APPLICATION OF SMARTPHONE-BASED BIOPHOTONIC INSTRUMENTATION FOR GLUCOSE SENSING USING COLORIMETRIC METHOD

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UNIVERSITI SAINS MALAYSIA

2024

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by

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Dissertation submitted in partial fulfilment of the requirements for degree of Bachelor of Health Sciences (Honours)

(Medical Radiation)

June 2024

ACKNOWLEDGMENT

All praises and gratitude to Allah, the Almighty, the Creator of all things in heaven and earth, for the opportunities and ideas He has provided, enabling me to complete this research work smoothly and successfully within the allocated time. Peace and blessings be upon Muhammad, His messenger of Islam. Firstly, I extend my sincere gratitude to my supervisor, Dr. Muhammad Nur Salihin bin Yusoff, for his invaluable advice, time, insight, and guidance in completing this research project.

I also wish to express my heartfelt thanks to my family for their unwavering support, deep understanding, and patience throughout the duration of my research. To my course mates, I am deeply appreciative of your help and support during this journey. Your kindness has been instrumental in making this project a reality. Lastly, to everyone who has contributed directly or indirectly to this research project, and to those I may have inadvertently overlooked, thank you. This project could not have been completed on time without your assistance. May all your kindness be repaid in the future.

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LIST OF ABREVIATIONS

GI	Glycemic Index
DIC	Digital image corelation
NIR	Near-Infrared
LED	Light Emitting Diode
CMOS	Complementary metal-oxide-semiconductor
CCD	charge-coupled devices
GOx	Glucose oxidase
HRP	horseradish peroxidase
TMB	3,3',5,5' Tetramethylbenzidine
ATP	adenosine triphosphate
H_2O	Water
$FADH_2$	Flavin adenine dinucleotide
APP	Application
UV	Ultraviolet
R	Red component
G	Blue component
B	Green component
L	Litre
mL	Millilitres
dL	decilitre
g	gram
mg	Milligram
µL	Microliter

POC	Point-of-Care
D-glucose	Dextroglucose
L-glucose	Levoglucose
α	Alpha
β	Beta
NADH	nicotinamide adenine dinucleotide hydrogen
CO_2	carbon dioxide
acetyl-CoA	acetyl coenzyme A
I	Intensity
T	Transmittance
A	Absorbance
JPEG	Joint Photographic Experts Group
BMP	Bitmap
PNG	Portable Network Graphics
GIF	Graphics Interchange Format
TIFF	Tagged Image File Format
HSB	Hue, Saturation, Brightness
CIE L*A*B*	Commission Internationale de l'Eclairage L*, a*, b
MP	Megapixel
ISO	International Organization for Standardization
AF	Auto Focus
EV	Exposure Value
ROI	Region of Interest
R^2	regression coefficient

ABSTRAK

Glukosa berfungsi sebagai sumber tenaga utama bagi tubuh manusia, yang diperoleh terutamanya daripada karbohidrat dalam diet harian kita. Ia memainkan peranan penting dalam menyokong pelbagai proses fisiologi, termasuk metabolisme selular dan fungsi otak. Menjaga tahap glukosa yang optimum adalah penting untuk kesihatan, kerana tahap yang terlalu tinggi atau rendah boleh mengakibatkan akibat serius. Walau bagaimanapun, di pasaran hari ini, makanan yang dicemari sering mengandungi gula tersembunyi dan bahan tambahan berbahaya, menyukarkan usaha untuk menguruskan pengambilan gula harian dengan berkesan. Kajian ini bertujuan untuk meneroka potensi penggunaan telefon pintar untuk pemantauan diri glukosa menggunakan diod pemancar cahaya inframerah (NIR) pada 940 (nanometer) nm sebagai sumber cahaya, digabungkan dengan ImageJ untuk analisis warna RGB. Sampel dengan pelbagai kepekatan glukosa disediakan dalam dua julat: tinggi (0-100 g/dL, kenaikan 10 g/dL) dan rendah (0-300 mg/dL, kenaikan 50 mg/dL). Gambar setiap sampel, yang diletakkan dalam bekas hitam kecil yang tertutup, ditangkap menggunakan kamera telefon pintar CMOS dengan tetapan kamera tetap. Kepekaan kaedah ini dinilai dengan menganalisis cerun dan pekali regresi (R^2) bagi lengkung kalibrasi. Lengkung kalibrasi secara konsisten mengesahkan bahawa kaedah yang dicadangkan selaras dengan undang-undang Beer-Lambert, mengikut jangkaan dan konsisten dengan kajian terdahulu. Kepekaan yang tinggi dalam mengesan kepekatan glukosa yang tinggi dapat ditunjukkan dengan baik berbanding dengan julat kepekatan rendah, kemungkinan disebabkan oleh ketidakseragaman semasa penyediaan sampel, pergerakan kecil instrumen, atau turun naik cahaya ambien sepanjang setiap rakaman yang mempengaruhi turun naik titik data. Kajian ini menekankan kebolehan dan kebolehlaksanaan analisis glukosa kuantitatif menggunakan ImageJ dan telefon pintar sebagai alternatif yang kos efektif kepada kaedah tradisional yang melibatkan reagen dan enzim mahal. Alur kerja yang dibangunkan, melibatkan pemprosesan dan analisis imej, menawarkan kesederhanaan dan kelajuan dalam ujian kepekatan glukosa. Kesimpulannya, kajian ini menonjolkan kemungkinan penggunaan teknologi telefon pintar sebagai pengesan kolorimetri yang berpatutan untuk pemantauan glukosa yang tepat dan mudah. Dengan menghapuskan sebatian kolorimetri yang mahal, kaedah ini mengatasi isu yang berkaitan dengan tabiat makan moden dan meningkatkan kaedah untuk menguruskan kesihatan.

ABSTRACT

Glucose serves as the primary energy source for the human body, derived predominantly from carbohydrates in our daily diet. It plays a crucial role in fuelling various physiological processes, including cellular metabolism and brain function. Maintaining optimal glucose levels is vital for health, as both high and low levels can lead to serious consequences. However, in today's market, adulterated foods often contain hidden sugars and harmful additives, complicating efforts to manage daily sugar intake effectively. This study aims to explore the potential of using smartphones for self-monitoring glucose sensing using near-infrared (NIR) light emitting diodes (LEDs) at 940 (nanometres) nm as a light source, coupled with ImageJ for RGB colorimetric analysis. Samples with varying glucose concentrations were prepared across two ranges: high (0-100 g/dL, increment of 10 g/dL) and low (0-300 mg/dL, increment of 50 mg/dL). The images of each sample, placed in a small enclosed black container, were captured using a CMOS smartphone camera with fixed camera settings. The sensitivity of the method is evaluated by analysing the slope and the regression coefficient (R^2) of the calibration curve. The calibration curves consistently confirm that the proposed method aligns with the Beer-Lambert law, in accordance with expectations with previous studies. A high sensitivity in detecting high concentrations of glucose can be demonstrated well compared to low concentrations range, potentially due to inconsistencies during sample preparation, minor motions of the instruments, or fluctuations in ambient light throughout each take affecting the fluctuation of the data points. The study underscores the applicability and feasibility of quantitative glucose analysis using ImageJ and smartphones as cost-effective alternatives to traditional methods involving expensive reagents and enzymes. The developed workflow, involving image processing and analysis, offers simplicity and speed in glucose concentration assays. In conclusion, this study highlights the possibility of using smartphone technology as an affordable colorimetry detector for precise and convenient glucose monitoring. Through the removal of costly colorimetric compounds, this method tackles the issues associated with contemporary eating habits and enhances methods for managing health.

