LOW-COST, WIRELESS OPTICAL OXIMETER
FOR MONITORING OF BRAIN FUNCTION IN
HIGH-RISK PEDIATRIC POPULATION

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biomedical Engineering

by

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ABSTRACT


Nearly 4 million neonates die every year due to brain injuries, most caused by hypoxia. Neonatal ICU requires newborns with critical health conditions to be monitored continuously calling for the need of a non-invasive, compact and portable device. However, commercial devices are mostly bulky and/or expensive. The neonatal mortality is higher in under-developed countries, where such expensive and large devices are not affordable. Hence, my thesis focuses on building first generation compact, inexpensive, and wireless device that can monitor and provide feedback to clinicians during intervention.

This approach is based on light absorption by oxy- and deoxy-hemoglobin chromophores. The total cost of this device is less than $100, which is lower than commercial devices (> $10K). The device has SNR of 65 dB. It was successfully tested by the arm occlusion protocol, which allowed measuring real-time oxygenation changes in *vivo*. Overall, the first generation device shows promise for future clinical studies.
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NIRS</td>
<td>Near Infrared Spectroscopy</td>
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<td>NIR</td>
<td>Near Infrared Region</td>
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<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>PET</td>
<td>Photon Emission Tomography</td>
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<tr>
<td>EEG</td>
<td>Electroencephalographic</td>
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<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
</tr>
<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
</tr>
<tr>
<td>$\text{HbO}_2$</td>
<td>Oxy-hemoglobin</td>
</tr>
<tr>
<td>$\text{HHb}$</td>
<td>Deoxy-hemoglobin</td>
</tr>
<tr>
<td>$[\text{HbO}_2]$</td>
<td>Oxy-hemoglobin Concentration</td>
</tr>
<tr>
<td>$[\text{HHb}]$</td>
<td>Deoxy-hemoglobin Concentration</td>
</tr>
<tr>
<td>$[\text{HbT}]$</td>
<td>Total Hemoglobin Concentration</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CBV</td>
<td>Cerebral Blood Volume</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to Noise Ratio</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>SDS</td>
<td>Source Detector Separation</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>Absorption Coefficient</td>
</tr>
<tr>
<td>$\mu_s'$</td>
<td>Scattering Coefficient</td>
</tr>
<tr>
<td>MBLL</td>
<td>Modified Beer Lambert Law</td>
</tr>
<tr>
<td>DPF</td>
<td>Differential Pathlength Factor</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Extinction Coefficient</td>
</tr>
<tr>
<td>BV</td>
<td>Blood Volume</td>
</tr>
<tr>
<td>$\text{StO}_2$</td>
<td>Oxygen Saturation</td>
</tr>
<tr>
<td>CW</td>
<td>Continuous Wave</td>
</tr>
<tr>
<td>FM</td>
<td>Frequency Modulated</td>
</tr>
<tr>
<td>TR</td>
<td>Time Resolved</td>
</tr>
<tr>
<td>TS</td>
<td>Time Domain</td>
</tr>
<tr>
<td>FD</td>
<td>Frequency Domain</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of Flight</td>
</tr>
<tr>
<td>TPSF</td>
<td>Temporal Point Spread Function</td>
</tr>
<tr>
<td>LD</td>
<td>Laser Diode</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>---------</td>
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<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
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<tr>
<td>DOS</td>
<td>Diffuse Optical Spectroscopy</td>
</tr>
<tr>
<td>APD</td>
<td>Avalanche Photodiode</td>
</tr>
<tr>
<td>PMT</td>
<td>Photomultiplier Tube</td>
</tr>
<tr>
<td>IEEE</td>
<td>Institute of Electrical and Electronics Engineers</td>
</tr>
<tr>
<td>IC</td>
<td>Integrated Circuit</td>
</tr>
<tr>
<td>SPST</td>
<td>Single Pole Single Throw</td>
</tr>
<tr>
<td>TX</td>
<td>Transmission</td>
</tr>
<tr>
<td>RX</td>
<td>Reception</td>
</tr>
<tr>
<td>DAQ</td>
<td>Data Acquisition Card</td>
</tr>
<tr>
<td>MC</td>
<td>Microcontroller</td>
</tr>
<tr>
<td>ADC</td>
<td>Analog to Digital Conversion</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
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Dedicated to

my parents and Rahman for all their support
Chapter 1 - Introduction

This chapter introduces the optical imaging technique- Near Infrared Spectroscopy (NIRS), which is the basis of this thesis. The motivation and objective behind this study and the thesis outline are also included in this chapter.

1.1 Near Infrared Spectroscopy

Near infrared spectroscopy (NIRS) is an optical imaging technique that uses light in the near infrared (NIR) region of the electromagnetic spectrum, which is ranges from approximately 600nm to 1000nm, to monitor functional activity of the brain. NIR light is used because it has higher penetration depths into the tissues than light in the visible or mid-infrared region. When light is incident on the scalp, it penetrates into the skull down to the grey matter. Different photons follow different paths within the head. Some of the photons get absorbed by the various components of tissues, called chromophores, and others get scattered back to the surface. In NIRS, this spectra of the back scattered light is used to derive information about the brain activity. Although other imaging techniques such as functional magnetic resonance imaging (fMRI), Computed Tomography (CT), Photon Emission Tomography (PET) are available, the major advantages of NIRS is its
portability, which allows for bedside monitoring, its relatively high temporal resolution and low cost [1].

