Direct Absorbance Bilirubin Spectrometer: Analysis of Bilirubin Content Using Direct Absorbance Spectroscopy

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***Abstract* — Neonatal hyperbilirubinemia, or jaundice, is a common but potentially dangerous condition in newborns wherein elevated concentrations of bilirubin causes yellow discoloration of the skin and eye whites. The Direct Absorbance Bilirubin Spectrometer (DABS) is designed to determine whether the bilirubin levels are threatening or safe by measuring light absorbance through a sample. The absorbance is calculated with the Beer--Lambert Law using currents generated from a photodiode by incident light from two monochromatic laser diodes transmitted through a sample. The DABS device is capable of providing approximate results that can indicate whether bilirubin levels are safe, elevated, or whether immediate medical attention is recommended.**

***Index Terms* — Central processing unit, optical wavelength conversion, diode lasers, data analysis, temperature control, amplifiers.**

I. Introduction

Neonatal hyperbilirubinemia, or jaundice, is a common condition in newborns wherein elevated concentrations of bilirubin causes yellow discoloration of the skin and eye whites. Bilirubin is the product of hemoglobin being broken down. When the neonate liver is incapable of processing the bilirubin because of increased production, decreased excretion, or other impaired mechanic, an excess concentration can occur. Levels resulting in visible discoloration occur in almost 60% of all neonates. At higher levels (308 μmol/L), the bilirubin is toxic to the neonate brain, and encephalopathy or brain damage (kernicterus) can occur without treatment. For less extreme cases, sunlight exposure is enough to aid the neonate in processing bilirubin. Phototherapy treatment is successful in treating hyperbilirubinemia in most extreme cases; therefore, the key issue is timely diagnosis and treatment.

Bilirubin concentration levels usually rise to dangerous levels 3-4 days after birth. Many infants and mothers are discharged from hospitals 2 days after birth, so hyperbilirubinemia is often not detected until the first pediatric checkup, often 5-7 days after birth. This means more serious cases of hyperbilirubinemia can go undiagnosed for days. The DABS project is a device capable of quickly measuring bilirubin levels from a small sample of whole blood and determining whether the bilirubin levels are “low”, “borderline”, or “dangerous”.

II. Goals and Objectives

DABS is designed for clinical use, so that a pediatric clinic can provide worried parents with a quick, inexpensive determination of the severity of their newborn’s jaundice. DABS will be easy to operate and take under 5 minutes to calculate an output, so that an appointment would not be necessary for parents to make an appointment. Given a sample, DABS will determine if it is “safe”, “elevated”, or “dangerous”. DABS will use natural pigments in place of real hemoglobin and bilirubin but can be converted to clinical use with real human blood serum with small modifications and recalibrations.

III. Bilirubin Analysis Method

Bilirubin is a yellowish-orange and absorbs light at 440 nm. Hemoglobin also absorbs light at 440 nm but absorbs light at 528 nm in equal measure. In neonates, bilirubin absorbs light at 460 nm. No other substances present in blood at this age absorb light at this wavelength, besides hemoglobin. Hemoglobin also absorbs light at 460 nm and has an absorbance peak at 540 nm. The ratio of hemoglobin at both wavelengths in full-term infants is constant enough so that a hemoglobin absorbance at 460 nm can be calculated from the hemoglobin absorbance measurement at 540 nm. [1]

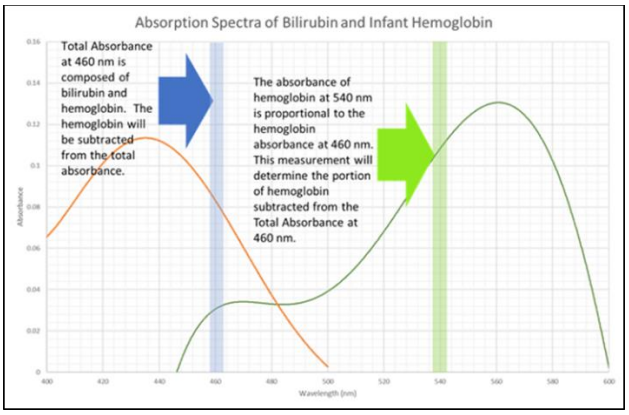

Fig. 1. Both substances contribute to the absorption taken at blue. The absorbance due to bilirubin can be determined by subtracting a projected absorbance value of hemoglobin at blue, based on its absorbance value at green.

