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An automated multiplexed turbidometric and data collection system for measuring growth kinetics of anaerobes dependent on gaseous substrates

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Abstract

Standard methods of monitoring the growth kinetics of anaerobic microorganisms are generally impractical when there is a protracted or indeterminate period of active growth, and when high numbers of samples or replications are required. As part of our studies of the adaptive evolution of a simple anaerobic syntrophic mutualism, requiring the characterization of many isolates and alternative syntrophic pairings, we developed a multiplexed growth monitoring system using a combination of commercially available electronics and custom designed circuitry and materials. This system automatically monitors up to 64 sealed, and as needed pressurized, culture tubes and reports the growth data in real-time through integration with a customized relational database. The utility of this system was demonstrated by resolving minor differences in growth kinetics associated with the adaptive evolution of a simple microbial community comprised of a sulfate reducing bacterium, *Desulfovibrio vulgaris*, grown in syntrophic association with *Methanococcus maripaludis*, a hydrogenotrophic methanogen.

Highlights

- The ODIn supports parallel quantification of up to 64 cultures under a defined gas headspace.
- High throughput culture monitoring resolved difficult to measure differences in growth rate.
- Real-time data visualization allows for immediate feedback on experiment progress.

Introduction

A variety of automated systems for monitoring microbial growth based on changes in turbidity or fluorescence are available, using small reactor systems (Takahashi et al., 2015; Toprak et al., 2013; Wong et al., 2018), microtiter plate readers (Duetz et al., 2000), or more specialized commercial systems (Vuono et al., 2019). Such systems however are generally not suitable for monitoring the growth of anaerobic microbial cultures, particularly those that require a gaseous substrate for growth or depend on a close hydrogen-based syntrophic coupling, upon which many anaerobic microbial food webs depend. In addition, the slow growth of many fastidious anaerobes requires that cultures be monitored continuously at regular intervals over multiple day periods. As part of ongoing studies on simple microbial communities composed of the hydrogenotrophic methanogen *Methanococcus maripaludis* S2 coupled with a facultatively syntrophic sulfate-reducing bacterium, *Desulfovibrio vulgaris* Hildenborough, we fabricated an automated multiplexed system to monitor growth. Essential advantages of the new system are compatibility with the Balch-type anaerobic culture tubes commonly used in the cultivation of fastidious anaerobes and a capacity for highly replicated data collection by simultaneous monitoring of up to 64 culture tubes at high temporal resolution. An important feature that distinguishes it from other available devices is the automated control of the sample holder platform, providing for both maximum gas exchange for culture growth in the down position and accurate optical density readings in the up position. Thus, the optical density readings and growth kinetics are directly comparable to data collected using the more cumbersome manual format.

The Optical Density Instrument (ODIn) was designed to consistently agitate sealed culture vessels, monitor growth of cultures via a noninvasive metric, and maintain relatively constant

conditions. This instrument will accommodate up to 64 pressurized tubes on a remotely controlled shaking platform, which automatically adjusts the angle of the tubes to accommodate periodic readings during continuous growth. To achieve good mixing and gas exchange of the culture medium during shaking, the tubes are positioned horizontally. Optical density is measured by temporarily arresting the shaking of the sample holder platform, raising the sample platform to a near vertical position, and then serially collecting optical density readings from all 64 channels using light emitter and sensor pairs on opposing sides of each culture tube. Sensor output data is reported automatically through a wired Ethernet connection and recorded on a remote database server where a custom data collection program displays the sensor data in real-time, converts sensor data to optical density, calculates changing growth rates, and offers direct comparisons of growth kinetics from individual cultures within or between different growth experiments. Thus, growth is quantified with very little disruption of the sample during monitoring periods that may be up to days for the slow growing cultures analyzed in this report. We here present a detailed description of the construction and operation of ODIn, demonstrating its application for quantifying and resolving small differences in the growth kinetics that arise through the adaptive evolution of a model microbial community composed of the hydrogenotrophic methanogen *Methanococcus maripaludis* growing in syntrophic association with *Desulfovibrio vulgaris*.

Materials and Methods

System overview. The ODIn system was constructed using commercially available electronic components, including sensing and control components operated by a microcontroller to

automate the collection of optical density measurements over a time series (Fig. 1). The system is designed to house 64 closed Balch tubes (18x150 mm, Bellco Glass Inc., Vineland, NJ), each tube sealed with a crimp-closed rubber stopper for gas retention. Tubes are housed in an array of eight identical machined modular resin tube racks, each designed to accommodate eight individual tubes in a linear array secured by a removable top cover and securing O-rings (Fig. 2). The racks are fastened to a shaker table either using compression straps or by insertion into a custom housing.

Using a microcontroller to direct operations, the optical density of each sample is quantified using individually paired light emitting diodes (LEDs) and receiving phototransistors (Optek Inc., Galena, OH) integrated into the base of each rack (Fig. 2B). The electrical leads for the sensor sets are attached to a custom printed circuit board (PCB, ExpressPCB, Mulino, OR) secured in a recess at the rack base by a removable bottom plate. The linear range of each sensor set was manually established prior to installation by quantifying the milliamps (mA) output at known optical density at 600 nm (Hach Co. Loveland, CO) using McFarland standards covering a range of optical densities (Fig. 3).

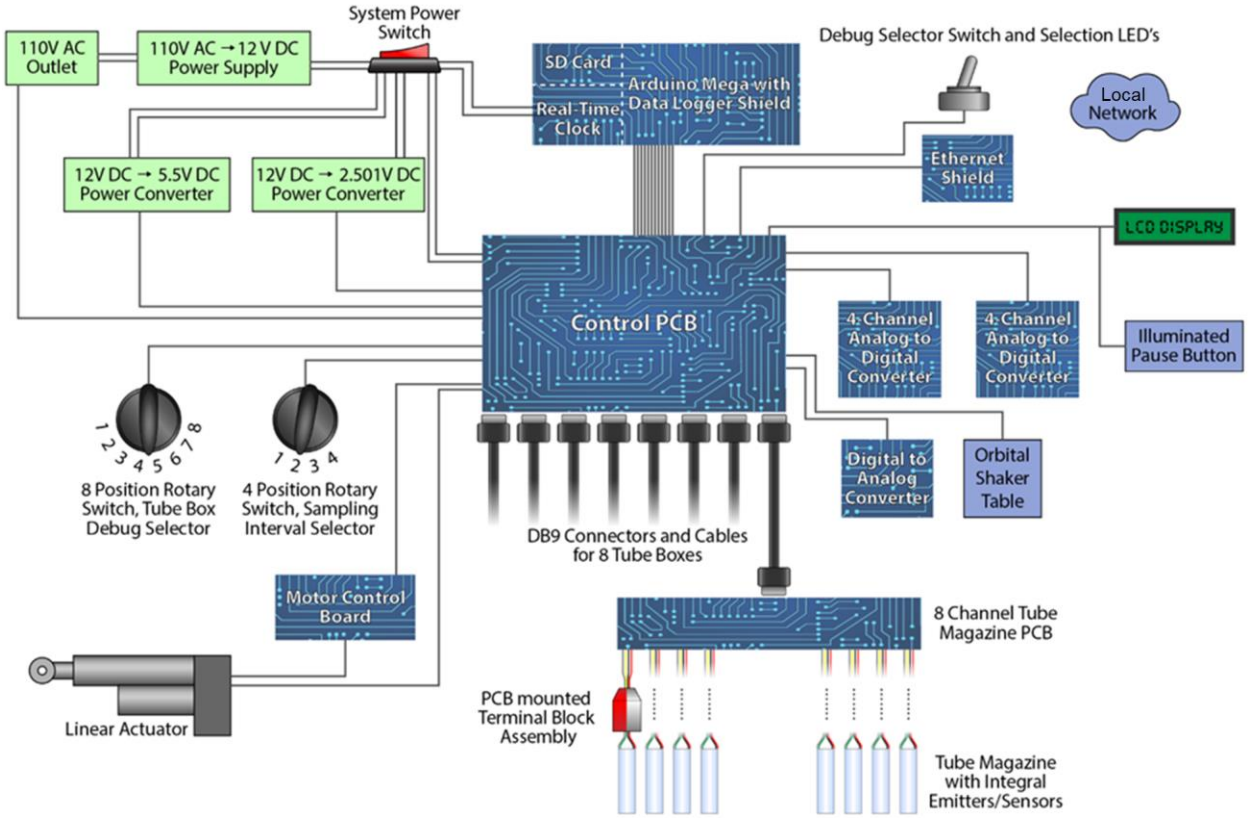


Fig. 1. Schematic diagram of control and sensing elements for the ODIn system. The system combines commercially available components with custom circuitry through an Arduino microcontroller platform to coordinate actions of the shaker platform, turbidity measurement frequency, and data reporting.