1.2 Advantages of NIRS

Some of the advantages of NIRS include and are not limited to [2]: 1) NIRS is completely safe, non-invasive and its target audience is not limited. It can be used for an individual of any age, 2) It is inexpensive, 3) It does not involve any fixture on the subject. It consists of sensors that are to be placed over the head, 4) It can be built compact such that it is portable and non-interfering making it ideal for intra-operative settings, 5) It is not associated with any acoustic noise, 6) It is portable, small in size and allows real-time data acquisition, which makes it highly suitable for bedside continuous monitoring.

1.3 Drawbacks of NIRS

Apart from all the advantages of NIRS imaging, it has certain drawbacks limiting its applications [2]: 1) light has to travel through several layers before it reaches the grey matter which leads to attenuation of the light. As a result, the penetration depth gets limited. Information of only the superficial brain matter can be extracted, 2) Electroencephalographic (EEG) waves are generated by the extra-cerebral regions cause physiological interferences in NIRS, 3) It’s resolution is limited to 1-3 cm when compared to the higher resolution of fMRI which is 3-6 mm.
1.4 Motivation

According to an article by Save the Children in 2001, nearly 4 million neonates die each year [3]. This is a major problem mostly in the developing countries, whose contribution is about 98% of the worldwide neonatal deaths [4]. South Asia alone contributes up to 2 million neonatal deaths each year. For every 1000 live births, an estimated 51 deaths in South Asia, 42 in Africa, 25 in Latin America and less than 10 in Europe and North America is reported (Figure 1.1) [4]. In a study conducted in India it was seen that out of 763 infants, nearly half developed, high-risk morbidities while 3/4\textsuperscript{th} suffered low-risk morbidities with high-risk conditions [5]. Research indicates few causes of neonatal deaths of which Birth Asphyxia or Hypoxia is the major contributor (Figure 1.2).

![Figure 1.1: The worldwide distribution of Neonatal Mortality rate per 1,000 Live Births. Image taken from http://www.sickkids.ca/PGPR/Symposia-and-Workshops/May2005/Perspectives-on-India/index.html](image-url)
Hypoxia is a condition in which oxygen cannot be delivered to the tissue, or tissue oxygen deficiency. Of all the organs, brain and eyes are the most sensitive to hypoxia [6]. Even if the child survives, hypoxic conditions during or after birth can lead to severe effects (Figure 1.3) in the long run, causing lifelong impairment. When the brain is deprived of oxygen it can lead to oxygen-related brain injuries in which brain cells are killed. This could even lead to conditions such as cerebral palsy (movement impairment), Autism, Attention Deficit Hyperactivity Disorder (ADHD) or even body paralysis [7,8,9]. This would also increase the average lifetime costs for the children and cause significant hardships for the child as well as the family.
Therefore, there is a need to continuously monitor the tissue oxygen saturation levels and hemodynamics, especially in neonates and infants. Lack of tissue oxygen can affect adults as well, but infants are particularly susceptible, since their tissues and organs would be still developing and lack of oxygen can hinder the growth and kill the cells. Even though oximetry has been around and commercialized for many years now, the commercially available devices (Table 3.3 and Table 3.4, Chapter 3) range from $10,000-$100,000 as shown in a study by Scholkmann et al [10]. Neonatal deaths are a major problem in low-resource settings such as under-developed and developing countries as well as rural areas, where they typically cannot afford such expensive instruments, so there is a need for a compact device which is inexpensive and portable for use in the rural areas where many home births occur due to lack of hospitals.
1.5 Objective

Neonatal Intensive Care Units (NICU) require newborns with critical health conditions to be monitored continuously to prevent any potential complications. Continuous monitoring of a neonatal brain requires the instrument to be non-invasive, compact and portable, such that it does not interfere with the other NICU devices [1]. Therefore, this study aims at quantifying oxy-deoxy hemoglobin concentrations allowing us to calculate the tissue oxygen saturation and total blood volume of a neonate in order to study the hemodynamic activity and functionality of their brain. It also aims at building a compact, wireless and inexpensive device such that it is non-interfering, NICU friendly and affordable by all.

1.6 Thesis Outline

In the second chapter the background and principle of Near Infrared Spectroscopy (NIRS) is discussed. The optical properties of absorption and scattering in tissues and the various chromophores contributing towards them are also talked about. The Beer Lambert Law and the types of NIRS systems are also mentioned.

The third chapter is a literature review talking about the history and evolution of the NIRS. It also talks about the instrumentation of NIRS systems, the sources, detectors, optode housing and spatial distribution of source and detectors used by studies from literature. Few commercial NIRS systems are mentioned and different types of system validation techniques used in literature

In the fourth chapter the instrumentation of the developed device is discussed. The block diagram of the device as well as the details of all its components are discussed.
Chapter five describes the device validation techniques used to test the device and also their results obtained.

Finally, the sixth chapter concludes the thesis with a brief discussion and conclusion followed with future directions.

The bibliography is at the end of the thesis with the references in the APA style.
Chapter 2 – Background

2.1 Propagation of Near Infrared Light in Tissues

Jobsis in the late 1970s showed that measurements taken of certain metabolites in the transmittance mode using near infrared (NIR) radiation could be useful in monitoring their oxygenation properties [11]. He was the first to successfully apply near infrared spectroscopy (NIRS) in-vivo, to study the oxygenation of a cat’s brain [12]. Consequently, the diagnostic potential and applications of optical methods was realized and since then a great deal of work has been done using NIRS.