Because this team does not have the resources to meet the conditions for procuring, storing, and handling human blood samples as dictated by code CFR 21 by the Human Research Protection Office, the DABS project will not be measuring real bilirubin from real neonatal human blood serum samples. Curcumin, a yellowish-orange pigment found in turmeric, absorbs light at 405 nm. Betalains, reddish-purple pigmentation found in beetroot, absorbs light at both 405 nm and at 532 nm. The DABS device will demonstrate the proof of concept by detecting curcumin content from betalain samples using the same principle proposed for human blood serum.

DABS will determine the concentrations of curcumin by illuminating a blank sample with two light sources, one at 532 nm and one at 405 nm, and recording this incident intensity. A blood sample or proxy will then be illuminated and the transmitted intensity at each wavelength will be recorded. The absorbance at each wavelength will be determined using the Beer Lambert Law equation.

$$A = log(I0/I) \qquad (1)$$

The absorbance at 405 nm due to curcumin will be determined by subtracting the betalain absorbance (405 nm), which was determined by its ratio to the betalain reading at 532 nm.

The DABS device will require a user to insert a blank cuvette filled with 100% isopropyl alcohol and then a cuvette filled with a beetroot solution containing unknown small concentrations of curcumin. It will calculate the absorbance due to curcumin in the sample, and deliver an output recommendation of "low", "borderline", or "dangerous".

IV. Sample Standards

In full-term neonates, bilirubin levels below 5.2 mg/dL are considered low, levels between 5.2 mg/dL and 15 mg/dL are considered elevated, and levels over 15 mg/dL are considered dangerous and in need of phototherapy [2]. Based on the molecular weight of bilirubin and a 1 cm optical pathlength, this translates to respective absorbance values of .5 and 1.4. Curcumin standards with equivalent absorbance values were developed by adding turmeric to isopropyl alcohol or IPA until the desired absorbance value at 405 nm was obtained with respect to the intensity transmitted through a blank sample and the Beer's Law equation in (1). Betalain solution was added to each standard in a 1-2 ratio. The absorbance values obtained through the MCU calculations, as shown in Table 1 and the calculated curcumin values became our standard absorbance values to deem whether unknown samples were "safe", "elevated", or "dangerous".

Table 1. Low and High threshold absorbance values were calculated from curcumin concentrations.

	Low Threshold	**High Threshold**
Measured Absorbance at 405 nm	1.45016	1.70543
Measured Absorbance at 532 nm	1.16975	1.30103
Calculated Betalain A at 405 nm	1.0856	1.20749
Calculated Curcumin A at 405 nm	**.36452**	**.49794**

The two standard curcumin solutions are depicted in Figure 2 and have respective absorbance values of .5 and 1.4. 1 mL of each solution was added to 2 mL of betalain solution, and the resulting absorbance values, .365 and .498 were coded as the high and low threshold barriers for future samples, with readings below and up to .301 corresponding to the "safe" output, readings above .369 and up to .498 corresponding to an "elevated" output, and readings above .498 corresponding as "dangerous".

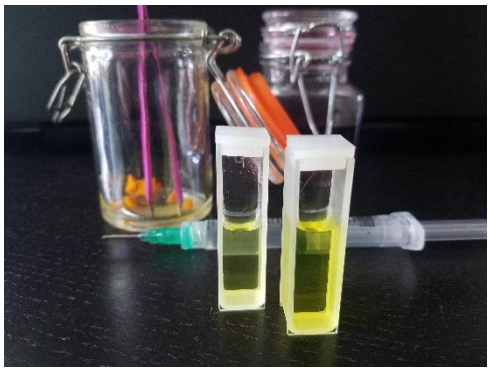

Fig.2. The two curcumin solutions with absorbance values of .5 and 1.4 were used to determine the low and high threshold values, respectively.

V. Optical System

The optical subsystem resides in its own light-containment compartment and comprise of the two light sources, a cuvette, and a photodiode. The light-containment compartment sits inside the outer DABS structure. Both laser diodes are stationary and are aligned so that both beams directly hit the surface of the photodiode.

