

1 **A low-cost DIY device for high resolution, continuous measurement of microbial**
2 **growth dynamics.**

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22

23 **ABSTRACT**

24 High-resolution data on microbial growth dynamics allow characterisation of
25 microbial physiology, as well as optimisation of genetic alterations thereof. Such data
26 are routinely collected using bench-top spectrophotometers or so-called plate readers.
27 These equipments present several drawbacks: (i) measurements from different devices
28 cannot be compared directly, (ii) proprietary nature of devices makes it difficult for
29 standardisation methods to be developed across devices, and (iii) high costs limit
30 access to devices, which can become a bottleneck for researchers, especially for those
31 working with anaerobic organisms or at higher containment level laboratories. These
32 limitations could be lifted, and data reproducibility improved, if the scientific
33 community could adopt standardised, low-cost and open-source devices that can be
34 built in-house. Here, we present such a device, MicrobeMeter, which is a do-it-
35 yourself (DIY), simple, yet robust photometer with continuous data-logging
36 capability. It is built using 3D-printing and open-source Arduino platform, combined
37 with purpose-built electronic circuits. We show that MicrobeMeter displays linear
38 relation between culture density and turbidity measurement for microbes from
39 different phylogenetic domains. In addition, culture density estimated from
40 MicrobeMeter measurements produced less variance compared against three
41 commercial bench-top spectrophotometers, indicating that its measurements are less
42 affected by the differences in cell types. We show the utility of MicrobeMeter, as a
43 programmable wireless continuous measurement device, by collecting long-term
44 growth dynamics up to 458 hours from aerobic and anaerobic cultures. We provide a
45 full open-source description of MicrobeMeter and its implementation for faster
46 adaptation and future development by the scientific community. The blueprints of the
47 device, as well as ready-to-assemble kit versions are also made available through
48 www.humanetechnologies.co.uk.
49

50 **INTRODUCTION**

51 One of the key measurements in microbiology, and the associated fields of systems
52 and synthetic biology, is the growth rate of individual microbial species. This
53 measurement provides qualitative confirmation on the types of substrates and
54 conditions a microbe can grow in, and can be used to infer quantitative growth rates
55 under such conditions (1, 2). The resulting information is essential for building
56 predictive models of microbial growth and understanding the impact of genetic or
57 environmental alterations, as well as the optimisation of media conditions or genetic
58 modifications. Therefore, measurement of microbial growth dynamics is utilised in all
59 microbial, synthetic and molecular biology laboratories, and within the
60 biotechnological and biomedical industries.

61 For the actual measurement of growth dynamics, scientists generally use
62 bench-top spectrophotometers or so-called plate readers (which implement similar
63 optics as spectrophotometers). These devices are used in measuring cell cultures'
64 absorption, typically at a wavelength of 600nm, and the resulting values are reported
65 as culture 'turbidity' or 'optical density (OD)'. Technically, however,
66 spectrophotometers are designed to measure light absorption of small molecules,
67 where the measured absorption value relates linearly to the concentration of the
68 molecule and the path length of the light through the container, as captured by the
69 Beer-Lambert law (3). It is well-known that this law does not fully apply to large
70 colloids suspended in liquid, such as microbial cells, as these larger particles can
71 display both absorption and scattering of light (4–6). Thus, reported turbidity values
72 are not absorption values, but rather a combination of absorption and scattering of
73 light through the cell culture (4, 5, 7). The ratio between scattering and absorption of
74 cell cultures can be affected by various factors such as cell size, type of cell
75 membrane, growth medium, cell density, path-length, and the presence of pigments
76 (5, 7–9). The scattering effects can also interact with the specific optical design of a
77 given spectrophotometer, producing differential effects on the resulting measurements
78 between spectrophotometers with different optical designs (4, 5, 7, 10, 11). As
79 expected from these considerations, turbidity measurements of the same cell culture
80 samples using different spectrophotometers are found to have significant variability
81 (sometimes more than 30%) (5, 10, 11). The relation between the turbidity and cell
82 concentration, while linear, is also found to vary from device to device (5–7). These
83 results show that both reported turbidity values and the turbidity-based calculation of
84 microbial growth rates are device specific.

85 This presents a significant challenge for reproducibility. In practical terms,
86 any experiment requiring exact modulation of microbial culture concentration cannot
87 be accurately reproduced, unless one uses the same spectrophotometer that is used to
88 generate the original concentration information. One possible solution to this
89 challenge is to 'calibrate' all spectrophotometers against a chosen standard solution.
90 This standard solution could be used for calculating the ratio between the turbidity
91 measurements obtained using two different spectrophotometers, and then applying
92 that ratio as a conversion factor between the devices. In practice, however, such
93 conversion factors are found to be highly specific to individual samples that are used
94 for obtaining them. For example, the conversion factor obtained from a standardised
95 solution, such as the McFarland standard (12), is not applicable in the case of
96 corrections required to be applied when measuring cell cultures (5, 7, 10, 11). This
97 creates a practical difficulty in establishing conversion factors between different
98 devices, where different conversion factors would need to be calculated among all
99 devices and on different samples.

100 An alternative approach to achieving standardisation and reproducibility of
101 microbial growth measurements would be for the scientific community to agree on
102 the use of a single photometric device for this measurement. This would allow
103 collection of data under the same optical design, and thereby reducing the
104 reproducibility problems that are associated with device-to-device variability. For it to
105 be adaptable by as many research groups as possible, such a ‘universal’ turbidity
106 measurement device should ideally be simple in construction, low-cost, open source,
107 and robust. These properties are not met by current bench-top spectrophotometers,
108 and the more high-throughput plate readers, as these are relatively high cost devices
109 and their manufacturing and optical design details are proprietary. In addition, the
110 optics of these devices are optimised for purposes other than just monitoring cell
111 culture dynamics, increasing the design complexity (and possibly the effects of
112 scattering). A simple, low-cost and open-source device designed solely for cell
113 growth measurement could thus allow increased reproducibility of experiments
114 relating to microbial growth and also open-up scientific analyses to larger groups of
115 people through reducing the cost of required scientific instrumentation, while
116 supporting the philosophy of do-it-yourself (DIY) science (13, 14).