The foundation of NIRS is on the fact that biological tissue, in the NIR region (600-1000nm), is relatively transparent to light. Many chromophores in human tissue have characteristic absorption spectra at these wavelengths, which can be used to determine their concentration in tissue. The concentration of some chromophores such as water, melanin and bilirubin remain constant with time, whereas the concentration of some chromophores such as oxygenated hemoglobin (HbO₂), deoxyhemoglobin (HHb) varies with respect to tissue oxygenation and metabolism [11]. Therefore, studying the changes in the concentration of these chromophores can help provide tissue information.

When light in the near-infrared region is incident on the tissue, it follows various paths. It may get transmitted, reflected, absorbed, or scattered by some chromophores as seen in
Figure 2.1. A small fraction of the photons gets sufficiently scattered within the tissue that they exit the surface as reflected light. The scattering and absorption depend on the wavelength and tissue optical properties [13].

![Diagram showing photon scattering and absorption](image)

Figure 2.1. The path followed by the photons when light is incident on the tissue. They can either 1) remain un-scattered, 2) undergo multiple scattering, get forward scattered and re-emit in the transmission mode, 3) get back-scattered and re-emit in the reflection mode.

### 2.2 Wavelength Selection

In the NIR range, larger amount of light can travel deeper into the tissue and hence high penetration depths of about 0.5 cm - 3 cm can be achieved in biological tissues. Light of wavelengths starting at 900 nm have high absorption due to lipid, water and for those below 700 nm, the scattering is dominant [13]. Therefore, 700-900 nm is considered the optimal wavelength range for optical imaging and is known as the therapeutic window, as shown in Figure. 2.2.
Information related to blood oxygenation can be obtained by NIR light propagation in tissue, where the HHb has higher absorption at wavelengths around 750nm and HbO₂ around 830nm. As shown in the Figure 2.2, at 808nm, the absorption due to both oxy- and deoxy- hemoglobin is the same, called the isobestic point [15]. In order to obtain information regarding the two chromophores, minimum of two wavelengths have to be used. Using more than two wavelengths can provide more accurate measurements. Conventionally, two wavelengths, one on either side of the isobestic point is chosen for NIRS to have better sensitivities on both Hb and HbO₂.

Wavelength selection is an important aspect and it should be such that there is minimum crosstalk and high contrast between [HHb] and [HbO₂]. The observation of Strangmann et al. was that there exists high cross talk and low seperability when both the wavelengths are above 780nm. In order to avoid this, they suggest using a wavelength below 720nm and one above 730nm. The wavelength combination suggested for better
focal changes during oxygenation measurements were 760nm or 690nm along with 830nm [16,17].

2.3 Human Brain

The brain and the spinal cord combined form the central nervous system (CNS). It has four major regions namely the cerebral hemispheres, the diencephalons, the brain stem and the cerebellum. The cerebral cortex, an approximately 5 mm thick outer layer of gray matter covers the cerebral hemispheres, the surface of which consists of ridges called sulci and deeper ridges called fissures. These folds increase the surface area of the cortex. The fissures separate the hemispheres into lobes [11]. The surface of the cortex is covered by pia matter, which in turn is enclosed by arachnoid matter and dura matter. The space between the pia and arachnoid matter is called the subarachnoid space, which contains the blood vessels serving the brain and is filled with cerebrospinal fluid (CSF) [18].

The brain is protected by several layers, which are, the scalp, skull, various membranes and CSF [18]. The brain merely amounts to 2% of the total body weight but it receives 15% of the heart’s output. As it is the major organ in the body, oxygen and glucose intake are also higher than the other organs [19].

When NIR light enters the brain, due to the scattering and absorption caused by the chromophores in the tissue, only a small fraction of the photons reaches the detector. The light that reaches the detector typically follows a path that is banana-shaped and in this region the concentration changes of hemoglobin are measured (Figure 2.3) [15]. The photons have to cross the scalp twice, as shown in the Figure 2.3, before being detected by the detector – once at the output of the light source and the second time before entering the photodetector [2].
Figure 2.3: The path of light in the tissue which is a characteristic “banana shape”. Image from NIRx NIRScout’s Manual [20]

Hemodynamic measurements typically range from 0.1Hz to 10Hz. However, the hemodynamic signals of interest are in the same frequency spectrum as other biogenic signals as shown in Table 2.1[21].

Table 2.1. The frequency ranges of various physiological parameters that interfere in NIRS. The fast signals measurements, which detect small changes in membrane and cytosolic index caused by neuronal activity, and the hemodynamic response are well above the frequency range of other signals. However, the “fast” signal is so weak as to be virtually undetectable [21].