The two laser diodes, 405 nm and 532 nm, each have 5 mW output and a working voltage of 3-5 Volts. Both laser diodes were purchased as modules with built-in current drivers. The emission peaks were measured with a spectrometer, and the blue and green diodes have 2 nm and 2.5 nm full width half max (FWHM) values, respectively. The narrow emission spectra is important to determine the absorption values at specific wavelengths.

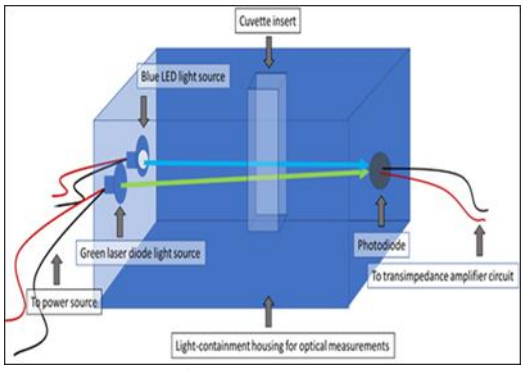

Fig .3. The layout of the optical subsystem within its light-containment housing.

The cuvettes are glass, as it was inexpensive and provided the needed transmission at our two wavelengths on the visible spectrum. The dimensions of the cuvettes are 12 mm x 12 mm x 45 mm and require a minimum 2.5 ml sample volume for the laser beams to pass through the solution. This is a satisfactory volume for the curcumin and betalain solutions prepared for this project but would not be a suitable sample volume to require of a neonate. Sub-millimeter pathlength cuvettes are available but expensive. If DABS were to go into production for use in a clinical setting with real blood, it would be a necessary expense.

The photodiode utilized by DABS is a BPW21R Photodiode from Vishay Semiconductors. This semiconductor has a slower response time when compared to other semiconductors on the market, but a higher sensitivity, which more closely meets the need of the project. The goal of the DABS device is to accurately measure varying unknown concentrations of turmeric in a betalain and turmeric solution that acts as the blood proxy. Since the sample is not changing at a relatively fast rate the response time was not as big of a factor as the sensitivity to determine the varying concentrations. The spectral range of the BPW21R is 400 nm – 675 nm, which includes both wavelengths of the laser sources being read. It also has the largest sensitive area allowing it to collect more light with a linear response to directly correlate to the Beer Lambert law to measure the correct concentrations of the samples.

VI. Electrical components

A. Transimpedance Amplifier

Since DABS measures the curcumin concentration using direct absorbance (i.e., measuring the absorbance of light), we used a circuit that links light to a meaningful voltage value. This is the purpose of the photodiode and transimpedance amplifier that was incorporated into DABS, Figure 4 gives a schematic illustration of this concept. For this, we used the OPA 170 by Texas Instruments.

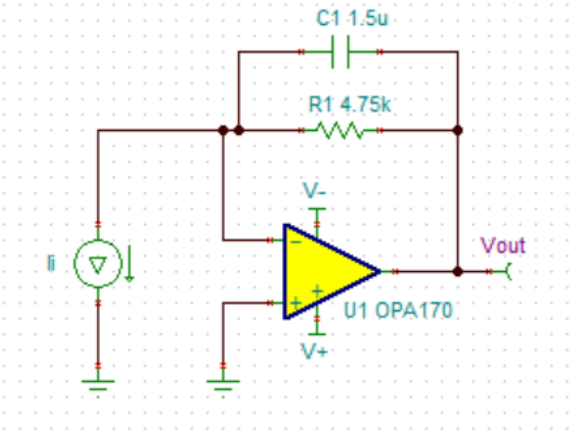

Fig. 4. Transimpedance amplifier.

In this figure, the photodetector output is modelled using the current source, the feed into the amplifier. The feedback resistor, R1, amplifies the current giving the circuit gain modeled in Figure 5.

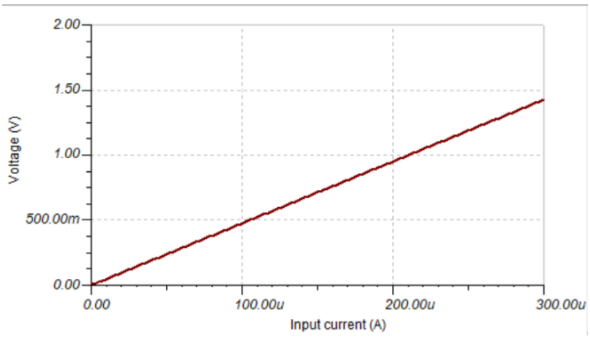

Fig. 5. OPA170 Simulation Output.

