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NEAR INFRARED SPECTROSCOPY (NIRS) APPLICATIONS IN MEDICAL: NON-INVASIVE AND INVASIVE LEUKEMIA SCREENING

Ruhaizan Ismail, Herlina Abdul Rahim*, Intan Maisarah Abd Rahim, Rashidah Ghazali

Department of Control and Mechatronics Engineering, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

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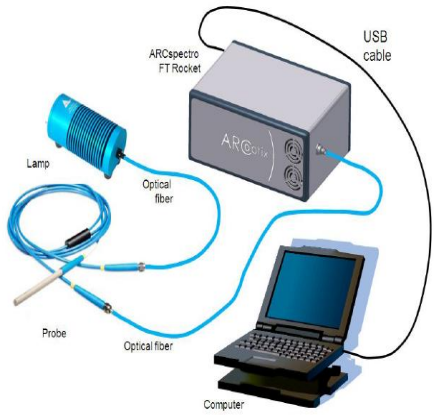
*Corresponding author
herlina@fke.utm.my

Graphical abstract



Abstract

Near Infrared Spectroscopy (NIRS) has been applied as analytical tool in numerous field of study due to its ability in non-invasive application. NIRS with the ability in providing the information on biological molecules shows a high potential as a diagnosis tool in medical as diseased related to biochemistry changes of the cell and tissue. This paper reviewed the application of NIR spectroscopy in leukemia screening and in other medical application. General comparison between invasive and non-invasive NIR spectroscopy method is provided. The author also proposed a new non-invasive NIRS method in leukemia screening and compared it with the previous invasive NIRS method.

Keywords: Near Infrared Spectroscopy (NIRS), biomarker, blood parameter, leukemia screening, non-invasive, invasive

1.0 INTRODUCTION

Near-infrared region was discovered by Herschel in the early 1800. However, it was ignored by spectroscopists due to it lacked of analytical interest. The earliest application of NIR spectroscopy were reported in the 1950s but there was a long hiatus until 1970s [1-2]. The slow growth in NIR spectroscopic studies is due to the complexity of spectral analysis and inadequate availability of NIR measurement tool. The application of NIR spectroscopy was increasing in numerous fields with the improvement of the equipment tool and advanced of computational method in processing the NIR spectral information. [1-2].

During 1993, spectroscopic techniques shows high potential but barely adequate in term of application. Then, in 1994 the application of NIR spectroscopic technique starts to increase in different fields [1]. NIR spectroscopy has become a widely used analytical method in various fields due to its ability in non-invasive analysis. NIR spectroscopy has been applied as analytical tool in numerous fields such as agricultural-food industries [2-10], petrochemicals industries [1, 11], medical/clinical sciences, pharmaceuticals industries, and environmental industries [1] and miscellaneous [1]. NIR spectroscopy is useful for quantitative analysis (measure the concentration of sample), qualitative analysis (determine the quality level of sample) and process control [11].

The agricultural and food industry was the first to adopt NIR spectroscopy method. NIR spectroscopy in this sector is focusing on the application of high

speed and non-destructive method [1]. NIR spectroscopy is also a convenient tool due to its online applicability [1, 11]. It also contributes in monitoring of production process as early detection of product flaws and directly eliminates the product to save time and money [1].

The NIR spectral ranges are varies in the literature. It had been defined from 500 to 2500nm [12], 700 to 2500nm [13], 800 to 2500nm [14], 750 to 3000nm [15], 750 to 2500nm [16], and from780 to 2500nm [1, 17]. However, based on the NIR spectrometer used in the new proposed method for noninvasive leukemia screening, the NIR range will be defined from 900 to 2600nm in wavelength or from 11000 to 3800 cm^{-1} in wavenumber. This spectral region has been actively studied for non-invasive diagnosis of disease based on the blood analytes [12, 18]. The greatest development of NIR spectroscopy technique in the clinical and biomedical field was during 1990s. This is because; it is a non invasive method which not involve biopsy or surgery [1]. NIRs with the combination of advanced computer aided multivariate analysis present a new non-invasive method in diagnosis of bimolecular [19].

NIR spectroscopy has been developed into non-invasive method for biomedical sensing and clinical diagnosis due to the ability of NIR light that can penetrate a great depth into biological tissues [20] and suitable to be used on blood and tissue as it has no effect on water band [21]. NIRs also can be applied directly at human body as NIR radiation is harmless to biological tissues [1]. NIR spectroscopy with fiber optic based device allows the spectra of samples with different characteristics to be recorded simply by selecting the most suitable mode for each sample [1]. NIRs principle is based on the understanding of cancer at the molecular level which played an important role in early cancer detection [19]. The rationale behind medical diagnosis using spectroscopy was based on the fact that diseases are related with the changes in the biochemistry of the cells and tissues that make up the different organs in our body and its ability in providing information on biological molecules [21]. Procedures of diagnostic and monitoring of diseases involves the measurement of blood parameter which also called biomarker. Biomarker is a distinctive biochemical indicator of a diseased [22]. Nowadays, there are various non-invasive measurement of data acquisition were done by directly applied NIR at human body. The value of NIR spectroscopy as a research tool is only beginning to be realized. The future should see increasing use of NIR spectroscopy for diagnosis in a broad range of clinical and biomedical applications [23].