117 Here, we develop such a DIY device for automated monitoring of microbial
118 growth dynamics under both aerobic and anaerobic conditions. This device, called
119 MicrobeMeter, is developed using open-source Arduino and 3D printing technology
120 and software, resulting in an estimated single-unit production cost under £150. These
121 specifications and the modular nature of MicrobeMeter’s design are expected to allow
122 further development and optimisation of the device by the research community, as
123 well as its possible adaption as a ‘universal’ turbidity measurement device. We
124 demonstrate the feasibility of MicrobeMeter to capture microbial growth dynamics by
125 measuring the turbidity of the serial dilutions of three different organisms: a
126 facultative aerobe (*Escherichia coli*), a heterotroph (*Shewanella oneidensis*), and a
127 yeast (*Schizosaccharomyces pombe*). MicrobeMeter produced linear measurements
128 ($R^2 \geq 0.99$) for the dilution series of all these cultures, and the slopes of the
129 measurements were ~92% close to the calculated theoretical slopes. Compared
130 against commercial bench-top spectrophotometers, MicrobeMeter measurements
131 produced the least variance in slope, indicating that the turbidity measurement of the
132 device is less affected by the differences in cell types. In addition, the device-to-
133 device variability among MicrobeMeter replicates was very low, indicating
134 robustness towards manufacturing errors. We also demonstrate the use of
135 MicrobeMeter as a continuous measurement device under aerobic and anaerobic
136 conditions, by collecting long-term growth dynamics of *E. coli* (under aerobic
137 conditions), and two strictly anaerobic microbes: a sulfate-reducing bacterium
138 (*Desulfovibrio vulgaris*) and a methanogenic archaeon (*Methanosarcina barkeri*).
139 Taken together, these results show that MicrobeMeter could be used as a reliable,
140 simple-to-construct and cost-effective photometer for the turbidity measurements of
141 cell cultures and derivation of kinetic modelling parameters relating to growth
142 dynamics. All information regarding the manufacturing of MicrobeMeter (3D models
143 of device casing, electronic circuits and component list, and software) is made
144 available for personal and academic non-commercial research use through this
145 publication and from a dedicated website (see *Device Availability* section), allowing
146 this simple photometer to be reproduced and adapted by the scientific community.

147
148

RESULTS

149 As the use of complex optics contributes significantly to the disparities between
150 commercial spectrophotometers (4, 5, 10, 11), an ideal universal measurement device
151 should be devoid of complex optics to increase its reproducibility and cost-
152 effectiveness. It is important to note that although spectrophotometers are capable of
153 taking measurements at different wavelengths of light, turbidity measurements of cell
154 cultures are widely conducted using 600nm wavelength (hence, most reports refer to
155 the resulting turbidity measurements as OD₆₀₀).

156
157 **MicrobeMeter has a simple design using low-cost components.** Using the above
158 fact allowed us to simplify the optics in turbidity measurement by using a single
159 601nm light emitting diode (LED), a suitable light-sensor (a silicon photodiode) and
160 two simple apertures as the sole optical components in MicrobeMeter (Figure 1A, and
161 see *Methods*). These components are integrated in a 3D-printed casing, which also
162 acts as a holder for the culture vessel (Figure 1B). The photodiode voltage is
163 measured using a purpose-built, Arduino integrated electronic circuit board that acts
164 as an analogue to digital converter (ADC) with high sensitivity (see below and also
165 *Methods*). Using the Bluetooth compatibility of the Arduino, the acquired data are
166 sent wirelessly to a computer, where they can be further analysed.

167 The current design is built around a standard test tube as the culture vessel,
168 rather than a quartz cuvette (note that the use of quartz cuvettes is primarily for
169 achieving better optics in absorption measurements, which is not the primary
170 measurement in cell cultures as discussed above). The use of a test tube allows for
171 larger culture volumes to be used, and for continuous measurements to be obtained
172 directly during growth experiments (minimum measurement interval is set at 6s; see
173 *Methods*). This approach also easily accommodates anaerobic experiments using
174 sealed test tubes with the dimensions of a standard test tube, such as Hungate tubes,
175 which we used as the standard for our current design (see *Methods*). The tube holder
176 is designed to shield the tube from external light to minimise the interference to the
177 measurement. Two identical apertures were created at the light source and sensor
178 sides. The aperture diameter was set to 2mm, which is possible to achieve with even
179 low-cost 3D-printers. The design of MicrobeMeter consists of ports for four-tubes and
180 accommodates all electronic components including a battery, and it is held in a
181 compact container for further protection (Figure 1B; see also *Methods*).

182
183 **MicrobeMeter is designed for robust turbidity measurements with high signal-
184 to-noise ratio.** To achieve high signal-to-noise ratio and low variability in
185 MicrobeMeter measurements, we implemented: (i) a state-of-the-art amplifier circuit
186 on the Arduino-compatible electronic circuit board, (ii) an averaging function into the
187 data acquisition program, and (iii) an optimised measurement routine that stabilises
188 the analogue to digital converter (ADC) output of the Arduino (see *Methods* for
189 details). The electronic circuit board design implements a chopper-stabilised
190 operational amplifier (15) connected as a trans-impedance amplifier with common-
191 mode rejection and differential input configuration. This configuration offers sensitive
192 measurements that are highly immune to external noises arising from electrostatic
193 coupling (16). This amplifier is set to have a gain of 10,200,000 and an output range
194 of 0-5V.

195 Given this amplifier and optimised measurement routine, we determined the
196 linearity of response in a technical quality control experiment. Using the
197 MicrobeMeter, we took independent measurements on each port, while reducing the
198 LED light intensity from maximum to minimum using Arduino's built-in pulse-width

199 modulation (31372.55Hz) feature. Performed with Hungate tubes containing 10mL of
200 distilled water, these controlled measurements mimicked growth of a biological cell
201 culture (and the resulting reduction in transmittance). Raw light intensity
202 measurements were converted to turbidity values using $-\log(I_t/I_0)$, where I_t is the light
203 intensity at time t , and I_0 is the light intensity at time 0. As shown in Figure 2,
204 measurements collected on each port demonstrated near-perfect linearity, while
205 variability between ports was low (see *Supplementary File 1* for raw values). The raw
206 light intensity values measured by each port was always within measurement range of
207 the device (see *Methods*). To allow users to check their MicrobeMeter unit, this
208 technical quality control experiment is integrated in the data acquisition program (see
209 *Methods*).

210
211 **MicrobeMeter based estimation of cell culture density from turbidity is**
212 **comparable to, or better than, bench-top spectrophotometers.** Having shown the
213 ability of MicrobeMeter to achieve high sensitivity with simulated turbidity
214 measurements of blank samples, we next tested the linearity of response using
215 biological samples. To this end, we used cell cultures of three different organisms that
216 are expected to have different scattering properties: *E. coli*, *S. oneidensis* and *S.*
217 *pombe* (see *Methods*). For each organism, two-fold serial dilutions were prepared by
218 harvesting the cells via centrifugation and re-suspending in standard phosphate-
219 buffered saline (PBS). Each cell dilution series was measured using MicrobeMeter
220 with three tubes containing the same culture sample and one tube containing PBS
221 only (i.e., “blank” measurement) and used for temperature correction (see *Methods*).
222 The same serial dilutions were also measured using three different bench-top
223 spectrophotometers (see *Methods*). For all tested cell culture dilutions, the resulting
224 turbidity measurements were linear both for MicrobeMeter and for the commercial
225 spectrophotometers ($R^2 \geq 0.99$) (Figure 3A). As expected, all devices resulted in
226 different actual values from each other (Figure 3A), confirming that the turbidity
227 measurements cannot be compared between devices (5, 10, 11).