The photodiode will read the incident light, as it passes through the sample, and then output a current that is dependent upon the sample concentration. This current will then serve as the input to the amplifier, similar to the figure shown above. In order to produce a voltage that can be read, and interpreted by the ADC of the microcontroller, we chose a resistor that gave a stable output over a relatively large range of inputs. Based on simulations, using the TI-TINA schematic editor and breadboard testing, we were able determine that a resistor value 4.75kOhms. This value gave us a useful voltage output that varies enough, over a range of input current.

B. Cooling system

To measure the temperature of the optical system, the Texas Instruments LM35 centigrade temperature is used to accomplish this. The LM35 was chosen as it is the most common temperature sensor for small to mid-level projects. The sensor measures the temperature of the lasers and sends an output voltage to an input pin of the MSP430 microcontroller.

A quick summary of the LM35 is given below:

- Linear +10-mV/°C Scale Factor
- Temperature range of -55 °C -to 150°C
- Operates from 4 V to 30 V
- Less than 60- uA current drain
- 0.5°C ensured accuracy

For supplying cooled air to the lasers, two 5 V fans are utilized at 13200 rpm. When the temperature is measured from the temperature sensor and sent to the microcontroller, the MCU sends an output voltage to two bipolar junction transistors. Once the threshold voltage is surpassed, the BJT will act as a short circuit and allow the fans to turn on from 5 Volts.

VII. MICROCONTROLLER SYSTEM

The microcontroller used in this project is the MSP430FR6989 produced by Texas Instruments. It acts as the coordinator of the system by mediating when subsystems are activated, consuming power. Such as not constricting the polling of touch response to only when a user has touched the screen. And powering down the display after a duration of idleness. The microcontroller also handles the computation and conversion of the analog readings from subsystems in the module. More specifically the readings from the touch display and the readings from the optical subsystem.

VIII. DISPLAY/TOUCHSCREEN INTERFACE

The primary interface for user interaction is the touch-LCD display. It is a resistive 4-wire touch interface and it allows for the user to operate the DABS module. It comes pre-compatible with the prototype board, so this display was a satisfactory choice and gives the functionality needed.

The structure of the resistive touchscreen also assists with the objective of making the module as energy efficient as possible. Instead of needing to run a constant loop that checks for a press-down the display allows for the system to simply detect a change once the it's been touched or un-touched. Regarding its use in operation it is illustrated in Figure 6. At start up the screen will display some information regarding its boot up. A touch calibration screen is a part of this

boot up process but is only show after a full reset of the system.

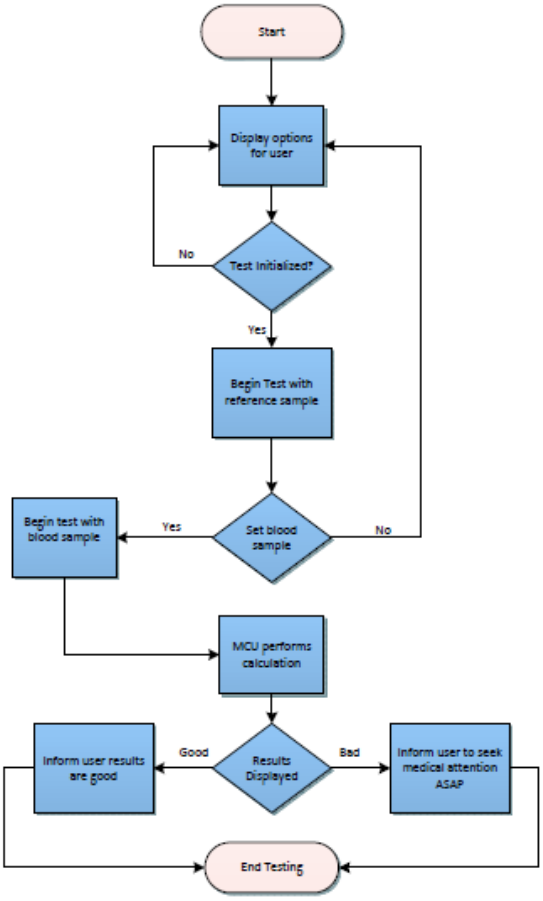

Fig .6. Flowchart of the operation of the DABS module device

Following the startup is the main menu where the user has the option to start the optical analysis, go into settings, put the device into sleep mode. Once the reference sample is inserted and the analysis starts the screen updates accordingly and indicate the running of this analysis until the it is complete.