1.1 NIR Spectroscopy: Measurement Principal and Operational Mode

Spectroscopy is defined as the interaction of light (near infrared) with matter. The interactions of light and matter are characterized by the energy of the radiation and its effects on materials. Each molecule produces a unique characteristic of IR spectrum that based on its atomic structure [24]. Infrared spectral provide information about biological components such as proteins, lipids, nucleic acids and carbohydrates [23].When a substance is irradiated with infrared light, absorption occurs in the molecule and then transition to high-energy level. Wavelength absorption band in the infrared region varies by functional group. Based on this, qualitative analysis is possible by checking the position of the absorption peak. By referring to the Lambert-Beer law, quantitative analysis can be performed as the absorbance of the sample concentration is proportional to the measured spectrum [25].

NIR spectral can be acquired in three different operation modes which are transmittance, diffuse reflectance, and transflectance [26]. Two common operation modes for NIRs are transmission and reflectance. The transmission type is where the light source and the detector are facing each other across the measurement site (e.g. ear lobe or finger). In reflectance type, both the light source and detector are in the same plane (e.g. forehead or forearm) with the source detector spacing typically less than 1cm [27]. Normally, reflectance mode is suitable for solids sample, transmittance mode is suitable for liquids sample, and transflectance mode is suitable for emulsion and turbid liquids sample [1].

Near-Infrared spectroscopy can be used to probe non-invasively deep into organ tissue (1-10mm) [12, 28].Three layers of human skin tissues consist of epidermis, dermis, and subcutaneous tissue. The epidermis layer consists of keratin and several lipids. The next layer is the dermis with mainly connective tissue consisting of collagen, sweat glands, blood vessels, lymphatic, nerves and others. The third layer is the subcutaneous tissues that consist mainly of fatty tissue [21, 29]. NIR spectroscopy is able to measure the blood parameter as the body fluids and soft tissues are relatively transparent at the range of near infrared wavelength [30]. NIR wavelength also can easily penetrate without blocked by blood and water. Based on the Figure 1 NIR wavelength can penetrate deeply until the blood capillary vessel in the dermis [31]. After NIR penetration at the target area of the skin, light will be partially absorbed and scattered due to the interaction with the chemical components inside the skin tissues. Some of the light will be reflected out of the tissues or transmitted through it [32].

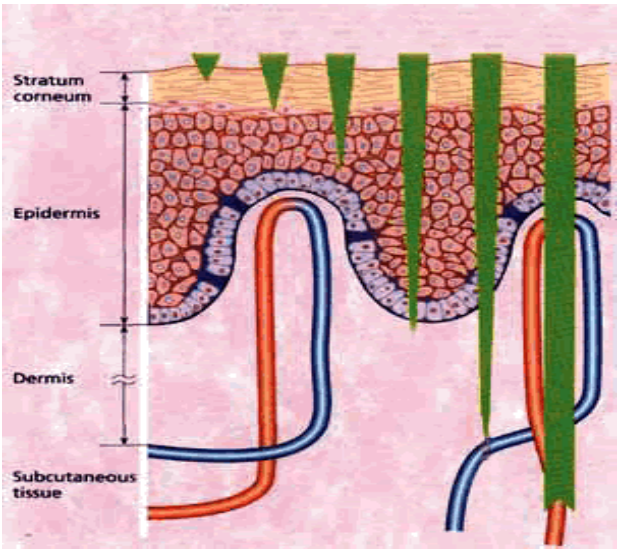


Figure 1 Depth Penetrations in Human Tissue

1.2 NIR Spectroscopy Measurement Influence Factor

There are several influence factors in NIR spectroscopy measurement had been highlighted in the previous studied. Measurement influence factor are related with the sample, sampling procedures (NIR technique), and reference methods used [33]. Other measurements influence factor of NIR spectroscopy may be caused by the different of the weather, humidity, temperature, environment, and human factors [17]. Some of the influence factor in diffuse reflectance spectra measurement is light scattering effects, spectral path-length effects, and multicolinearity. Scatter variation are dependent on the physical nature of the sample, while spectral path-length depend on the size of the sample [33]. It is pointed in the review that the lowered the extinction coefficients which is the size or bands intensities, and the decreased in scatter coefficients of NIR region results in a greater penetration depth into human tissue compared to visible or mid-infrared regions [12].

In the non-invasive application of NIRs, the transmitted light through the target area (finger tip, forearm, abdomen, thigh, ear lobe, lip) as is modified by the tissue and produce an optical signature based on the tissue's content. Different proportion of light scattering and absorption depends on the tissues structured such as the thickness, location, layers, tissue fluid, and blood vessel distribution and also influenced by the tissues chemical components such as melanin, water, hemoglobin, fat, and glucose [32]. Moreover, Scheeren *et al*. [34] stated that NIR measurement may get influenced by the skin pigmentation.