<table>
<thead>
<tr>
<th>Measured Parameter</th>
<th>Response Timescale</th>
<th>Frequency Band</th>
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<tbody>
<tr>
<td>Heart rate</td>
<td>~ 1 sec period</td>
<td>1-3 Hz</td>
</tr>
<tr>
<td>Respiration</td>
<td>~ 5 sec period</td>
<td>0.2-0.3 Hz</td>
</tr>
<tr>
<td>Mayer waves</td>
<td>~ 10 sec period</td>
<td>0.08-0.12 Hz</td>
</tr>
<tr>
<td>“Fast” signal (intrinsic scattering)</td>
<td>~ 0.050 sec period</td>
<td>20+ Hz</td>
</tr>
<tr>
<td>Hemodynamic response</td>
<td>~ 0.2-10 sec period</td>
<td>0.1-5 Hz</td>
</tr>
</tbody>
</table>
Heartbeat or breathing can affect the global concentration of hemoglobin and cause interference in the NIRS measurements. Heartbeat, changes in blood pressure, partial pressure of $CO_2$ and respiration are some physiological interferences that affect NIRS measurements. Among these, heartbeat has the highest frequency ($\sim$1.2Hz). By having a NIRS system of sampling frequency higher than 2.4 Hz, this interference can easily be eliminated. Current NIRS systems have sampling frequencies of 2-250 Hz [22].

### 2.4 Blood circulation in human body

The cardiovascular system is comprised of two major parts: the systemic circuit and the pulmonary circuit, which consists of arteries and veins [19]. Arteries are responsible for the transport of oxygenated blood, which then travels to arterioles and to capillaries to provide oxygen and nutrients to all the organs and tissues of the body. The deoxygenated blood, on the other hand, travels through the veins and venules and returns to the right atrium of the heart [19]. In the lung capillaries, the blood flowing gets oxygen in exchange of carbon dioxide from the alveolar air. The amount of oxygen absorbed across the lungs depends on the amount of oxygen consumed by the surrounding tissues during metabolism [19].

### 2.5 Blood circulation in the Brain

Arterial blood to the brain is supplied by two carotid and two vertebral arteries of which carotid arteries are more important since they provide 80% of the total perfusion [19]. Each common carotid artery divides into an external carotid artery, which supplies
blood to the neck, esophagus, pharynx, larynx, lower jaw and face; and an internal carotid artery that delivers blood to the brain [19]. The deoxygenated blood is returned from the brain through superficial cerebral veins, deep cerebral veins and cerebral venous sinuses that combine and drain into the internal jugular vein [18].

2.6 Brain neural activity

Neurovascular coupling is the connection between neuronal activity and changes in the vascular system [23]. An external stimulus is followed by neuronal activity in the related area of the brain (e.g. finger tapping stimulus activated the motor cortex of the brain) and that causes changes in local cerebral blood flow and blood volume [23]. This leads to changes in the concentration of blood and also its oxygenation level in cells and molecules in the capillaries around the neurons and this is detected by the fNIRS [23] as shown in Figure 2.4. From these hemodynamic measurements, the level of neuronal activity can be inferred.

Figure 2.4. An external stimulus causes neuronal activity which leads to changes in the hemodynamics. Image from O. J. Arthurs and S. Boniface [23]
2.7 Effect of intravascular events on Blood Oxygenation, Blood Flow and Blood Volume

Local brain activity causes the local arterioles to dilate, also called vasodilation. When the local arterioles dilate, the cerebral blood volume (CBV) and the cerebral blood flow (CBF) increase [24]. The increase in the CBF and oxygen delivery is more than the local oxygen consumption. Hence, the local cerebral blood oxygenation increases. Therefore, changes in [Hb] can be explained as those related to the oxygen supply and oxygen demand, whereas changes in [HbO$_2$] are related to alterations in CBF [24]. At the onset of neuronal activity, the concentration of oxygenated hemoglobin may decrease but as the activation continues it will increase and overshoot due to rise in the blood flow [24].

2.8 Hemoglobin

Hemoglobin, both oxy and de-oxy, is a major chromophore responsible for the changes in the attenuation of NIR light. Hemoglobin is a protein that contains iron and it has a molecular weight of 64450 g/mol. It consists of 4 subunits made up of 4 possible protein chains, α, β, δ, and γ [25]. Studies show that 97% of hemoglobin in adults is in the HbA form (αβ chains), ~2.5% in the HbA2 form (αδ chains) and less than 1% in the fetal HbF form (αγ chains) [25,26].

The role of hemoglobin in the oxygen transport chain is to exchange oxygen between the lungs and the tissue and transport carbon dioxide back to the lungs. All four of hemoglobin’s subunits, in its active ferrous form, can combine with an oxygen molecule and become oxygenated [25]. Thus oxygenated hemoglobin is formed when 1 mole of Hb
combines with 4 moles of oxygen. This gives rise to the definition of hemoglobin saturation, percentage of the total oxygenated hemoglobin, as \[25\]

\[
StO_2 = \frac{[HbO_2]}{[HbO_2] + [HHb]} \times 100, \text{ [units: %]} \tag{2.1}
\]

Arterial blood is about 98% saturated with a bright red color and venous blood about 70% saturated with a purple color [15].

### 2.9 Optical properties of tissue

#### 2.9.1 Light absorption

The quantitative relation between the absorption of light in a non-scattering, absorbing medium and the thickness of the medium was first determined by Bourger in 1729 and then by Lambert in 1760. According to them, when intensity of light \(I\) is incident on a medium having successive layers of thickness \(\delta d\), a fraction \(dI\) of the incident intensity \(I\) is absorbed [11]. This is shown in Figure 2.5.

