**Developing an Automated Spectrophotometer Using Arduino Microcontroller**

Ethan Cha  
Bergen County Academies  
United States

**Abstract**

This research used RGB led, LDR sensors, 3D printed sampling cartridge and Arduino mega to create a spectrophotometer with automation software to select wavelength, calculate calibration parameters, and report concentration of the materials being studied for. Spectrophotometers are finding applications across many different fields, including clinical laboratories, pharmaceuticals, molecular biology, biochemistry, and even within the medical realms. This study assembled an Arduino mega system coupled with software for automatic selection of best-fit color light, calibration curve and actual concentration interpolation processes. Dilution solutions from multiple chemical materials were evaluated to examine their linearity of calibration curve. Our study demonstrated that the chemical samples tested revealed a significant regression coefficient reaching up to 0.9945 for serial dilution solutions. Relative concentrations were measured with remarkable precision, exhibiting less than 10% error when compared to a commercial spectrophotometer. These findings not only highlight the viability of the RGB spectrophotometer but also emphasize its potential with the promise of further refinement in development.

# Introduction

A spectrophotometer is the most popular analytical instrument used to quantitatively evaluate the absorption or reflection of visible light, UV light, or infrared light. A spectrophotometer quantifies the amount of light transmitted through a substance and becomes an essential analytical device in various science fields. Determining the concentrations of chemical compounds would be easy now due to the development of the spectrophotometer. Spectrophotometers boast the most variety of applications in clinical laboratories and other multidisciplinary fields such as pharmaceutical, molecular biology, biochemistry, and even medical areas.

Most biomedical companies maintain great spectrophotometers for quantitative measurement in diverse research. Scientists can measure the effect of metabolic intermediates on a cell by measuring the color of the dye in each section. Even the degree of antigen-antibody reaction by the COVID-19 virus might be determined when it is properly configured.

The monitoring of pollution in sewer networks can be at different levels, from merely taking some periodical samples to be analyzed in the lab, to the continuous monitoring of pollutants. The measurement of continuous values of turbidity, pH, conductivity, temperature, nutrients, and organic compounds through spectrophotometric probes today constitutes robust techniques that enable the mobilization of pollutants to be characterized throughout the day, on-line and continuously [4,5,14,15]. Furthermore, on-line monitoring helps in the planning of actions and infrastructures and is required in order to comply with existing legislation on wastewater treatment [16,17]. Nowadays, to address the CSOs’ pollutant dynamics, time-continuous transmittance measurements have been used with satisfactory results. Measurements are taken at several wavelengths, like those of the visible and 254 nm UV wavelengths [9,18,19]. Spectroscopy devices capable of working in the visible spectrum, such as those made of RGB-LED, will help to increase knowledge of pollutants’ movement and water quality monitoring during CSOs [20].

Cost-effective spectrophotometers based on LEDs are being developed and spread to determine wastewater pollution with high accuracy [21,22,23,24], and their comparison with transmittance calculated from classical devices based on incandescent lamps shows good agreement [25].

Low-cost RGB light-emitting diodes (RGB-LEDs) are nowadays used in the construction of simple and compact spectrophotometers for molecular absorbance measurements in analytical chemistry. For instance, RGB-LED-based sensors have been developed to measure different parameters on-line, such as the microalgae-biomass concentration, within a photo-bioreactor, with a 2% error [26]. The use of RGB allowed the study of on-line chromatic values without the cost spiraling throughout the winemaking process [27]. An RGB sensor was also used to obtain information about the color of the sample, detecting the movement of phytoplankton [28]. In the case of tap water, RGB sensors through a web cam were also utilized to control the concentration of parameters, such as ortho-phosphate and aluminum (III) [29]. In the case of wastewater, a portable RGB diode was utilized for the on-site determination of nitrite and iron in river waters [30]. The results of visible spectrophotometry in the near infrared region (NIR) using an RGB diode and a 360- to 740-nm spectrophotometer were compared when characterizing parameters, such as the ammonia concentration and electrical conductivity values [31].

RGB-LED has also been used to calculate the dense packing of bacterial cells in sample solutions in well plates, obtaining good accuracy [32]. The RGB-LED assumes the superposition of different wavelengths, as opposed to conventional spectrophotometers that apply reduced wavelengths for each measurement. This fact introduces an increased susceptibility to interference. However, several authors have shown RGB to be effective, simple, compact, and at a low cost. As stated by [33], its performance indicates that it could be suitable as a replacement for conventional spectrophotometers used in photometric analytical procedures. A loss of sensitivity will usually be encountered, and users will have to decide if this is not significant, since the accuracy continues to be high enough.

The present work includes an experimental campaign in which transmittance is measured from an RGB-LED diode through multiple water samples with different dyes and compounds that cover a broad range of transmittance. The results are compared and discussed with those obtained from a commercial spectrophotometer. An RGB-LED is operated with different equations to vary the emitted superposition of wavelengths, so as to cover the widest possible range within the visible spectrum of light, making use of the combination of the three small individual LEDs contained within the RGB-LED.