CHAPTER 1 INTRODUCTION

1.1 Background Of Study

1.1.1 Glucose And Its Complication

Sugar is the simplest form of carbohydrate, known by various names based on its chemical structure, such as glucose, fructose, sucrose, and lactose. Glucose, a fundamental building block of many sugars, serves as the primary energy source for human cells (Julie, et al., 2023). Unlike other forms of sugar, glucose does not break down into simpler forms; it is directly absorbed into the bloodstream from the small intestine, thereby immediately affecting blood sugar and insulin levels. According to American Health Association (AHA) (2024) in food, sugar is classified into two main types based on origin and composition: natural sugars and added. For health benefits, it is crucial to prioritize the consumption of natural sugars, such as those found in fruits and milk, which come with additional nutrients and moderate sugar levels, thus have lower GI (glycemic index) level (Harvard Health, 2023). Overconsumption of added sugars which has a high GI level can contribute to health problems such as obesity, type 2 diabetes, and heart disease (Centers for Disease Control and Prevention, 2024).

To avoid such complications, the Dietary Guidelines for Americans recommend that added sugars should be comprise less than 10% of total daily caloric intake (Food and Drug Administration (FDA), 2024). Added sugar refers to sugar incorporated into foods during cooking, processing, and adulteration (AHA, 2024). Food products have been adulterated with cheap artificial sweeteners to compromise their quality for economic gain (Momtaz, et al., 2023). The widespread availability of these adulterated foods makes proper glucose management nearly impossible without stringent care and appropriate knowledge. Therefore, it is crucial, especially for individuals from low-income backgrounds who may choose inexpensive adulterated foods, to identify fraudulent products to prevent excessive sugar intake, which can lead to significant health problems as previously mentioned.

1.1.2 NIR Spectroscopy: An Optical Method in Glucose Sensing

As shown in Figure 1.1, over the years, several techniques have been proposed, studied, and commercialized for glucose sensing and spectroscopic techniques are superior by their non-invasive nature and ease of sample preparation (Zhang and Abdulla, 2022). This approach involves analysing the electromagnetic radiation absorbed, transmitted, or scattered by a sample to ascertain its composition, physical and electronic structure, and properties (Sapkota, 2022). NIR spectroscopy utilizes the NIR (800-2500 nm) region of the electromagnetic spectrum to analyse molecular structure based on its unique absorption patterns (Biswas and Chaudhari, 2024).

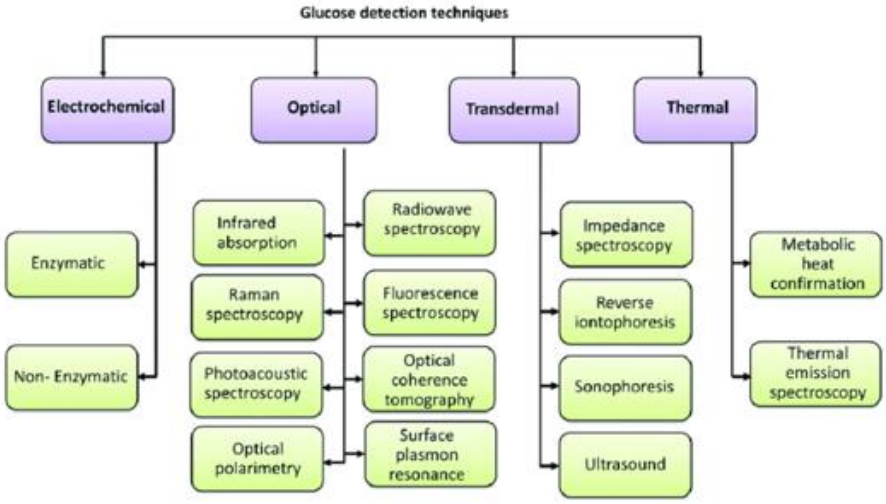

Figure 1.1 The glucose detection techniques (Das, et al, 2022)

As shown in Figure 1.2, each molecule exhibits distinct vibrations and overtones of bonds, leading to specific absorption bands. These distinct absorption bands can be considered molecular fingerprints, occurring when a molecule absorbs light within a specific wavelength range, causing electrons to transition to higher energy levels (Coslett, 2023). This property of light absorption by molecules forms the basis for quantifying glucose concentration in solutions using NIR spectroscopy, following the fundamental principle of Beer-Lambert's law, which establishes a direct mathematical proportionality (Ramachandran, et al., 2023). NIR spectroscopy is an optical method that has gained prominence in food analysis and quality

control over the past four decades due to its cost-effectiveness, rapidity, and non-invasive nature, preserving sample integrity and enabling comprehensive characterization of chemical composition and physical properties (Beć, et al., 2022). According to Narkhede, et al. (2016), glucose exhibits several absorption peaks at NIR wavelengths, specifically at 940 nm, 970 nm, 1197 nm, 1408 nm, 1536 nm, 1688 nm, 1925 nm, 2100 nm, 2261 nm, and 2326 nm. In this study, an NIR LED with a wavelength of 940 nm was utilized to detect glucose molecules in an aqueous solution. Despite being the lowest recommended NIR wavelength for glucose detection, it offers minimal optical signal attenuation by other components, such as water (Narkhede, et al., 2016)

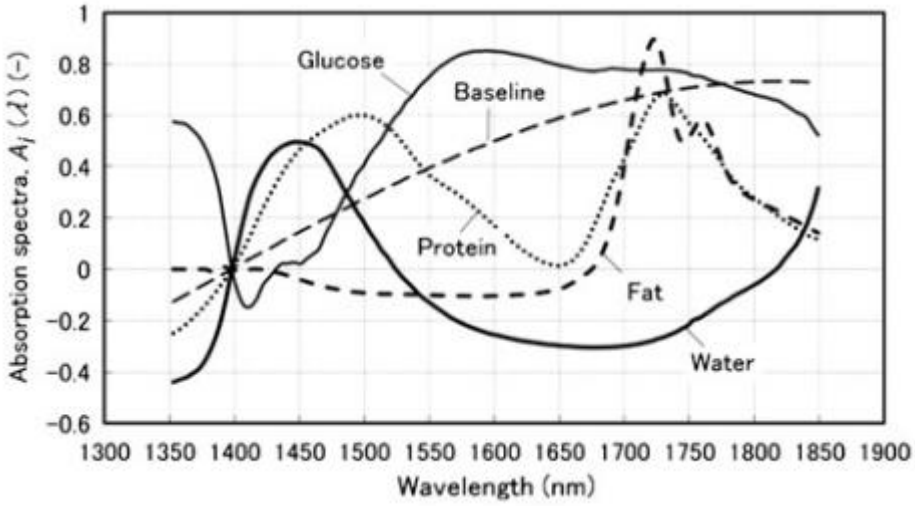

Figure 1.2 Absorption spectrum of different molecules in blood at NIR region (Maruo and Yamada, 2015)

1.1.3 Application Of Smartphone in Biophotonic

The analytical procedures within biophotonic focus on developing sensitive, low-cost, and non-destructive methods for analysing the concentration of analytes (Firdaus, et al., 2022). According to Wargocki (2022), smartphone was first proposed as biophotonic devices in 2008. By equipping the necessary peripherals and leveraging the smartphone as a readout sensor, the author asserts that the smartphone camera can excel as a highly effective portable biophotonic device and analytical biosensing platform. This capability extends to various point-of-care (POC) applications, including disease diagnostics, environmental monitoring, and food screening (Wang, et al, 2021). The integration of CMOS technology in smartphone imaging sensors, which offers superior image resolution compared to charge-coupled devices (CCD), positions smartphone as powerful tool in contemporary molecular diagnostics, such as glucose measurement.

