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ODX - A fitness tracker-based device for continuous bacterial growth monitoring

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Abstract

Continuous monitoring of bacterial growth in aqueous media is a crucial process in academic research as well as in the biotechnology industry. Bacterial growth is usually monitored by measuring the optical density of bacteria in liquid media, using benchtop spectrophotometers. Due to the large form factor of the existing spectrophotometers, they cannot be used for live monitoring of the bacteria inside bacterial incubation chambers. Additionally, the use of benchtop spectrometers for continuous monitoring requires multiple samplings and is labour intensive. To overcome these challenges, we have developed an optical density measuring device (ODX) by modifying a generic fitness tracker. The resulting ODX device is an ultra-portable and low-cost device that can be used inside bacterial incubators for real-time monitoring even while shaking is in progress. We evaluated the performance of ODX with different bacterial types and growth conditions and compared it with a commercial benchtop spectrophotometer. In all cases, ODX showed comparable performance to that of the standard benchtop spectrophotometer. Finally, we demonstrate a simple and useful smartphone application whereby the user is notified...
when the bacterial concentration reaches the targeted value. Due to its potential for automation and mass production, we believe that the ODX has a wide range of applications in biotechnology research and industry.

**Introduction**

Bacteria present a cheap and easily scalable platform for the synthesis of a wide range of biological products[1]. For centuries, food processing and fermentation industries have driven the commercial markets of bacterial based products. The advent of recombinant technology paved the way for various engineered enzymes and novel protein-based therapeutics[2]–[4]. In all those mentioned above, clinical, scientific, and commercial settings, monitoring of the population, and growth kinetics of bacteria plays a crucial role[5], [6]. Each species and strain of bacterium has unique growth kinetics[7]. These growth kinetics depend on various parameters such as oxygen availability, temperature, medium in which the bacteria are grown, pH, culture vessel, the volume of the culture etc[8]. Working with these microorganisms typically requires continuous monitoring of growth patterns. In many cases, microbiologists monitor the microbial growth regularly to ensure that the population does not exceed pre-set thresholds or to maintain the population at a particular level. Traditionally, several methods such as plate counting[9], direct counting[10], biomass measurement[10], [11], and light scattering have been used to measure bacterial growth. At present, optical density measurement based on the scattering of light from individual bacterial cells remains the gold standard [12].

**Optical density-based methods for bacterial growth**

When light passes through a sample consisting of bacterial cells, the transmitted light from the sample is logarithmically correlated to the concentration of the cells, and the concentration is expressed as the optical density (OD). The mathematical description of this correlation is given by the Beer-Lambert law [13],[14]. With the significant advantages of being quick, accurate, and non-destructive, the OD measurement is the most convenient
analytical technique for estimating bacterial concentrations. For decades, spectrophotometers have been deployed for OD measurements in laboratory[12]. While the last few years have seen tremendous evolution of spectrophotometers, most of these devices are costly, bulky and remain benchtop based and so cannot be taken inside the incubator for real-time monitoring of OD measurements[15]. Furthermore, the usage of these instruments requires significant user interaction with the analyte and lack both versatility and flexibility due to their large form factor. These spectrophotometers also come with the penalty of high labour costs and introduce contamination risks (See Figure 1,a-d).

In recent years, researchers have built several portable continuous bacterial growth monitoring device prototypes based on optical density measurements. These prototypes take advantage of the availability of inexpensive electronic components and 3D printing technologies. For example, Sasidharan et al., have developed an optical density meter based on the Arduino platform[16]. A similar device for live monitoring the bacterial growth was reported by Kutschera et al[17]. Hoang et al., developed a smart centrifuge tube that can provide real-time optical density during centrifugation[18]. However, most of these devices cost about $100 or more to build, require many components and significant knowledge of sourcing the materials and assembling them.