In the study done by Haiquan Ding *et al*., 2014 [35] strong light scattering of human tissues is said to be the cause of the wrong prediction measurement. Therefore, a fixed finger device was used to helps fixed the position of human finger on the light spot. This device helps improved the stability of the spectra by eliminate the slight uncontrollably shake and artificially displacement.

Moreover, Chen *et al*., 2005 [36] stated that the diffuse reflectance spectra obtained from the non-invasive measurement are influenced not only by the absorption coefficient but also by the scattering coefficient, anisotropy factor, and refractive index, which are normally nonlinear with the concentrations on interested parameter.

1.3 The Advantageous and Disadvantageous of NIRS

There are several advantageous of NIR spectroscopy that had been discussed and pointed out by researchers in the literature reviewed such as:

1. It is applicable for non-invasive (direct applied NIR at human body and avoid the blood draw) measurement in medical. This is easier and more comfortable for both patient and doctor.
2. NIR spectral allows transmission through one to two mm of tissues. Non-invasive measurement could be achieved through the web between the thumb and index finger or possibly through the ear lobe [37].
3. It can be used for non-destructive analysis which means that it is applicable for in-situ (in the natural or original place) sampling, and only requiring a little or no sample preparation [2, 11].
4. It can direct analyze the spectra for solid and liquid samples that have various shape and thickness with no pretreatment [1-2]
5. It does not require any wet chemical for sample preparation (free from hazardous chemicals as it is a dry lab system) as it involved non-invasive and non destructive method [11]. Therefore, continuous and repeated measurement on the same sample can be done [38].
6. It allows determination of non-chemical (physical) parameters like density and viscosity [1, 11].
7. It is suitable for high-throughput at-line, on-line and in-line analyses [2, 11].
8. It allows the fast and simultaneous analysis of several chemical parameters from a single spectrum. These attributes make it especially attractive for straightforward, speedy characterization of samples [1, 11].
9. NIR spectrum provides the information of nearly all types of organic molecules, such as carbon-hydrogen (C-H), nitrogen-hydrogen (N-H), and oxygen-hydrogen (O-H) bonds [2].
10. NIR spectroscopy have a really flexible measurement technique as it instrumentation can incorporate a variety of devices (reflectance, transmission, tranflectance) depending on the sample and the particular analytical conditions and needs (such as speed, sample complexity and environmental conditions) [1]

11. Safety aspects can be seen as one of the advantages due to the safe measurement probes and fiber optics [39].

However, there are still some limitations of NIR spectroscopy being discussed and highlighted in previous researches such as:

1. Chemical compound in NIR spectrum cannot be directly interpreted [2].
2. The complexity of NIR spectra due to the broad and overlapping absorption bands (most of the wavelengths in NIR region contain similar information) needs complicated chemometric techniques in order to extract meaningful information from the spectra and to calibrate the data (statistical relationships between the spectral data and reference data) [2].
3. Accurate, robust calibration models are at time difficult to obtain as their construction entails using a large enough number of samples [11].
4. The need to incorporate the physical and chemical variability of sample types, and hence more than one model per analytes [11].

1.4 Leukemia

Leukemia is a blood forming cells cancer in the bone marrow [40]. It is a cancer of white blood cells (WBC) and characterized by an abnormal multiplication or mitosis of white cells in the bone marrow [41-42]. In normal condition, WBC reproduces in an orderly and controlled manner but in Leukemia patient the process is disturbed and cells continue to divide but not mature. Leukemia patients have high white blood cell counts. However, these cells are defective and cannot perform their normal role in protecting the body from infection [42].

Leukemia can be acute (fast growing) or chronic (slow developing). In acute leukemia the bone marrow cells cannot mature properly while in chronic leukemia the bone marrow cells can mature partly but not completely. Myeloid leukemia is a type of leukemia that starts in early forms of myeloid cells (the cells that make white blood cells other than lymphocytes). Lymphocytic leukemia is a type of leukemia that starts in immature forms of lymphocytes (one type of white blood cell) [43]. There are four types of Leukemia which are Acute Lymphocytic Leukemia/Acute Lymphoblastic Leukemia (ALL), Acute Myelogenous Leukemia/Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), and Chronic Myelogenous Leukemia (CML) [42, 44].

1.5 Standard Leukemia Diagnosis Method

Leukemia diagnosis starts with complete medical history and physical examination before proceed to peripheral blood film examination (full blood picture) and bone marrow assessment. Blood samples will be taken to perform full blood count (FBC) automatically using hematology analyzer machine in order to measures the total white blood cells (TW), hemoglobin (Hb), hematocrit (Hct), and platelet (ptt). Then, a blood smear which also called full blood picture (FBP) will be done on the same sample to look for presence of any abnormal white cell or "blast'' under the microscope. A bone marrow aspiration will have to be carried out to confirm the diagnosis, determine type of leukemia as well as prognostication. This procedure involves the insertion of a needle into the posterior hipbone to obtain a sample of the marrow. Additional tests will be carried out on this sample to determine the specific type or subtype of leukemia, as well as for prognostication, cytogenetic analysis, and molecular study. This information will help doctor in determination of the best treatment for the patient [45].