Unlike conventional molecular diagnostic methods, smartphone do not require expensive instruments, highly skilled personnel, or extensive time (Devadhasan, et al., 2015). Although smartphone cameras are not originally designed for scientific use (Wargocki, 2022), many researchers have successfully developed innovative ways to utilize smartphones as colorimeters, spectrometers, fluorometers, voltmeters, and more (Firdaus, et al., 2022). The aim of this study is to develop and validate a smartphone-based biophotonic instrument for glucose sensing through colorimetric analysis. This approach seeks to leverage the accessibility and functionality of smartphones to create a cost-effective, portable, and user-friendly device capable of accurately measuring glucose levels.

1.2 Problem Statement

An effective glucose intake in our daily diet is very important to look after. Furthermore, according to Zia (2022), it is essential to monitor blood sugar levels in order to avoid long-term health complications. This is crucial for everyone who is at risk as well as those who have diabetes. Advances in light-based technologies have resulted in innovative and transformative tools to study and manipulate biological systems at the subcellular, cellular, tissue, and organ levels (Marcu, et al., 2017). The development of self-monitoring devices for glucose sensing has undergone significant advancements, transitioning from enzyme electrochemical-based methods which is invasive to more innovative optical-based methods which is non-invasive to allow a proactive intervention can be implemented to prevent or delay potential problems by identifying abnormal glucose levels early on.

Researcher has been working on to develop a NIR-based non-invasive technology (Aminah and Wala, 2022). Recently, colorimetric analysis has brought great attention as it is one of the easiest techniques that can be performed with a smartphone as a signal reader (Granica, 2019). However, most current research uses colorimetric methods for detecting glucose with expensive and easily decomposing enzymes like glucose oxidase (GOx) and horseradish peroxidase (HRP), chromogenic pigment such as 3,3',5,5' Tetramethylbenzidine (TMB) and noble metal nanoparticles such as silver and gold nanoparticles (Firdaus, et al., 2022) to produce colour change that is detectable to the human naked eye (Shrestha and Shrestha, 2023). Thus, this research aims to evaluate how sensitive the use of smartphone-based digital image colorimetry for glucose detection employing NIR spectroscopy without necessitating any chemical reactions.

1.3 Objectives

1.3.1 General Objective

To assess the feasibility, sensitivity, and applicability of applying smartphone-based biophotonic instrumentation to measure glucose concentrations in aqueous solution through colorimetric method.

1.3.2 Specific Objectives

1. To design and construct smartphone-based biophotonic instrumentation using its CMOS camera, NIR LED, and lens accessories configured for colorimetric detection
2. To prepare sample with different glucose concentrations in aqueous solution
3. To capture sample with different glucose concentration images and perform colorimetric analysis using ImageJ.
4. To analyse the sensitivity, and applicability of the constructed instrumentation and method for glucose sensing

1.4 Significance Of Study

The significance of this study is to simplify the process of glucose sensing by leveraging smartphone, the most widely used pocket-sized reader device for digital image colorimetry. The availability, affordable nature and widespread of smartphone can be an excellent platform to implement a glucose sensing technology. Next, this study allow glucose sensing to be done in various settings including home environments or remote areas where a laboratory facilities access is limited which then enhance the efficiency of glucose monitoring programs. Besides that, this study also able to address the important of the advancement in biophotonic technologies as it promote sustainable development and provide solutions to global challenges in health.

CHAPTER 2 LITERATURE REVIEW

2.1 Glucose

2.1.1 Physical Properties of Glucose

According to an article by Shendurse and Khedkar (2016), in 1747, Andreas Marggraf isolated glucose from raisins and in 1838, Jean Dumas named glucose as "gleucos" from the Greek word meaning ‘sweet’ or ‘sugar’. Scientifically, this compound is known as 2,3,4,5,6-pentahydroxyhexaldehyde and is also referred to as D- (+)-glucose or dextrose. Conventionally, its molecular formula is represented as $C_6H_{12}O_6$. As shown in Figure 2.1, the structure of glucose can be depicted using different projections, such as the Fischer and Haworth projections.

Glucose exists in two stereoisomeric forms, D-glucose, and L-glucose, which are derived from the molecule glyceraldehyde. These isomers have the same chemical formula and physical properties but differ in the arrangement of the hydroxyl (-OH) groups. As illustrated in Figure 2.2, D-glucose has the -OH group on the right side of the carbon chain, while L-glucose has it on the left side. The molecular weight of glucose is 180.19 kDa. It appears as a colourless crystalline substance with a melting point of 150°C and a density of 1.5620 g/cm^3 at 18°C. Due to its multiple hydroxyl groups, glucose is highly soluble in many solvents, including water (with solubility dependent on temperature), acetic acid, pyrimidine, and others, and is slightly soluble in methanol and ethanol (Shendurse and Khedkar, 2016).

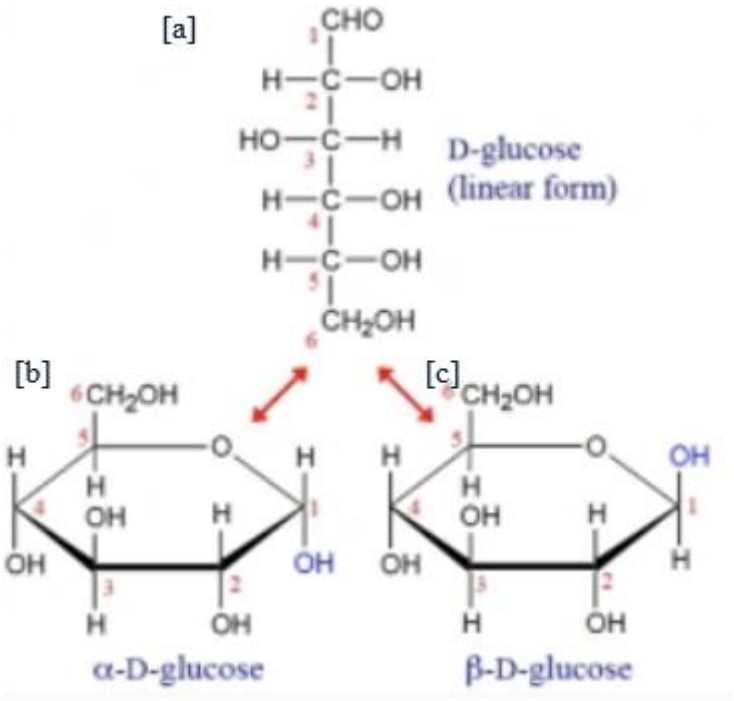

Figure 2.1 [a] represent the Fisher projections of glucose. Meanwhile [b] and [c] represent the Haworth projections of glucose. (Nahmany and Strino, 2004)

