

Exploring the Potential of an Affordable and Portable Colorimeter for Optical-Based Biological and Chemical Analyses: A User-Friendly Solution



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ABSTRACT

Microplate readers are fundamental instruments widely used in various scientific fields that include molecular biochemistry, biodiagnosis and bioengineering to conduct colorimetric and spectrophotometric measurements of biological samples enabling the quantitative detection of different molecular targets. With their high accuracy, sensitivity and throughput, the microplate plate readers have notably enhanced the robust screening of patient samples and the reliable detection of diseases. However, their utilisation in resource-limited third-world countries has been greatly constrained by their complex, expensive and bulky nature. To address this concern, we suggest an inexpensive device that is able to conduct optical analysis to a standard similar to that of lab-based equipment. Specifically, we have designed and fabricated a low-cost, portable and user-friendly microplate reader to support colorimetry. This colorimeter can be directly used at the point of care and to illustrate its capability, we assessed its analytical performance by detecting the concentration of dye in preparation for its potential use in diagnosing different diseases through measuring the concentration of their protein biomarkers in experimental and clinical ELISA reactions. We compared the results obtained from our device with those from a commercial microplate reader. With the consideration of portability, inexpensive nature due to the use of off-the-shelf components and ease of use due to the implementation of popular open source software like Arduino, we believe it represents an acceptable proposition in enabling high precision laboratory-level analysis in remote and point-of-care settings.

INTRODUCTION

The recent COVID-19 pandemic has highlighted the importance of early disease diagnosis in addressing global health issues. While it showed the rapid transmission of infectious diseases in our increasingly interconnected world, it also shed light on existing challenges within the healthcare industry, including the need for timely detection of various diseases. One such example is cancer, which, although not classified as a pandemic disease, poses a significant health burden worldwide. Early detection is crucial for effective treatment and improved patient outcomes. Unfortunately, the accessibility of diagnostic equipment has been a longstanding crisis, particularly in resource-constrained regions. This issue was exemplified during the early stages of the COVID-19 pandemic when many third-world countries faced a shortage of equipment, hindering the timely diagnosis of not only infectious diseases but also other life-threatening conditions like cancer. The lack of widely available, commercial-grade diagnostic equipment means that patients may receive a cancer diagnosis at an advanced stage when treatment options are limited. This delay in detection can have devastating consequences for individuals and their families. To address this challenge, there is a pressing need to develop affordable and accessible diagnostic methods that can enable early detection of diseases. The objective is to supply all countries with the required lab-based equipment that enable early diagnosis of diseases but this is not always possible considering their expensive nature hence the need for scientists to explore the path of reengineering, miniaturisation and translation of the industry lab-grade methodologies into cheap, user-friendly and portable analytical versions while maintaining the accuracy of these devices. One of the predominantly used lab-instruments is a microplate reader which facilitates biochemical analysis of solutions using various readout strategies ranging from colorimetry to spectrometry. Nevertheless, despite its efficiency which stems from its ability to process multiple samples at the same time and its capacity to process low-concentrated biological samples, a microplate reader is generally bulky and expensive. This places a huge constraint on the procurement of an adequate number of these instruments, particularly in third-world countries. Focusing on the microplate's colorimetric capability, which is one of the most used techniques to determine the concentration of biological samples through the measurement of either the absorbance or transmittance of light passing through a coloured sample solution, we will scrutinise the use of cheaper off-the-shelf components to fabricate a colorimeter with a similar performance to the high-grade laboratory microplate reader. In this quest, some of the valuable tools to be used include 3D printing, an Arduino toolkit which comes with a customisable open-source software together with essential electronic components like

LEDs and microprocessors. 3D printing permits the fabrication of cheap products. LEDs act as light sources that can be adjusted to meet the different requirements that may be needed in the fabrication of this portable device. Lastly, the open source Arduino software allows for the chance to code customisable programs to be used for analysis of the various parameters that allow for the calculation of the absorbance values needed for the colorimeter's intended function. We use these techniques to fabricate a colorimeter that is able to perform analysis on biological samples and compare the results of this analysis with those obtained from a commercial microplate reader.