228 The average of the slopes of the three serial dilutions was compared against
229 the calculated slope of the serial dilution, which is expected to be $\ln(2)$ given the two-
230 fold dilutions performed. The averages of the slopes produced by the three ports of
231 MicrobeMeter displayed lower variability compared to slopes obtained from
232 commercial spectrophotometers (Figure 3B). These results show that the turbidity
233 measurements obtained using MicrobeMeter allows estimating cell density as
234 accurately as commercial spectrophotometers and provides less variability across
235 estimates. The latter finding suggests that MicrobeMeter is less sensitive to the
236 differences in scattering caused by the different tested cell types in comparison to the
237 spectrophotometers tested.

238
239 **MicrobeMeter can be used for long-term continuous monitoring of growth**
240 **dynamics under aerobic and anaerobic conditions.** Following on from the
241 measurements of cell culture serial dilutions, we next tested the ability of
242 MicrobeMeter to be used in continuous monitoring of cell culture growth. This
243 overcomes the need for manual sampling and allows automated acquisition of high-
244 resolution growth dynamics. Furthermore, the wireless capability of MicrobeMeter
245 allows it to be placed in an incubator, clean bench, containment or anaerobic chamber
246 without needing to run wires for power supply and data acquisition.

247 To test long-term growth dynamics measurements, we used MicrobeMeter
248 with open or sealed Hungate tubes. We then collected high-resolution turbidity data

249 under aerobic conditions for the facultative organism *E. coli* (Figure 4), and under
250 anaerobic conditions for the strictly anaerobic organisms *D. vulgaris* and *M. barkeri*
251 (Figure 5). Measurement periods for these organisms were approximately 30.5, 75,
252 and 458 hours, respectively, allowing us to collect unprecedented growth dynamics
253 data. For the case of *E. coli*, we compared this high-resolution data with
254 measurements of the technical replicates of the same cultures, obtained using a
255 commercial bench-top spectrophotometer (see *Methods*). Applying an appropriate
256 conversion factor (see *Methods*), the measurements using the commercial
257 spectrophotometer and MicrobeMeter showed near perfect overlap for the *E. coli* data
258 (Figure 4). This demonstrates the reliability and potential application of
259 MicrobeMeter as a continuous turbidity measurement unit to be used in the laboratory
260 setting, under both aerobic and anaerobic conditions.

261

262 **DISCUSSION**

263 Here, we present a low-cost DIY photometer called MicrobeMeter. It consists of
264 LEDs, photodiodes, and a 3D-printed casing that can hold four test tubes, a battery
265 and a purpose-built Arduino-compatible electronic circuit board for signal
266 amplification, noise cancellation, and data logging. We show that MicrobeMeter can
267 perform linear measurements on a range of serial dilutions of microbial cells cultures,
268 and that these measurements are as good as, or better than those obtained from
269 common commercial bench-top spectrophotometers. We demonstrate the utility of
270 MicrobeMeter by collecting long-term continuous measurements of microbial
271 cultures both under aerobic and anaerobic conditions. These measurements can be
272 taken in a shaking incubator or anaerobic cabinet, given the small footprint of
273 MicrobeMeter. The fully disclosed design and construction information of
274 MicrobeMeter bring these features to any researcher or citizen scientist at a cost of
275 around £150, presenting MicrobeMeter as a potentially adaptable device for turbidity
276 measurements by the scientific community.

277 Besides its cost advantage, the open-source and simple nature of the presented
278 device can have significant impact in terms of data reproducibility. Despite the wide
279 spread use of turbidity measurements of microbial cultures, this measurement remains
280 as one that cannot be directly compared between devices (Figure 3) (4, 5, 10, 11).
281 Given its low cost and simplicity, MicrobeMeter, or similar devices, could become
282 ‘universal’ in the sense that they can be employed across many research labs and
283 allow the acquisition of comparable turbidity measurements. To this end, we note that
284 several other DIY-style photometers have also been described. For example, a simple
285 photometer design is implemented on a flow cell for monitoring the turbidity of
286 continuous and/or fed-batch microbial cultures (5). Another simple design was
287 developed for the measurement of flask and/or test tube grown cell cultures (11). The
288 latter device offers also UV-Vis spectroscopy capabilities; however, it makes use of
289 several proprietary components.

290 The presented device, MicrobeMeter, is designed to be cost effective, open-
291 source and customisable. These features allow it to be adapted by low-income, as well
292 as DIY biology research groups. Both communities are indeed in need of reliable and
293 accurate measurement tools at a low cost and with an open source nature (13, 14). The
294 latter is an important feature, as users should be able to modify such devices in a
295 variety of ways to fit their needs. MicrobeMeter provides such adaptability and
296 flexibility as its electronics are built on the popular and cost-effective Arduino
297 platform, and its physical design can be readily modified in different ways. The 3D
298 casing, for example, can be replaced by simpler and cheaper wood or cardboard

299 design, or its tube-holders can be adapted for different cell culture vessels or use it as
300 flow cells. Similarly, the electronics can be integrated with a display panel to use it as
301 a traditional photometer, while measurement spectrum can be extended by inclusion
302 of LEDs with different wavelengths.

303 The open-source nature of DIY devices such as MicrobeMeter can also
304 encourage innovation in education and in scientific research. In particular, it is not
305 uncommon that measurement devices developed for a particular measurement with a
306 particular experiment in mind can outperform multi-purpose devices or open up new
307 measurement areas (e.g. see (17)). In the case of MicrobeMeter, we hope that the
308 wireless data communication and programmable continuous measurement features
309 will allow for collection of high-resolution microbial growth data, which can be
310 shared at online databases and contribute to the development of better mathematical
311 models of microbial physiology. Such resources, as well as the DIY nature of the data
312 collection devices, could help train interdisciplinary scientists who have a better grasp
313 of measurement techniques, the resulting data, and their possible uses across scientific
314 borders.

315

316 **METHODS**

317 **Design of MicrobeMeter casing.** The MicrobeMeter casing was designed using
318 OpenSCAD version 2015.03-3. It was then manufactured using a 3D-printer
319 (Ultimaker 2/2⁺, 0.4mm nozzle; Ultimaker B.V., Geldermalsen, The Netherlands) and
320 polylactic acid filaments (2.85mm diameter; Ultimaker B.V., Geldermalsen, The
321 Netherlands). The 3D-printer was operated as per manufacturer's instructions and
322 using the following parameters: 0.1mm layer height, 20% infill and with support
323 structure enabled. The photodiode and LED were placed in tunnel-shaped holding
324 bays, with identical apertures at both ends (2mm in diameter and 3mm in length),
325 while the culture tubes were placed in cylindrical holders with an inner diameter of
326 18.5mm. Thus, the total light path length is 24.5mm. The centre of apertures was
327 placed 15.9mm above from the bottom of the culture tubes (Figure 1A). The tube
328 holder size was optimised to fit a standard Hungate tube (CLS-4209-10, anaerobic
329 culture tubes, Chemglass Life Sciences, New Jersey, USA) with an outer diameter
330 18mm, which is also the diameter of several other regular test tubes. The 3D design
331 information of MicrobeMeter are provided for personal and academic non-
332 commercial research use through a dedicated website (see *Device Availability*
333 section). The casing design specifications can be changed if needed by the user (e.g.
334 to fit different growth vessels and tubes).