1.6 Leukemia Biomarkers

In the early study done related to leukemia, researchers studied the different characterization between leukemia and normal sampled (cell, blood plasma, full blood). Mostly, the characterization studies were done by applied infrared spectroscopy on the cell sampled [46-49]. The common biochemistry parameter measured using spectroscopic technique in characterization between normal and leukemia cell are lipids, protein, and DNA [46, 49]. However, it requires sample preparation (centrifuges process, replicate films preparation, and involved the use of some chemical reagent) and highly trained person [49].

The study done by the author is focusing on the acute leukemia patients. Leukemia is known as the cancer of white blood cell (WBC). The abnormal WBCs continue to accumulate as leukemia progresses because they do not go through the normal cell life cycle to death [50]. In literature, white blood cell count is stated as one of the prognostic factor in acute lympoblastic leukemia (ALL) [51] and acute myeloid leukemia (AML) [52]. According to Aguayo *et al*., it is stated that acute myeloid leukemia (AML) patients had higher white blood cell compared to other patients [53].

From the study done by Abraham Kornberg *et al*. [54] there is high level of LDH in Acute Lymphoblastic Leukemia (ALL). LDH level is said to be correlated with the number of immature blood cells (blast). Study done by Bierman, H., B. Hill, *et al*. [55] shows a correlation between LDH levels with the leukemia patient clinical status. Based on Hafiz *et al*. [56] measurement of serum LDH level can be accepted in early prediction of children ALL and in monitoring the chemotherapy efficiency.

In the study done by Abu Zaid *et al*. [57] it is prove that level of hemoglobin was low in leukemia patient compared to the normal range. In leukemia cancer, the ability of the bone marrow to produce red blood cells is affected due to the abnormal production of blast [58]. Anemia (a condition of blood lacks enough healthy red blood cells or hemoglobin) is one of leukemia symptom [59]. About 80-90% of acute myeloid leukemia (AML) cases are adults [60].

Adwani *et al*. [61] analyzed the prognostic factors for the acute lymphoblastic leukemia (ALL) in India using a single treatment regimen. The multivariate analysis of prognostic factors result showed that WBC, HB and LDH were significant risk factors. The risk increased as the value of WBC and LDH increases and Hb decreases.

After further discussion with the medical doctor is made, the proposed new noninvasive method in leukemia screening will be focusing on the total lactate dehydrogenase (LDH), total hemoglobin (HB), and total white blood cell (TW) as the biomarker for leukemia screening.

2.0 APPLICATION OF NIRS IN MEDICAL

Based on the literature reviewed, there are widespread applications of NIRs in medical field. The effectiveness of NIR spectroscopy applied in medical is already proven for the determination of blood biochemical's that are used as biomarker of specific diseases and for monitoring tissues oxygen level during surgical processes [1]. This method successfully applied in determination of albumin, globulin, cholesterol, urea, glucose, total protein, and hemoglobin in whole blood or blood serum [19]. Besides, there also many studies have been done related to non-invasive blood glucose measurement, cancer detection, body fat measurement, and others even though most of them are not yet clinically reliable [62].

According to Arimoto *et al*. [62], the measurement of oxygen saturation is the most successful application of NIR spectroscopy and Kraitl *et al*. [20] stated that oximetry is an example of well established non-invasive application of NIR spectroscopy that is used in oxygen saturation measurement. The other application of NIR spectroscopy that already applied in medical is the pulse oximetry that is used to measure heart beat or pulse. This is possible because during systolic arteries tend to contain more blood due to an increase in diameter. As a result, the absorbance of light in tissues with arteries increases as the number of hemoglobin (absorber) is higher and light passes through a longer optical path. The intensity changes produce the PPG wave [63].

The studies related to glucose sensing using NIR spectroscopy has been done for quite long time. Measurement and monitoring the blood glucose level is an ongoing field of research in clinical analysis which play an important role in controlling and preventing diabetes. Glucose is the major energy carrier in human organism and fast growing technology helps in the development of painless non-invasive method in glucose measurement either for single or repeated reading [64]. Blood glucose level helps in diagnosis or monitoring of diabetic level[65].NIR spectroscopy have been proposed and developed for non-invasive method in glucose measurement to replace the conventional method which involved finger pricking.

There is a few works done related to non-invasive blood glucose monitoring using NIR spectroscopy. In 1993, Marbach, *et al*. [66] took measurement of blood glucose noninvasively at human lower inner lip using diffuse reflectance spectroscopy. Inner lip is a tissue that rich with capillary blood vessel and well temperature control. The maximum absorbance spectrum of glucose is stated at 6350 cm^{-1} (1.6 µm/ 1600nm) which is the overtone bands of OH- and C-H stretching modes. Fei, S., *et al*. [32] determined the blood glucose concentration using transmission mode of NIR spectroscopy applied at finger. The absorption band for glucose absorption was obtained at 940nm. Studies done by Huang Zhen-hao *et al*. [65] showed that it is possible to measure the glucose concentration using near infrared reflectance spectroscopy applied at forefinger. In this studied, near infrared wavelength at 1.2µm (1200nm) and 1.35µm (1350nm) is correlated with the glucose concentration range of 30 to 300mg/L. Yoshinari, H., *et al*. [25] developed a non invasive method in glucose measurement using NIR. This study proved a high correlation between glucose NIR spectrum measured at human fingertip and on the quartz cell with glucose aqueous solution. Glucose absorption is confirmed at peak of 1550nm (combination bands of CO stretching vibration and OH stretching vibration of glucose) and 2150nm (OH bending vibration of glucose).