![Figure 2.5](image)

Figure 2.5. The effect of absorption of the medium on the incident light of intensity \(I_o\). The light travels the distance between the source and detector, \(d\), and is emitted with a lower intensity \(I\).
The expression known as Lambert-Bougner was given as

\[ \frac{dl}{l} = -\mu_a \delta d \] (2.2)

Integration gives,

\[ I = I_o \exp(-\mu_s d) \] (2.3)

Where \( \mu_a \) is a constant called absorption coefficient expressed in cm\(^{-1}\). The absorption coefficient is defined as the photon’s absorption probability per unit length of its travel. The inverse of \( \mu_a \), \( 1/\mu_a \), is called the absorption path length.

### 2.9.2 Absorbing compounds in the brain

Biological tissue can be considered to be a homogeneous combination of compounds or chromophores, whose overall light absorption in the tissue depends on the type and the amount of chromophore present, at a given wavelength. Each chromophore has its own characteristic absorption spectrum. In NIRS, the chromophores of interest are those whose absorption changes with oxygenation. However, the chromophores whose concentration does not vary with oxygenation also contribute to the attenuation of light. Therefore, it is important to know the spectrum of both types of chromophores (figure 2.6)
2.9.2.1 Water

Since major part of the human body comprises of water, it is a dominant chromophore and knowing its absorption spectra is important. The amount of water in humans varies with the tissue type, gender and age. In a neonate’s brain, water is about 90% by mass which is more than that in an adult skeletal muscle, which is around 74% [27,28]. If the attenuation due to water is more than 1 OD/cm, it would be difficult to get any useful information about the tissue. Therefore, the wavelength cut off for spectroscopy measurements is at 1.35 µm [29]. The extinction coefficient of water is less than 0.001/cm being almost negligible for wavelengths between the ultraviolet region up to 600nm [29]. Its absorption is gradually increases between the wavelengths 600nm to 1.35 µm. due to
high losses caused by the water absorption above 900 nm, depth of measurement of the tissue would get limited.

2.9.2.2 Lipids

Present in the subcutaneous tissue, lipids constitute about 2.6% in newborns, 6.1% in infants (18 months) and about 11.6% in children between 3-18 years and adults [26]. The absorption spectrum of lipid and water is nearly the same as seen in Figure 2.6. However, the water content is much higher than that of lipids in human brain, which account to approximately one tenth proportion compared to water. Hence absorption due to the latter is considered negligible [28].

2.9.2.3 Melanin

When light is incident on any tissue it has to pass through the skin first. The first layer of the skin, the stratum corneum, is 10 µm thick and has negligible impact on tissue absorption in the visible and NIR region. The next layer, the epidermis, is about 100 µm thick and contains the melanin [30]. The pigment melanin is found in the skin and hair and has high absorption of light in visible and NIR region. Both hair and skin color are due to melanin. These colors depend on the relative concentration of the two forms of melanin: eumelanin (dark brown) and pheomelanin (red) [15]. The effect of melanin, optically, on skin reflectance is significant. The skin reflectance of people with darker skin is approximately half of that of people with lighter skin in the 600-1000 nm wavelength range [30]. Due to the attenuation of light by melanin, the sensitivity of the instrument has to be
increased. The attenuation of light by melanin is higher in people with darker skin, which reduces the SNR of the measurements. Thus the intensity of the light source has to be increased for people with darker skin to have the same SNR as people with lighter skin [1].

The concentrations of the above mentioned compounds, water, lipid and melanin, do not change considerably and hence they are considered to be static optical absorbers [15].

2.9.2.4 Hemoglobin

Hemoglobin is present in red blood cells, or erythrocytes, and account for 40-50% of the total blood volume. The remaining is plasma [31]. Oxygenated and deoxygenated hemoglobin are the two main chromophores in NIRS. They provide functional contrast in the brain with respect to changes in the oxygen levels, referred to as BOLD (blood oxygen level dependent) effect [32]. Figure 2.6 shows the absorption spectra of HbO₂ and HHb in the NIR region. The two spectra differ significantly. Looking at the absorption spectra of hemoglobin we can determine the shortest usable wavelength in order to transmit light through the tissue. Considering an OD of 1/cm of tissue, the shortest wavelength beyond which light cannot penetrate a tissue few cm thick can be estimated as approximately 600nm [33].

Besides oxygenated and deoxygenated hemoglobin there are other forms of hemoglobin in the blood such as carboxyhemoglobin (HbCO), hemiglobin (Hi) (also known as methemoglobin) and sulfhemoglobin (SHb), but their concentrations are low [25,26]. The specific extinction coefficient of HbCO is low making it effect negligible in NIRS [26].
2.9.3 Light scattering

Refractive index mismatches at a cellular level are the reason for light scattering in tissue. Major scattering is caused due to refractive index variations between the cell membranes and internal cell organelles as they account for a large proportion of the solid content of tissue. On the other hand, red blood cells are ~2% of the solid content and their contribution is thought to be low [26]. Scattering is the dominant mechanism in light propagation through the tissue. Photons that enter the tissue are most likely to get scattered several times before reaching the boundary, even for thin tissues of submillimeter thickness [11].

![Figure 2.7: Path followed by light when it is incident with an intensity $I_o$, in a medium having both absorption and scattering properties. The photons undergo multiple scattering, increasing their pathlength (>d), and are emitted with a decreased intensity I.](image)

Scattering causes photons to deviate from their path, increasing their pathlength and also increasing their probability of being absorbed in the tissue (Figure 2.7). The factors that determine the scattering characteristics of brain tissue are: 1) gestational age 2) tissue oxygenation and 3) wavelength of light source [26]. Scattering in the brain tissue is highly
affected with age. From birth to adulthood, the total lipid and protein content increases two folds while in the white matter, lipid concentration increases 7 fold. This increases the scattering significantly. In newborns, the protein content is more than the lipid content but as myelination begins, the lipid has a higher growth rate than protein [34].