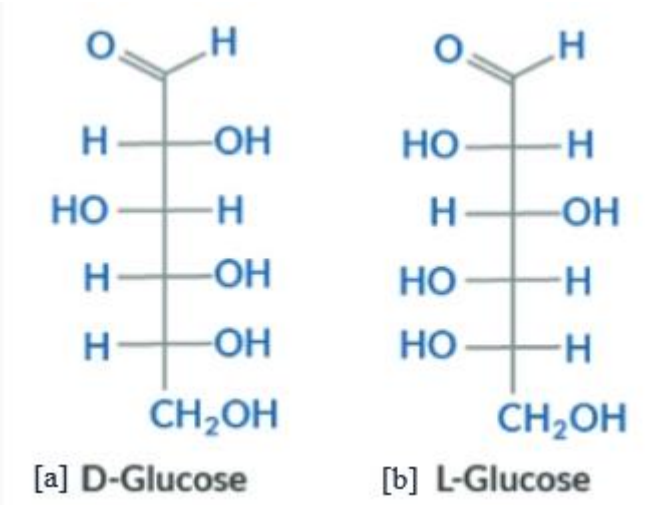

Figure 2.2 The Fisher projection of D-glucose [a] and L-glucose [b] (Young, 2023)

2.1.2 Glucose in The Human Body

Consuming glucose is very important for human as it is the primary source of energy for cells in the human body along with proteins and fats, it is important for metabolic regulation,

brain function and biosynthesis (Zia, 2024). According to Tarantino (2022), glucose provides its energy to the human cell in the form of packets of chemical energy called as adenosine triphosphate (ATP) which can be yield from the process known as cellular respiration. There are 3 stages involves producing ATP from food molecules. Stage 1 involve the digestion process of the food macromolecules such as polysaccharides into its simple subunits which is sugars which occurs either in intestine outside cells or by the lysosome organelle within the cells. Next as shown in Figure 2.3, in stage 2 [a], as it absorbed into the cells, the glycolysis process take place where the glucose is then further breakdown into 2 pyruvate, 2 ATP and 2 nicotinamide adenine dinucleotide hydrogen (NADH). And lastly in stage 3 [b], which take place in mitochondria cell, when there is a present of oxygen, the pyruvate is further converted to 2 acetyl- coenzyme A (CoA). The acetyl-CoA is then oxidized in the citric acid cycle and converted to water (H_2O), carbon dioxide (CO_2), 6 NADH, 2 Flavin adenine dinucleotide ($FADH_2$) and 2 ATP. Through the oxidative phosphorylation, NADH and $FADH_2$ yield 15 and 3 ATP respectively (Tarantino, 2022). Thus, one molecule of glucose often produces about 32 ATP (Melkonian and Schury, 2023)

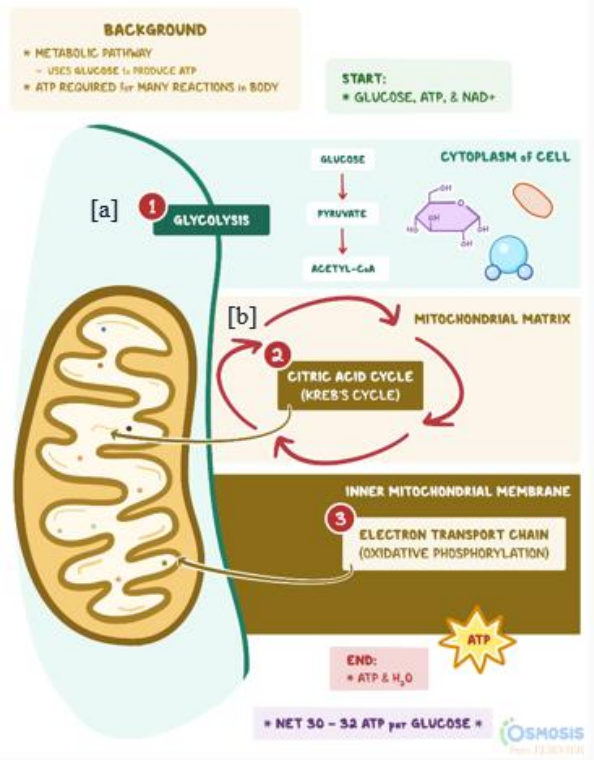

Figure 2.3 A chain reactions of cellular metabolism where [a] is the stage 2 and [b] is the stage 3 (Tarantino, 2022)

Glucose however did not go through the first stage as it can directly absorbed into the bloodstream and human cell to further breakdown for energy because it is already in the simplest form (monosaccharide). Many hormones are involved in regulating glucose such as insulin, glucagon, somatostatin, cortisol, epinephrine, thyroxine, growth hormone and adrenocorticotropic hormone (Hantzidiamantis, et al., 2024). According to Rahman, et al. (2021), insulin is an important hormone that is naturally produce from the β cells in the pancreatic islets of Langerhans to maintain the glucose level in the bloodstream. Following a meal, as blood glucose levels rise to approximately between 2 mM and 4 mM (36.03 – 72.06 mg/dL), insulin is started to produce by the pancreas. Once the blood glucose level rises above 5 mM (90.08 mg/dL), the produced insulin is secreted into the bloodstream to promote glucose absorption into adipose, muscle, and liver tissues, thereby reducing blood sugar levels. Conversely, insulin secretion decreases in response to a drop in blood glucose levels (Utiger, 2024). When there is an abnormality in insulin production, it can lead to elevated blood glucose levels (hyperglycemia), which can result in either type 1 or type 2 diabetes decrease in blood glucose levels (hypoglycaemia).

2.1.3 Glucose in The Food

According to Alexander (2020), carbohydrate or crabs is a one of the macronutrients that need to be taken by human body as it has the nutritious components that are required by body for energy and to maintain the structure and system of the body. In food, carbs can be classified into simple, and complex based on its chemical structures. Sugar such as glucose, fructose and lactose are simple carbs, meanwhile starch and fiber are complex fiber. As mentioned by Dr Bajpaiee (Gupta, 2022), consuming carbs is not giving negative effect to body except simple carbs. This is because simple carbs such as sugar are easily broken down and can cause blood sugar levels to rise quickly which then increase the risk of cardiovascular disease (Harvard Chan, 2023) than complex carbs such as those found in whole grains as it broken down into simple sugar slowly and allow blood sugar to rise gradually and enough time or body to regulate them effectively (Benton, 2017). Thus, it is important to minimize the consumption of simple carbs than complex carbs. According to Thompson (2024), Carbohydrates also can be classified based on its glycemic index (GI). It is a rate that tells how fast the food raise the blood sugar levels after eating. Foods are ranked on a scale from 0 to 100 with pure glucose given a reference value of 100. Delage (2016) noted that foods with high GI (70 or above) such

as baked potato are rapidly digested and cause substantial fluctuations in blood sugar. Meanwhile, foods with a low GI (55 or less), like whole oats, are digested more slowly, prompting a more gradual rise in blood sugar. The GI is a useful tool for understanding how different foods affect blood glucose levels.