335

336 **Optical and electronic components.** The complete MicrobeMeter parts list, along
337 with the electronic circuit diagram is provided for personal and academic non-
338 commercial research use through a dedicated website (see *Device Availability*
339 section). A LED with specific wavelength of 601nm (L-53SED, Kingbright, New
340 Taipei, Taiwan) was used as the light source and a silicon photodiode (BPW21R,
341 Vishay, Selb, Germany) was used as the light sensor. Both the LEDs and the
342 photodiodes were controlled (i.e., illumination and measurement, respectively) using
343 an electronic circuit that consists of regulators for LEDs, amplifiers for photodiodes, a
344 temperature sensor, and a Bluetooth data communication module. The electronic
345 circuit is designed as a "shield" to work with Arduino Mega microcontroller (Arduino
346 Mega 2560 Rev3). Each MicrobeMeter unit was powered by a dedicated battery (TL-
347 PB10400, 10400 mAh Power Bank, TP-Link, Reading, UK) which, along with the
348 device, can be packed in a plastic container (PJSCPP1.75LW_BUNDLE; height:

349 165.3mm; diameter: 126.2mm) (see Figure 1B). In multiple tests, the maximum
350 runtime of MicrobeMeter on a fully charged battery was ~100 hours (measurement
351 frequency was set to 5 minutes). A step-by-step instruction of the MicrobeMeter
352 assembly is provided for personal and academic non-commercial research use through
353 a dedicated website (see *Device Availability* section).
354

355 **Data acquisition and processing.** MicrobeMeter can acquire data in an autonomous
356 fashion and send it to a computer. This is achieved by the above said Bluetooth
357 enabled Arduino Mega shield and two dedicated programs, written in a server-client
358 architecture. The server component is written using Arduino Software version 1.8.3
359 and is interfaced with a client component that is written using Perl version 5.18.2. The
360 client program is currently optimised to run on macOS version 10.13.1 and Windows
361 version 8 & 10, and can be readily adapted to other operating systems. The server
362 program runs on the Arduino microcontroller and allows wireless connection to the
363 client program to send the measurements made. Source code for both programs are
364 provided for personal and academic non-commercial research use through a dedicated
365 website (see *Device Availability* section).

366 The Bluetooth connection between MicrobeMeter and a computer can be
367 established using standard methods as per its operating system (tested using MacBook
368 Pro and MacBook Air with macOS version 10.12 & 10.13, and desktop and laptop
369 computers with Windows version 8 & 10). The default Bluetooth device name of
370 MicrobeMeter is “HC-06” and the password is “1234”. The Bluetooth connection
371 needs to be configured only once, when connecting with the same computer. Once a
372 Bluetooth connection is configured, the client program can be executed on the
373 computer to start individual or long-term measurements with MicrobeMeter. When
374 the program initiates, it proceeds first with setting up user-defined specifics, such as
375 experiment name and time between measurements, then initiates the collection of
376 ‘blank’ and sample measurements. The measurement is taken from one port at a time
377 and the minimum interval between each set of measurements (i.e., from four ports) is
378 set at 6s. This setting can be altered by the user, if desired. The measurements will
379 continue until the user terminates the client script or turns off MicrobeMeter. In case
380 of Bluetooth connection failure or interruption at the computer side, the client
381 program will seek and re-establish connection with MicrobeMeter when the computer
382 is back online. At the beginning of an experiment, the client program creates two files
383 that store the connection status and the measurements. The latter file contains the
384 version of the device, experiment name, measurement time, temperature, and the
385 measurement data. While users can develop appropriate post-analysis approaches for
386 this data, an R script for basic analysis and plots is provided for personal and
387 academic non-commercial research use through a dedicated website (see *Device*
388 *Availability* section).
389

390 **Measurement range and conversion factor.** The maximum light intensity that the
391 device can measure is effectively its “measurement saturation point”. Given its use of
392 the Arduino platform, this point is theoretically at 5V (corresponding to an integer
393 reading of 1023) for MicrobeMeter. The design is optimised, so to have a maximum
394 of light reading below 1023 (on the Arduino platform). To ensure this, the Arduino
395 shield circuit is implemented in a way so that when using MicrobeMeter with a
396 Hungate tube containing 10mL distilled water, the maximum intensity light setting
397 produces an integer reading between 600-1000. This allows the device maxima to be
398 below the saturation point of 1023, while still allowing a minimum integer reading

399 range of 1-600 (or up to 1-1000), which corresponds to more than 2.78 turbidity units
400 (e.g., $-\log(1/600)$). In addition, this setting allowed us to account for the observed
401 lensing effects from water-filled Hungate tubes, which can cause approximately 1.6-
402 fold increase in light intensity. As shown in Figures 3 to 5, the resulting measurement
403 range is sufficient to cover detection of microbial cultures' growth from very early
404 stages well into stationary phase, and the measurements are linear in this range.

405 For two photometers producing two different measurements for the same
406 sample, a simple conversion factor can be calculated by taking the ratio of the two
407 measurements (10). However, as photometers are differently sensitive to the optical
408 properties of the samples, the conversion factor can vary as the sample changes its
409 properties, for example as changes occur to the cells and medium during a growth
410 experiment (7). Such a normalisation based on a conversion factor was used in Figure
411 4, where the average of conversion factors over all time points was used for the
412 normalisation.