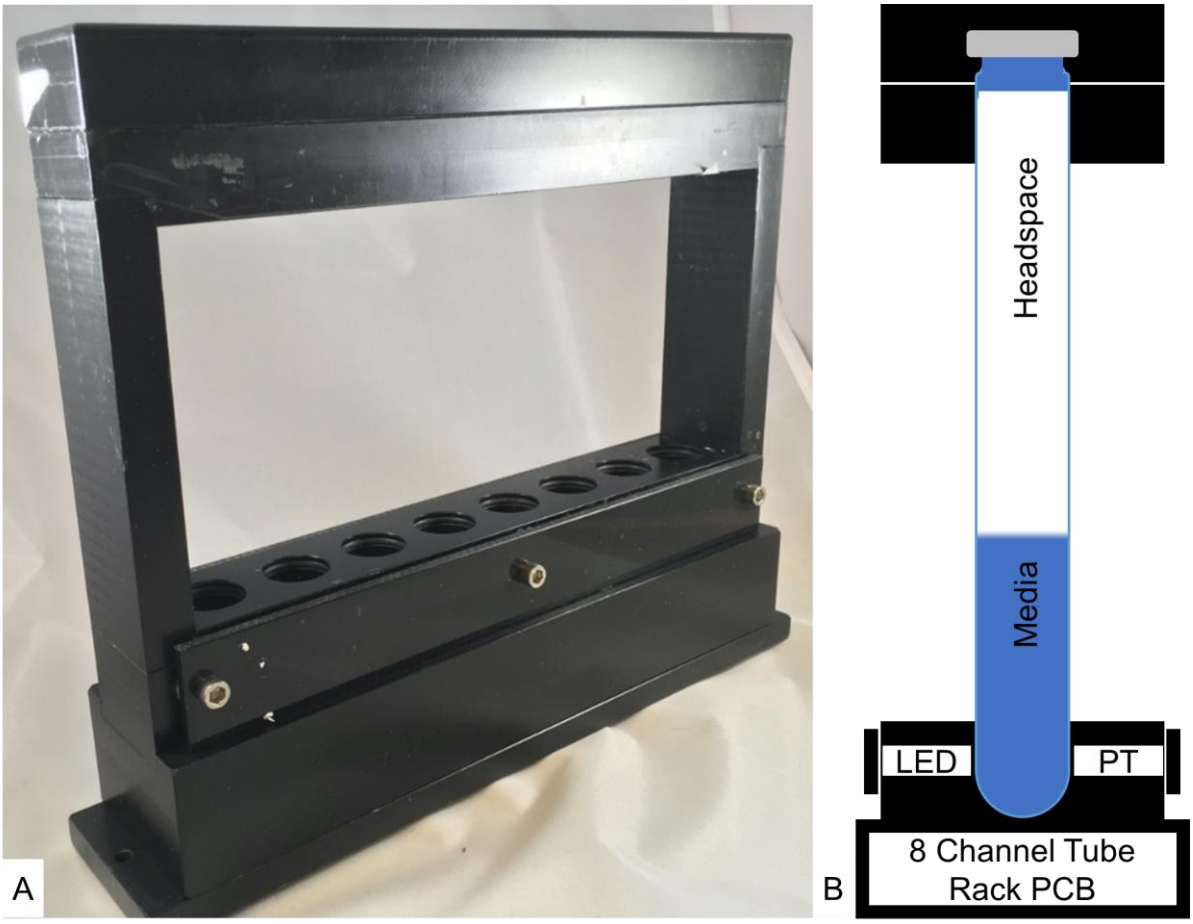


Fig. 2. Tube rack (**A**) machined from acetyl resin to house eight culture tubes in individual sensor channels. Sensors consisting of a LED and phototransistor (PT) are positioned opposite one another near the bottom of each rack (**B**) and connected to a PCB embedded in the base of the rack.

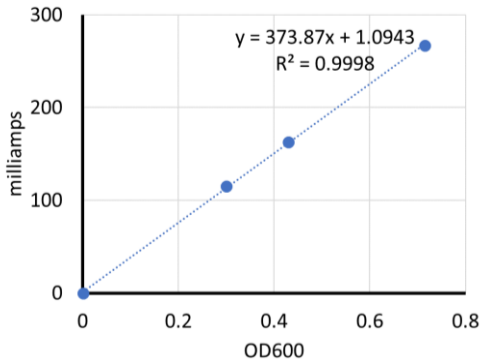


Fig. 3. Relationship between sensor output (milliamps) and corresponding spectrophotometric readings of McFarland turbidity standards.

The tube racks are secured to a hinged acrylic lid attached to a raised platform (Fig. 4) secured on a New Brunswick Innova 2300 orbital shaker (Eppendorf, Hamburg, DE). The lid of the platform is manipulated by a linear actuator (Servocity, Winfield, KS) controlled by a separate circuit board (Canakit, North Vancouver, BC) through a tethered cable connector. During shaking, tubes are maintained in a horizontal position. At user defined intervals, the shaker is stopped, and the tubes are raised to approximately 50-degrees above the horizontal prior to taking OD readings. Sequential readings of the 64 sample tubes requires a period of two-three minutes before lowering the platform and reinitiating shaking. A minimum of 5-mL of medium is needed for an accurate reading of culture turbidity. Reading frequency is controlled by microcontroller software using programmed default settings of 5-, 20-, 60-, and 120-minute reading intervals selected with a rotary knob on the system control box (Fig. 5).

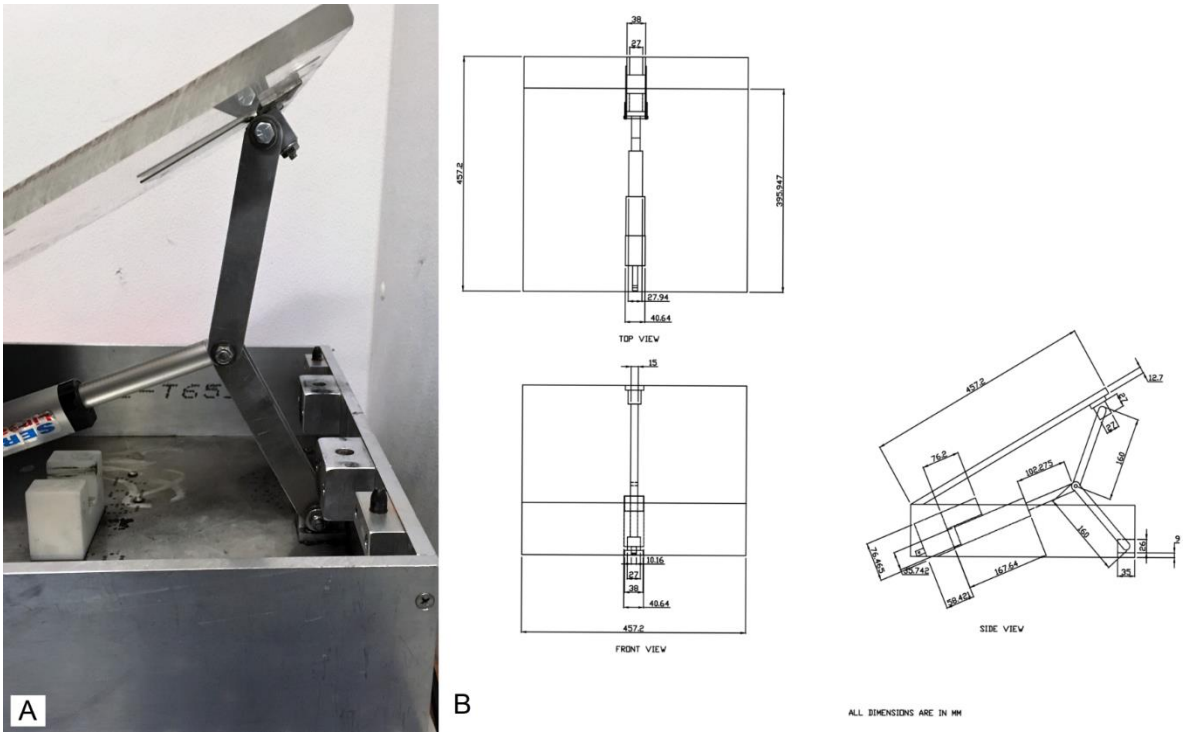


Fig. 4. Construction of platform (**A**) and linear actuator (**B**) for raising tube racks in preparation for periodic measurements of turbidity. Following shaker table deactivation, the linear actuator unfolds a scissor hinge, transitioning tubes from a horizontal to a near vertical position.