The contributions of this work are as follows: First, we developed a novelty calibration process, in order to measure transmittance values between 510 and 645 nm using a single RGB-LED, without optical devices, and with a high level of accuracy. As indicated in the study [25], 18 individual LEDs are needed to be able to analyze water samples between 510 and 645 nm; therefore, a single RGB-LED enables us to significantly reduce the cost of the equipment. Secondly, we provide a simple way to define the red, green, and blue RGB-LED intensity combinations to be used in order to measure the transmittance values that are in agreement with those obtained using the commercial equipment. The novelty lies in using the RGB combination to produce a chemical response in water samples; this can be correlated with that obtained by equipment based on incandescent lamps, where a single wavelength passes through the sample.

Despite the importance of the spectrophotometer as an analytical instrument, it has not been possible to access many STEM science laboratories because of their monetary limitations at institutional administration. The idea of creating an automated spectrophotometer with an Arduino microcontroller inspired us to understand the feasibility of low-cost assembling procedures, which will give us an in-depth comprehension of spectrophotometry and accessibility for our educational purposes.

Our system will have 3 unique properties that most other spectrophotometers don’t offer. We will try our automated RGB light sources to find the most optimal wavelength color for passing through the samples. Secondly, the system will automatically calculate its calibration curve and regression coefficients and report. Thirdly, it will report the final concentration report for the users.

# Experimental Methods

## Specifications of Each Electric Parts

### Arduino Mega and Uno

The Arduino MEGA 2560 is designed for projects that require more I/O lines, more sketch memory and more RAM. With 54 digital I/O pins, 16 analog inputs and a larger space for your sketch it is the recommended board for 3D printers and robotics projects. This gives your projects plenty of room and opportunities maintaining the simplicity and effectiveness of the Arduino platform. This document explains how to connect your Mega2560 board to the computer and upload your first sketch.

The Arduino Mega 2560 is programmed using the Arduino Software (IDE), our Integrated Development Environment common to all our boards and running both online and offline.

### RGB LED

An RGB LED is an LED module that can produce almost any color using these three primary additive colors: Red, Green and Blue. The simplest version of an RGB LED has a combination of 3 separate light-emitting diodes in one package, housed under a clear protective lens. This LED package will have 4 pins, one for each of the three colored diodes and one common anode (+) or cathode (-). The 3 primary color LEDs use the principle of additive color mixing we talked about above to make more colors than we can imagine. LEDs are dimmable by nature which allows each red, green and blue color to produce all the different hues of that color.

Technically, each colored LED can produce 256 shades. It takes a high quality and premium DMX controller to actually get every shade possible but we will go over that more in the controllers section. For now, you can see that light manufacturers combine the 3 primary LEDs with their 256 shades (256 x 256 x 256) to come up with the ‘16.7 million different colors’ slogan that so many RGB lights come with.

### LDR FUNCTIONS & SENSITIVITY

Photoresistors, also known as light dependent resistors (LDR), are light sensitive devices most often used to indicate the presence or absence of light, or to measure the light intensity. In the dark, their resistance is very high, sometimes up to 1 MΩ, but when the LDR sensor is exposed to light, the resistance drops dramatically, even down to a few ohms, depending on the light intensity. LDRs have a sensitivity that varies with the wavelength of the light applied and are nonlinear devices. They are used in many applications, but this light sensing function is often performed by other devices such as photodiodes and phototransistors. Some countries have banned LDRs made of lead or cadmium over environmental safety concerns.

### STEPPER MOTOR

A stepper motor is an electric motor whose main feature is that its shaft rotates by performing steps, that is, by moving by a fixed amount of degrees. This feature is obtained thanks to the internal structure of the motor, and allows to know the exact angular position of the shaft by simply counting how may steps have been performed, with no need for a sensor. This feature also makes it fit for a wide range of applications.

### OBSTACLE SENSOR

A black circuit board with blue and white buttons

Description automatically generatedThe KY-032 Obstacle Avoidance Sensor module is a distance-adjustable, infrared proximity sensor designed for wheeled robots. Also known as AD-032.

The sensor detection distance ranges from 2cm to 40cm, it can be adjusted by turning the potentiometer knob. Its operating voltage is 3.3V – 5V so it is suitable for a variety of micro-controllers like Arduino, ESP32, Teensy, ESP8266, Raspberry Pi, and others.

It has strong adaptability to ambient light and it is fairly accurate to sense changes in the surrounding environment.

### KEYPAD

A close-up of a keypad

Description automatically generatedA keypad for Arduino is a user input device used to provide numerical or alphanumeric input to an Arduino microcontroller. It typically consists of a set of buttons arranged in a grid, similar to a telephone keypad, with each button representing a specific character or function.

Keypads for Arduino usually come in various configurations, such as 3x3, 4x4, or even larger matrices, depending on the number of buttons required for your project. These keypads are interfaced with the Arduino using digital input/output pins or using specialized libraries and modules to simplify the process.

When a button on the keypad is pressed, it makes an electrical connection, allowing the Arduino to detect which button was pressed based on the change in the electrical signal. The Arduino can then interpret this input and perform the desired actions based on the programmed logic.