413
414 **Further specifications of MicrobeMeter.** The additional aspects of the device are as
415 follows; **Response time:** The response time of MicrobeMeter (i.e., the time required
416 for the device measurement to equilibrate after a full rise or fall of signal) was
417 determined by exposing the photodiode for 10s each to the maximum and minimum
418 light intensity of the LED, allowed by the MicrobeMeter circuit (i.e., a cycle). These
419 measurements were done using a Hungate tube containing 10mL distilled water, and
420 with the cycle being repeated for six times at room temperature (see *Supplementary*
421 *File 2* for the raw data). The response times are determined by taking the average of
422 the device output during the second half of each signal as the target value, and
423 identifying the time when that target value is reached after a maximum/minimum
424 signal is originally initiated (a moving average with window size ten was used to
425 smooth the signal). The longest response times recorded among any of all tested ports
426 were 57ms and 31ms, respectively, for raising (i.e., minimum to maximum) and
427 falling (i.e., maximum to minimum) signals. These results suggest that 88ms plus the
428 time for measurement should be the minimum time gap between two independent
429 measurements on MicrobeMeter. Currently, the default value for this gap is set to
430 0.9s, well-above this suggested minima. This setting can be altered by the user, if
431 desired. **Temperature effects and correction:** As MicrobeMeter is a portable device
432 designed to be placed on a desk at room temperature, or inside a heated incubator, or
433 even to carry around for fieldwork, it is important to identify the stability of its
434 measurements at varying temperatures. This was tested by taking continuous
435 measurements using Hungate tubes containing 10mL of distilled water.
436 Measurements on a 1-minute interval were started and the device was kept at room
437 temperature for 30 minutes, before being moved into an incubator (30°C; Heratherm,
438 Thermo Scientific, MA, USA) for 15 hours and 6 minutes. This data showed
439 approximately 5% decrease when the device was moved from room temperature to
440 30°C (*Supplementary File 3*). This change was very similar for all ports, indicating
441 that the measurements can be corrected for the temperature effect by using one port
442 with a “blank” sample (i.e., multiplying the measurements of a port using the ratios
443 between the first measurement of that port and the measurements of the “blank” port).
444 This approach was used in the data shown in Figure 3 to 5 and *Supplementary File 3*.
445 **Temporal stability:** The data presented in the above section also showed that the
446 temperature corrected measurements of MicrobeMeter are highly stable over time. To
447 test this aspect further, a similar separate test was conducted using pre-heated
448 MicrobeMeter (24 hours at 30°C prior to starting the test) to determine the stability of

449 the measurement over a much longer period of time (65 hours 13 minutes; 3913
450 measurements; at 30°C). The results showed that the variability among turbidity
451 values was approximately 0.0003 for each port (see *Supplementary File 4* for the data
452 and calculations).

453 **Biological samples and experiments.** The biological results were obtained using
454 different microbes and culturing protocols as follows. **Cell cultures:** The following
455 species were used in the presented experiments: *Escherichia coli* K12 (substr.
456 MG1655; originally obtained from the German Culture Collection, DSM18039),
457 *Shewanella oneidensis* MR-1 (originally obtained as a gift from Susan Rosser's
458 research group at the University of Edinburgh), *Schizosaccharomyces pombe* MBY
459 102 (genotype: ade6-210 ura4-D18 leu1-32 h+; originally obtained as a gift from
460 Mohan Balasubramanian's research group at the University of Warwick),
461 *Desulfovibrio vulgaris* (originally obtained from the German Culture Collection,
462 DSM644), and *Methanosarcina barkeri* (originally obtained from the German Culture
463 Collection, DSM800). **Serial dilutions:** The *E. coli* and *S. oneidensis* starter cultures
464 were prepared by inoculating 100µL of thawed cryo-stock and liquid culture (grown
465 in lysogeny broth (LB) medium without glucose (18)) into 100mL of LB medium,
466 respectively. These two starter cultures were incubated overnight at 37°C and 30°C,
467 respectively, with 150rpm shaking. Starter culture of *S. pombe* was prepared by
468 picking individual colonies from YEA medium (19) agar plates, dissolving these in
469 100mL of YEA medium, and incubating for 48 hours at 30°C with 240rpm shaking.
470 After the turbidity values of the starter cultures reached above 2.5, three 33mL-
471 aliquots of each starter culture were transferred into 50mL-centrifuge tubes. The cells
472 were then pelleted by centrifuging at 2000 relative centrifugal force (rcf) for five
473 minutes at 4°C, the supernatants were removed, and each pellet was re-suspended
474 using 23mL PBS. The re-suspended pellets of each organism's replicate cultures were
475 combined (final volume of 69mL each) and diluted further using PBS to achieve a
476 turbidity measurement between 2.3 to 2.4 before blank subtraction. Turbidity was
477 measured using disposable cuvettes (Fisherbrand, FB55147, Fisher Scientific, UK)
478 and a bench-top spectrophotometer (Spectronic 200, Thermofisher, MA, USA). Two
479 sets of two-fold serial dilutions (1 to 1/16 dilution) were prepared using PBS for each
480 organism. Note that each set contains three technical replicates. The volume of each
481 dilution was 1mL and 10mL in the first and second sets, respectively. The former was
482 used for turbidity measurement using disposable cuvettes, whereas the latter was used
483 for turbidity measurement using Hungate tubes. The turbidity of the well mixed serial
484 dilutions was measured at 600nm using Spectronic 200, Jenway 7305 (Cole-Parmer,
485 Staffordshire, UK) and Novaspec Pro (Biochrom Ltd., Cambridge, UK). Note that the
486 Hungate tube samples were measured only using Spectronic 200 equipped with an
487 adapter for Hungate tubes, and MicrobeMeter. The blanks were prepared using 1mL
488 and 10mL of PBS for the cuvette and Hungate tube samples, respectively. **Cultures**
489 **for continuous growth measurements:** All measurements were done using a pre-
490 heated MicrobeMeter (overnight at 37°C) with three inoculated and one sterile tube
491 (the 'blank'). After starting the measurements, the MicrobeMeter was moved into a
492 shaking incubator set at 30°C or 37°C (Stuart SI600C, Cole-Parmer, UK or MaxQ
493 4000, Thermo Scientific, MA, USA). ***E. coli* experiment:** The starter culture was
494 prepared by inoculating 100µL of *E. coli* K12 from thawed cryo-stock into 50mL of
495 LB medium and incubating at 30°C with 150rpm shaking overnight. Each of the six
496 sterile Hungate tubes containing 10mL of LB medium was then inoculated using
497 100µL of the starter culture. Two sterile Hungate tubes containing 10mL of fresh LB

498 medium were used for obtaining blank measurements. All tubes were sealed with
499 sterile air-permeable membranes (AeraSeal, LW2783, Alpha Laboratories, UK). The
500 measurements using MicrobeMeter were taken every minute for approximately 30.5
501 hours at 30°C with 250rpm shaking. Technical replicates (a set of three inoculated
502 tubes and one sterile tube) were also moved into the same incubator and turbidity
503 measurements were taken using Spectronic 200 equipped with an adapter for Hungate
504 tubes every 30 minutes for three hours. *D. vulgaris experiment*: The cell cultures were
505 prepared by inoculating 500µL of a starter *D. vulgaris* culture from its late-log phase.
506 The starter culture was grown anaerobically for four days in a defined medium
507 described in (20), and containing 30mM Na-Lactate and 10mM Na₂SO₄. The 500µL
508 inoculum was diluted into sterile Hungate tubes containing 10mL of the same defined
509 medium (three replicates were used, with one un-inoculated blank). Measurements
510 were taken using MicrobeMeter every five minutes for approximately 75 hours at
511 37°C with 250rpm shaking. *M. barkeri experiment*: The cell cultures were prepared
512 by inoculating 500µL of *M. barkeri* from its late-log phase culture into each of the
513 three sterile Hungate tubes containing 10mL of a defined medium described in (20)
514 (one un-inoculated tube was used as blank). The medium was adapted for *M. barkeri*
515 growth by adding 100µL of 50% (v/v) methanol as sole carbon source and 200µL of
516 100mM Na₂S as reducing agent. Furthermore, NaHCO₃ was omitted as it precipitates
517 during the anaerobic medium heating and degassing processes. Measurements were
518 taken using MicrobeMeter every five minutes for approximately 458 hours at 37°C
519 with 300rpm shaking (high speed shaking was used for avoiding sedimentation of
520 granules).

