Introduction

Photometric methods of analysis are heavily required within the field of analytical microbiology. The determination of microorganism numbers in liquid media is such an assay, where the cells themselves scatter light. The physicist John Tyndall explored this scattering phenomenon during the 19th century, leading to the effect being widely known as the Tyndall effect. A modern theoretical description was provided for this phenomenon (Mie, 1908), and later applied to microbiological samples providing the theoretical foundation for interpreting the scattering of light (Koch, 1968). By comparison of the light transmitted through a sample containing microorganisms to a blank reference containing no cells, the relative light scattering can be determined.

When incident light interacts with a sample-containing test tube, the light may be absorbed, reflected, or scattered. If a wavelength of light that is not readily absorbed by the microorganisms is selected, and reflection effects are insignificant, the amount of transmitted light is largely dependent on the scattering effect of the sample. In this situation, the transmitted light has a logarithmic correlation to the concentration of cells, with respect to Beer's law. The cell density in the liquid media can be referred to optical density (OD) in photometric analyses (Koch, 1968). This is the base 10 logarithm of the incident to detected light ratio. A photometer is generally used in absorption mode to determine the scattering of light by microorganisms in liquid media.

The use of table-top spectrophotometers, or micro well-plate readers is standard practice for determining OD. Many of these require start-up and calibration time for the illumination system and optical detection system, respectively, before the instrument can be used. Although micro well-plate readers can perform live tracking of culture growth, spectrophotometers generally lack the ability to perform straight-forward live monitoring of cultures. Both of these table-top machines are generally expensive, and therefore may not be a viable option for many laboratories. Alternatively, an inexpensive instrument capable of cell density determination may be of interest to many researchers. Especially if it is rapid, precise, and produces live results, with the instrument being compact enough to fit within a culture incubator to maintain optimum growth of the microorganisms.

Light-emitting diodes (LEDs) have been embraced by scientific instrument manufacturers in recent years, due to their ability to provide stable monochromatic light, requiring low voltages and power. They are small components, allowing their use in portable, and small devices dedicated to

photometric analysis (Flaschka et al., 1973). The growth in the available variety of fixed wavelength LEDs has led to a variety of instruments being developed for many analytical purposes (Dasgupta et al., 2003; Lamb et al., 2012, 2013; Lamb et al., 2015; Liu & Dasgupta, 1996; O'Toole & Diamond, 2008).

The system-on-a-board (SOAB) design is implemented in the construction of many commercial spectrophotometers. This SOAB design incorporates separate integrated circuits (ICs), as well as many other components, that are used to implement many peripheral circuits (e.g., power supply, data storage, system monitor, analog to digital converter, real-time clock, and communication). Although flexible, such a design can be costly and large, and can be prone to electrical interference due to the multiple ICs used within the construction.

The single-integrated-circuit (SIC) is a design alternative to the SOAB design, containing almost all requirements (e.g., instrumental control, data acquisition, data processing, and communications), within one SIC. The swift development of mixed-signal microcontroller circuits in recent years has facilitated the growth of SIC-based instrumentation. Coupled with low power and voltage components like LEDs and monolithic photodiodes, powering of such components can be achieved directly from a mixed-signal microcontroller, eliminating the need for a peripheral power supply. The performance of such microcontroller SIC based photometers has been shown to be comparable to that of commercial table-top instruments (Cantrell & Ingle, 2003; Lamb et al., 2013).

This report aims to describe how to construct a SIC-based modular live monitoring photometer, highlighting the performance parameters, as well as demonstrating practical analytical microbiology applications. The observed change in OD of microbiological cells suspended in liquid media determined using the instrument described correlates well with OD values determined by table-top instrumentation, for the bacteria *Escherichia coli* and *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000), as well as *Saccharomyces cerevisiae* yeast.

Materials and Methods

Cell Growth

E. coli DH5α, *Pst* DC3000 and *S. cerevisiae* AH 109 cultures were grown in Luria-Bertani Broth (LB), King's B (KB) or yeast peptone dextrose adenine (YPDA) media, and incubated at 37 °C, 26 °C, 30 °C, respectively. Continual mixing was achieved with ~250 rpm shaking on an orbital culture shaker.