The amount of carbohydrates consumed must be carefully considered since it greatly affects the body (Alexander, 2020). The Dietary Guidelines for Americans propose that 45% to 65% of one's daily calorie intake be carbohydrates. According to FDA guidelines, if a person consumes 2000 calories a day, their daily carbohydrate intake should be 275 g (Gunnars and Sharon, 2023). However, many find it difficult to follow this advice given the prevalence of contaminated food. The intentional addition or replacement of ingredients in food that may jeopardize its safety, quality, or nutritional value is referred to as "food adulteration" (Haji, et al., 2023). It can appear in several ways, as Figure 2.4 illustrates. Artificial sweetener adulteration in food is a practice motivated by marketing low-calorie items including diet drinks, regular soft drinks, and low-sugar products, as well as economic considerations. While artificial sweeteners offer a calorie-free, sweeter flavour and can help save calories (Pang, et al., 2021), their secret insertion raises questions regarding consumer rights and potential health consequences (Haji, et al., 2023). In order to reduce the hazards connected with this type of adulteration and empower consumers to make educated decisions about the foods they eat for their health, regulatory control and consumer education are essential.

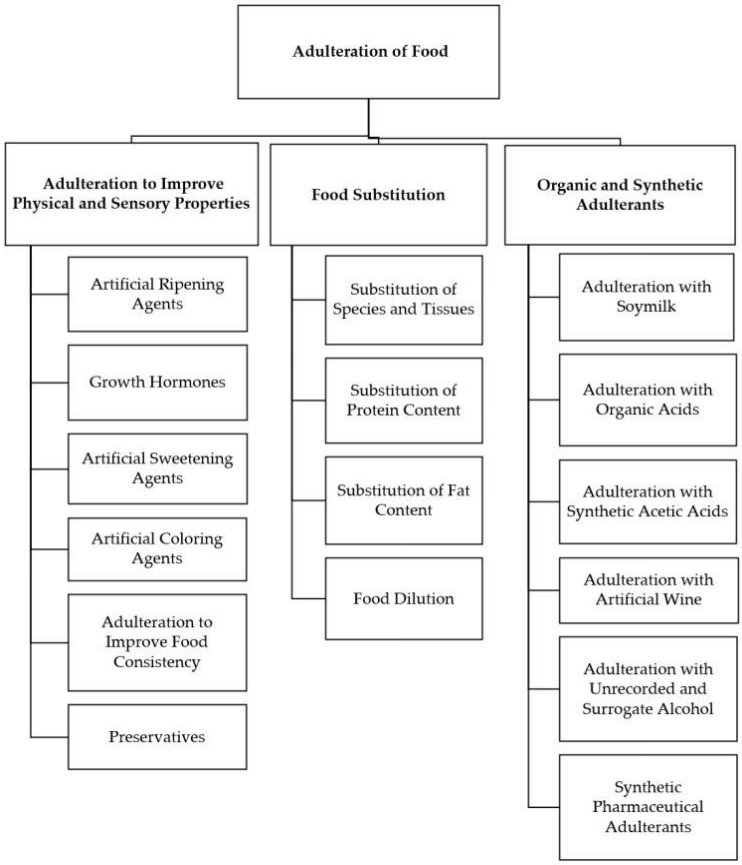

Figure 2.4 Major forms of food adulteration (Momtaz, et al., 2023)

2.2 Biophotonic

2.2.1 Definition

Biophotonic is the study of biological materials through the use of light-based technology. This includes the radiation that is produced, detected, absorbed, reflected, modified, and created by biomolecules, cells, tissues, organisms, and biomaterials (Oncea, 2024). The Greek terms "bios," which means life, and "phos," which means light, are the source of the phrase. According to Jürgens, et al. (2013), photonics encompasses a broad range of methods and tools that make use of light from all parts of the electromagnetic spectrum, including visible, infrared, ultraviolet, and terahertz radiation. Light has several beneficial qualities that make light-related technologies perfect for use in the biological and medical sciences. First of all, light has a large spatial scale, as shown in Figure 2.5, making it easier to observe and work with things that are between nm and centimetres (cm) in size. Light also

makes reliable, long-term monitoring possible, allowing for ongoing study of processes and structures. Furthermore, light can be used to evaluate the morphological, chemical, mechanical, and mobility characteristics of molecules, cells, and tissues. Moreover, because it provides non-invasive diagnostics, it is very adaptable and useful for a range of applications (Jürgens, et al., 2013).

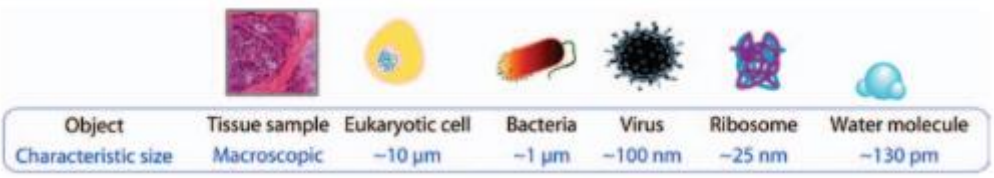

Figure 2.5 Light can detect objects ranging in size from nm and cm. (Jürgens, et al., 2013)

In the seventeenth century, the first biophotonic device, the light microscope, was invented and was further advanced in the nineteenth century by key figures such as Carl Zeiss, Ernst Abbe, and Otto Schott in Jena, Germany. Today, the light microscope remains an essential tool in the life sciences (Jürgens, et al., 2011). This history of invention leads the continuous development of biophotonic technologies, and it has generated significant interest due to their potential to play a crucial role in next-generation diagnostic, analytical, and therapeutic modalities (Marcu et al., 2017). These advancements are expected to drive innovations and improve the precision and effectiveness of medical and biological research. There are few optical methods that can be used to analyse the biological sample such as photometric, spectrometric, colorimetric, and fluorometric techniques.

2.2.2 Optical Methods

2.2.2 (A) Photometric

According to Gollor (2022), photometry is a technique used to measure light intensity at specific wavelengths. In biophotonic, photometric methods quantify the concentration of substances in a sample by analysing the absorption or transmission of light. Applications of photometry include measuring enzyme activities, monitoring environmental changes, and detecting biomolecular processes in solutions. This method is based on the Beer-Lambert law, which states that the intensity of transmitted light decreases as it passes through a sample containing an absorbing substance.

2.2.2 (B) Spectrometric

Spectrometry is a technique for quantitatively measuring the light spectrum—specifically, the intensity of light at different wavelengths—that is absorbed, emitted, or scattered when interacting with a sample (ATA Scientific, 2020). According to Mishra (2022) there are several types of spectrometric techniques that are used in determine the food quality, including UV–visible, fluorescence, infrared, Raman, and nuclear magnetic resonance spectroscopy. UV spectroscopy uses electromagnetic radiation with wavelengths ranging from 100 to 750 nm and detect sample according to the Beer-Lambert law. On the other hand, fluorescence spectroscopy uses the fluorophores that are already present in the sample to identify fluorescence. Next, infrared spectroscopy is divided into three regions: far-infrared, NIR, and mid-infrared based on the wavelengths range from 78 nm to 1 mm employed. Besides that, Raman spectroscopy is a vibrational spectroscopy technique that uses the Raman effect to identify compounds by measuring their atomic vibrations. According to Illy and Karlsson (2018), this approach is often applied at wavelengths of 780 nm and 1064 nm. Furthermore, nuclear magnetic resonance spectroscopy takes advantage of the interaction between the magnetic characteristics of atoms and molecules and an applied magnetic field, typically ranging from 6 to 24 T (Raja and Barron, 2024).