For a medium having single scattering component, the probability of a photon being scattered per unit length of travel is given by the scattering coefficient, \( \mu_s \). The intensity of light, \( I \), obtained at the output of a non-absorbing sample of thickness \( d \) is given as

\[
I = I_o \exp(-\mu_s * d)
\]  

(2.4)

The reciprocal of \( \mu_s \), \( 1/\mu_s \), is the scattering pathlength which is defined as the average distance a photon travels between consecutive scattering events [11]. In a simplistic model, the overall path of a photon can be approximated as the linear combination of scattering and absorption. In an ideal case scenario of a single scattering event the transport coefficient, \( \mu_t \), is given as [35]

\[
\mu_t = \mu_a + \mu_s
\]  

(2.5)

In reality for many systems like the biological tissue, the photon undergoes multiple scattering and hence the assumption of a single scattering event becomes invalid.

![Figure 2.8: Phase diagram, showing the angular probability \( \alpha \) of a photon undergoing multiple scattering. Image taken from [11]](image)
If $\hat{s}$ is the unit vector representing the incident photon that is scattered. The angular probability of it being scattered in the direction of the vector $\hat{s}'$ is given by the normalized phase function $f(\hat{s}, \hat{s}')$, as shown in figure 2.8. For random, soft tissues it is assumed that the probability distribution function is a function of angle between the incident and scattered photon. Therefore, the phase function, $f$, can be expressed as a function of the cosine of scattering angle $\hat{s} \cdot \hat{s}' = \cos \theta$. The phase function is represented as the mean cosine of the scattering angles and is called the anisotropy factor, $g$ [11]. Scattering is thus characterized by a factor called the reduced scattering coefficient, $\mu'_s$. The reduced scattering coefficient is the reciprocal of one photon random walk step, or the distance the photon travels before it is completely randomized [35]. It is the scattering coefficient reduced by a factor of (1-g):

$$\mu'_s = \mu_s (1 - g) \quad (2.6)$$

For an ideal tissue, $g=1$, where all radiation travels forward in the incident direction and for $g=0$ the scattering is completely isotropic. In human tissue, $g$ lies in the range 0.69-0.99 [36]. Hence the transport attenuation coefficient becomes:

$$\mu'_t = \mu'_s + \mu_a \quad (2.7)$$

### 2.9.3.1 Types of scattering

There are three types of scattering in biological tissue, i.e., Mie, Rayleigh and Debye scatterings [37,38].
Rayleigh scattering occurs when photons of wavelength $\lambda$ interact with particles having radii significantly smaller than the wavelength, i.e, $r \ll \lambda$. It is inversely (fourth power) related to wavelength. Hence it is more dominant at lower wavelengths [15].

Debye scattering occurs when photons interact with particles of radii smaller than their wavelength, i.e, $r < \lambda$ but bigger than the particles producing Rayleigh scattering [38].

Gustav Mie in 1908 developed the Mei theory. It is valid for small sized spherical particles (less than $\frac{1}{4}$ wavelength) [26]. Mie scattering is a result of the interaction of particles with radii larger than the wavelength of the photon, i.e, $r > \lambda$ [37,38]. As the wavelength increases, scattering coefficients decrease and anisotropy factor, g, increase as a result of decrease in Rayleigh scattering and increase in Mie scattering [15]. Mie scattering dominates in NIRS as cells and other particles of tissues have larger radii than the NIR light [37].

2.10 Penetration depth

In NIRS, the light source and detector are placed on the surface of the head and have limited penetration depth, receiving information only from the superficial structures of the brain. The penetration depth can be increased to some extent by increasing the source-detector separation (SDS) and thus increasing the information content obtained. However, as the SDS increases, the intensity of the detected light and also the spatial resolution decreases, creating a trade-off between SDS and penetration depth. This is shown in figure 2.9. Patil et al conducted phantom experiments with source detector separations of 2, 3, 4 cm and found the penetration depth to be 1, 1.3, 1.5 cm respectively [39]. Gervain et al
reports a penetration depth of 10-15 mm into the cortex of newborns and 3-5 mm into the cortex of adults at a SDS of 3 cm [40].

Figure 2.9: Penetration depth varies with the distance between the source and detector. Larger the source detector separation, more the penetration depth of the light inside the tissue. [40]

Light emitted on to the head, from the light source, has to pass through the scalp, skull, dura and CSF before it could reach the brain and the scalp itself is composed of skin, fat and muscle. The scalp is 5-7 mm thick and the skull is 7-8 mm thick in human adults [15]. That’s a combined thickness of 12-15 mm. Hence, using surface measurements to study the brain function can be challenging. In the case of a neonate brain the combined thickness of scalp and skull is around 5 mm, making it easier for measurements. Abdo et al. [37] studied the penetration depth of NIR light into the rat peripheral nerve and brain cortex using an 830 nm laser source and a GaAs PIN photodiode. They found that the penetration depth was 0.35 ± 0.023 mm in the rat sciatic nerve, 0.35 ± 0.026 mm in the white matter and 0.41 ± 0.029 mm in the gray matter. These were based on their definition of penetration depth as the distance at which the total optical power reduces to 37% of the incident light.
According to a study on human gray matter, it was reported that the NIR light has a penetration depth of around 1.6 mm at 850nm [38], which is much larger than that in the rat. This may imply that the peripheral and the brain cortex in the rat attenuate the NIR light more than the human.