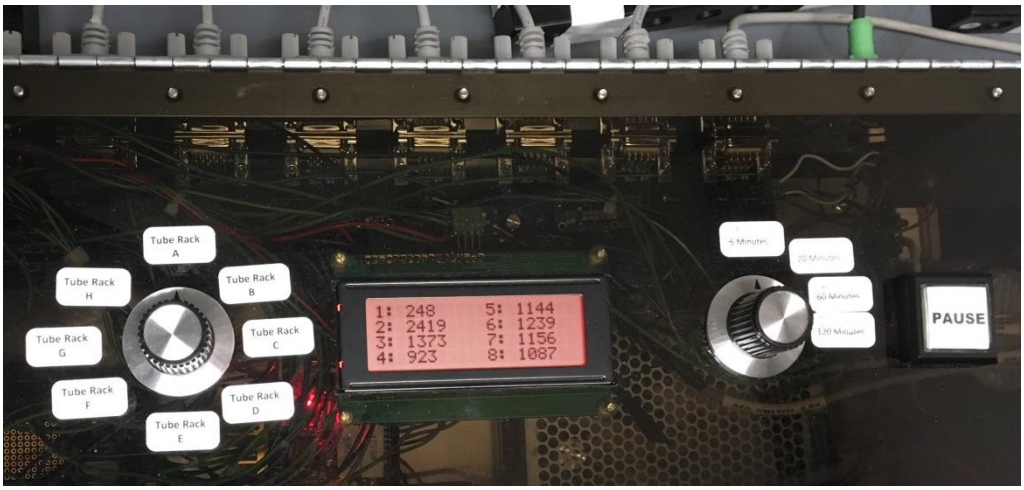


Fig. 5. Control box. Controls for different functions are located on the lid and include a system status feedback display LCD screen, a knob controlling a four-position rotary switch for selecting preset sampling time intervals, an eight-position rotary knob to select a single tube rack for display of sensor output values during run and debug modes, and a push button "Pause" switch for system pausing and activation.

The system control box is separate from sensing components of the system (Fig. 5). A custom acrylic box houses all system control components including the microcontroller, communication devices, power supply, motor controller, and control interfaces, all of which communicate through a custom PCB controller (ExpressPCB, Mulino, OR). Access to internal components is by a hinged lid on the control box. Communication with each tube rack is through a serial cable connection to eight DE-9 female receptacles (NorComp, Charlotte, NC) located on the side of the control box (Fig. 1). System power is provided by a 12VDC 100W panel mount power supply powered through a standard external 120 VAC female appliance coupler to a 120 VAC wall outlet. Operation control interfaces are located on the control box lid and include a system status feedback display LCD screen (Adafruit Industries, LLC New York, NY), a four-position rotary knob for selecting preset sampling time intervals, an eight-position rotary knob to select a single tube rack for display of sensor output values during a debugging mode, a two position toggle switch for selecting between device modes "Run" and "Debug", and a push button "Pause" switch for system pausing and activation. Direct communication with the Arduino Mega microcontroller is through the USB Type-B embedded port (Adafruit Industries, LLC New York, NY) accessed on the side of the control box. Local data storage is performed using an SD card Data Logger Shield (Adafruit Industries, LLC New York, NY), which provides an SD card

receptacle for a standard SD card accessed on the side of the control box. An Ethernet connection for data output is through an Ethernet Shield (Adafruit Industries, LLC New York, NY) using a static IP address programmed into the Arduino and accessed on the side of the control box. The orbital shaker is plugged into the side of the control box and controlled by a AC interrupting relay via the Arduino.

During normal operation, the interval between OD readings is controlled by presets on a selectable rotary knob. At the selected time intervals, the microcontroller deactivates the orbital shaker. Following a ten second delay to allow the orbital shaker to come to a full stop, the hinged acrylic platform lid is raised, and sensor readings are taken sequentially for the 64 channels. Those data are stored on a local SD card as well as reported to a remote server through the Ethernet connection. The data can be visualized and archived in real-time for all 64 channels using custom software developed on FileMaker Advanced version 14 (Claris International, Inc., USA), the host application has been tested through FileMaker Server 15 and the client application has been tested through FileMaker Pro 17 (Fig. 6A). The FileMaker algorithm processes ODIn data collected through a server interface but operates independently from the ODIn hardware. Upon completion of the data acquisition and reporting procedure, the linear actuator retracts, and the shaker reactivates. A full description of the device, fabrication, and operation is provided in supplementary information.

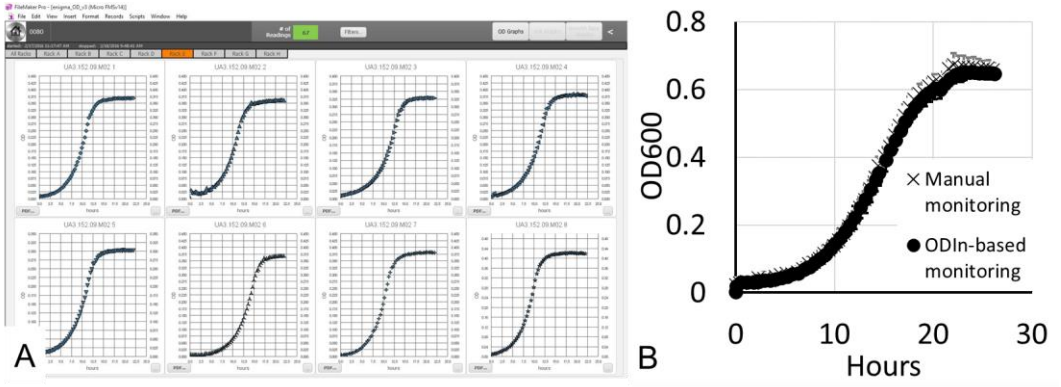


Fig. 6. Continuous monitoring of growth using the FileMaker interface provides direct feedback of experimental progress (**A**) and is indistinguishable from manually collected measurements (**B**). Example FileMaker display of growth curves for eight channels of a single tube rack where data can be displayed as milliamps or converted to optical density values after completion of the experiment. Growth is tracked in real-time and updated as new data packets are received from the sensor channels.

System operation. Before initiating a growth study, the system is held in a paused state to allow LED light output to stabilize at the operating temperature. This occurs within four hours when the ambient temperature is 37°C. A two-position toggle switch on the lid of the control box is used to select between the operational mode used for data collection during an experimental run and a debugging program mode for continuous displaying of sensor output in real-time on the control box system status LCD screen. In the debugging routine, the tube racks are elevated using the hinged lifting platform and the output from each tube in a tube rack is continuously displayed on the control box LCD screen, with selection of readings from each of the eight racks controlled by a rotary knob on the control box (Fig. 5). This procedure allows for immediate sensor value data to be displayed from any sensing channel and is a useful diagnostic tool for identifying faults and determining optical working ranges.

An experimental run is initiated by depressing the pause button on the control box when the toggle switch is in the “up” position. This action raises the hinged lifting platform acrylic lid and initiates the calibration procedure on each of the 64 sensing channels. Once calibration is complete, the program takes the first measurement of each sample, and then reads the status of the four-position rotary switch on the control box that determines the sampling interval of programmable preset values, either 5, 20, 40, or 60 minutes. These intervals are configurable in the program software and may be readjusted during an experiment by switching to any of the four values using the selectable dial. The linear actuator then retracts, lowering the lifting platform acrylic lid to return to a horizontal position and a countdown commences until the next data collection point. The control box system status LCD screen displays the selected interval time in minutes and the active countdown in milliseconds until the next sampling. If the systems pause button is depressed during an active experiment it will continue to count down to the set data collection time but will not collect data until the system is resumed. This allows samples to be removed during an active experiment for external sampling.

When the time interval between samplings is reached, the orbital shaker is depowered through the interrupting relay and the tube racks are raised for the next measurement. To mitigate the effects of random spikes in sensor readings, a total of 20 ADC values are taken for each sensing circuit at each sampling point and averaged for the reported value. All 64 data points, along with the elapsed time since testing began, are sent to the data collection server via UDP datagram transmission over an Ethernet network connection. The measurements are also recorded on an

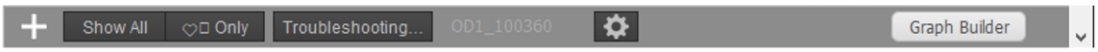
onboard SD card located in the SD Data Logger Shield in the control box as a backup in a timestamped .CSV file. An onboard real-time clock with coin cell battery backup keeps track of the current date and time. Data is both reported and recorded in the .CSV file format and arranged with the output of each channel's reading in separate columns. Output data are milliamp values as recorded by the ADC. Upon completion of the experiment, each culture is measured at 600-nm in a spectrophotometer and pre- and post-experiment OD_{600} values used to convert milliamp values to an OD_{600} (Fig. 6). A step-by-step protocol of general operation for an experiment using the FileMaker interface is described with images below.