2.2.2 (C) Colorimetric

Shrestha and Shrestha (2023) explain that colorimetry refers to the measurement of light in the visible range of electromagnetic radiation which is between 320 and 700 nm. In this method, a monochromatic beam of light from a light source is permitted to pass through a sample holder containing the analyte in the solution; the intensity of the light transmitted is lower than the light passing through the sample in the cuvette. The amount of light absorbed is proportionate to the analyte's concentration. The sample's colour is either an inherent property of the solution or can be changed by adding the right reagents. The colour intensity is proportional to the concentration of the chemical (analyte) responsible for producing the colour which accordance to the Beer Lambert law. It is a technique that is cheap and easy to handle to quantitatively determine a coloured substance.

2.2.2 (D) Fluorometric

According to Paudel (2024), fluorometry is a technique used to measure the fluorescence emitted by a sample when excited by a specific wavelength of a monochromatic light at range of between 160 nm to 1000 nm which is dependent on the type of the source of light. When molecules absorb light, usually in the ultraviolet range, they become excited to a higher energy state. As they return to their ground state, they emit light at a longer wavelength. The intensity of this emitted light is directly proportional to the concentration of the fluorescent species in the sample. Fluorescent are categorized into organic dyes, biological fluorophore, and quantum dots. Due to its high precision, fluorometry can detect lower concentrations of analytes compared to similar techniques.

2.3 Smartphone-Based Biophotonic Instrumentation Using Colorimetric Method

2.3.1 Near-Infrared Led

Recently, NIR spectroscopy has emerged as a prominent optical method alongside Raman spectroscopy, photoacoustic spectroscopy, and fluorescence (Yang, et al., 2024) for non-invasive and non-destructive detection of biomolecules, owing to its significant optical penetration depth (Tanaka, et al., 2021). NIR spectroscopy for glucose detection relies on the absorption of near-infrared light by molecular bonds within glucose molecules. Specifically, when NIR light interacts with glucose, specific wavelength is absorbed which then causes bonds such as C-H, and O-H which is abundant in glucose molecules (Figure 2.1)—to stretch and bend due to the first overtone and combination vibrations of that bonds (Pires and Martins, 2024). These absorption spectra yield both chemical and physical insights into glucose molecules, facilitating their identification and quantification in solutions (Mekonnen, et al., 2020).

Since only a portion of the NIR light is absorbed by the molecule, the rest is typically measured using one of two primary measurement modes during detection: transmittance or reflectance (Ahmed, et al., 2022). Transmittance measures the intensity of light that passes through a tissue and exits on the opposite side, while reflectance assesses the intensity of light scattered back from the tissue's surface (Ramachandran et al., 2023). Transmittance is commonly used in spectroscopy to quantify the absorption of light by glucose molecules in solution, as it exhibits an inverse relationship with absorption: as light absorption increases, transmittance (T) decreases, and vice versa (Philips, 2023). This relationship is mathematically expressed as:

$$A = -\log_{10} T$$

These principles underscore the effectiveness of NIR spectroscopy in biochemical analysis, offering precise and sensitive measurements crucial for biomedical and clinical applications.

2.3.2 CMOS Camera

Smartphone-embedded CMOS camera has been employed and studied by many researchers as a light detector sensor to create a diagnostic system that is affordable by many. According to Iyer (2024), by the mid-2000s, the image sensors used in smartphones have generally transitioned from CCD to CMOS technology. The transition from CCD to CMOS sensors in smartphones has been driven by advancements in technology, improved performance metrics, cost considerations, and the need for compact, energy-efficient components that can deliver high-quality imaging capabilities in a mobile environment (Cockett, 2022). It has the sensitivity to detect a wide range of wavelength including NIR spectrum.

The basic component of the CMOS sensor are pixel array, photodiode, and transistor. As shown in Figure 2.6, each grid of pixels or photosites containing photodiode and 3 transistors which used to perform different tasks like amplification, resetting, and readout of the electrical signal generated by the photodiode (Elprocus, 2024). The principle behind this image sensor is based on the conversion of light into electrical signals using semiconductor devices. As written by Yoon (2019), when light hits the photodiode in each pixel, photons are absorbed and creating free electron-hole pairs. Thus, creating an electrical charge which is proportional to the intensity of the light. The charge is then converted to a voltage signal by transistor and transfer to the analog-to-digital convertor (ADC) to convert the analog signal (voltage) to a digital form that can be read (Haraoubia, 2019).

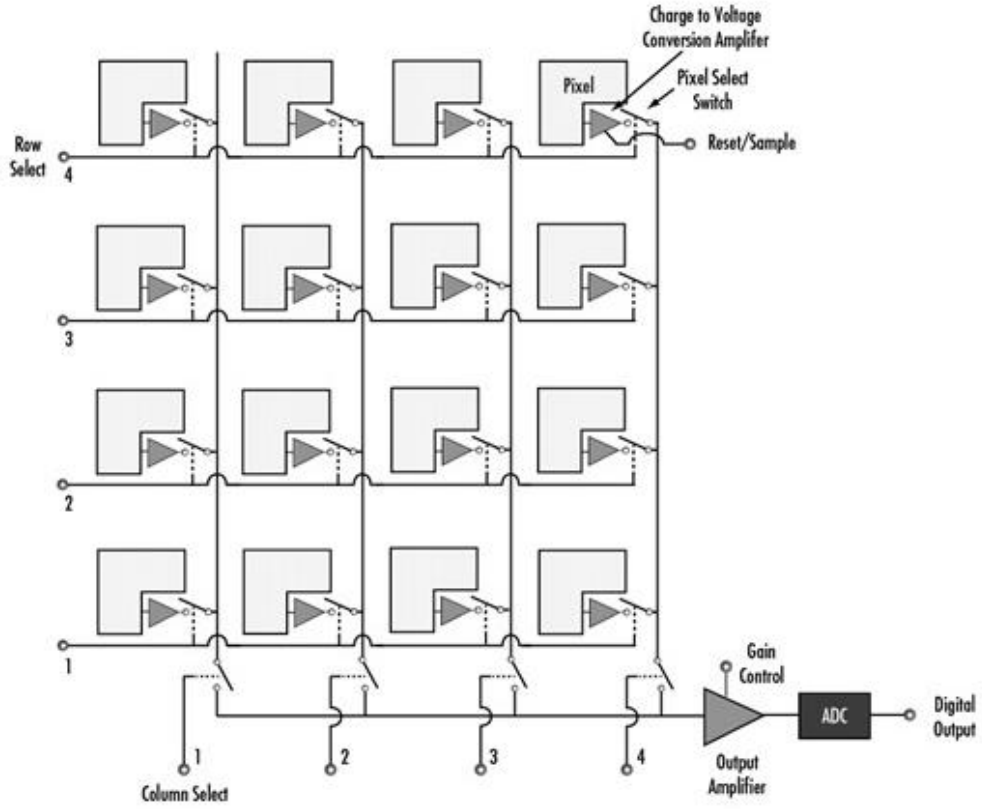

Figure 2.6 CMOS sensor design (Elprocus, 2024)