**ODX - A fitness tracker based optical density measuring device**

To overcome these challenges, we sought to develop a low-cost ($25) and portable live bacterial growth monitoring device based on a commercially available fitness tracker. Recent advances in electronic miniaturization have paved the way for various types of wrist-worn low-cost fitness trackers[19]. These fitness trackers typically track, monitor and analyse various activities such as physical movement, sleep, and heartbeat rate, facilitated by various sensors such as an accelerometer, heart rate monitor, ECG, GPS etc. The output of these sensors is processed and stored by a small but powerful microprocessor. Fitness trackers transmit the result of the activities directly to the built-in OLED screen as well as to smartphones via Bluetooth. Despite having many sophisticated
sensors, a powerful microprocessor, and highly miniaturized design, these fitness trackers are priced as low as $10 on the consumer market[20]. Of all the sensors in fitness trackers, the optical heart rate sensor is particularly interesting[21]. The heart rate sensor consists of LEDs, one or more photodiodes, the essential components required of an OD meter. In this work, we take advantage of the considerable development effort that has gone into developing fitness monitors, and we present the hardware and firmware changes required to convert a fitness tracker into a low-cost OD meter for live monitoring of bacteria. Furthermore, we have developed a smartphone application to make the ODX device easy to use remotely and log the bacterial growth data (See Figure 1,e-g).

Figure 1 A schematic comparing the traditional method to the ODX device-based method for continuous optical bacterial growth monitoring. (a) The tube containing appropriate culture medium inoculated with bacterial cells. Traditional (b) Bacterial cultures in a shaking incubator (c) Laminar airflow chamber (all the biological sampling is done inside a laminar airflow chamber to reduce the potential risk of contamination) (d) Culture sample collected in a cuvette and OD measured using a commercial benchtop spectrophotometer. ODX (e) The sample is inserted into the ODX device and placed in a shaking incubator. (f) Basic components of ODX. (g) Data collected via Bluetooth are processed on ODX smartphone app and readouts displayed.
Materials and Methods

Optical and mechanical design of ODX device and choice of materials

The ODX hardware consists of the following parts: 1. A generic fitness tracker 2. The 3D printed enclosure 3. An orange LED. 4. A voltage regulator and a current regulator

Fitness tracker

An ID107HR branded (Shenzhen DO Intelligent Technology Ltd) fitness tracker was chosen for the work presented in this article as it is inexpensive ($10 to $25) and widely available through online retailers. More importantly, it contains an nRF51822 microprocessor from Nordic Semiconductors Ltd (Figure 2a), which has well-documented open-source firmware development tools for modifications. In particular, a dedicated Arduino based firmware has been developed to program the nRF51822 microcontroller. Furthermore, this fitness tracker has marked Serial Wired Digital Programmer (SWD) pads (Figure 2b) on the main PCB that allows the custom developed firmware to be easily uploaded.

Figure 2 The internal circuitry of the ID107HR fitness tracker. (a) Rear view of the circuit board showing SWD programming connections to upload the firmware into the fitness tracker (b) Front view of the circuit board showing nRF51822 microcontroller and OLED display. (c) Heart rate sensor consisting of visible and IR photodiodes. (d) The spectral response of the visible (blue line) and IR (red line) photodiodes of the heart rate sensor (Si1143)[22] in the ID107HR fitness sensor. The emission spectrum of the LED is represented by the orange line.

The heart rate sensor (Si1143, Silicon Labs Ltd) in the ID107HR fitness tracker has two photodiodes[22] (Figure 2c): one to cover the visible spectral range and the other to cover the infrared (IR) spectral range as indicated with blue and red lines respectively in Figure 2d. Raw readings from both PDs can be accessed using the modified firmware. These two PDs provide two complementary measurements for each optical density measurement of bacteria, thus resulting in more accurate OD values than OD meter designs that have only a single PD.
LED

The optical density of bacteria is usually measured at a wavelength of 600 nm as most bacteria and growth media in which bacteria are incubated known to have negligible absorption at that wavelength[23]. Therefore, we chose an orange colored LED (C503B-AAN-CY0B0251, CREE) with peak emission at a wavelength of 596 nm as the light source for the ODX device as it has low power consumption, small size, low weight, high robustness, and acceptable monochromaticity. This external orange LED is powered through the MOSFET that originally drove the vibration motor in the fitness tracker. The power output of the MOSFET depends on the charge of the battery. Therefore, power is not uniform over time. However, it is crucial to maintain a constant luminosity of the LED throughout the OD measurement experiment. Therefore, a small external circuit that supplies a uniform power to the LED is built using a current regulator (NSI45020AT1G, ON Semiconductors Ltd) and a voltage regulator (TPS709B33DBVT, Texas Instruments) as depicted in the circuit diagram (Supplementary section 3).

![Figure 3 3D printed enclosure of ODX device](image)

(a.) 
Falcon tube holding space

(b.) 
Rear view showing the groove for an orange LED.