Hemoglobin is one of the important blood parameter that helps in the assessment of physiological condition and oxygen transportation in the blood [63]. The predominant organic absorber of nir light in tissue is hemoglobin. The oxygenation and de-oxygenation of hemoglobin greatly vary the infrared spectrum of hemoglobin [28]. Previously, Kraitl, Jens, *et al*. [63] applied NIR at artificial finger model before directly applied NIR at the area on fingertip skin for the noninvasive measurement of blood hemoglobin (Hb). The significant spectrum band for Hb in the studied done using artificial finger was found at 980nm and 1310nm. When NIR is directly applied at human fingertip, it is stated that the oxygen saturation can be calculated at 670nm (absorbance of deoxy-hemoglobin greatly exceeds the absorbance of oxy-hemoglobin) and at 905nm (absorbance of oxy-hemoglobin greatly exceeds the absorbance of deoxy-hemoglobin) transmission signal.

Anemia is a common diseased that occurred due to low hemoglobin level (low red blood cell count) and Ding, H., *et al*. [35] proves the potential of non-invasive diagnosis of Anemia using NIRs with range of 600-1050nm. Anemia was diagnosed by applied NIRs in transmission mode at the fingertip. The hemoglobin (Hb) level can be measured at the wavelength of 900nm.

A study on tumor prognosis is done by measure the concentration of oxygen-hemoglobin [67]. The vascular bed of rat (implanted with breast tumor &

prostate tumor) was used as sample. Oxygen-hemoglobin concentration measured at 758nm and 782nm using NIR spectroscopy showed a prompt rise followed by a gradual persistence throughout the intervention.

Assessment of Acute Lung Injury (ALI) using in-vitro model was done by Shibata *et al*. [68]. The in-vitro model simulates the spectrophotometric characteristics of the lung, water, and hemoglobin (hb) oxygenation. The spectrum shows elevation at 975 nm (water) and at 760 nm (deoxy-hb).

NIR spectroscopy also applied in medical for monitoring tissues oxygen level during surgical processes [1]. In literature, there is a few work related to brain monitoring during surgery were done. This application is possible as NIR photons are able to transmit into the brain as the skin, scalp, and skull are relatively transparent to NIR light [69]. Human head consists of four layers which are one layer of scalp and skull, CSF, grey matter, and white matter. The change of signal of different states of brain is mainly derived based on the absorption changes in the grey matter. In active state, there will be an increase of blood supply (oxygen) into the brain tissues. This will result into the change of grey matter absorption coefficient [70]. Ali, M.S. [69] applied NIRS in non-invasive monitoring of brain activity during adult and pediatric surgery. In monitoring the brain activity, NIRs provides information on the concentration of cerebral oxyhaemoglobin (HbO2), deoxygenated haemoglobin (HHb), total haemoglobin (THb), and oxidized cytochrome aa3 (CytOx). The measured ratio of HbO2/THb is reported as "regional cerebral saturation" (rSO2) or as "tissue oxygen index" (TOI).Z. Guo, *et al*. [70] using their four-layered model for near infrared light propagation in a human head based on the Monte Carlo method stated that the suitable wavelength for monitoring human brain activity using four-layered model of human head is at 1300 nm with a high absorption sensitivity of grey matter and improved spatial resolution.

The hydrogen ion concentration (H^+) is refer to pH and normally reported as –log [(H^+)] [12, 18]. In previous observation, hydrogen ion is stated not to have infrared bands as it is more towards an ion rather than a molecule [28]. The spectroscopic changes after varying the hydrogen ion is said to be due the changes in the water absorption band. However, non invasive measurement of blood pH is still possible using NIR spectroscopy because hydrogen ion will bind to other species in solution that are infrared active. So, a correlation for pH will be made based on the secondary spectroscopic effects [12]. As a result, Rosen, N. A., *et al*. [28] suggested that histidine (a crystalline essential amino acid $C_6H_9N_3O_2$ formed by the hydrolysis of most proteins) residues of hemoglobin that provide the spectral variation necessary for pH modeling.

For example, Alam, M.K. *et al*. [12] applied NIR spectroscopy at human fingertip to determined pH in human tissue by utilizing histidine spectral data at 2060-2115 nm, 2160 nm, 2225-2235 nm and 2360 nm [12]. Previously, the first non invasive pH measurement was demonstrated by Soller, B.R. *et al*. [71]. Deep tissue pH is measured in skin-covered muscle on the back of the rabbit with blood flow easily controlled by ligation of a single artery using NIR reflectance spectroscopy with the range of 700-1100 nm [71]. Thomas *et al.* were able to noninvasively estimate blood pH in the leg of a lamb using transmission near-infrared spectroscopy (500–1000 nm) [72]. Alam *et al.,* using transmission near-infrared spectroscopy in the spectral range 1500 to 1820 nm, were able to estimate the pH of lysed blood [73]. They further demonstrated that spectral changes over the wavelength range from 1500 to 1785 nm can estimate the pH of whole blood [74]. Their work suggested that it is the histidine residues of hemoglobin that provide the spectral variation necessary for pH modeling [28, 73].