RESULTS AND DISCUSSIONS

Colorimetry Tests

To conduct our colorimetric experiments, we assembled the necessary equipment that included a dark blue dye, Arduino microcontroller, cuvette, red LED light, light sensor, computer, and a box. The decision to use a dark blue dye as our reagent was specifically due to the principles of the ELISA reaction - a technique used for detecting and quantifying soluble substances like proteins. As many diseases are characterised by the presence of abnormal concentrations of specific proteins in the blood, proteins serve as biomarkers for various diseases thus the ability to measure protein concentrations is of great essence. An important step in the ELISA reaction is the use of the HRP enzyme to amplify the detection signal of a particular protein in the blood. It achieves this amplification effect by catalysing the reaction between a protein and a specific substrate, resulting in the production of a coloured detectable product. However, in our colorimetry experiments, we are not directly using the HRP enzyme or a substrate. Instead we are using the blue dye as a surrogate to mimic the colorimetric response observed in the ELISA reaction. By successfully detecting and quantifying different concentrations of the blue dye in our colorimetric experiments, we can indirectly assess the potential of our device to detect signals produced in actual ELISA reactions, which are commonly used for detecting protein biomarkers associated with different diseases. From our blue dye solution, we prepared eight 2000 micro-litre solutions of varying concentrations through further dilutions using water. To ensure precision, we utilised a pipette for these measurements. Each solution was carefully prepared and placed in a transparent glass cuvette.

With our eight solutions prepared, we proceeded to measure the absorbance of red light passing through each solution. This step allowed us to generate the concentration and absorbance data required for constructing our calibration curve.

For the light sensor, we employed the TCS3200 light-to-frequency sensor and programmed it through the Arduino microcontroller to generate frequency values which corresponded to the intensity of light transmitted through each solution. These frequency values were displayed on a computer. To eliminate any potential distortion of results due to the presence of background light in the experimental environment, we housed the cuvette, light sensor, and the LED in a dark and opaque 3D-printed container (**Figure 3**).

For each solution, we measured light transmittance three times and calculated the average value to ensure accurate readings. Additionally, we adhered to a standardised 90-second waiting period after placing the cuvette in the container to allow the solution to stabilise before taking the absorbance readings. This helped to ensure repeatability, comparison, and consistency of experimental results. Upon completing these readings using our prototype setup, we employed a 96-well laboratory-grade microplate reader to measure the absorbance of the same solutions. We used a pipette to extract 10 micro-liters of each solution and carefully dispensed them into the wells of a 96-well microplate. The microplate was then inserted into the microplate reader for generation of the absorbance signals.

For our second colorimetric test, instead of using a glass cuvette and 2000 micro-litre solutions, we fabricated an eight-well microfluidic chip with each well housing 90 micro-litres of solution. Using a similar prototype setup, we placed the microfluidic chip on top of the sensor and aligning the LED light with one well at a time, we measured the light transmittance 3 times and calculated the average light transmittance. This equipment was also housed in an opaque 3D printed casing.

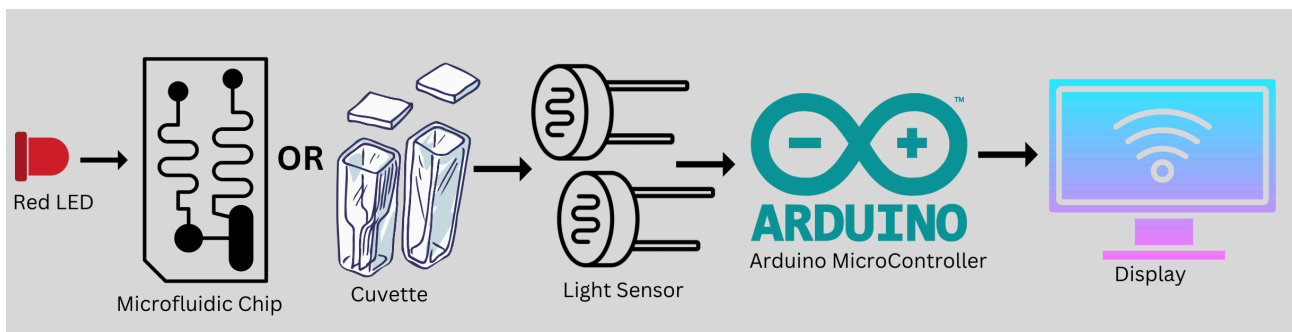


Figure 1: Overview of the System

Data Analysis of Colorimetry Tests

Because our portable colorimeter reader only displays frequency values directly proportional to the intensity of the transmitted light, we needed to calculate the absorbance values of each of these solutions. The equation to calculate the absorbance of the solutions is as follows:

$$A = \log\left(\frac{F_z}{F}\right)$$

A refers to the absorbance of a solution, F_z refers to the recorded frequency when red light passes through the solution and F refers to the background frequency which is the frequency recorded when red light passes through an empty cuvette or empty well of a microfluidic chip. We plotted a calibration curve (**Figure 2**) with results from the 3 experiments.

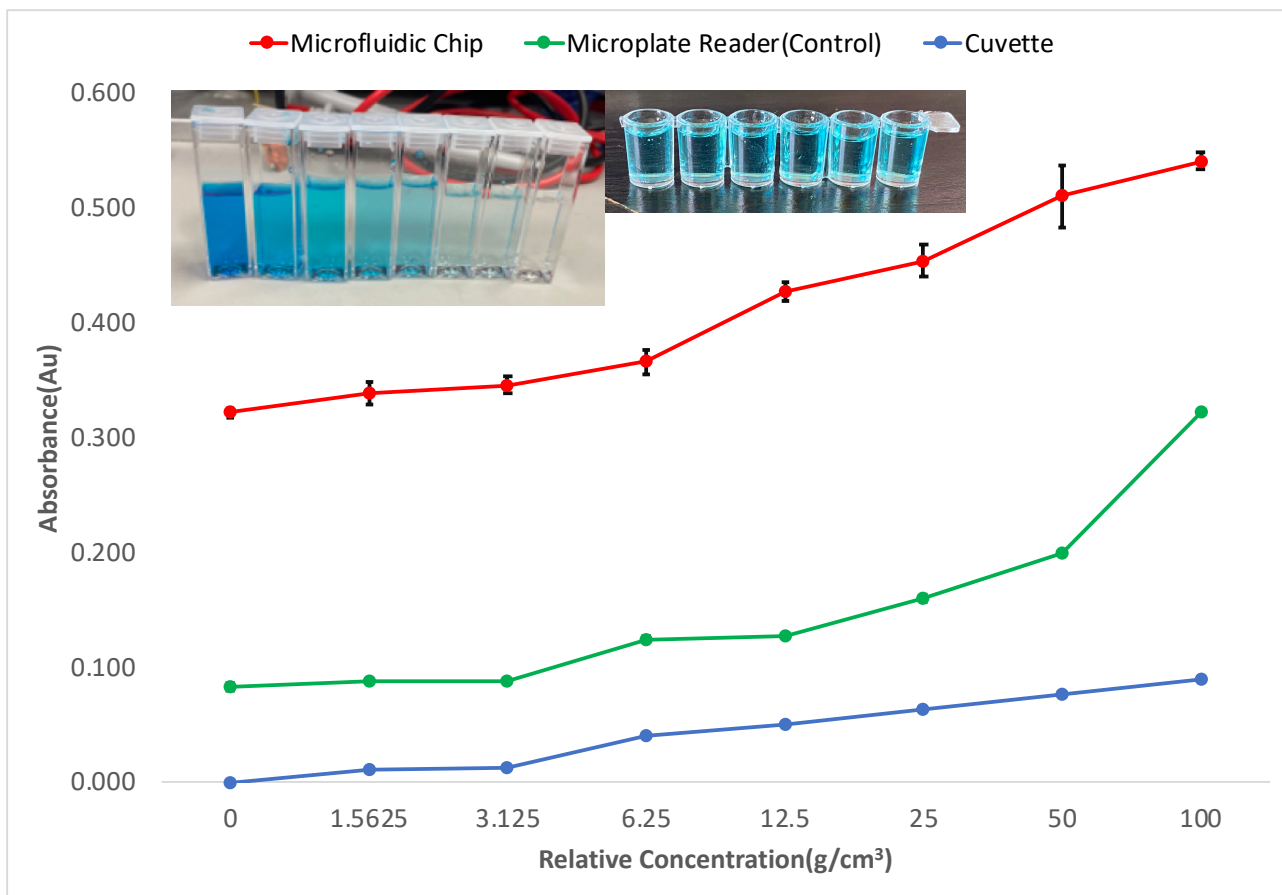


Figure 2: Calibration Curve

Mechanical Design

Our portable colorimeter is a rectangular shaped box housing 3 important hardware components: light sensor, LED and a slot for inserting our microfluidic chip/cuvette. Firstly, the Arduino LED light is connected in series with a 220 Ohm resistor to prevent excess current that could burn out the LED. For the cuvette, the sensor is fixed on the wall of the box on one side while the LED is fixed on the opposite side. To optimise the functionality of the device, we ensured that the slotting channel had sufficient space to facilitate the easy and stable placement of the cuvette. With regards to the microfluidic chip, we placed the LED light directly above it and the sensor was placed on the bottom of the box to measure the light transmitted. We ensured that the slotting channel in this case allowed for the free movement and sliding of the microfluidic chip. This design consideration also meant that we were only able to measure the signal of one well of the

microfluidic chip at a time. Additionally, we used a lid to stabilise the lighting conditions in the box enhancing the accuracy and quality of the results. Since our sensor has over 8 pins that need to be connected to the Arduino microcontroller, we incorporated a breadboard to increase the connection points. As our breadboard was significantly long, we decided to not place the microcontroller and the breadboard in the box to maintain the versatility of the colorimeter.

The commercial components used are commercially available and the total cost of the device is below HKD\$500. To be noted, we did not include the price of the computer that acts as our display interface as the brand and the price vary in different places in the world.