The type of tissue and wavelength along with absorption and scattering affect the penetration depth. This is seen in a developing human brain, where the penetration reduces with maturity (due to increase in scattering coefficient) and decreasing wavelength (due to increased absorption coefficient) [41]. Myelination also increase scattering reducing the penetration depth explaining why an infant’s brain is more transparent than that of an adult [26]. Penetration depth is also less for darker skin (highly pigmented) individuals due to higher absorption.

2.11 Governing law of NIRS

2.11.1 Beer-Lambert Law

Studies show the process of decrease in the intensity of light in two stages. Within the first 8 mm, the rate of decrease is very high as the collimated input beam gets scattered into a diffused beam. Following this, the intensity drops less rapidly and the rate of decrease depends on the chromophores in the tissue. After the first few millimeters, the intensity of light drops linearly, thus the penetration depth can be calculated as the length over which the space irradiance drops by 1/e on a log_e scale [26,41].
According to the Beer-Lambert law, in trans-illumination mode, there exists a linear relationship between the changes in oxyhemoglobin and deoxy-hemoglobin concentration and the logarithm of the detected light [15]. This law gets its name after the German physicist/mathematician, August Beer, and Swiss mathematician, Johann Lambert. It was introduced by them in their book Einleitung in die höhere Optik (Introduction to the Higher Optical) in 1854 even though the law was first discovered 100 years earlier by Bougner [15].

According to the law, if \( I \) is the intensity of detected light and \( I_o \) is the intensity of the incident light, then optical density (OD), which is proportional to the concentrations of the light absorbing chromophores of the tissue is given as:

\[
OD = - \log \frac{I}{I_o},
\]  

(2.8)

OD is related to the concentration \([C]\) as:

\[
OD = \varepsilon \ast [C] \ast d,
\]  

(2.9)

Where \( \varepsilon \) is a physical constant that is related to the light absorption capability of the compound known as the molar extinction coefficient of the solution. It is dependent on wavelength of light, chemical state of the compound, physical parameters like temperature, pH, or osmolality [15]. When light passes through a solution its intensity decays exponentially which is related to the absorption coefficient (\( \mu_a \)). This can be seen in the Figure 2.10. Typical absorption coefficient for the adult brain at 800 nm is 0.4 \( cm^{-1} \) [15].
Therefore, considering the medium to be non-scattering, the intensity changes according to the law are given as:

\[ I = I_0 e^{-\mu_a d}, \quad (2.10) \]

\( \mu_a \) is related to the concentration as:

\[ \mu_a = \ln(10) \varepsilon_\lambda [C], \quad (2.11) \]

\[ I = I_0 10^{-\varepsilon_\lambda [C] d}, \quad (2.12) \]

where \( \varepsilon_\lambda \) is the molar extinction coefficient (\( L \text{ cm}^{-1} \text{ mol}^{-1} \)) of the sample at wavelength \( \lambda \), \([C] \) (\( \text{mol}^{-1} \text{L}^{-1} \)) is the solution concentration and \( d \) is the source detector separation [15].

Thus, the Beer-Lambert law is rewritten as

\[ OD = -\log \frac{I}{I_0} = \varepsilon \times [C] \times d, \quad (2.13) \]

As the tissues have more than one chromophores, for multiple chromophores OD or absorption (A) can be written as:

\[ A = OD_\lambda = \sum_i \varepsilon_{i,\lambda} [C]_i d, \quad (2.14) \]
However, the description of light propagation in tissue is more complex. This limits the use of the Beer-Lambert law in real applications as [15]: 1) It considers the tissue to be a homogeneous medium which is not true for biological tissues; 2) Atomic effects such as multi-photon absorption, optical saturation, or stimulated emission have not been considered; 3) This law assumes the incident light to be a set of parallel rays, not considering the scattering phenomena that occurs in tissues. For these reasons the modified Beer-Lambert law was introduced.

2.11.2 Modified Beer-Lambert Law (MBLL)

The attenuation of NIR light in a tissue is due to both absorption and scattering. In fact, scattering is more dominant than absorption. Scattering causes approximately 80% of the total attenuation whereas absorption causes the remaining 20% [11]. When light travels in a turbid medium like a biological tissue, photons undergo multiple scattering in random directions increasing their total pathlength as can be seen in figure 2.11.