Near-infrared spectroscopy can be used to probe noninvasively deep into organ tissue (1–10 mm). The feasibility of reflectance NIRS in measuring blood pH in vitro was investigated found that spectral changes in the wavelength range 650–1050 nm were directly related to changes in pH. The spectral variations seen are due predominantly to changes in oxygen saturation and hemoglobin. The effects of pH cannot be visually identified and are discernible only with multivariate analytical techniques [28].

NIR spectroscopic method also had been applied in many other medical diagnoses. Joelle wallon, et al., 1994 in his study of identification of breast carcinomous tissue, cancerous tissue-normal surrounding fibro-glandular tissue (Breast cancer), cancerous tissues/Cancer cells, four wavelength intervals were found to be different between normal and cancerous tissues at spectral range of 1208-1242, 1746-1788, 2012-2048 and 2326-2368nm [75]. R.A. Shaw *et al*. [76] diagnosed arthritis by applied NIR spectroscopy at the synovial fluid of arthritis patients. The diagnosis is done by monitoring and analysis the albumin (a type of protein) spectrum data. Albumin referred are at wavelength of 1691, 1737, 2056, 2019, 2168, 2292, and 2347 nm. Other, urine analytes also can be measured using NIRs. Pezzaniti, J. Larry, *et al*. [77] measured urine analytes (urea, creatinine, glucose, protein, ketone) of normal urine using NIR spectroscopy. The spectral signatures of urea, creatinine, glucose, ketone, and protein in the 1350-1800 nm and 2050-2375 nm range are sufficiently strong and unique for accurate measurements.

3.0 APPLICATION OF NIRS IN LEUKEMIA SCREENING

Infrared spectroscopy has developed into a novel biomedical tool in hematology field and has been used in the studies related to leukemia for example in the diagnosis and prognosis of leukemia, characterize differentiation between sampled (cell, blood plasma, full blood, etc) obtained from normal and leukemia patient, and to monitor the

effectiveness/response of certain drugs or chemotherapy [49, 78].

Infrared spectroscopy was applied in characterize differentiation between leukemia and normal sampled to see the spectroscopic changes of certain biochemical biomarkers [49]. The studies about this had been done by several researchers.

Schultz *et al*. [46-47] has compared the pheripheral blood mononuclear cells (PBMCs) of normal individuals with the purified B cells of chronic lymphocytic leukemia (CLL) patients using infrared spectroscopy (900-1800cm^{-1}) by observing the DNA, protein, and lipids levels. Leukemia cells had greater DNA and lower lipids content compared to normal cells.

Benedetti *et al*., 1997 [48] observed the nucleic acids and protein content between leukemic and normal lymphocytes (a type of white blood cells). The measurement is done using infrared microspectroscopy (900-1800cm^{-1}) by comparing the spectra of DNA/RNA/protein mixtures with the spectra of lymphocytes obtained from B-chronic lymphatic leukemia (B-CLL) patients and normal donors. The analysis done result showed that there was different in band profile and intensities related with the absorption of proteins (1080cm^{-1}) and nucleic acids (1540cm^{-1}) between leukemia and normal cells. There in an increased in DNA/RNA ratio in leukemia lymphocytes compared to normal lymphocytes.

Then, there also a study done on the characterize differentiation between healthy human T-cells (peripheral blood mononuclear cells, PBMCs) and leukemia-T cell line (Jurkat) with the purposed to distinguish spectral changes of the related biochemical biomarker. Jurkat cells were obtained from American Type Culture Collection and PBMCs were collected from donators. The spectral was recorded using Nicolet 380 spectrometer (Thermo Scientific Nicolet, Waltham, MA) with spectral range of 4000-900cm^{-1}. The biomarkers used to helps in differentiate between Jurkat cells and PBMCs in this study are proteins (Amide I (1651.8 cm^{-1}), Amide II (1542.6 cm^{-1})), lipids (2962.2 cm^{-1}) and DNA (1084.8cm^{-1}) [49].

In 2005, Erukhimovitch *et al*. [79] used transmission mode of Micro-FTIR Spectroscopy (600-2000 cm^{-1}) to examine plasma sample of leukemia patients. Blood plasma samples were taken from males age from 25 to 45 consists of 10 CLL patients and 10 normal subjects. Cluster analysis was performed on the spectral obtained using OPUS software. From the analysis it showed that peaks at 1056, 1270, 1592 cm^{-1} each represent carbohydrates, amide III, and amino acid is lower in CLL patients.