521

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524 with the prototyping of electronic circuit boards, Mohan Balasubramanian for
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527 current members of the Soyer research group for insightful discussions on device
528 development.

529

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535

536 **AUTHOR CONTRIBUTIONS**

537 OSS and KS designed the research. OSS contributed to device and electronics design,
538 AVM contributed to device design, JC and TF contributed to culture work, and KS
539 designed the system and electronics, implemented the device, and performed the
540 analyses. OSS and KS wrote the manuscript, which was approved by all co-authors.

541

542 **CONFLICT OF INTEREST**

543 OSS and KS declare conflict of interest in the form of affiliation (as co-founders) with
544 Humane Technologies Limited, a spin-out company set up for the development and
545 distribution of DIY devices such as, and including, MicrobeMeter.

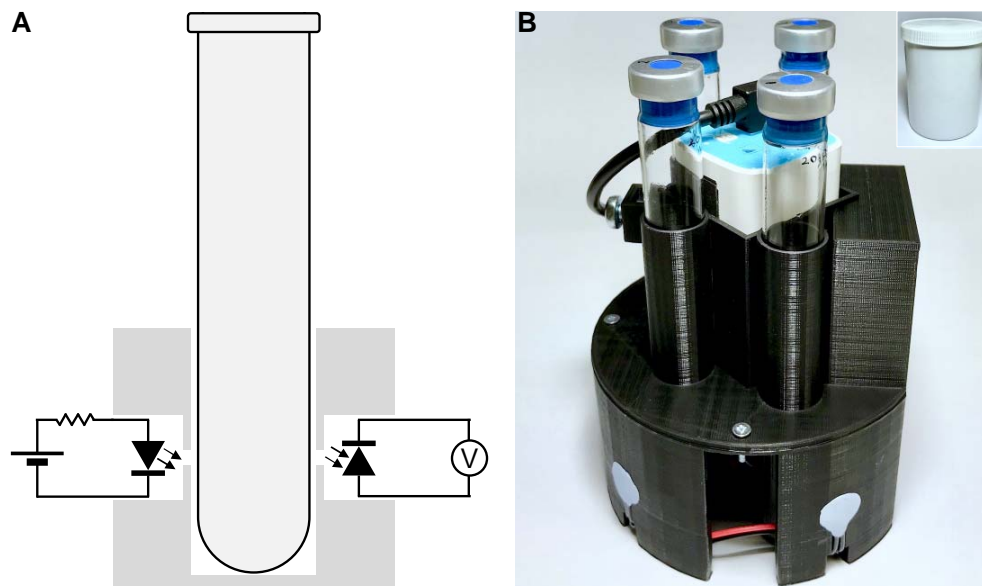
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547 **DEVICE AVAILABILITY**

548 The blueprints and additional details (such as data acquisition and analysis software)
549 of MicrobeMeter are made publicly available under a personal and academic non-
550 commercial use licence at www.humanetechnologies.co.uk.

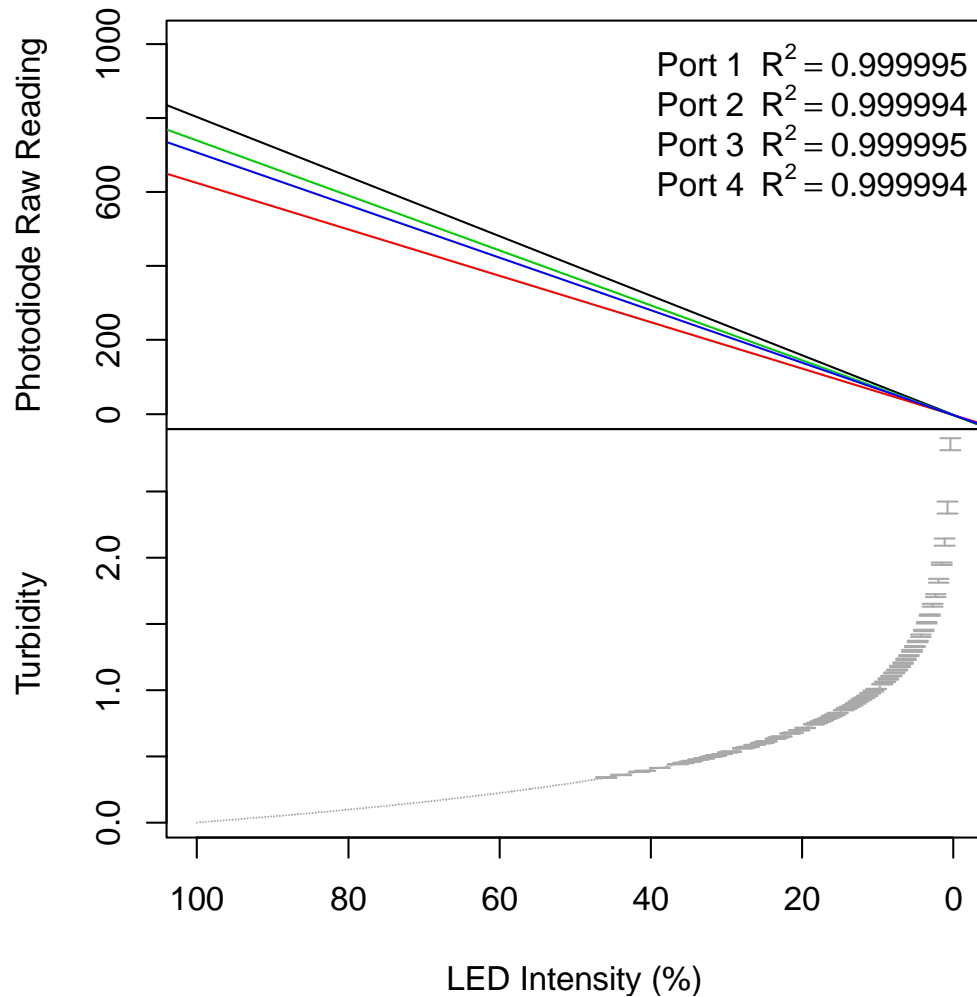
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FIGURES