Following its completion, the next screen gives the user the option to restart the process or continue and run a similar process for the next sample after the blood sample has been placed in the device.

The final screen of the process gives back a summary of the results of the analysis along with any recommendation accordingly. Such as whether treatment or medical attention is required. Figure below displays the welcome screen that displays the start button and settings for users to choose. The start button begins the testing analysis.

Fig. 7. Welcome screen on LCD touchscreen.

IX. POWER INPUT

The main power source is a lithium polymer-based battery capable of outputting a voltage of 4.2 Volts when fully charged and a current compacity at 2500 mAh The battery system will be able to recharged using a USB Type-A connection to an external power source either from a wall outlet using a USB adapter or any computer system that has a USB connection port.

The configuration shown in the schematic for the charging circuit allows the device to be power by an external power source while charging the battery (Figure 8). So, two power source options will be available for the functionality of the device.

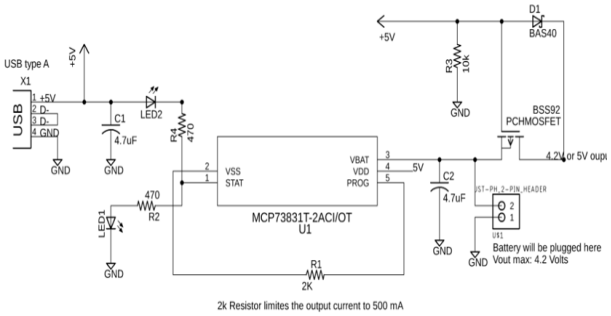

Fig. 8. Power input and battery charging circuit.

From the schematic, a USB port was soldered on the PCB for a USB connection for optional power input. The main chip that will charge the battery is the MCP73821 from Texas Instruments.

This chip was chosen for the reliability and safety of charging the main battery system at 4.2 volts and a programmable charge current rate of 500 mA. The range of supply voltage to this chip is from 3.75 to 6 volts max which is safe operation since most USB type connections are rated at 5 volts.

The design also has 2-pin JST-XH connector that was soldered on the PCB. This will allow the battery to simply connect to the board and be secured tightly and not have a loose connection. The battery can also be replaced if the battery can no longer be functional.

The schematic also includes a Schottky diode that connects between USB output to the drain pin on the P-Ch MOSFET. The diode is there to prevent any leakage current from the device.

A P-Ch MOSFET is used to open the circuit from battery to a DC-DC converter or a buck booster whenever there is power coming from the USB connection. When there is no power from an external power source, the P-Ch MOSFET will close the switch between source and drain and allow the battery to provide power to the system.

For the low-powered microcontroller to function properly, a voltage input of 3.3 volts is required as specified the MCU datasheet (Figure 9) . A buck converter is used to accomplish this feat. The input voltage of this circuit will either be 4.2 volts from the lithium-polymer battery when fully charged or 5 volts from an external power source using USB connection. The IC used to provide 3.3 V to the controller is the TPS6300x. This chip is rated to take an input from 1.8 to 5.5V and output 1.2 to 5.5 Volts at 1200 mA or less. In our project, the IC is set to provide 3.3 V at 600 mA.

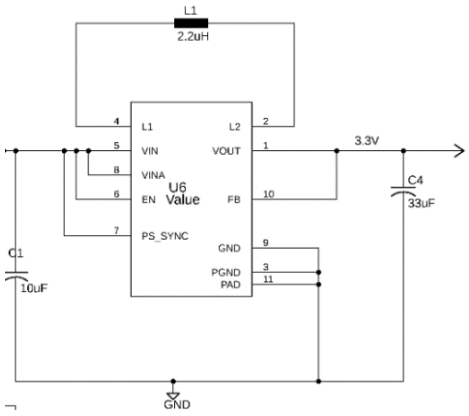

Fig. 9. 3.3 V DC-DC converter.