(c.) 
Bottom view showing the space for LED controlling circuit. A one-euro coin was placed for size comparison to ODX device.
**3D printed enclosure**

We designed the enclosure for the ODX device with an open source parametric CAD software (OpenSCAD version 2015.03-2). The CAD design of ODX has provisions for holding the fitness tracker, a culture tube (Figure 3a), an orange LED (Figure 3b) and the additional circuitry powering the LED (Figure 3c). The CAD design of the ODX enclosure was fabricated using a 3D printer (Ultimaker 3) with black coloured Polylactic Acid (PLA) material. For this purpose, a GCODE is generated from the (Stereolithography) STL file using a slicing software (CURA version 3.2.1) with the 3D printer settings and the following print settings: 0.4mm nozzle, 0.1mm layer height, 20% infill and support structure disabled. The OpenSCAD file for the 3D printed enclosure can be found in the GitHub link in supplementary information section 1.

**Firmware**

We used the Arduino integrated development environment (IDE) to program the nRF51822 microcontroller inside the ODX. The firmware is based on an excellent Arduino compatible toolset for nRF5 series microcontrollers developed by Sandeep Mistry[24]. Furthermore, we installed a specifically developed wrapper in Arduino IDE to seamlessly access the 1D107HR device within Arduino IDE[25]. Also, to access each of the peripherals (i.e. OLED screen, Bluetooth Low Energy radio, an optical sensor) attached to the microcontroller in the fitness tracker, we installed the corresponding Arduino libraries in the Arduino IDE. With the use of these libraries, it was possible to display the ODX values on the OLED screen of the fitness tracker and also transmit them via Bluetooth. Finally, the above-developed custom firmware was uploaded to the fitness tracker through the soldering pads GND, VCC, SCL, and SDIO on the fitness tracker’s circuit board. For this firmware upload, a Black Magic Probe Mini V2.1 (1BitSquared Ltd) program was used (see Supplementary information Section 5).

**Smartphone app development**

An Android app was specifically developed using an open source platform (App Inventor) to transfer the data from the ODX device to a smartphone via Bluetooth. The app then
processes the data and displays the OD on the screen. Moreover, the app displays the growth of bacteria graphically in terms of OD and saves the data in a text file inside the smartphone’s internal memory for further analysis. However, the primary role of the app was to let the users create alerts informing them when a bacterial culture reaches the required growth stage. The working mechanism of the firmware and the app are shown schematically in Figure 4a and Figure 4b, respectively, while Figure 4c shows the user interface of the ODX app.

![Figure 4](image)

**Figure 4** Functional workflow of ODX device (a) Firmware (b) Smartphone app (c) ODX app user interface.

**Bacterial culture**

*E. coli* Nissle was grown on Luria Broth agar plates and 25ml inocula made in LB broth from single colonies, before shaking at 37 °C overnight. *S. aureus* (ATCC 25923) was cultured on Trypsin soy broth (TSB) agar plates and 25 ml inocula made in TSB broth from single colonies, before shaking at 30 °C overnight. *S. agalactiae* was grown on Trypsin soy broth (TSB) agar plates and 25 ml inoculum made in TSB broth from single colonies. Bacteria were sub-cultured in 50 ml falcon tubes by adding 100 ul overnight bacterial
culture to 30 ml of fresh broth. For batch measurements, bacterial cultures were diluted, and OD readings given by ODX device were recorded. For continuous measurement, the falcon tube containing freshly inoculated broth was inserted into the ODX device. The measurements were logged into a text file, which was later used for optimizing the ODX app. In all the cases, OD was cross verified by a standard spectrophotometer (Eppendorf BioPhotometer) in biological and technical replicates. Continuous growth monitoring was performed by seeding the bacteria into Luria-Bertani broth (LB) and monitoring the growth pattern over 10 h.

Results

Calibration of ODX device with biological samples

To convert the ODX device output into optical density values, it was necessary to determine the empirical relationship between the 'Visible and IR' photodiode values of the heart rate sensor and the OD values given by a benchtop spectrophotometer. For this purpose, ODX was calibrated using three bacterial samples. Overnight cultures of *E. coli* Bl21, *S. aureus* and *S. agalactiae* were diluted to five different concentrations. Each sample was measured using the ODX as well as a traditional benchtop spectrophotometer and these results are plotted in Figure 5. A logarithmic function is fitted to these data sets following the Beer-Lambert law [13],[14],[17]. The quality of fit (R²) obtained for the visible and IR photodiodes were above 0.9 for all the three bacterial solutions, indicating the excellent quality of fit and showing the accuracy of above 96% (assuming that the benchtop spectrometer has minimal error). For each of the bacterial solution, the logarithmic functions of the photodiodes are shown in Table 1.
Figure 5 Response of the IR photodiode (blue dots, top x-axis) and Visible photodiode (orange dots, bottom x-axis) for different optical density solutions (y-axis). ODX was calibrated using three different bacterial strains (a) *Escherichia coli* Nissle, (b) *Streptococcus agalactiae* and (c) *Staphylococcus aureus*. The respective coloured lines represent the logarithmic trend lines.