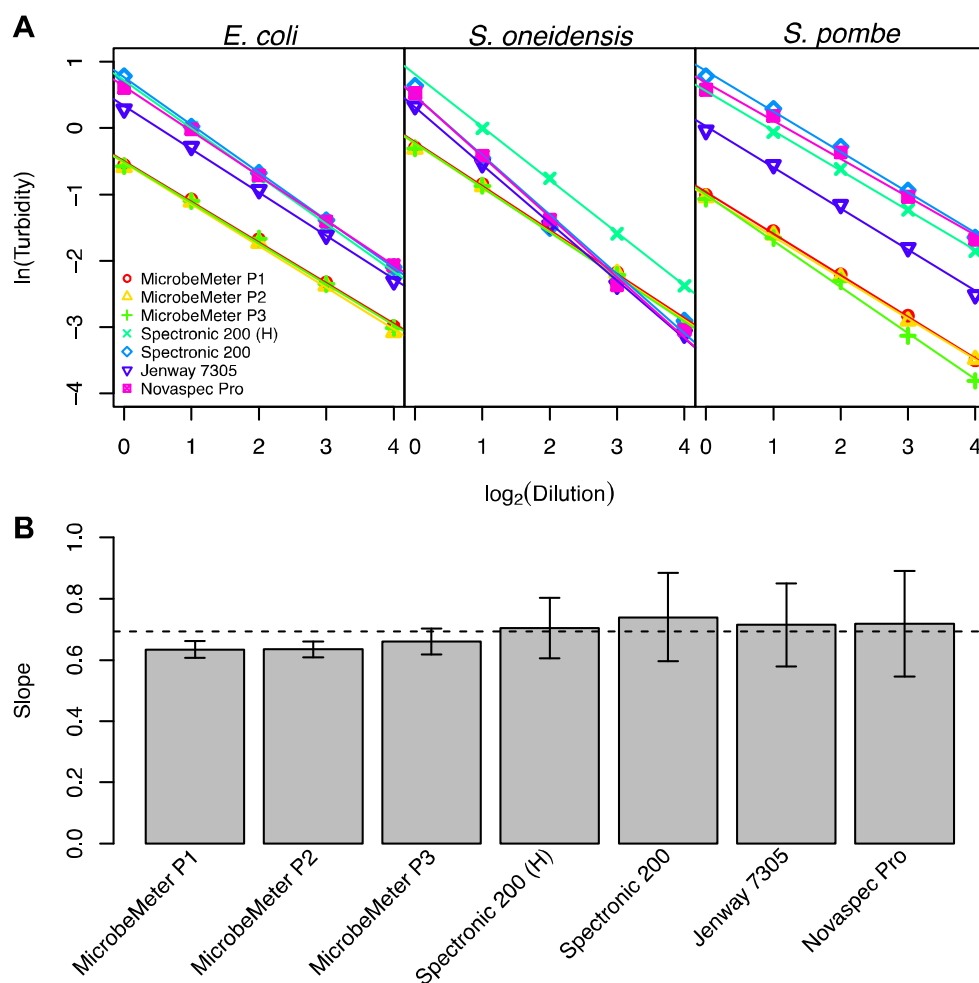
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556 **Figure 1: A: Schematic showing the cross section of a simple photometer.** The
557 dark grey region shows the plastic body of the device that holds a LED (left), a
558 photodiode (right), and a test tube (middle). The LED is connected to a battery via a
559 resistor. The photodiode is connected to a volt meter for measuring the voltage
560 generated by the photodiode upon the incident of the light from the LED through the
561 apertures. **B: The design of MicrobeMeter.** The picture shows (in black) the 3D-
562 printed casing of MicrobeMeter, holding the measurement tubes (i.e., samples and
563 blank), battery, and Arduino compatible electronic circuit board. The casing and
564 electronic circuit designs are available for personal and academic non-commercial
565 research use through a dedicated website (see *Device Availability*). Insert at top-right
566 corner shows MicrobeMeter placed in a white plastic jar for further protection.
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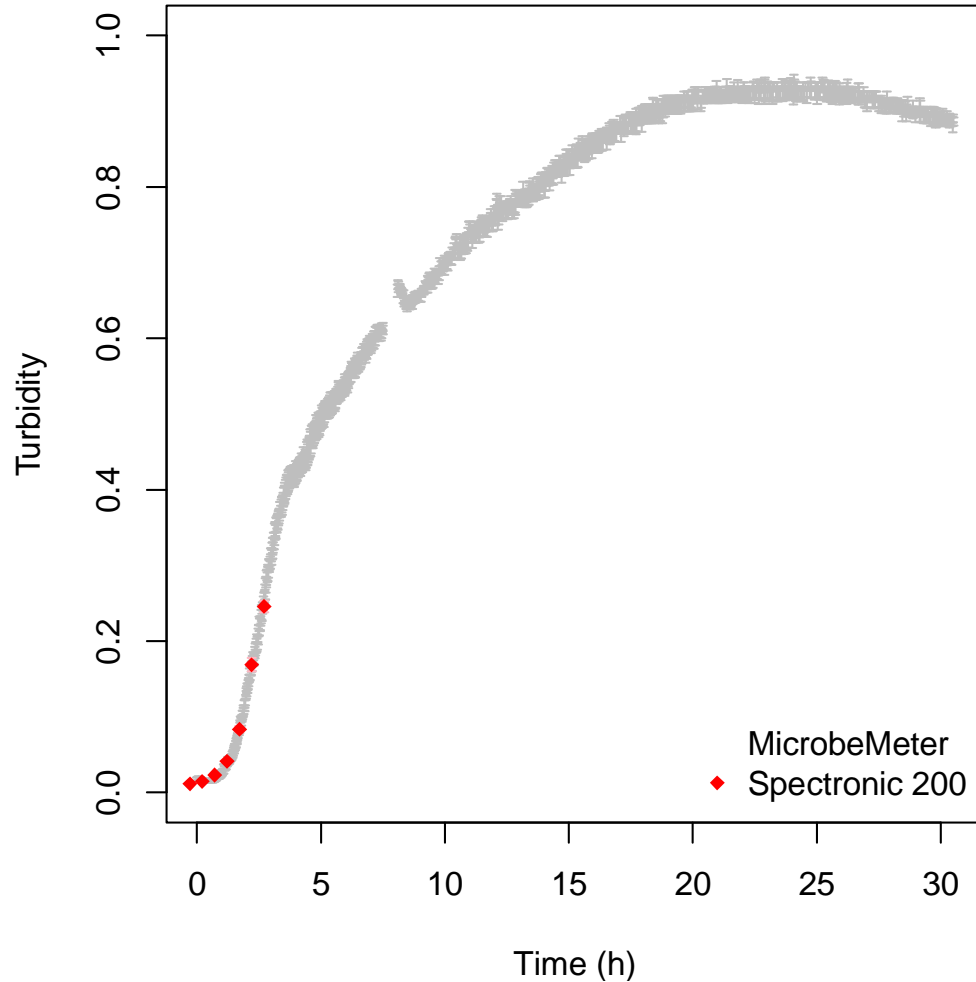
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570 **Figure 2: Linearity of MicrobeMeter response to light.** The linearity of the
571 response of the four ports of MicrobeMeter was determined by lowering the light
572 intensity from its maximum to minimum by adjusting the duty cycle of the signal that
573 drives the LED (x-axes), while recording the photodiode raw readings (y-axis of top
574 panel). The coefficient of determination (R^2) is shown in the legend. The raw
575 photodiode readings from each port (top panel) were converted to turbidity values
576 (see main text), the average (black dots) and the standard deviation (grey bars) of
577 which are shown in the bottom panel.
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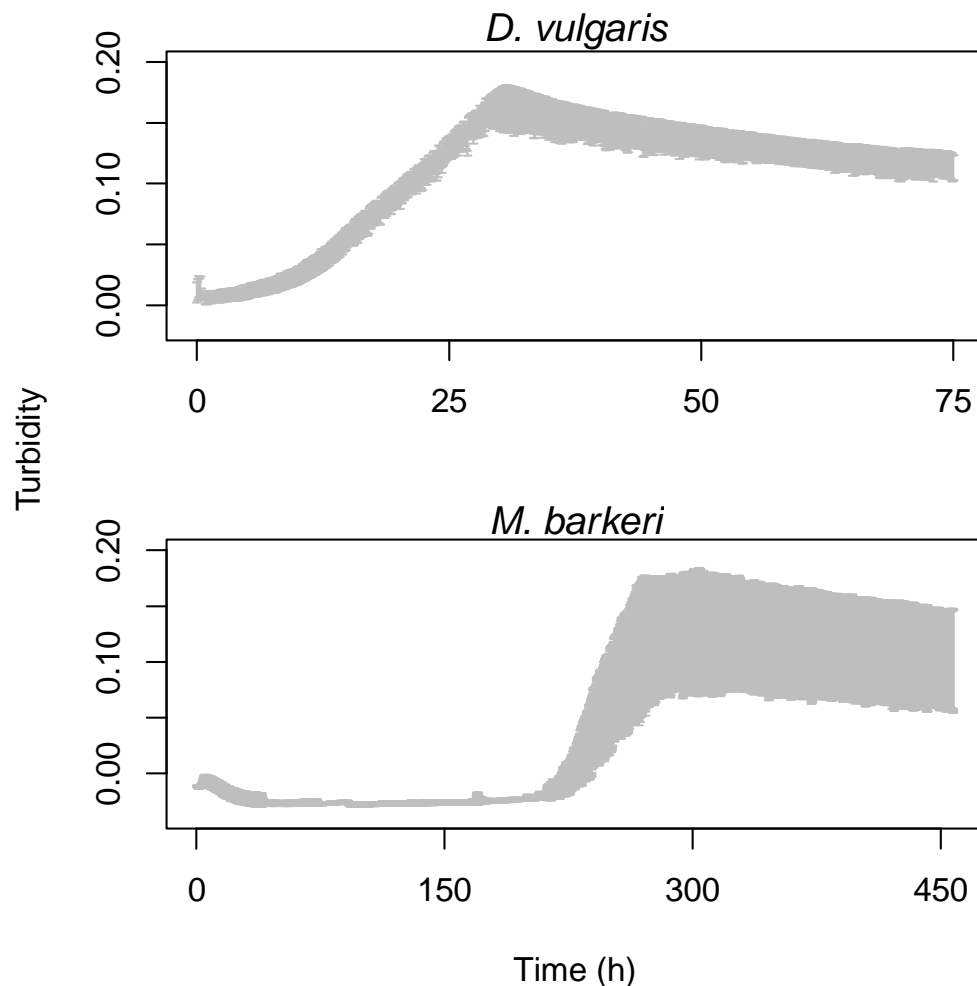
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Figure 3: A: Regression analysis of the turbidity measurements taken from the serial dilutions of three different cell cultures (plot titles). Measurements are obtained using the MicrobeMeter (P1, P2 and P3 indicate the three ports of MicrobeMeter) and three commercial spectrophotometers. The x-axes show culture dilution levels, while the y-axis shows the natural logarithm of the blank subtracted and path-length corrected turbidity values. Regression line was calculated for each dataset as shown. The coefficient of determinations (R^2) of all datasets were greater than 0.985 (not shown in the figure). The Spectronic 200 was used for obtaining measurements from samples kept both in cuvettes and Hungate tubes (H). **B: The slopes of the turbidity measurements of the serial dilutions.** Each bar shows the average and standard deviation (error bar) of the slopes of three different cell cultures shown in panel A. The dotted line indicates the calculated slope of the serial dilutions (as described in the main text).