Two other DC-DC converters were used for a 5 Volt and a 3.7 Volt output. The two converters and can be seen in Figure 10 and Figure 11. All the DC-DC converters were created using Texas Instrument WEBENCH software tool.

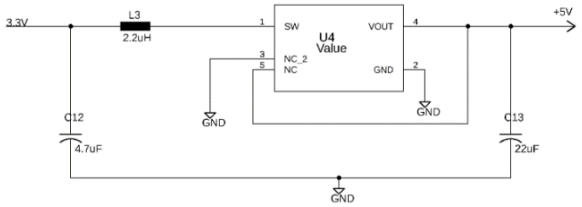

Fig. 10. 5 V DC-DC converter.

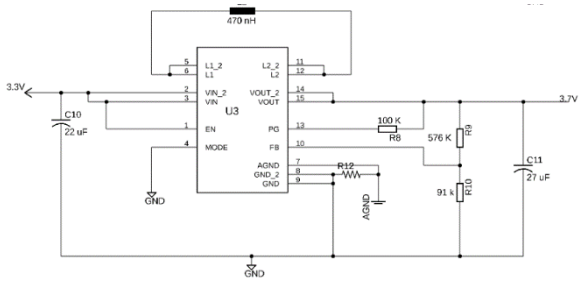

Fig. 11 3.7 DC-DC converter.

X. PROCEDURE

A betalain solution was created by soaking 1 oz of diced fresh red beets (beta vulgaris) in 2 oz 100% isopropyl alcohol (IPA). Various amounts of IPA were added to cuvettes with betalain solution to create multiple concentrations. The absorbance of each solutions was recorded at 532 nm and 405 nm. A line was fitted to the results, passing through the origin, to give the absorbance of betalain at 405 nm as a result of the absorbance at 532 nm.

A line was fitted through the datapoints and the origin to give the absorbance of blue as a function of betalain absorbance at green (Shown in Figure 12).

The curcumin concentrations were prepared by submerging peeled turmeric into 10 ml of 100% IPA for 5 seconds.

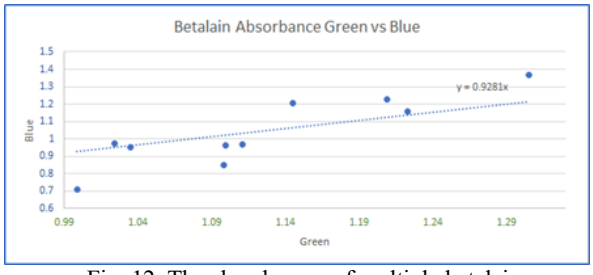

Fig. 12. The absorbances of multiple betalain concentrations at 532 nm (green) and 405 nm (blue).

Three samples of this this solution with descending concentrations were prepared by diluting subsequent mixtures with 10 ml IPA. The absorbance of each sample was measured at 405 nm for validation. After the target values were recorded, 2 ml of betalain

solution was added to 1 ml of each curcumin concentration to form the 3 samples.

The microcontroller calculated the absorbance of each sample at 532 nm and at 405 nm by recording 4 input values: the voltage values from the transimpedance op amp generated by currents from the photodiode at each wavelength for the incident light through a blank cuvette containing pure IPA (I_{405nm} and I_{532nm}) and the transmitted light through the sample (T_{405nm} and T_{532nm}). The absorbances were calculated with the Beer's Law equation

$$A_{405nm} = log(I_{405nm}/ T_{405nm}) \quad (2)$$

$$A_{532nm} = log(I_{532nm}/ T_{532nm}) \quad (3)$$

The absorbance at 405 nm for the betalain alone was calculated using the absorbance at 532 nm and the equation from the fitted line in Figure 12,

$$A_{B405nm} = A_{532nm} * .9281 \quad (4)$$

A_{B405nm} was subtracted from the measured absorbance of the whole solution, A_{405nm}. The remainder was the absorbance value attributed to curcumin, A_{C405nm}.

$$A_{C405nm} = A_{405nm} - A_{B405nm} \quad (5)$$

XI. RESULTS

Three curcumin solutions with unknown concentrations were prepared and 1 mL each was added to 2 mL of betalain solution, maintaining the same ratio as the standards. Table 2 shows the

Table 2. The incident and transmitted inputs at 405 nm and 532 nm and the calculated absorbances for each of the three sample concentrations.