In the study done by Ranjit Kumar Sahu, *et al*. [80] a continuous monitoring of white blood cell (WBCs) biochemistry in an adult leukemia patient was done using advanced FTIR-spectroscopy consist of FTIR microscope IRscope II with liquid nitrogen cooled mercury cadmium-telluride (MCT) detector coupled to FTIR spectrometer (Bruker Equinox model 55/S, OPUS software). White blood cells (WBCs) sample were taken from adult MLL, children ALL, and normal adult. All patients used in this study are under treatment. Second derivative of spectra is used to check the exact wavenumber. After treatment, the phosphates absorbance from nucleic acids and the lipid-protein ratio in WBC decreased immediately.

The characterizations of sample collected from patient that undergo chemotherapy or treated using certain drug were done to monitor the effectiveness or responds. During 2002, Ramesh *et al*. [81-82] had been studied about the progress made with chemotherapy by monitoring B-cell and T-cell of pediatric acute lymphoblastic leukemia (ALL) patients. The spectra obtained were provided by FTIR microspectroscopy (600-4000cm^{-1}) that operated using transmission mode. The result showed the decreased of nucleic acids content after chemotherapy [82]. In the next study, Ramesh *et al*., monitor the progress made with 7 days of chemotherapy in 1 B-cell and 2 T-cell obtained from pediatric ALL patients. The result from the cluster analysis showed good classification between samples with and without blasts. It is acceptably correlated with clinical data [81].

4.0 COMPARISON BETWEEN INVASIVE AND NONINVASIVE METHOD

NIRS is a technology that successfully applied in non-invasive diagnosis and monitoring of diseases. A truly non-invasive diagnostic method is a method that does not physically breach the skin or enter the body deeply through an external orifice. Diagnosis method involving blood draw and endoscopy is considered minimally invasive as it requires sterile technique and local or general anesthesia for some cases is considered as minimally invasive [83]

In the non-invasive measurement, NIR was directly applied at human body for spectral data acquisition [25, 32, 65-66]. It does not involve any wet chemical for sample preparation which means no hazardous chemicals is used. The laboratory/clinical data are used as the reference data for validation. This laboratory data's were obtained using the standard procedure that involves blood draw.

Blood parameters are essential in diagnosis and monitoring of diseases. Sometimes there is a need for a frequent and continuous blood parameters measurement to check the condition of the patient. The applications of noninvasive method allow for continuous and repeated measurement to be taken as it is easier, simple procedure, not cause pain, non destructive [35], fast [35], without chemical reagents [35], multi-component detection method [35] and more comfortable for both patient and doctor.

Previously, noninvasive NIR method had allowed the continuous measurement of hemoglobin concentration, oxygen saturation and pulse [63]. However there is some limitation in noninvasive application of NIRs compared to invasive method.

For example in brain monitoring study, NIRs only able to detect regional cerebral oxygenation, the optical path length is also difficult to identify, arterial versus venous changes are not distinguished, the effect of extra cranial tissue on NIRs signal is unknown, absolute quantification is lacking, vulnerable to light and movement artifacts, and cannot measured dissolved oxygen [63].

Non-invasive glucose monitoring allows repeated measurement without any pain and bleeding. This approach is applicable in diabetes management to adjust their calorie intake and/ or administer insulin injections [65]. However, the noninvasive procedure which involves blood draw had caused pain, stress, and blood infection. So, the frequent monitoring of blood glucose is hard to achieve. Noninvasive measurement procedure using near infrared helps in frequent monitoring of blood glucose with a high speed and no blood infection [25, 36]. Besides, non-invasive measurement also contribute in reducing the enormous costs of the disposal test strips for finger-prick blood sample in the conventional method which are required by the glucometer (glucose meter) that had been used for glucose home monitoring [66, 84].

The current method for blood pH and other blood analytes measurement the use of blood analyzer equipped with electrodes to measure the desired analyte. But this method requires a lot of work as the electrodes used must be thoroughly washed after the blood analysis to prevent protein buildup on the electrode surfaces and the analyzer must be calibrated at least every two hours. Even the pH and the other blood analytes can be measured with this analyzer for only two minutes, due to the size and expense of the blood analyzers it is only kept in central locations in most hospitals. Therefore, blood sample need to be transported to the analyzer [18].
Blood test requires a blood draw using a needle while a bone marrow aspiration involves insertion of a needle into the posterior hipbone to obtain the samples needed. These methods require extensive sample preparation, staining technique, and an expert to examine the samples obtained. The diagnosis took quite some time as it needs to be done in the laboratory by an expert [1]. Blood analysis using the blood analyzer cannot perform continuous monitoring for a critically ill patient even the analysis can be made in a few minutes because it is an invasive procedure that involve blood draw which causes discomfort and pain. The blood sample must immediately place on ice right after the arterial blood withdrawn before transported to the clinical chemistry laboratory in the hospital to inhibit red blood cell metabolism that would change the sample's blood gas parameters and lead to an incorrect measurement of the patient's blood gas values including the pH value. After analyzed using the blood analyzer, the results are entered in the hospital computer and made available to the physician for interpretation. Therefore, it is hard to achieve continuous real-time monitoring as the blood analysis took some period during which patient status can change [18]. Diagnosis procedure that involved blood draw can lead to the spread the blood and body fluid infectious. Besides, blood draw also inconvenient for neonates as the blood vessels are narrow and blood volume is low [35].