General operational protocol

Step 1: Turn on the ODIn control box and leave "Paused" for equilibration

Step 2: Setup a run in the FileMaker interface

Step 2.1: Click the "+" button in the bottom left corner of the interface



Step 2.2: Enter a unique name for the experiment

Step 2.3: Return to the home screen and click "start" for the experiment

Step 3: Setup the ODIn

Step 3.1: Prepare sealed Balch tubes with at least 5-ml of media

Step 3.2: Record first manual OD_{600} measurements for 2-point calibration

Step 3.3: Load Balch tubes into the ODIn tube racks

Step 3.4: Press the "Pause" button on the ODIn control box to initiate ODIn

Monitor mA values reported at intervals set on the control box for "live" feedback of run

Step 4: Terminate the experiment

Step 4.1: Click the "Stop" button in the FileMaker interface

Step 4.2: Turn off the ODIn control box and remove Balch tubes

Step 4.3: Take second manual OD_{600} measurement for 2-point calibration

Step 4.4: Enter metadata under the appropriate experiment using the FileMaker interface

Step 5: Analyze data for desired parameters

243

244 **Cultivation and data analysis.** Culture media were prepared as previously described (Lim et

245 al., 2014) containing 7.5 mM lactate and 5 mM sulfite, 30 mM lactate and 15 mM sulfate, and 10

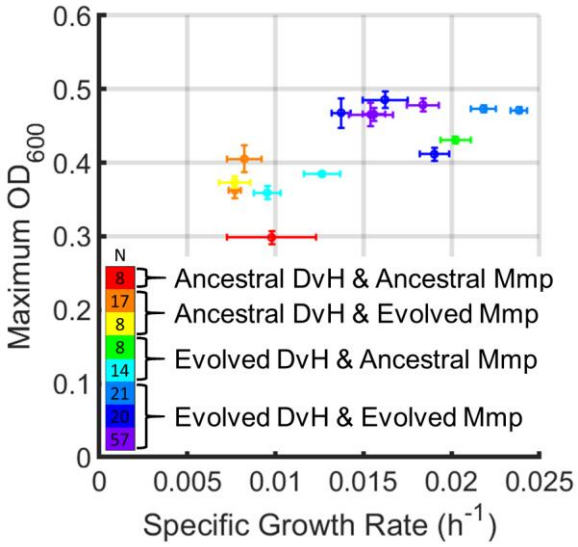
mM acetate or 30 mM lactate to facilitate growth of *D. vulgaris* and *M. maripaludis* monocultures and co-cultures, respectively. Balch tubes were filled with between 10-mL and 20-mL of anaerobically prepared liquid media with a headspace of 80% N_2 /20% CO_2 for both *D. vulgaris* and syntrophic cocultures and 80% H_2/20% CO_2 pressurized to 30 psi for *M. maripaludis* cultures. Replicate culture tubes were inoculated with cultures recovered from freezer stocks as previously described (Hillesland and Stahl, 2010) and grown at 37 °C with continuous shaking at 300 rpm. Sensor readings (mA) collected at 20-minute intervals were converted to optical density (OD_{600}) by relating a spectrophotometric measurement taken for each culture tube at run completion with the final mA value. Growth kinetics were analyzed using the logistic fit option of the grofit (Kahm et al., 2010) packages developed for R-project (R version 3.2.3, https://www.R-project.org/).

Results and Discussion

The utility of the system was evaluated using comparative analysis of available cultures previously shown to exhibit minor to significant differences in growth rate and yield. Using this sensor design the ODIn system can characterize growth patterns with a precision and sensitivity difficult to achieve by manual reading (Fig. 6). The system was also used to examine changes in growth kinetics associated with the evolution of a simple microbial mutualism established between *D. vulgaris* Hildenborough and *M. maripaludis* S2 (Turkarslan et al., 2021). Prior studies have shown that this forced syntrophic mutualism, based on interspecies hydrogen transfer, improved rapidly through adaptive evolution, increasing several fold in growth rate and yield within a few hundred generations of initial pairings (Hillesland and Stahl, 2010). Ongoing

analysis of mutations that have accumulated in replicated cultures suggests that both common and divergent patterns of mutation accumulation in different populations are associated with growth improvement. For example, many *D. vulgaris* in replicated evolution lineages lost the ability for sulfate respiration due to nonsense mutations in genes coding for sulfate activation and reduction to sulfite (Turkarslan et al., 2021).

Resolving the contributions of those and other mutations to community improvement has been complicated by the large number of mutations present at low frequency in each evolution lineage reflecting by the emergence of multiple genotypes of evolved *D. vulgaris* and *M. maripaludis* populations (Hillesland et al., 2014). In order to identify mutations within a single evolved genotype contributing to community growth improvement, clones of evolved *D. vulgaris* and *M. maripaludis* at different generations of evolution were isolated from different replicated evolution lineages. Growth kinetics of individual clones in monoculture, as well as when syntrophically paired within and between different evolution lineages were measured using the ODIn system (Fig. 7). Unlike the highly reproducible kinetics of growth in monoculture (Fig. 6), growth was much more variable when individual evolved organisms were paired, presumably reflecting minor differences in initial conditions. Thus, analysis of many replicates, as was made feasible with the ODIn system, was essential for resolving minor differences in growth kinetics.



288

289 **Fig. 7.** Growth parameters for the slow growing *Desulfovibrio vulgaris* Hildenborough (DvH)
290 and *Methanococcus maripaludis* S2 (Mmp) cocultures were determined for different ancestral
291 and evolved strains indicating fitness benefits of coevolution challenging to resolve through
292 manual measurements. Colors indicate different combinations of strains and error bars indicate
293 one standard error of measurement with number of replicates (N) indicated.

294

295 The ODIn system automates the collection of optical density readings over the extended growth
296 periods of days for these slow growing cultures, eliminating the constraints of experimenter
297 fatigue and variability of performing manual measurements. An exemplary growth curve of *D.*
298 *vulgaris* using ODIn data and manual measurements over 26 hours demonstrates the agreement
299 of the new device and traditional methods (Fig. 6B). Thus, in addition to a much greater
300 capacity for multiplexing than other available systems for monitoring the growth of anaerobes,
301 the current format fully replicates that of established manual methods. The format also opens
302 other developmental opportunities, for example implementing tunable wavelengths in the device

for monitoring of optical density at more specialized wavelengths, such as 550, 650, and 660 nm, designed to avoid confounding signals from substrate or products or optimized to differentiate biomass components. The highly replicated collection of growth data made feasible by the ODIn system is now providing an essential foundation to identify mutations and combinations of mutations contributing to improved mutualistic growth of the evolved co-cultures. More generally, the system offers broad utility for microbiological studies quantifying the growth kinetics of fastidious slow growers and those dependent on gaseous substrates for growth, such as methanotrophs and Knallgas bacteria, and those dependent interspecies exchange of gaseous metabolites as examined in this demonstration of the ODIn system operation.

Acknowledgements

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Supplementary Information

Sensor Circuit Design. The sensor system for each culture tube contains a snap-in mount 880-nm LED, a paired phototransistor and a four-section amplifier bias circuit (Fig. S1), which together are used to measure signal attenuation resulting from increasing culture turbidity. One section of this circuit uses a 2.501 VDC power supply to deliver a constant 833.67 μA current to the LED. This constant current supply to the emitter provides constant light output throughout the experiment. The phototransistor produces a direct electrical current, proportional to the amount of light being transmitted through the culture tube by the LED. A clear culture tube would cause the phototransistor to produce a large electrical current, whereas a very turbid culture tube would result in only a small electrical current being generated. Each LED/phototransistor pair in the device is characterized and selected so that no more than 2.48 mA of current is generated for a tube of uninoculated medium and at least 0.54 mA is generated for a culture of 1.350 OD_{600} (the highest density for the target experiments). A transresistance amplifier converts each 1 mA of current input to a 1 VDC + 2.501 VDC output. The added 2.501 VDC is then immediately removed in the next stage of the analog circuit to provide electrical isolation from power supply fluctuations. The signal at this stage of the analog circuit is 1 VDC for every 1 mA of current generated in the phototransistor. The signal is then amplified, and finally subtracted from the calibration voltage set for that sensor at the start of each experiment. This adjustment is done both to maximize sensor resolution by generating a signal that accommodates the range of voltages read by the 16-bit 5 VDC analog to digital converter (ADC), as well as to invert the signal so that small and large ADC values correspond to low and high turbidity, respectively. Between the sensing circuit and the ADC, an eight-channel multiplexer is used to sequentially measure each of the eight cultures in each tube

rack. Suppliers and components used are described in the supplemental file "Notes_on_Materials".