Another component that is essential in CMOS sensor technology is the color filter array (CFA) that can be found over each photosite. According to Jain (2020), as photodiodes in image sensor are made of semiconductor, it only governed the rules of solid-state physics, which means they lack the inherent ability to distinguish between different wavelengths of light. Colour filters address this limitation by allowing the sensors to capture colour-specific light, enabling the differentiation of colours in the final image by filtering the light by wavelength range. Most CMOS sensor use a Bayer filter pattern (Rentals, 2021). The filter can provide information about the intensity of light in red, green, and blue (RGB) wavelength regions. As shown from Figure 2.7, the sensor has a distribution of 50% green, 25% red, and 25% blue photosites where green photosites are surrounded by red and blue photosites. This distribution is chosen because the human eye is more sensitive to green light, which helps in capturing more luminance detail (Jain, 2020). Each photosite captures RGB light intensity in only one colour, corresponding to one pixel in the final image (Rentals, 2021) which is initially incomplete and

in a raw format. A demosaicing algorithm then processes this raw data to produce a full-colour image by estimating the missing colour information for each pixel (Jain, 2020).

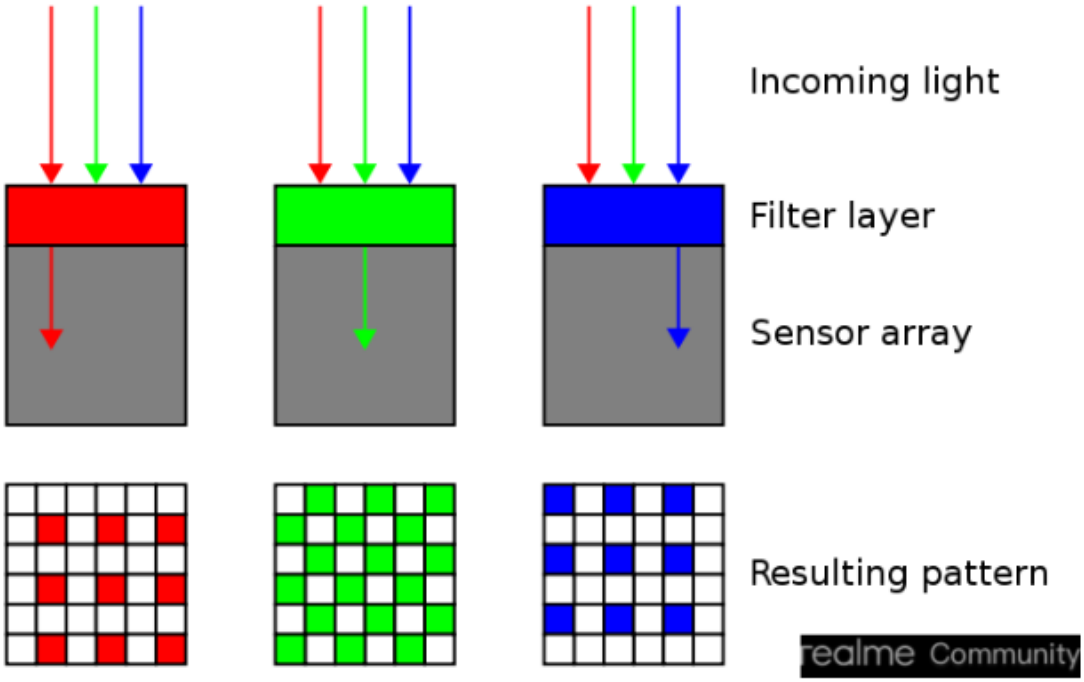

Figure 2.7 The arrangement of Bayer filter pattern (Jain, 2020)

2.3.3 ImageJ Software

According to Woolf, et al., (2020), ImageJ, a free image processing software developed using Java, was created by the National Institutes of Health (NIH) in collaboration with the Laboratory for Optical and Computational Instrumentation. Since its early version, NIH Image, was introduced in 1987 and the public release of ImageJ in 1997, this software has become a powerful and widely used tool in the scientific community. ImageJ is accessible and easy to use, even for those without prior experience in image analysis. Additionally, it is compatible with various computer platforms and supports most common image formats such as JPEG, BMP, PNG, GIF and TIFF (Woolf, et al., 2020). ImageJ also offers a wide range of functions, such as noise reduction, background subtraction, smoothing, sharpening, contrast adjustment, region of interest detection, and intensity quantification. Moreover, it enables measurements of areas, volumes (stacks), distances, and angles (Elagamy, et al., 2023). ImageJ is a flexible software that is also capable of measuring a variety of colour spaces such as RGB, HSB (Hue, Saturation, Brightness), CIE L*A*B* (L represent separate luminance from chromatic

components (A and B). In analytical chemistry, both RGB and HSB (hue, saturation, and brightness) colour spaces are commonly used for reporting colorimetric results. The RGB model is a logical starting point for analysis due to its ubiquity and simplicity, consisting of red, green, and blue components. Each component is typically represented as an 8-bit value ranging from 0 to 255, allowing for a numerical representation of over 16 million colours in total. This extensive range facilitates reproducible and precise colour measurements in various applications.

2.4 Review of Previous Studies Related to Optical Sensing of Glucose

2.4.1 Optical Sensing of Glucose in Food Using RGB Colorimetric Method and Smartphone as The Detector

A study from Su, et al. (2023), reported that, there are many methods that has been studied and documented for the analysis of glucose. Among them are high-performance liquid chromatography, electrochemical methods, spectrophotometry, fluorescence, chemiluminescence and electrochemiluminescence. The spectrophotometry methods are one of the methods that is get high interest among researchers as it exhibits good sensitivity, easy to operate, can give a faster response and cost-effectives when the method is implemented with HRP and GO_x enzyme. The reaction of this enzyme with glucose can produce a colour change when combined the enzyme and any chromogenic substrate such as TMB (Wang, et al., 2020) which is then allowing for the colorimetry analysis to be done through naked eye or a smartphone. A study made by Wang, et al., (2020) prove that the colour changes of the HRP-H_20_2-TMB with smartphone as the RGB camera detector can give a high accuracy and linear response over 0.039 milligram/millilitres (mg/mL) to 10 mg/mL. Besides that, they are also a study prove that the colorimetry analysis can be done without the use of the mentioned enzymes. Firdaus, et al., (2022) use a nanozymes, gold nanoparticles as a substitute for the HRP and GO_x enzyme. They report that when the glucose and the gold nanoparticles react, it can give an observable colour change because the of the nature of its surface plasmon resonance. They created a smartphone application (APP) called glucose analyser and designed the Android Studio platform (DIC-Smartphone). The suggested technique exhibits good linear range (R^2= 0.9984) selectivity and sensitivity from 0 to 40 mirco Molar (µM).

CHAPTER 3 METHODOLOGY

3.1 Research Tools And Design Setup

3.1.1 Materials and Reagents

All the reagents used were a pharmaceutical grade where the 500 grams (g) of D (+)-Glucose Monohydrate (Dextrose) powder with formula of $C_6H_{12}O_6$ and the 1 liter (L) of distilled water were both obtained from ABM Intellect Resources (Malaysia).