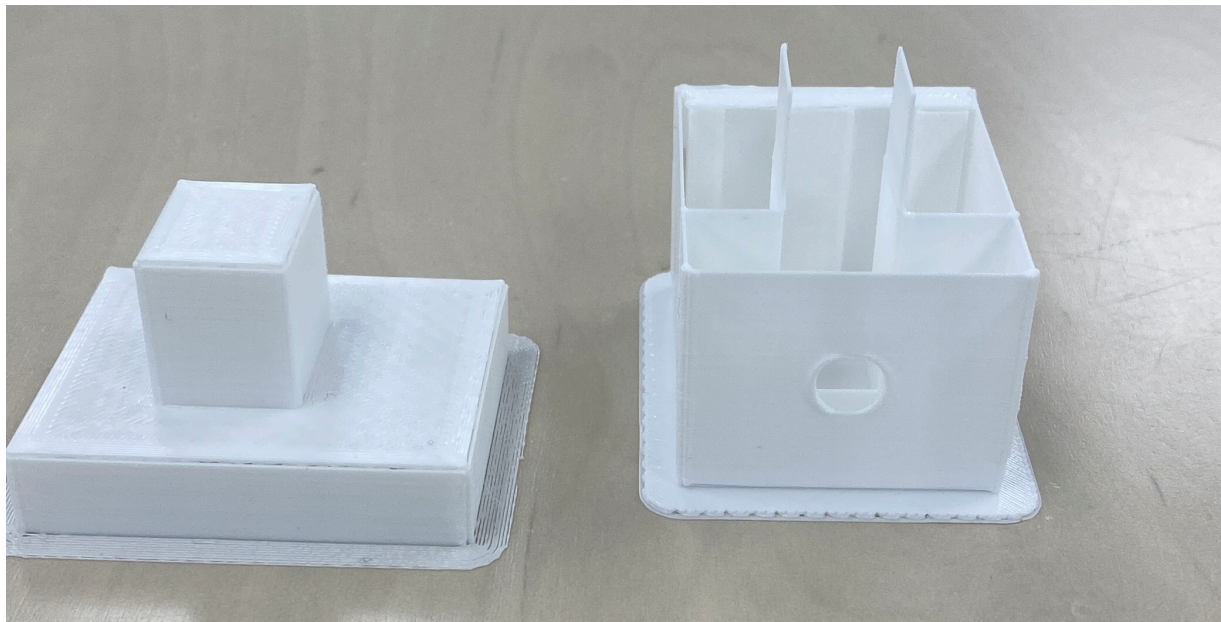
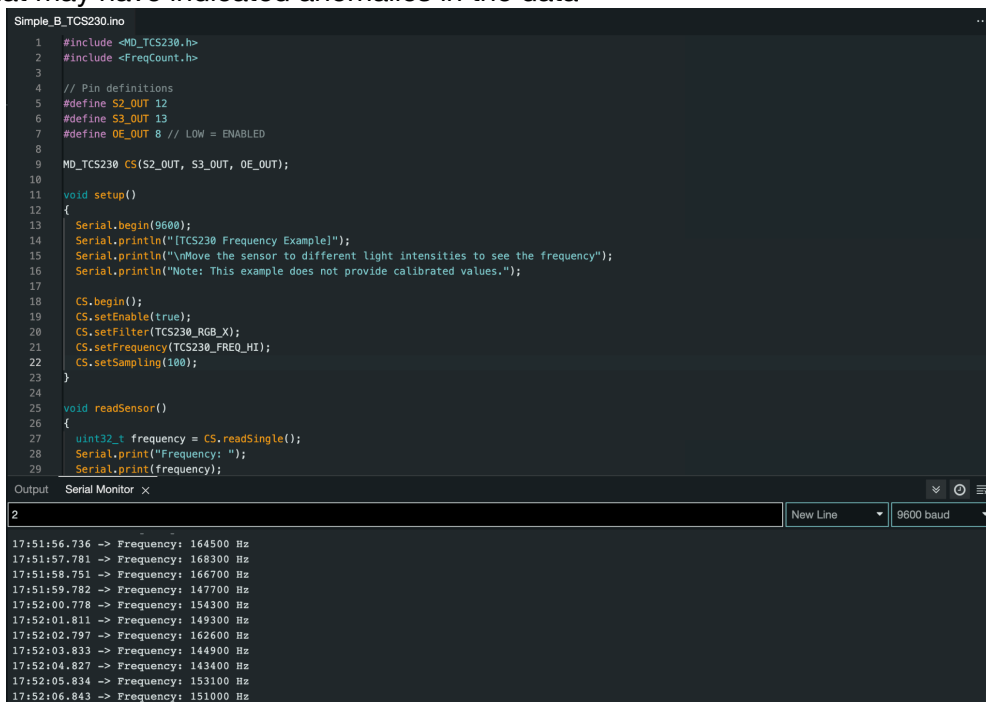


Figure 3: 3D Printed Casing

Interface Design

As the Arduino microcontroller serves as the central component of our device, we utilised the Serial Monitor tool in the Arduino IDE to showcase the frequencies that corresponded to the light absorbance of each solution. This tool allowed us to display the frequency values on the screen in real-time. We sampled the frequencies at a rate of once per second, ensuring accurate conversion of the analog light intensity signal detected by the sensor to discrete frequency values. To initiate the colorimetric measurements, we established a connection between the microcontroller and our laptop using a USB cable. Once connected, we launched the Arduino IDE and associated the connected board through the drop-down menu. With the board properly selected, we executed the program responsible for reading and converting signals from the digital pins of the Arduino into frequency values. The program ran continuously, providing real-time results that are immediately displayed on the Serial Monitor. This allowed us to observe any changes or fluctuations in the frequency values, providing instant feedback on the varying light absorbance within the device. By monitoring the frequency values, we can analyse and interpret the optical properties of the solutions being tested.