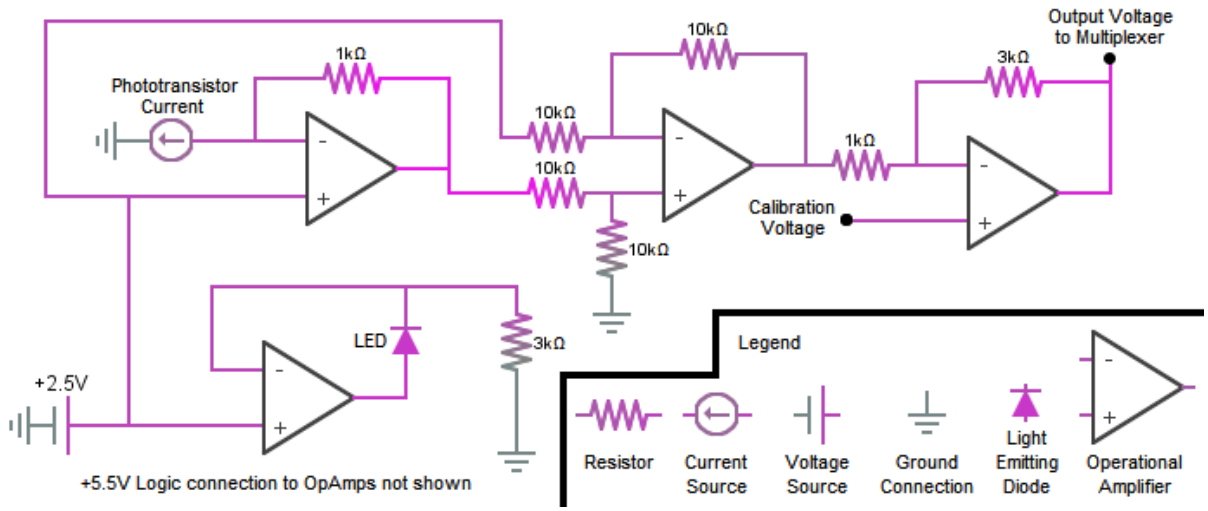
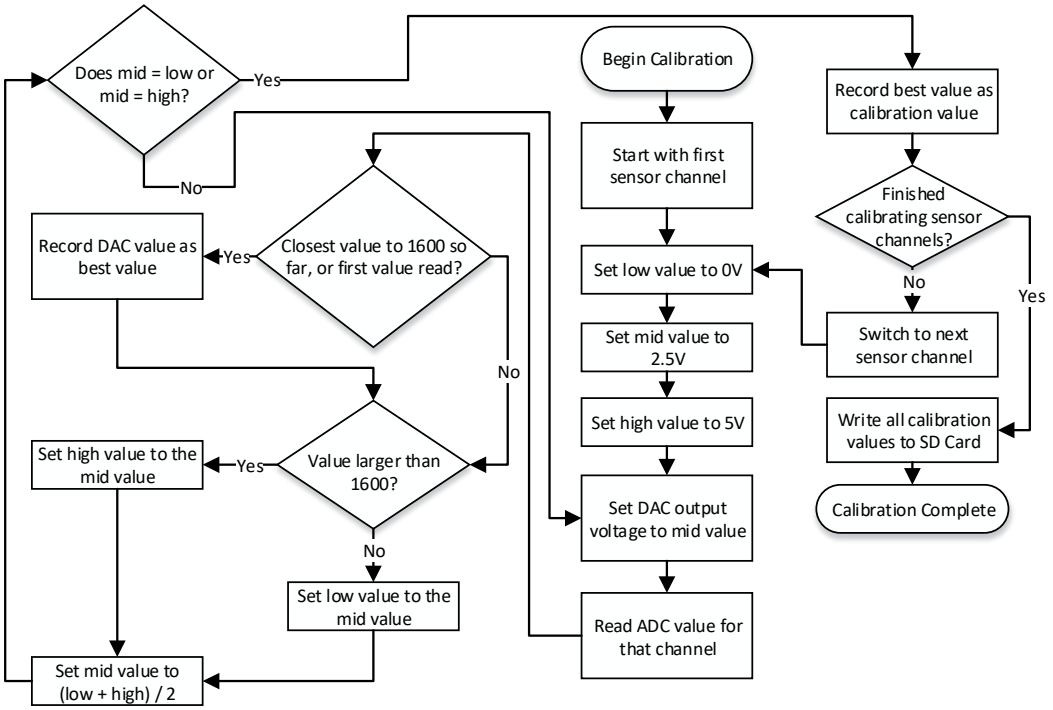


Fig. S1. Circuit Diagram for a single sensing channel. The emitter is powered by a constant current to maintain consistent illumination of the tube sample. The resulting current produced by the sensor is converted to a voltage, isolated from any noise polluting the signal, and then inverted and adjusted by the calibration voltage before being fed into the ADC for measurement.

Sensor calibration. At initiation of each growth study, all 64 LED/phototransistor sensors are powered and allowed to reach a stable operating temperature by maintaining the system in the "Paused" state for a minimum of four hours (overnight is recommended). The inoculated culture tubes are then secured in tube racks and each sensor calibrated independently against its sample tube, with the objective of adjusting sensing circuit outputs to be near 0.3 VDC, corresponding to an ADC value of approximately 1600. By calibrating all sensors to begin each growth study at an output of 0.3 VDC, a small buffer is provided to compensate for possible small decreases in OD_{600} at the beginning of operation. The calibration voltage for a sensing circuit was set using a digital to analog convertor (DAC) with a programmable range of 0 VDC to 5 VDC. Calibration begins with the DAC set to provide half of its maximum voltage (2.5 VDC) to the sensing circuit

being calibrated, and the ADC value of the sensor circuit output is recorded. If the ADC value is lower than the target of 1600, then the final calibration voltage cannot be lower than the voltage that was just tested, and the DAC is then reprogrammed to provide a voltage that is halfway between the previous voltage tested and the maximum possible voltage for this calibration. A similar adjustment is made if the ADC value is higher than the target of 1600. Once the adjustment to the calibration voltage being tested is made, the ADC value is once again measured and recorded. Calibration continues in this manner until the minimum and maximum possible calibration voltages converge on a voltage that results in a sensor output that corresponds closest to the target ADC value of 1600. Following this calibration, the value used to program the DAC to the identified calibration voltage for the sensor circuit is stored in program memory so that the calibration voltage for each sensing channel can be set during each measurement taken during the growth study (Fig. S2). The final ADC values following calibration are sent to the data collection server as the first data points. Individual sensor sets commonly calibrate to consistent beginning ADC values over the course of many experiments, therefore deviations from usual calibration values can be used to identify faults occurring on a specific sensing channel.



414

415 **Fig. S2.** Program logic for calibrating each sensor channel. Each sensor is calibrated in series
416 until the ADC reports the value closest to 1600, within the range of voltages available for the
417 DAC to input into each sensor channel.

418

419 **Data collection.** The ODIn database archives the data generated by the ODIn system hardware
420 and facilitates its analysis. The database, developed with FileMaker Advanced version 14 and
421 hosted by FileMaker Server v14, operates independently from the ODIn hardware. FileMaker
422 Advanced is a cross-platform, relational database development application published by
423 FileMaker, Inc (Santa Clara, CA). The FileMaker Server hosts databases created with FileMaker
424 Advanced making them available to multiple simultaneous users via FileMaker applications for
425 Macintosh, Windows, iOS, or browser-based clients. At its top level, the database is organized
426 by individual experiments. Each experiment corresponds to a single run of the ODIn hardware

with sensing channel output data collected for each of the 64 Balch tubes. Metadata for each experiment includes experiment name, description, start and stop timestamps, and the number of measurements collected. This information is displayed in the database's experiment view. Each experiment in the ODIn database is organized by rack, and each of the eight racks can be given additional descriptive metadata as inputted by the user. In the database structure, each of the eight racks is divided into records for each of its eight Balch tubes. More detailed metadata can be entered for each tube, including description, media composition, electron donor, electron acceptor, and organism(s). In addition, a notes field is available for each tube, allowing essentially unlimited text entry. Associated with the notes are three image fields, which can be used to store photographs or other images related to a tube. A variety of auto-fill tools have been created to facilitate the user entry of metadata for each of the 64 culture tubes. These tools include functions for replicating a tube's metadata entry, with automatic sequential numbering and a "fill-down" button, which will copy the current tube's metadata to the remaining tubes in the rack. A clone of FileMaker interface and database containing presented data is available at 10.5281/zenodo.4646431.