Materials and Instrumentation of Home-Built Test Tube Photometer

An Arduino Uno R3 microcontroller board was used to power the LEDs and photodiodes, and to digitize the detected light signal by an integrated 8-bit, high precision analog to digital converter. The in-system programmable FLASH memory was programmed to control the LED illumination and data acquisition using the Arduino integrated development environment. The measuring module can communicate, and be controlled by, external single-board computers (Raspberry Pi, Raspberry Pi Foundation, United Kingdom) via a USB cable. Alternatively, the measuring module can operate autonomously using a LCD screen display (an HD44780-driver-compatible, 5 V, 20×4 Character LCD).

Black PLA (Polylactic acid) plastic was used by a 3D printer (Ultimaker Original+, Ultimaker, The Netherlands) to construct the test tube chambers that housed the LEDs and photodiodes (measuring unit). The microorganism-containing sample is subject to a monochromatic LED light source with a spectral half-bandwidth of 30 nm centered at 740 nm (dome-topped clear epoxy 5 mm diameter LED, 740-01AU, Roithner Lasertechnik, Vienna, Austria). The LED was powered by the microcontroller board's 5 V power pin. A 220 Ω resistor was used to modulate the voltage supplied to the LED. The amount of light that penetrates the sample without being scattered is detected by a DIP photodiode (OPT101, Texas Instruments, Texas, USA) positioned opposite to the light source. The photodiode was also powered by the 5 V power output pin of an Arduino. An external resistor of 1 MΩ was connected to pin 2 and pin 5 of the photodiode, thereby achieving a DC gain of 2 million volts per ampere. This amplification provided a good range of voltages for cell density determination in the range of 0.01–1.0 of determined O.D.

Results

The live monitoring of the OD of a liquid cell culture is of importance as an analytical parameter in many biological fields. We have designed a modular instrument that can obtain live OD values, which utilizes LEDs and photodiodes to determine the scattering of light by microbial cells. This report details the performance of the modular instrument, as well as providing the technical background to allow researchers with limited background in electronics to construct such a photometer.

Microcontroller Choice

The use of compact mixed-signal microcontrollers facilitates the design of scientific instruments. For this modular instrument, the Arduino Uno R3 mixed-signal microcontroller was used for powering both the photodiodes and LEDs, as well as storing and digitizing the data. The microcontroller chip (ATmega328P) has an analog to digital conversion (ADC) successive-approximation-register on-chip. This offers a true, linear, 8-bit accuracy that is satisfactory for the requirements of the modular photometer.

LED Choice

The use of LEDs for small laboratory instruments has become the industry standard as they prove to be an ideal light source for their size. Their benefits have been widely reported, with the availability and range of monochromatic LEDs expanding their use for many applications (O'Toole & Diamond, 2008). A monochromatic LED with a 740 nm peak wavelength was chosen as most microorganisms have negligible absorption of light at this wavelength. This ensures that the changes in light transmitted through the sample is effectively due solely to the scattering of light by the microorganisms, rather than light absorption.

Photodiode Choice

An OPT101 photodiode was chosen for use in the modular photometer. It is an inexpensive photodiode containing an on-chip operational amplifier, and responds to light in the range of 300–1100 nm (linear dependence between 400–800 nm). The benefit of the onboard operational amplifier is

the reduction of potential noise as the signal is amplified before leaving the photodiode chip, reducing the likelihood of interference.

Modular Design

The test tube photometer setup is highly modularized to enable simple expansion of the measuring system. The base module consists of a microcontroller and one measurement cell. Depending on the number of analog input pins of the microcontroller, further measurement cells can be added. A schematic for the measuring setup is presented in Fig. 1.