Table 1 Calibration of the ODX using bacterial samples. Best fit functions and corresponding $R^2$ for the IR, and Visible photodiode values as a function of OD.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Polynomial regression function</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>$\text{OD}_{\text{IR}} = -0.47 \ln[\text{IR}] + 4.6351$</td>
<td>0.9923</td>
</tr>
<tr>
<td></td>
<td>$\text{OD}_{\text{VIS}} = -0.625 \ln[\text{Vis}]+4.9436$</td>
<td>0.9944</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>$\text{OD}_{\text{IR}} = -0.344 \ln[\text{IR}] + 3.3413$</td>
<td>0.9903</td>
</tr>
<tr>
<td></td>
<td>$\text{OD}_{\text{VIS}} = -0.396 \ln[\text{Vis}]+ 3.1196$</td>
<td>0.9865</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>$\text{OD}_{\text{IR}} = -0.563 \ln[\text{IR}] + 5.4173$</td>
<td>0.9878</td>
</tr>
<tr>
<td></td>
<td>$\text{OD}_{\text{VIS}} = -0.674 \ln[\text{Vis}]+5.2519$</td>
<td>0.9737</td>
</tr>
</tbody>
</table>

These logarithmic functions were used to obtain the optical density corresponding to each photodiode ($\text{OD}_{\text{IR}}$ and $\text{OD}_{\text{VIS}}$). The final optical density of a bacterial solution is obtained by calculating the weighted average of corresponding individual optical densities of IR and Vis photodiodes, i.e. $\text{OD}=0.5(\text{OD}_{\text{IR}}+\text{OD}_{\text{VIS}})$. Accordingly, these logarithmic calibrating functions are programmed into the persistent memory of the fitness trackers microprocessor, so that raw readings of the photodiodes will directly output the OD values.

**Continuous bacterial growth monitoring**

The ultimate aim of this work was to deploy ODX for continuous monitoring of bacterial growth. Therefore, the performance of the ODX device was evaluated with three bacterial
strains *E. coli*, *S. aureus* and *S. agalactiae*. Bacteria were inoculated in a 50ml tube containing growth media. The tube was inserted into the ODX device and placed in a shaking incubator. OD readings corresponding to the bacterial strains were collected wirelessly via Bluetooth-enabled smartphone and recorded using the ODX Android app. OD readings were measured every 8sec and data extracted to plot the data representing the growth curves of the three bacterial strains. The measurement periods for these organisms were approximately 10h, allowing the collection of complete growth dynamics data. The results for batch-wise bacterial growth monitoring is shown in Figure 6. The three major growth phases of bacterial growth, the lag, log, and stationary, were clearly evident in Figure 6.

![Figure 6 Growth curves of (a) *Escherichia coli* Nissle, (b) *Staphylococcus aureus* and (c) *Streptococcus agalactiae* obtained with the ODX device were plotted. In all the cases, ODX was placed in a shaking incubator and the OD measurements (once every 8 sec) were recorded on a Bluetooth enabled smartphone. The resultant data is plotted after averaging the values for every 400sec to avoid the slight variations in the OD values resulting from the shaking of tubes inside the incubators.](image)

**Real-time bacterial growth phase reporting**

Bacteria-produced compounds are classified into two kinds of metabolites: 1. primary metabolites, which are required for growth, development, and maintenance of cells (produced predominantly in the lag phase); 2. secondary metabolites, which are not directly involved in their growth or maintenance (produced predominantly in the log phase). While the biotech industries capitalize both on primary and secondary metabolites, growth phase and bacterial cell concentration play an important role in regulating the biosynthesis of these primary and secondary metabolites. Harvesting the bacterial cells at an appropriate growth phase and cell concentrations is essential to obtain high yields of
the desired product and also ensures minimal cell death due to nutrient depletion or bacterial quorum quenching.

