, *79* (3), 931–941.
5. Toprak, E.; Veres, A.; Yildiz, S.; et al. Building a Morbidostat: An Automated Continuous-Culture Device for Studying Bacterial Drug Resistance under Dynamically Sustained Drug Inhibition. *Nat. Protoc*. **2013**, *8* (3), 555–567.
6. Zwietering, M. H.; Jongenburger, I.; Rombouts, F. M.; et al. Modeling of the Bacterial Growth Curve. *Appl. Envir. Microbiol*. **1990**, *56* (6), 1875–1881.
7. Palmer, K. L.; Aye, L. M.; Whiteley, M. Nutritional Cues Control *Pseudomonas aeruginosa* Multicellular Behavior in Cystic Fibrosis Sputum. *J. Bacteriol*. **2007**, *189* (22), 8079–8087.