Additionally, we utilised another built-in tool called the Serial Plotter in the Arduino IDE. This tool offers real-time data visualisation and graphical representation of the data received from the microcontroller. One important aspect of using the serial plotter is its ability to help us detect any random outliers or irregularities in the frequency readings over time. By plotting the frequency data on the Serial Plotter, we can observe the trend of the readings visually. This graphical representation allowed us to easily identify any unexpected spikes or drops in the frequency values that may have indicated anomalies in the data



```
Simple_B_TCS230.ino
1 #include <MD_TCS230.h>
2 #include <FreqCount.h>
3
4 // Pin definitions
5 #define S2_OUT 12
6 #define S3_OUT 13
7 #define OE_OUT 8 // LOW = ENABLED
8
9 MD_TCS230 CS(S2_OUT, S3_OUT, OE_OUT);
10
11 void setup()
12 {
13   Serial.begin(9600);
14   Serial.println("[TCS230 Frequency Example]");
15   Serial.println("\nMove the sensor to different light intensities to see the frequency");
16   Serial.println("Note: This example does not provide calibrated values.");
17
18   CS.begin();
19   CS.setEnable(true);
20   CS.setFilter(TCS230_RGB_X);
21   CS.setFrequency(TCS230_FREQ_HI);
22   CS.setSampling(100);
23 }
24
25 void readSensor()
26 {
27   uint32_t frequency = CS.readSingle();
28   Serial.print("Frequency: ");
29   Serial.print(frequency);
30 }
31
32 int main()
33 {
34   readSensor();
35 }
```

Output Serial Monitor x

```
17:51:56.736 -> Frequency: 164500 Hz
17:51:57.781 -> Frequency: 168300 Hz
17:51:58.751 -> Frequency: 166700 Hz
17:51:59.782 -> Frequency: 147700 Hz
17:52:00.778 -> Frequency: 154300 Hz
17:52:01.811 -> Frequency: 149300 Hz
17:52:02.797 -> Frequency: 162600 Hz
17:52:03.833 -> Frequency: 144900 Hz
17:52:04.827 -> Frequency: 143400 Hz
17:52:05.834 -> Frequency: 153100 Hz
17:52:06.843 -> Frequency: 151000 Hz
```

Figure 4: Sample Arduino IDE Output

CONCLUSIONS

The above demonstration highlights the feasibility of designing and fabricating a portable and relatively accurate colorimeter device using low-cost hardware, open-source software, a computer and readily accessible technologies. The device showcased the ability to distinguish solutions of different concentrations accurately, as indicated by the non-overlapping error bars on the calibration curve (**Figure 2**). The intended use case for this device is the detection and measurement of protein concentrations in the blood for diagnosis of different diseases.

While significant progress has been made in this project, there are areas for further improvement to enhance the device's operation and capabilities. Specifically, sensitivity and diagnostic outcomes can be further improved. Currently, the device produces frequency values corresponding to the absorbance of the solutions, and a calibration curve has to be manually constructed and used to determine the concentrations of subsequent solutions. This process could be enhanced by designing an app that takes the frequency values generated by the Arduino as input, automatically plots the calibration curve, and accurately determines the concentration of some protein biomarkers, for example epidermal growth factor receptor (EGFR) protein for cancer and amyloid beta protein for Alzheimer's disease. Additionally, incorporating a machine learning algorithm could improve the accuracy of predicting a positive diagnosis based on the concentration values.

Another challenge with the current device is its compactness despite being portable. The use of a breadboard and wires can introduce connection issues and instability in functionality especially when the device is subjected to excessive movements. To address this, designing a dedicated circuit board and soldering all the electrical components onto it would eliminate the fragility associated with a breadboard setup.

Furthermore, the current setup can only read the absorbance of one solution/well at a time, requiring manual movement of the microfluidic chip and alignment with the optical system for subsequent readings. This introduces potential parallax errors and affects the accuracy of the readings. To overcome this, a casing could be designed with a motor that facilitates the movement and alignment of the microfluidic chip with the optical system, ensuring the repeatability of experimental results.

In conclusion, although commercial microplate readers exist that offer higher accuracy, they lack customisation. With our portable reader, not only is it more affordable, but it also provides the opportunity to automate the entire process from absorbance measurement to biodiagnosis. This automation could reduce the potential for manual errors that could be introduced at each stage of the biodiagnosis process. Affordable and accurate medical devices are crucial for timely diagnosis, as seen during the COVID-19 pandemic. The portable reader has the potential to be highly valuable, particularly in developing countries where access to medical equipment is limited, leading to prolonged waiting times for diagnoses. By prioritising affordability in the design of laboratory equipment and medical devices, we can increase the accessibility of affordable medical care worldwide.

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