In all experiments, data from ODX was transmitted continuously to the ODX app via Bluetooth and throughout the process, it could be accessed in real-time. This real-time access to bacterial growth phase and optical density would be a highly valuable asset for fine tuning the process efficiency in biotechnology industries.

Discussion

Monitoring of bacterial growth represents a staple process in every bacterial or biological engineering focused laboratory. Plotting the growth characteristics of various bacteria is important to harvest cells for protein production and to study the effects of various test substances on bacterial growth. The traditional method to measure OD and monitor bacterial growth is a time-consuming process, uses a huge amount of plastic consumables and adds risk of contamination. In this study, we showed that the ODX device has successfully overcome most of the problems and challenges posed by the traditional OD measuring methods.

Bacteria are extensively used in research and industry. In most cases where bacteria are used as bio factories, the products (proteins, secondary metabolites etc.) are produced in the log phase of the bacterial growth. In such settings, the bacterial OD is maintained between 0.4 and 0.8. For cloning DNA, bacterial OD of between 0.6 and 0.8 is preferred. ODX showed similar lower limit of detection to the benchtop spectrophotometer (as low as 0.01 OD, approx. 10^6 CFU).

By combining a generic fitness tracker and a smartphone-aided data reporting system, ODX forms a complete continuous bacterial monitoring system. In this study, ODX has been tested on three different bacterial strains and their growth was monitored continuously for over 10h. The resultant growth curves are shown in Figure 6 resemble the typical bacterial growth curves[26].
Our ODX device presents several advantages over the existing commercial and DIY spectrophotometers. Since the ODX is ultra-portable, it could be used in various biological settings such as shaking incubators, anaerobic incubators or sterile laminar airflow chambers, thus eliminating the potential chances of contamination. The heart rate sensor (Si1143, Silicon labs Ltd, USA)[22], employed in a fitness tracker is a standard component widely used in most smartphones as a proximity and brightness sensor. Therefore, it has undergone many rigorous tests and has been shown to work in all type of harsh conditions, ensuring the high performance for the ODX devices in laboratory settings. Moreover, this heart rate sensor has two photodiodes, which therefore provides two complimentary readings for each measurement. The ODX could also be used as a regular benchtop spectrophotometer as it can display the OD values on the OLED Screen without requiring a smartphone or a computer. ODX works with a range of standard sample containers. The current device was tested using a standard cuvette and a generic test-tube (data not shown), thus eliminating the need for specific consumables.

One of the key aspects of ODX is the availability of low-cost fitness trackers, that makes it affordable. Today, fitness trackers are available at a retail price of less than $10. These fitness trackers have all the electronic components required to make OD meters. The same components, when bought individually, are the main contributors to the high costs of the currently proposed prototypes. More importantly, in the case of fitness trackers, all these components come preassembled on a circuit board, further reducing the cost and labour required. This is a significant advantage for both DIY and large-scale manufacturing of ODX devices when compared to the already existing DIY OD meter designs.

Although the current paper explores the potential of ODX primarily in an academic lab setting, the scope of ODX is not only limited to academic labs. Biotech industries such as the recombinant protein production industries, fermentation industries, dairy, and food-based industries, use turbidity and optical density monitoring for both batch and continuous quality monitoring. The portability and modularity to adopt in many settings expand the potential of ODX into any biotech industry setting. The ability to continuously log the data with unmanned supervision (via Bluetooth) reduces the risk of data fraud and data loss. This data log file could later be used for retrospective inspections[27]. Using wireless Bluetooth based systems avoids sophisticated wiring systems and decreases physical
maintenance costs in the industry. OD methods are also widely used in clinical labs and hospitals for various blood, urine, and other body fluid analyses[28], [29]. The ODX could be deployed as an NPD (Near patient diagnostics) which reduces the burden on personnel on the health sector. The data logging system could form a very helpful feature for patients who require regular monitoring of body samples. This eliminates the manual errors in clinics where analyses are still done by physical examination by a staff member.

In this paper, we have shown the ability of ODX to continuously monitor the bacterial growth, which is not possible with the current benchtop spectrophotometers. With all the features such as portability, versatility and the customisability ODX can be a valuable tool for monitoring bacteria in a wide range of academic and industrial settings.

Acknowledgments

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Associated content: GitHub link for project files, Bill of materials, detailed instructions for building ODX, tools used and raw data for Figure 6 have been included.

References


[22] "Si1141/42/43 PROXIMITY/AMBIENT LIGHT SENSOR IC WI T H I 2 C INTERFACE Features Applications Description," 2015.