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Figure 4: Continuous turbidity measurements of *E. coli* cultures using MicrobeMeter. Long term turbidity measurements were taken using three technical replicates of *E. coli* culture. The black dots and grey bars show the average and standard deviation of measured turbidity values, respectively. The x-axis shows the time in hours. These data were compared against manually collected data on an additional set of three technical replicates of *E. coli* culture, obtained using a commercial spectrophotometer (Spectronic 200; average: red rhombus; standard deviation: light red bars). Note that the Spectronic 200 measurements were multiplied using a conversion factor (as described in the main text) to fit with the MicrobeMeter measurements. All turbidity values are blank subtracted and path-length corrected. The MicrobeMeter measurement frequency was one minute. A gap was introduced in the measurements, at about 8h, when the cultures were removed for visual inspection.



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612 **Figure 5: Continuous turbidity measurements of *D. vulgaris* and *M. barkeri***
613 **cultures using MicrobeMeter.** Long-term turbidity measurements were taken using
614 three technical replicates of *D. vulgaris* and *M. barkeri* cultures. The black dots and
615 grey bars show the average and standard deviation of measured turbidity values,
616 respectively. The x-axes show the time in hours. All turbidity values are blank
617 subtracted and path-length corrected. Smoothing, using a moving average with
618 window size of ten, was performed on the measurements of *M. barkeri* cultures. The
619 MicrobeMeter measurement frequency of *D. vulgaris* and *M. barkeri* cultures was
620 five minutes.

621

622 **Supplementary Files (provided as a compressed file).**

623 Supplementary File 1: Data from the technical quality check experiment using pulse-
624 width modulation as explained in the main text.

625 Supplementary File 2: Data from experiment to determine the response time of
626 MicrobeMeter as described in *Methods* section.

627 Supplementary File 3: Data from the experiment to identify the effect of temperature
628 on MicrobeMeter measurements as described in *Methods* section.

629 Supplementary File 4: Data from the experiment to identify the temporal stability of
630 MicrobeMeter as described in *Methods* section.

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