Ease-of-use was a paramount design concern, with the intention of achieving greater accuracy and metadata entry completion. The ODIn database's rack view (Fig. S3) is designed to quickly show the metadata for each tube in a rack. Many experiments include biological replicates, in which two or more tubes may be inoculated from the same sample. While it can be valuable to view results for each replicate individually, it is preferable to average the results of replicates and display them as one. The ODIn database simplifies the grouping of replicates together and a color-coding system makes the replicate-grouping apparent to the viewer. Up to 32 replicate

groups can be created for each experiment. When working in the ODIn database experiment view, clicking the right-arrow button associated with an experiment triggers two events. If the experiment is still running, the ODIn database will query the data collection server to determine if new data is available and if so, that data will be imported. Subsequently, or if the experiment has already been completed, ODIn will switch to a summary view, showing data for all 64 tubes, organized into eight graphs, one for each rack. The ODIn database can graphically display the summary of results, organized by rack, a single rack's results, a single tube's results, or the results of any combination of tubes and replicate sets from a single or from multiple experiments. Additionally, a user can choose between graphs and specify filters that restrict the graphs to specific time intervals or that specify minimum and maximum values for OD_{600}, mA, or growth rate. Any combination of filters may be applied simultaneously, including removal of spurious reading and linear vs log axes. The growth rate graphs display the change in growth rate over time and the values are averaged using a user-selectable number of readings before and after each time point. Finally, the ODIn database includes an export tool that allows one-click exportation of raw data, OD values, or mA values to save on a local machine as a .CSV file for manual data analysis.

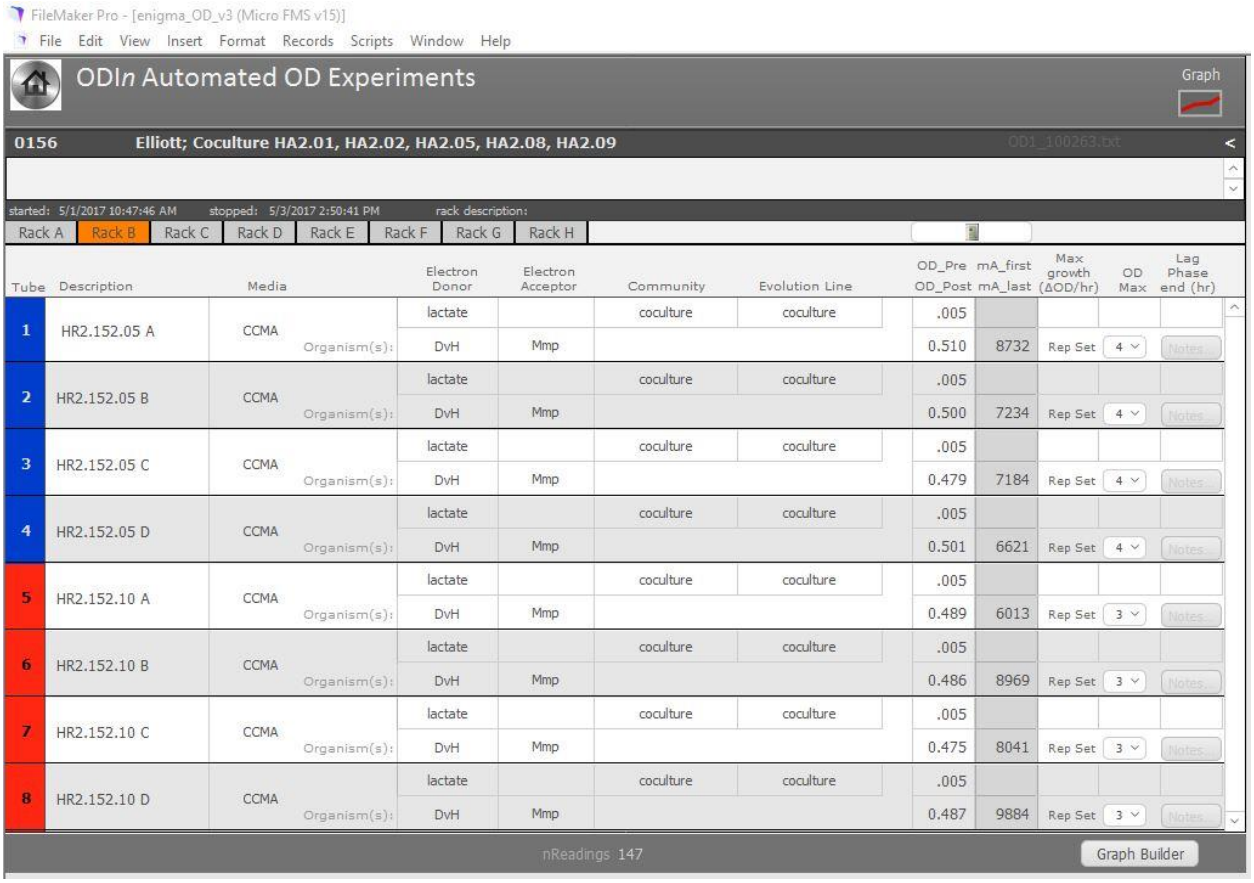


Fig. S3. Tube rack sample metadata display. Selectable tabs detail information for all tube rack channels for a complete eight channel rack. Entry fields are provided for sample description, media composition, organism(s) grown, electron donor, electron acceptor, initial OD_{600}, Final OD_{600}. Row headings are selectable for grouping by assigning colors to those within a group.

The FileMaker algorithms and database constructed for use with the ODIn apparatus is available at 10.5281/zenodo.4646431. The data server software is available at 10.5281/zenodo.4619754.

Commercially assembled system components. Atmel ATmega2560V microcontroller in an Arduino Mega 2560 and Arduino Ethernet Shield (Adafruit Industries, LLC New York, NY) are used for sensor operation, sampling protocol, data acquisition, data reporting and mechanical

operations. One IEC 60320 C13 120 VAC female panel mount connector (Qualtek Electronics Corp., Mentor, OH) is provided for AC power supply and one NEMA 5-15P 120 VAC outlet (Molex LLC, Lisle, IL) connected to a G2R PCB mount relay (Omron Corporation, Kyoto, Japan) for shaker table activation/deactivation. One L298 H-Bridge dual bidirectional motor controller (Canakit Corporation North Vancouver, BC, Canada) is used for operation of an HDA4-30 4-inch stroke linear actuator (Servocity, Winfield, Kansas). A Delta Electronics PMT-12V100W1A 100 W AC/DC converter (Delta Electronics, Inc., Taoyuan, Taiwan) is used to provide 12 VDC power for the linear actuator power source, and to be down converted for use by the system. Two Texas Instruments PTN78060WAH DC-DC converters (Texas Instruments Incorporated, Dallas, Texas) take 12 VDC and supply 5.2 VDC power for the sensor circuit and PCB power source, and 2.501 VDC to drive the emitters and provide a reference voltage for power isolation. ADS1115 16-Bit 4-channel Analog to Digital converter and MCP4725 12-bit Digital-to-Analog converter (Adafruit Industries LLC, NewYork, NY) are used to take measurements and provide calibration voltages, respectively. A full list of all materials used is available in the bill of materials file titled “Notes_on_Materials.xlxs”. Circuit boards production files are available for both the control box board and tube box board as “Control Board mark 1.pcb” and “Tube_Rack_Boards.zip” respectively. Electrical connections between all control box system components were made using solid core 22AWG hookup wire and detailed in the available file “ODIn Control Box Pinout.xlsx”.