Microcontroller Interfacing

The schematic representation in Figure 2 displays the interfacing of the microcontroller with the external components of interest (LED, photodiode, LCD screen and PC). The chosen Arduino microcontroller has 16 ports (labeled A0–A15), which are analog input ports. The analog inputs A1, A3, & A5 (test tube module 1, 2, & 3, respectively) were chosen for the input signals from the OPT101 chips (the signal come from pin 5 of the respective OPT101 photodiodes). Pin 1 of the photodiode is directly powered by a 5 V DC pin on the microcontroller, with pin 8 being connected to the ground pin on the microcontroller. The LEDs are connected directly to 5 V DC digital output pins (D7, D8, & D9; test tube module 1, 2, & 3, respectively) on the microcontroller, as the Arduino can provide enough power to drive the LEDs. The power to the LEDs is then modulated using a 220 Ω resistor, and grounded by connecting to the ground pin on the microcontroller. Test tube module one was used as the blank reference module, whereas modules 2 and 3 were used as the sample modules. The LCD screen can be powered directly from the 5 V pin on the microcontroller, and grounded by connecting to the ground pin on the microcontroller. The RX pin from the LCD screen connects directly to the TX pin (pin 1) of the microcontroller to transmit the data to be displayed. Monitoring can also be achieved using a PC or single-board computer. The microcontroller connects to the Raspberry Pi single-board computer through a USB cable, allowing the live monitoring of the modules output via serial communication.

Microcontroller Algorithm and Software

The microcontrollers software was written using the Arduino compiler, and utilizes a similar program flow described by Lamb et al., (2013) (Lamb et al., 2013). Upon power up, the microcontroller will

start the measuring script and will send the live data through the serial port to the LCD screen, or a PC. Additional updates of the firmware installed onto the microcontroller can be performed in this connected mode.

The OPT101 photodiodes used produce a small amount of current even when there is no light incident on the sensors. To compensate for this, the dark signal is subtracted from the sample signal. The firmware installed is set to turn on the photodiodes indefinitely upon instrument initialization. The LEDs are set to be turned off in the beginning of a measurement cycle and the dark signal in each attached measurement cell is determined. LEDs are then turned on and the light signal is measured. Each measurement is repeated numerous times within one measurement period to ensure high accuracy of the OD readings. The light transmission through the sample interacts with the microorganisms and is scattered, resulting in a logarithmic reduction in light transmission as a function of cell concentration. This can be calculated by using the modified equation:

$$OD = -\log\left(\frac{\sum_{i=1}^{n} Sample - Dark}{\sum_{i=1}^{n} Blank - Dark}\right)$$

Here, the *OD* is the optical density of the culture; the *Sample* is the average of the signals from the sample containing modules achieved when the LED lights are on; whereas, *Blank* is the average of the signal from the blank reference module achieved when the LED light is on. The *Dark* refers to the average signals from the respective module, and is the average signal when the LED light is turned off. This algorithm allows for the use of the photometer with very simple ambient light barriers, avoiding interference of unwanted light with the measurement (Yang et al., 2008). A standard technique of oversampling and averaging of the photodiode signals was used to increase both the resolution and the signal-to-noise of the ADC. Consecutive sample values from the ADC are added together, then divided by the number of samples taken to determine accurate photodiode outputs.

Measurement of Microorganisms

To characterize the performance of the modular photometer, a series of standard cell densities within liquid growth media we made (0.0, 0.01, 0.1, 0.3, 0.5, 0.7, 1.0 and 1.5, as determined using a spectrophotometer (Ultrospec III, Pharmacia LKB, Sweden, Uppsala) set at a wavelength of 740 nm) for the various bacteria. The solutions were then used to evaluate the measuring characteristics of the

modular photometer, which uses LEDs with a peak wavelength of 740 nm. A cell-free blank of the same liquid growth media was used in all modules to determine the OD. The performance of the modular photometer is comparable to that of the table-top spectrophotometer used, when measuring the OD of the bacteria *Pst* DC3000 (Fig. 3). A small deviation between the OD measured by the two instruments may be apparent as the OD value increases, but this seems to be due to the differing arrangements of optical components in the instruments used (Koch, 1970).

Live Monitoring of Microorganisms

In order to evaluate the live monitoring performance of the modular photometer, two test tubes containing cell cultures of the bacteria *Pst* DC3000 at an OD of 0.01, and one cell free blank test tube, were placed within the modular photometer that was inside a culture incubator. Their OD was constantly monitored for 50 hours, with the OD of the cultures also being measured in a 24 well plate every 10 minutes using a well plate reader (Tecan infinite M200, Tecan Group Ltd., Switzerland, Männedorf) set at a wavelength of 740 nm. The live performance is different to that of the well plate reader used (Fig. 4). Interestingly it seems that growth conditions are better in the test tube photometer because a detectable cell amplification starts earlier and the bacteria can grow to a higher density in the modular test-tube system. This may be due to our design being able to be housed within an incubator, allowing optimal growth conditions to be maintained.