The standard leukemia diagnosis method is considered minimally invasive as it involved blood draw which requires sterile technique [83].The standard procedure in leukemia diagnosis is mainly dependent on the morphological examination of pheripheral blood and bone marrow sample, cerebrospinal fluid analysis, and complemented with cytogenetics, immunophenotyping, and molecular studies [78]. Even though these standard procedures may give an accurate diagnosis, it is time consumed and required highly trained person.

NIR spectroscopy represented a good technique for automated diagnosis of leukemia [78]. Figure 2 above showed the comparison between invasive and non invasive method in leukemia screening using NIR spectroscopy. In the minimally invasive method which involve blood draw, NIRS was applied at the sample (cell, full blood, blood plasma, etc) obtained from normal and leukemia subjects for data acquisition. NIR was applied for the spectral biochemical characterization of the sample. The common biomarkers pointed out by researchers in characterization between normal and leukemia cell are lipids, protein, and DNA [46, 49]. There is a need for sample preparation before performing the measurement (normally for normal sample as the leukemia sample directly obtained from laboratory). Sample preparation involved the centrifuges process, replicates films preparation, and involved the use of some chemical reagent [49].

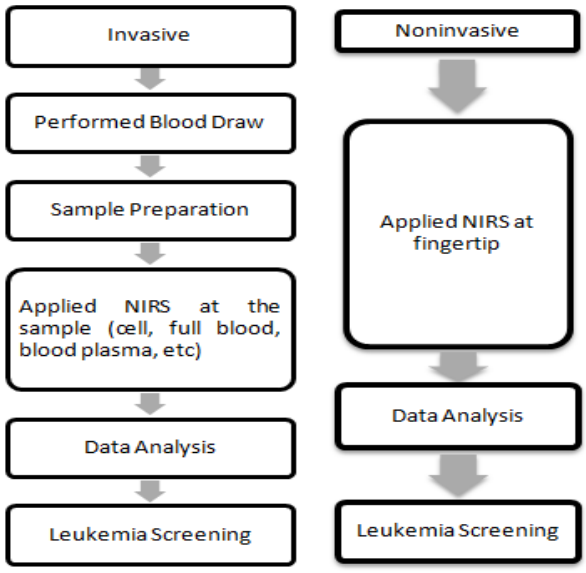


Figure 2 The comparison between invasive and noninvasive NIRS procedures in Leukemia Screening

In the non-invasive measurement, NIR was directly applied at human body for spectral data acquisition [25, 32, 65-66]. It does not involve any wet chemical for sample preparation which means no hazardous chemicals is used. The laboratory/clinical data are used as the reference data for validation. This laboratory data were obtained using the standard procedure that involves blood draw.

5.0 CONCLUSION

Development for a better diagnostic method is needed for a faster diagnosis process without analysis error. Early diagnosis is essential so that a right treatment can be given on right time in order to saves more lives. Diagnosis and monitoring methods has developed from invasive towards non-invasive to avoid feeling of pain, discomfort, prevent the chance of blood infection, improve the diagnostic accuracy, decrease unnecessary biopsies, simplify and faster the diagnosis procedure, and applied for repeated and continuous monitoring of blood parameters. Recently, many efforts have been directed towards utilization of near- infrared spectroscopy NIRs for cancer and pre-cancer detection. NIR spectroscopy is a potential method for non-invasive biochemical detection due to its good penetrability for body fluid and soft tissue. The technique used is based upon an understanding of cancer at the molecular level.

Many studies done involving non-invasive method (NIR was directly applied at human body) but none in leukemia screening. Moreover, the earlier studied related to leukemia only focus on spectral biochemical characterization between normal and leukemia sample. It is concluded that there are some similar studies have been done in screening of leukemia using infrared spectroscopy but they were done on samples or specimens (full blood, blood plasma, cell, etc) instead of directly applied on human body.

NIRS is a new non invasive diagnosis tool that can be used as a new alternative in an early screening of leukemia with just a modest cost, simpler method and faster diagnosis time [1]. The new proposed method of leukemia screening is a noninvasive method. The NIR Spectroscopy used in this study was using fiber optic that operates in reflectance mode. Reflectance is the suitable mode to be used as NIR is applied noninvasively at human fingertip. This is because it is easier and more comfortable for both patient and doctor. With NIR, the diagnosis can be performed repetitively with a simpler and faster procedure without any blood draws needed.

Early screening of leukemia is the main focus of this research. In this work, NIR is directly applied at human body (fingertip) non-invasively without any sample preparation included. In the non-invasive measurement, NIR was directly applied at human body for spectral data acquisition [25, 32, 65-66]. The obtained spectral will be analyzed to identify the useful biomarkers in classification between the normal and leukemia patients. It does not involve any wet chemical for sample preparation which means no hazardous chemicals is used. Three blood parameters (biomarkers) which considered to be used are total white cell (TW), total hemoglobin (HB), and total lactate dehydrogenase (LDH).

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