Keypads are commonly used in projects such as security systems, access control systems, password entry systems, and various other applications where user input is required. They provide a convenient and intuitive way for users to interact with Arduino-based projects without the need for more complex input devices.

### LCD

LCD stands for Liquid Crystal Display. It's a flat-panel display technology commonly used in electronic devices such as television screens, computer monitors, and digital watches. In the context of Arduino or other microcontroller projects, an LCD refers to a specific type of display module that utilizes liquid crystal technology for displaying alphanumeric characters or graphics.

Arduino-compatible LCD modules typically consist of a liquid crystal display panel, a backlight for visibility in low light conditions, and a driver circuitry that allows the microcontroller to control what is displayed on the screen. These modules often come in various sizes, such as 16x2, 20x4, etc., indicating the number of characters each line can display and the number of lines.

LCDs are popular for displaying text-based information in Arduino projects due to their simplicity, low power consumption, and ease of use. They provide a convenient way to output data, sensor readings, messages, and other information in a format that is easily readable by users.

Interfacing an LCD with an Arduino typically involves connecting it to the microcontroller using digital input/output pins and utilizing a library (such as the LiquidCrystal library for Arduino) to control the display. This allows programmers to write code to display text, numbers, and symbols on the LCD screen, making it a versatile tool for creating user interfaces in various projects.

## Assembly of Our Electric Parts

Draw a diagram with Arduino mega; Connect obstacle sensor to D2, stepper motor to D14, D15, D16, D17, and Keypad connection to D3, D4, D5, D6, D7, D8, D9, D10, light sensor LDR to A3, buzzer to D46, RGB LEd to D50, D15, D52

An orange plastic wheel with wires and wires

Description automatically generated

A diagram of a circuit board

Description automatically generated

## Our Software and Algorithm

A white background with black text

Description automatically generated

## Software Development

**Steps**

* Turn on Spectrophotometry
* Greeting; Hi, EH Spectrophotometer
* What is DF (2~10)?
* Place all sample and press C
* Reading STD 1
* Reading STD 2
* Reading STD 3
* Reading STD 4
* Reading STD 5
* Reading STD 6
* Chosen Color:
* R2:
* Reading Smp 1
* Reading Smp 2
* Reading Smp 3
* Reading Smp 4
* Reading Smp 5
* Reading Smp 6
* Reading Smp 7
* Reading Smp 8
* Reading Smp 9
* Reading Smp 10
* Now calculating
* Concentration here
* Smp 1 =
* Smp 2 =
* Smp 3 =
* Smp 4 =
* Smp 5 =
* Smp 6 =
* Smp 7 =
* Smp 8 =
* Smp 9 =
* Smp 10 =
* Slope= R squared=
* Analysis Done

# RESULTS

## Red Color Dye 1:10 Serial Dilution with 6 Point Calibration Curve

With RGB light and LDR sensor, serially diluted red dye solutions were measured in our preliminary prototype model. When the relative concentrations were 6, the regression coefficient was moderately dispersed from 0.42 to 0.58 as in Fig.1.a. However, when the concentration points were reduced to 4, their regression coefficient from the calibration curve were satisfactorily high from 0.96 to 1.00 as seen in Fig.1.b

A graph of an analogogram

Description automatically generatedFig. 1.a Red Color Dye 1:10 Serial Dilution with 6 Point Calibration Curve. R2 was moderately low at 6 points; b Red Color Dye 1:10 Serial Dilution with 4 Point Calibration Curve. The 4 point calculation was far higher, R2 near to 1.

A comparison of graphs with numbers

Description automatically generated with medium confidence

Fig.2 presents the calibration curve from Green Color Dye, calculated with 6 points.

A graph of a function

Description automatically generated with medium confidence

Fig.3 presents the calibration curve from green color dye, but, calculated with 4 points, which showed higher R2.

## Blue color dye with RGB color illumination

A graph of a function

Description automatically generated

Fig.4 presents analog absorbance with respect to the relative concentration, in which R2 showed a variable from 0.3074 to 0.9944, calculated with 6 points.

A graph with numbers and a line

Description automatically generated

Fig.5 presents analog absorbance with respect to the relative concentration, in which R2 showed higher from 0.5183 to 0.9679, calculated with 4 points.

# Conclusion

# References

<https://testbook.com/maths/dot-regression-coefficients#:~:text=Using%20the%20formula%20to%20find,x>

<https://www.physicsclassroom.com/class/light/Lesson-2/Light-Absorption,-Reflection,-and-Transmission>

<https://byjus.com/chemistry/spectrophotometer-principle/>

<https://biochromspectros.com/media/wysiwyg/support_page/UV-Visible_Spectrophotometry.pdf>

<https://www.researchgate.net/publication/321017142_UVVIS_Spectrophotometry_-_Fundamentals_and_Applications>

<https://www.instructables.com/Science-With-Rainbows-Spectrophotometry/>

<https://en.wikipedia.org/wiki/Calibration_curve>

<https://www.uomus.edu.iq/img/lectures21/MUCLecture_2021_12123477.pdf>

<https://www.mdpi.com/1424-8220/19/13/2951>

<https://www.sciencedirect.com/science/article/abs/pii/S0039914022000406>