Further experiments were conducted to monitor and compare cell amplification of *E. coli*, *S. cerevisiae* and *Pst* DC3000. Each experiment was performed with two technical replicates and repeated three times for the respective microorganism. Results indicate a high reproducibility of the measurements and revealed organism-specific growth curves (Fig. 5).

Discussion and Conclusions

This report has shown a live monitoring modular photometer that can precisely determine test tube cell concentrations in liquid media. The measurement of the OD recognizes the light scattering caused by the cell concentration in the liquid. The OD value can then change proportionally to the concentration of cells within the liquid media. Despite the strong correlation between the observed OD in the modular test-tube apparatus, and the commercial spectrophotometer, small deviations may appear at higher cell concentrations. The LED choice was crucial in the modular photometers design, as to avoid light absorption, and reduce error and providing precise determination of cell concentration.

This modular photometer is based on 3D-printed chambers, and an inexpensive, widely obtainable microcontroller platform for powering of the LEDs, the LCD and photodiodes. The microcontroller also converted the photodiodes analog signal into a digital signal, and communicated this data to a PC. The modular photometer has a low cost for around $50-100 US to build, making it an economically appealing option for many research laboratories.

Only a single calibration is required after building the instrument. This is because LEDs have a high stability over time, with only a 2–5% loss of light intensity expected after 1,000 h of use. Given that, the LED is only turned on and off for short periods, this calibration should remain sufficient for the lifetime of the modular photometer. Despite this, calibrations performed for one type of organism may not be suitable for others, as the shape of the cell may be different and therefore change the scattering characteristics of the light. Therefore, regular calibration of the instrument should be performed. Despite this, the adaptation of this instrument to organisms other than those tested in this report should be straight forward, as well as being applied to other photometric analytical measurements.

The Arduino compiling software is free and has been made available for Microsoft Windows, Linux, and MacOS platforms. The design of the Arduino boards facilitate the use and design of electronic instruments by non-experts. Furthermore, the ease of firmware designing and programing allows greater flexibility for the end-user to tailor the modular photometer to meet their specific experimental requirements. This can all be achieved using a standard PC with a USB port.

An advantage of this instrument is its simplicity. Although it is possible to obtain a variation in the amount of light detected between different instruments dependent on how the components of the instrument are arranged (Koch, 1970), the modular photometer uses identical components in exactly the same arrangement, allowing reproducible results from multiple photometers. This allows the live monitoring of multiple cultures in the lab simultaneously.

The test-tube photometer holds further applications and can facilitate lab work in various ways. For example, it can be used as a regular photometer to determine culture densities within test tubes. This avoids the otherwise necessary opening of the tube to take a sample and thus not only avoids a possible source of contamination but also accelerates the measuring process. Further development of the test-tube photometer will focus on online real-time plotting and analysis of the OD measurements. This might for example facilitate monitoring cell cultures to grow to a certain density for cell harvest.

In summary, the design, construction, characterization, and calibration of a live monitoring modular photometer for determining the cell concentration of microorganisms in liquid growth media has been presented. It provides an easy-to-use, modular, and reliable solution to OD measurements, allowing the live monitoring of liquid cultures. The presented instrument will help many laboratories produce simultaneous live culture measurements without the financial requirement of large, commercial designs.

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Figure Legends

Figure 1.

Schematic drawing of the top-view (A) and side-view (B) of a measuring module.

Figure 2.

Schematic drawing of the interfacing of the microcontroller with the measurement modules and a single-board computer via serial connection.

Figure 3.

Performance comparison of the test-tube photometer and a commercial photometer. Mean and standard deviation of measurements that were repeated at least four times for each measurement cell and cell density. The linear graphs represent the linear regression of the values of the respective measurement cell.

Figure 4.

Evaluation of the live monitoring performance of the modular photometer in comparison to a commercial well plate reader. Mean and standard error of the mean of the measured culture densities (test tube photometer: n = 2, well plate reader: n = 18).

Figure 5.

Cell amplification of E. coli (A), S. cerevisiae (B) and Pst DC3000 (C) measured with the test tube photometer. Mean and standard error of the mean of the culture density measurements (n = 6)

Conflict of Interest:

The authors declare that they have no conflict of interest.

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