Tube rack custom printed circuit board. Tube rack PCBs were manufactured by ExpressPCB.com using the digital file “OD_mark4_revision_2.pcb” (Fig. S4). The following components were used to complete the assembly; 10K ohms resistors, 3K ohms resistors, 1K

ohms resistors (Susumu International USA, Palisades Park, NJ), opamps (Microchip Technology Inc., Chandler, AZ), female DE-9 connector (TE Connectivity Ltd., Schaffhausen, Switzerland), and 8-channel multiplexer (Texas Instruments Incorporated, Dallas, Texas). Resistor placement is described in Table S1 and Fig. S5. Each opamp is placed on the PCB to position the circle indicator (see Fig. S6) to the closest edge of the circuit board relative to its solder pads. All eight opamps are positioned in the same orientation. The multiplexer is placed in a position to locate the gold bar indicator on one short edge of the chip (see Fig. S7) closest to the silkscreen "Output Test" on the board. Terminal blocks (Phoenix Contact USA, Middletown, PA), as delivered, are block sets of two separate spring-loaded wire connectors; red and white. Sets of four blocks are connected by removal of the red baffle plate on the red terminal block, revealing molded attachment pins, and connected to another terminal block of two by the newly exposed pins. This action is repeated until a set of a total of eight blocks is fabricated. This set of eight blocks now has 16 separate wire connectors and can be attached to the circuit board by pacing the wire leads on the bottom of the terminal blocks through holes proximal to the opposite board side of the PCB. The terminal block assembly wire input holes face the resistor array on the PCB. The female DE-9 serial connector is placed in the middle of the PCB with the serial cable connector side facing out towards the opamps. All connections were made using 60/40 solder. Tube rack circuit boards were also printed and assembled at Technical & Assembly Services Corporation (TASC, Seattle, WA) using the files ("OD_mark4_revision2") and bill of material ("BOM_of_OD_mark4_revision2") provided.

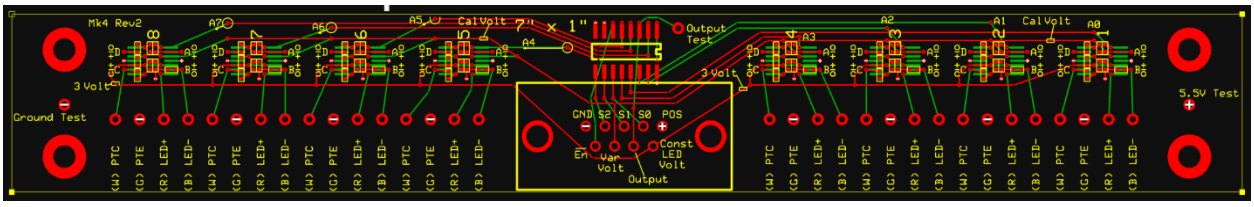


Fig. S4. Tube rack PCB. Front side layout of tube rack PCB with silkscreen text displayed. PCB produced from "OD_mark4_revision_2.pcb" file and assembled from the components listed on the "Tube Rack PCB" sheet of the "ODIn_BOM.xlsx" file.

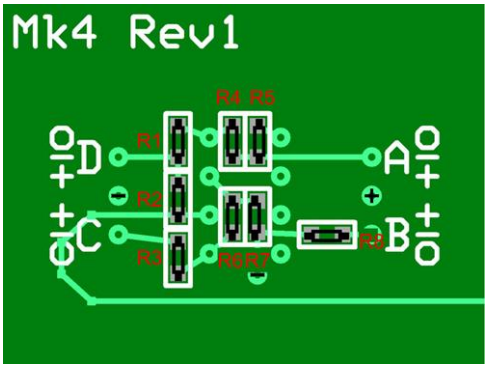


Fig. S5. Resistor placement for each sensing channel circuit on Tube Rack PCB. Resistor values and part numbers are provided on the "Tube Rack PCB" sheet of the "ODIn_BOM.xlsx" file and the assembly placement detailed in Table S1.

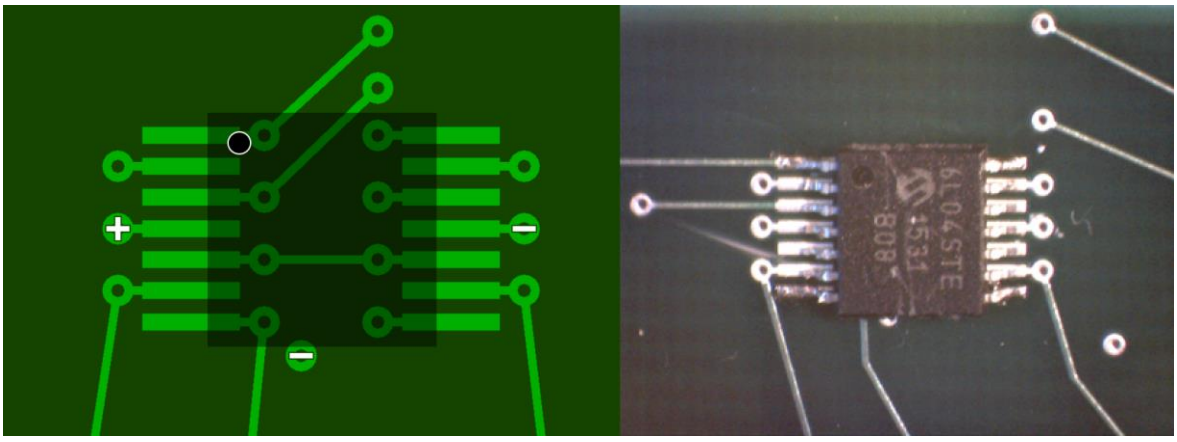


Fig. S6. Opamp solder Tube Rack PCB placement. Sixteen leads from the opamp are soldered on to each of the eight solder pads on the Tube Rack PCB. Circle indicator on top side of opamp is oriented toward the closest edge of the PCB.

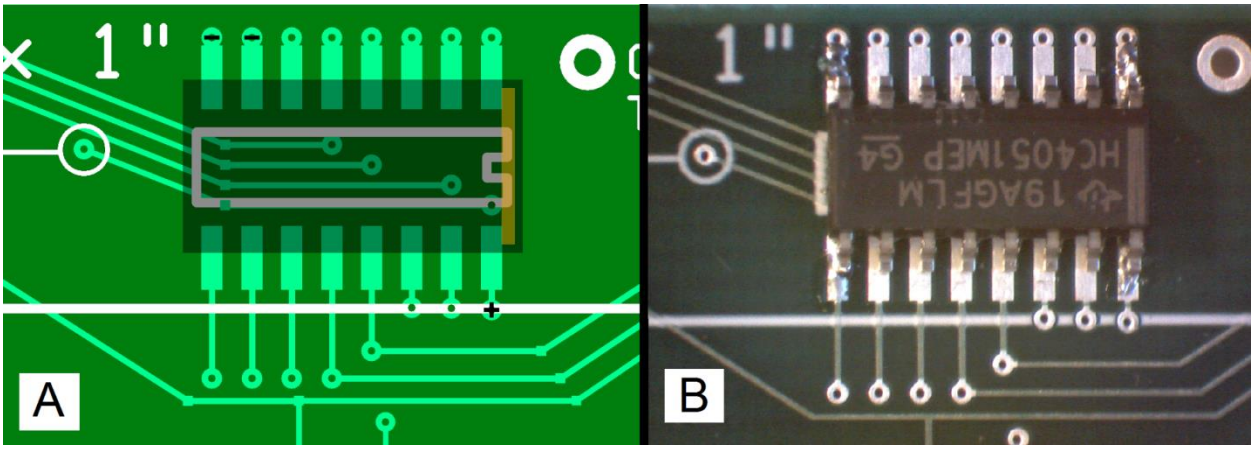


Fig. S7. Multiplexer placement on Tube Rack PCB. Sixteen leads from multiplexer are soldered to the single centrally located solder pad on each Tube Rack PCB. Multiplexer is oriented with the gold bar indicator oriented opposite the "7" x 1"" silkscreen text.

Table S1. Susumu (Palisades Park, NJ) resistor placement on Tube Rack PCB

Resistor	Ω Value	Part Number	Manufacturer
R1	10K	RR05P10.0KDDKR-ND	Susumu
R2	10K	RR05P10.0KDDKR-ND	Susumu
R3	1K	RR05P1.0KDDKR-ND	Susumu
R4	1K	RR05P1.0KDDKR-ND	Susumu
R5	3K	RR0510P-302-D	Susumu
R6	10K	RR05P10.0KDDKR-ND	Susumu
R7	10K	RR05P10.0KDDKR-ND	Susumu
R8	3K	RR0510P-302-D	Susumu

LEDs and phototransistors are connected to the terminal blocks by securing the leads to the terminal blocks in the order specified by the silkscreen on the PCB. Phototransistor (OPB-100Z) leads are green and white and connected to "(W) PTC" and "(G) PTE" labeled terminal blocks.

LED (OPB-100) leads are red and black and connected to the "(R) LED+" and "(B) LED-" labeled terminal blocks.