Sample #	**A_{532nm}**	**A_{B405nm}**	**A_{405nm}**	**A_{405nm}- A_{B405nm}**
1	1.1915	1.1059	1.3231	.217
2	1.1697	1.086	1.4706	.385
3	1.1154	1.035	1.8925	.857
Sample #	**A_{532nm}**	**A_{B405nm}**	**A_{405nm}**	**A_{405nm}- A_{B405nm}**
1	1.2578	1.1674	1.4154	.248
2	1.2607	1.1701	1.6011	.431
3	1.0345	.9601	1.9201	.960

measured absorbance logged for each sample at green and blue, and the calculated absorbance due to curcumin. Each sample was run two times in order to verify that the values were similar for samples tested twice in a row.

Using this methodology, the resulting curcumin absorbances were consistent and matched values expected based on the yellow coloration of the curcumin solutions. The resulting values maintained the same relative scalars as the expected values, and the correct "dangerous", "elevated", and "safe" values were identified, shown in Table 3. When curcumin was added to samples reading "safe" or "elevated", the measured absorbance went up and the output indicator changed accordingly.

Table 3. The results from three unknown solutions, and the output values printed for each.

Calculated Curcumin Absorbance	**Output**
.217	Safe
.385	Elevated
.857	Dangerous

XII. CONCLUSION

DABS successfully determined whether the curcumin absorbance of a given sample was safe, elevated, or dangerous in relation to the threshold values input. Consecutive runs of the same sample contained some variance, but the output category for each value remained consistent. There would be clinical benefit if the concentration of each sample could accompany the output, but the small discrepancies between consecutive runs would translate into larger variance in concentration values and the results would be less meaningful. However, the primary objective of DABS was to provide a quick, general reading so that parents could be reassured in cases of mild jaundice and be prompted to send further samples to a lab for more accurate testing in the case of dangerous levels. Given the appropriate threshold values, DABS successfully achieves the objective.

For DABS to be used to calculate the bilirubin absorbance from real human blood serum, a few modifications would be necessary. The current cuvettes require a minimum of 2 mL to ensure the laser beam passes through a sample. This is too high a volume to be taken from a newborn to meet the objectives of safety and ease of use. Custom .05 mm pathlength cuvettes are available that would require only 4 or 5 drops of blood for a sample. Secondly, the laser modules used were inexpensive, but for more consistent results, DABS would use higher-quality laser modules with electrical attenuation to reduce noise in the signal. Readily available wavelengths 405 nm and 532 nm were sufficient to capture curcumin and betalain on blue and isolate betalain on green, but

a 460 nm wavelength would be needed to capture the absorption of bilirubin. Finally, blood standards would be used to recalibrate new threshold values. With these three modifications, the DABS prototype could successfully meet its goals and objectives in a pediatric clinic setting.

ACKNOWLEDGEMENTS

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BIOGRAPHY

Bernardin Dezius is a graduating Computer Engineer at the University of Central Florida. He plans to go into the industry right after graduating and hopes to become a Software/Hardware Consultant with a software firm. He also hopes to eventually return to school for a degree in one of multiple stem fields that interest him.

Juan Gonzalez is a graduating Electrical Engineer at the University of Central Florida. He plans to make his internship a full-time job as a Control Systems Engineer. He also plans to continue his education by obtaining his masters in a STEM related field at the University of Central Florida.

Kevin Kuzius is a graduating Electrical Engineer at the University of Central Florida. Currently a part of the College Work Experience Program partnership with Lockheed Martin. He plans to enter the industry upon graduation.

Andrea Wetteland is graduating with a Bachelor of Science degree in Photonic Science and Engineering from CREOL, The College of Optics and Photonics at the University of Central Florida. She has been working as a laser engineering intern at L3Harris Advanced Laser Systems Technology and plans to continue designing and testing laser resonators and optical systems upon graduation.

Kevin Landau is graduating with a Bachelor of Science degree in Photonic Science and Engineering from CREOL, The College of Optics and Photonics at the University of Central Florida. He plans to go into industry after graduating with ASML which works with photolithography machines for the semiconductor industry. He also hopes to eventually return to school to obtain a master's degree in a STEM related field.

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