3.1.2. Hardware and equipment (Sensing system)

During the experiments, a Realme C2 smartphone (BBK Electronics, China) equipped with a 13MP+2MP rear dual camera (CMOS sensor) was adapted for colorimetric measurement. To capture high-quality images of the samples, a 12 mm diameter macro lens with 10x magnification was mounted horizontally and fixed in front of the smartphone camera lens using a tripod. This setup allowed for sharp images to be taken from a distance of 2.5 cm. In comparison, the standard smartphone camera provides sharp images for objects situated at distances greater than 6 cm.

An accessory for the smartphone was designed using a small enclosed black container as a chamber to eliminate interference from the ambient light and with a tube featuring three holes to eliminate any movement from the camera lens, sample, and LED light (centre, top, and bottom). As depicted from Figure 3.1, the central hole was aligned to insert the Eppendorf tube, while the top and bottom holes were for the smartphone camera lens tube and the NIR LED, respectively. The optimal distance for capturing sharp images was determined by taking a series of photographs at progressively increasing distances from the Eppendorf tube, with the magnifying lens attached to the smartphone camera. The distance that produced the sharpest image was selected as the optimal one. The detailed dimensions of the accessory are shown in Figure 3.2.

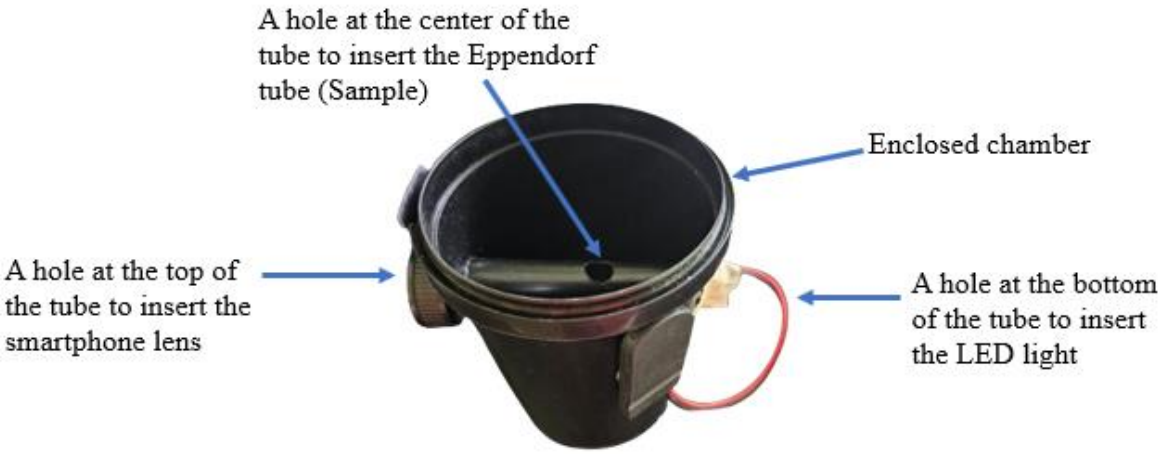

Figure 3.1 The design of the tube within the enclosed chamber for housing the lens, sample, and LED light from upper view.

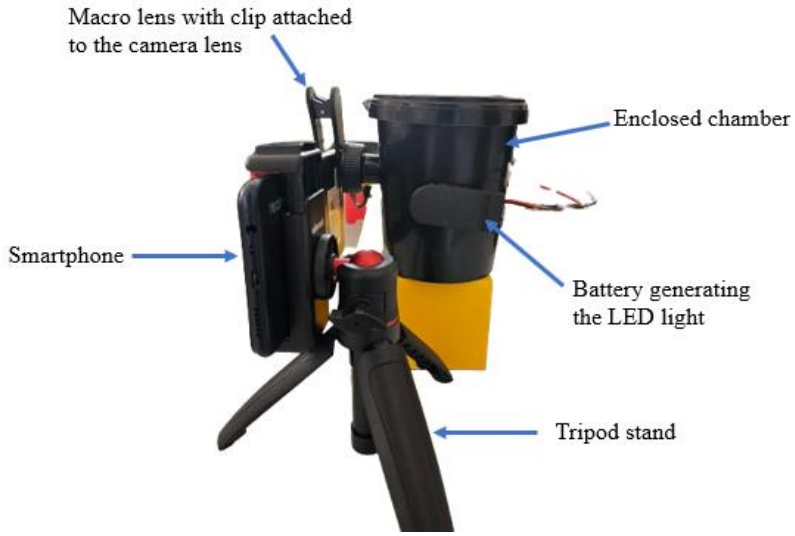

Figure 3.2 The design of the smartphone accessory used during the experiment

A monochromatic LED with a wavelength of 940 nm was inserted into the bottom side wall of the chamber at a 90-degree angle. The LED was powered by a 3V lithium coin battery. Samples with varying glucose concentrations were prepared by weighing, measuring, and diluting using a mini digital electronic scale with a capacity of 500 g x 0.01 g, a beaker, a measuring cylinder, micropipettes (10 µL and 1000 µL), and magnetic stirrer.

3.1.2 Software and analytical tools

ImageJ (Figure 3.3) is a software tool used to quantitatively analyse sample images by extracting the mean values of the RGB components (Red, Green, and Blue channels). The raw data obtained from ImageJ is collected and organized with the assistance of Microsoft Excel's (Figure 3.3) spreadsheet and data manipulation features. Variables such as glucose concentration, RGB mean pixel intensity, and standard deviation are manually transferred from ImageJ, then organized and formatted within the Excel workbook. Excel's extensive range of built-in functions, such as SUM, AVERAGE, and LOG10, allows for complex calculations including absorbance and transmittance from a large dataset to be performed quickly, accurately, and efficiently. Additionally, Excel's powerful data analysis tools, such as pivot tables and charts, provide valuable insights and graphical representations for data interpretation (Agarwal, 2024).

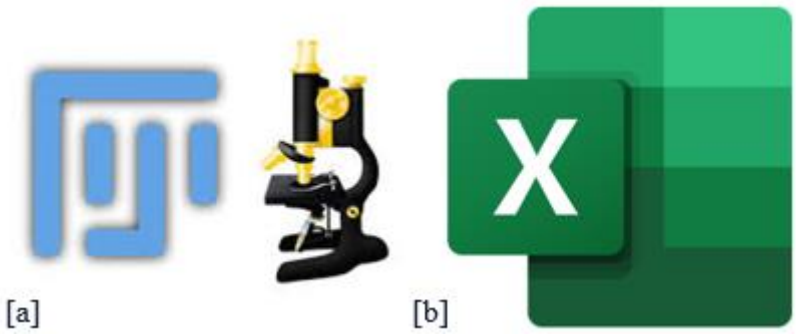

Figure 3.3 ImageJ (Fiji) [a] and Microsoft Excel [b]

3.2 Research Workflow

3.2.1 Glucose Sample Preparation

Figure 3.4 illustrates the procedure for preparing glucose samples of various concentrations. To facilitate the preparation of these samples using the available micropipette, both high and low concentration stock solutions were prepared first, then diluted to achieve a range of glucose concentrations. Using a mini digital electronic scale, 100.0 g (Figure 3.5 [a]) and 1.0 g (Figure 3.5 [b]) of glucose powder were measured and transferred into separate