Control box custom printed circuit board. A single control box PCB was manufactured by ExpressPCB.com using the digital file "Control board mark 2.pcb". Assembly of the circuit board uses the components listed in the "Control Box" tab of "Notes_on_Materials.xlsx". Components are placed according to the location specified by the silkscreen text on the PCB face. Unlike the Tube Box PCBs, the Control Box PCB identifies the component to be soldered to the board at each solder pad and through hole. Silkscreen text details the specific component to attach by its type and electronic value all of which are detailed in the spreadsheet. D-sub serial connectors were attached to the PCB by socket cap screws and nuts from the screw set on the "Tube Box Board" sheet of "Notes_on_Materials.xlsx".

Construction of tube racks and control box housing with interface components. All eight tube racks were machined using a CNC mill (Bolton Tools Corp. Cerritos, CA). The tube rack was designed to accommodate up to eight Balch tubes having dimensions of 18x150mm. All tube racks and tube rack cabinet were made using the submitted CAD file schematics; "tube_rack_base_opp.dxf", "tube_rack_base_cover.dxf", "tube_rack_base.dwg" and "tube_holder_assembly.dwg".

Placement of interface components (switches, knobs, and screen) was done without the use of a guide for specific placement but rather spaced evenly around the centrally positioned system status LCD screen located on the lifting lid of the control box. Component placement in this manner allowed unencumbered access to the underside of the interface components for attaching and soldering wire leads. Cutouts for attaching components to the acrylic were made using a CNC mill and cut to the specifications listed on the component datasheet provided with their delivery. The acrylic was cut with a rotary cutting tool to provide access to the Arduino stack interfaces (Arduino Mega 2560, SD Data Logging Shield, and Arduino Ethernet Shield (Adafruit Industries, LLC New York, NY)). The lid for the control box was attached to the box using the continuous hinge and secured using countersunk 3/16" sheet metal screws. System status LCD screen was attached to the lid using the brass screws provided with its delivery. Placement of the components in the interior of the control box was done using metal standoffs secured to the base of the box interior (Fig. S8). Components secured in this manner were the 12 VDC power supply, 120 VAC relay, and the Arduino stack. The Control Box PCB was secured inside the control box by attachment through the acrylic side wall of the right-angle D-sub 9 pin connectors using their provided mounting screws.

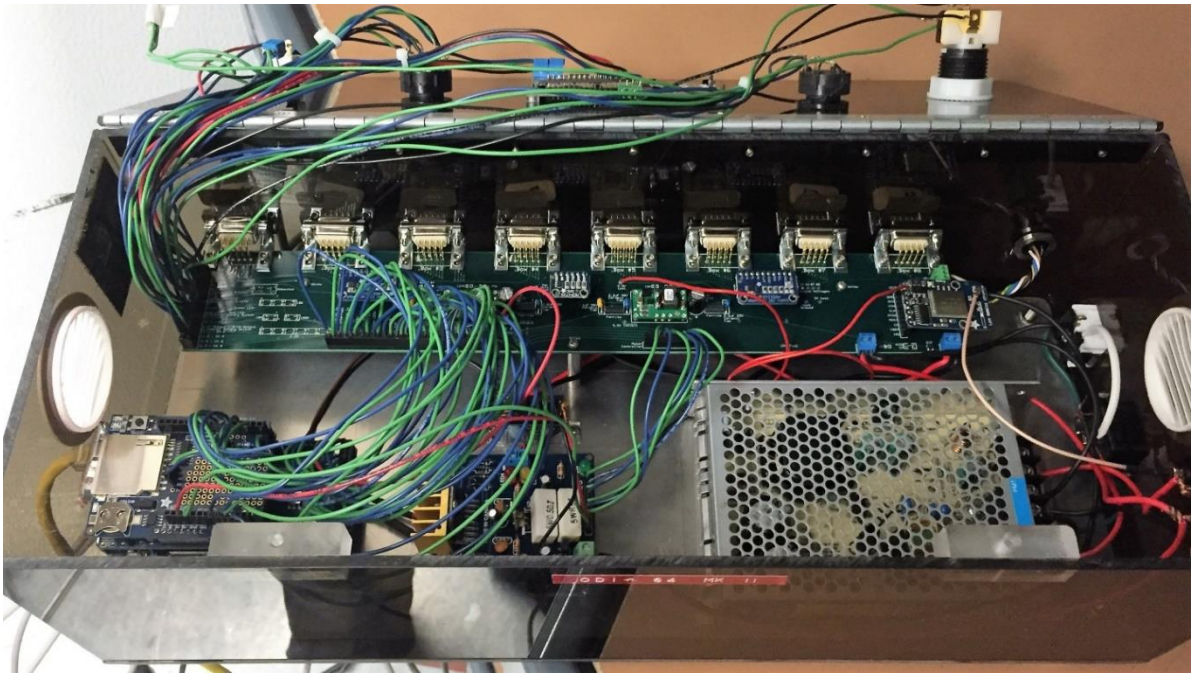


Fig. S8. Control box with lid open showing placement of all control box components.

Control box wiring. Solid core 22 AWG hookup wire was used for control box wiring. All components (motor controller, control box PCB, Arduino Mega, and SD Data Logging Shield) were connected to the PCBs using the header connectors (Sullins Connector Solutions, San Marcos, CA). The Canakit motor controller was modified by removal of the right-angle male pin header set and replacement with a straight through female header connector. All header connector positions were wired to specified components according to the connection guide in the file "ODIn Control Box Pinout.xlsx". Connections from the control box to the eight tube racks used 10' DE-9 M/F serial cables.

Construction of raised platform. The raised platform used to secure the tube rack to the New Brunswick orbital shaker was constructed according to the follow specifications. The dimensions of the box are 18" l x 18" w x 5" h. Side walls of the raised platform were made from ½" thick aluminum stock cut to lengths of 18" x 4" and 17" x 4" and assembled using countersunk sheet metal screws to achieve the 18" x 18" square frame. The base of the platform was produced by attachment of an 18" l x 18" w x 1/2" d sheet of stainless steel to the aluminum walled frame using countersunk stainless-steel screws. The lifting lid of the platform was constructed from an 18" l x 18" w x 1/2" d sheet of transparent cast acrylic and attached to the base of platforms aluminum wall frame using a continuous hinge secured to the acrylic and aluminum sidewall by self-tapping sheet metal screws.

Linear actuator and scissor hinge lifting mechanism. The linear actuator is positioned inside the raised platform opposite to the lifting edge of the acrylic lid with its stationary end attached by a linear actuator pivoting mounting bracket (Fig. 4 of the main text). To raise the lid via the linear actuator a scissor-hinge mechanism was manufactured from stainless steel stock. 3-mm stainless steel sheets were cut into rounded-end rectangular strips measuring 160-mm in length. Hinge mechanism was generated by drilling 4-mm holes in each end of the rounded-end rectangular steel strips and attaching two strips to each side of the linear actuator piston rod through hole using a 40-mm x 4mm threaded-end pivot pin and securing with a lock nut with wave washers and bushings positioned between each metal-metal contact. Aluminum cubes (27-mm) were machined and attached to the interior front edge of the lifting platform and lifting edge underside of the acrylic lid and used to attach the opposite end of each rounded end rectangular strip using 40-mm threaded end pivot pins, wave washers, bushings, and lock nuts. Anchoring

the steel strips to the platform interior base, the piston rod of the linear actuator, and the underside of the lifting platform acrylic lid (using pivot pins, bushings, and wave washers) produces the lifting action of linear actuator extension. The linear actuator is connected to the 5-pin Conec threaded connector (American CONEC Corporation, Garner, NC) using the datasheet delivered with the device and connected to the control box for operation.

Arduino software, code, and libraries. The “arduino-1.0.6-windows.exe” development environment was used to program operation of the Arduino Mega microcontroller. ODIn software project-specific libraries were developed for operation of the ADC, DAC, and LCD in place of libraries provided by the stock Arduino development environment, which if used will be incompatible with the ODIn software. The following actions are necessary to allow the programs to compile, and load on the Arduino: Locate where the Arduino program is installed on the computer communicating with the Arduino Mega (default 64-bit Windows location is “C:\Program Files (x86)\Arduino” Installation to the standard Program Files folder might be required on a 32-bit machine). Locate in that "Arduino" folder containing the Arduino software, re-name the existing "libraries" folder to "stock libraries" and from the provided .zip file, "NECESSARY LIBRARIES", unzip and copy the "libraries" folder found there to the "Arduino" folder where the Arduino software was installed. Finally, restart the Arduino sketch program and the software should compile with the newly installed libraries. All libraries and software for operation of the ODIn apparatus is available at 10.5281/zenodo.4663185.