![Figure 2.11: Presence of scattering in the medium causes the photons to undergo multiple scattering, decreasing the intensity of light exponentially and adding to the attenuation due to absorption. Image modified from [15]](image-url)
This increase in the pathlength due to scattering has to be incorporated in the attenuation equation. This gives rise to the MBLL. The attenuation of light in inhomogeneous layers of tissue can be approximated by MBLL to include an additional pathlength factor DPF, a dimensionless quantity known as differential pathlength factor (DPF), due to the effect of multiple scattering as [42]:

\[ A = OD = - \log_{10} l = \varepsilon * [C] * d * DPF + G, \quad (2.15) \]

Where G is the geometry and scattering dependent term that includes the losses due to scattering and other boundary losses. The d is the source detector separation and DPF is the differential pathlength factor. The product (d*DPF) is the “differential pathlength” or the “effective optical pathlength”, \( L_\lambda \) in cm. G is a highly measurement geometry dependent factor, making it difficult to obtain absolute value of attenuation. Also, the values of DPF, G and the number of chromophores is uncertain. Hence, changes in absorption or OD are used in continuous diffuse optical spectroscopy (DOS) as [15]:

\[ \Delta A = \Delta OD_\lambda = \sum_i \varepsilon_{i,\lambda} \Delta [C]_i d \ DPF_\lambda, \quad (2.16) \]

By considering changes in OD, due to subtraction of equations, the background effect and geometry factor gets eliminated. OD, DPF and extinction coefficient are wavelength dependent and thus their values must be known at different wavelengths [15]. In classical spectroscopy, HbO2 and HHb are the two main chromophores considered, therefore measurements for at least two wavelengths are required. If the number of wavelengths exceeds the number of chromophores of interest, the accuracy can be improved by using multi-linear regression to fit each chromophore spectrum [26].

For HbO2 and HHb, the equation at a particular wavelength becomes:
\[ \Delta OD_\lambda = (\varepsilon_{\lambda,HbO_2} \Delta [HbO_2] + \varepsilon_{\lambda,HHb} \Delta [HHb]) dDPF_\lambda, \]  

(2.17)

The DPF is defined as [43]:

\[ DPF_\lambda = \frac{1}{2} \left( \frac{3 \mu_s' \lambda}{\mu_a \lambda} \right)^{1/2} \left[ 1 - \frac{1}{1 + d(3 \mu_s' \lambda \mu_a \lambda)^{1/2}} \right], \]  

(2.18)

For a particular wavelength, \( \Delta OD \) is given as:

\[ \Delta OD_\lambda = \Delta OD_{2,\lambda} - \Delta OD_{1,\lambda}, \]  

(2.19)

\[ \Delta OD_\lambda = \log \frac{l_2}{l_1} - \log \frac{l_0}{l_1}, \]  

(2.20)

\[ \Delta OD_\lambda = \log \frac{l_0}{l_2} \cdot \frac{l_1}{l_0}, \]  

(2.21)

\[ \Delta OD_\lambda = \log \frac{l_1}{l_2}. \]  

(2.22)

Where \( l_1 \) and \( l_2 \) are detected light intensities at times \( t_1 \) and \( t_2 \) [15]. By calculating the changes in OD at two different wavelengths and solving the system of linear equations defined by Equation 2.17, we can calculate changes in the concentration of oxy-hemoglobin and deoxy-hemoglobin.

\[ \Delta OD_{\lambda_1} = (\varepsilon_{\lambda_1,HbO_2} \Delta [HbO_2] + \varepsilon_{\lambda_1,HHb} \Delta [HHb]) dDPF_{\lambda_1}, \]  

(2.23)

\[ \Delta OD_{\lambda_2} = (\varepsilon_{\lambda_2,HbO_2} \Delta [HbO_2] + \varepsilon_{\lambda_2,HHb} \Delta [HHb]) dDPF_{\lambda_2}, \]  

(2.24)

\[ \Delta [HbO_2] = \frac{\Delta OD_{\lambda_2} \frac{\Delta OD_{\lambda_1}}{DPF_{\lambda_2}} - \Delta OD_{\lambda_1} \frac{\Delta OD_{\lambda_2}}{DPF_{\lambda_1}}}{(\varepsilon_{\lambda_1,HHb} \varepsilon_{\lambda_2,HbO_2} - \varepsilon_{\lambda_2,HHb} \varepsilon_{\lambda_1,HbO_2}) \cdot d} \]  

(2.25)

\[ \Delta [HHb] = \frac{\varepsilon_{\lambda_2,HbO_2} \frac{\Delta OD_{\lambda_1}}{DPF_{\lambda_1}} - \varepsilon_{\lambda_1,HbO_2} \frac{\Delta OD_{\lambda_2}}{DPF_{\lambda_2}}}{(\varepsilon_{\lambda_1,HHb} \varepsilon_{\lambda_2,HbO_2} - \varepsilon_{\lambda_2,HHb} \varepsilon_{\lambda_1,HbO_2}) \cdot d} \]  

(2.26)
Studies like Kim et al [44], Y. Fatmehsari [22], used these equations. Studies such as M. Papademetriou [11], Bozkurt et al [1] simplified the equations and solved them as matrices:

\[
\begin{bmatrix}
\Delta [\text{HbO}_2] \\
\Delta [\text{HHb}]
\end{bmatrix} = \bar{M}^{-1} \begin{bmatrix}
\Delta O\bar{D}_{\lambda 1} \\
\Delta O\bar{D}_{\lambda 2}
\end{bmatrix}
\] (2.27)

Where \( \bar{M} \) is a constant matrix defined as:

\[
\bar{M} = d. (\bar{\varepsilon} \ast \bar{DPF})^T
\] (2.28)

\( \bar{\varepsilon} \) and \( \bar{DPF} \) are known from literature and are defined as

\[
\bar{\varepsilon} = \begin{bmatrix}
\varepsilon_{\lambda 1, \text{HbO}_2} & \varepsilon_{\lambda 2, \text{HbO}_2} \\
\varepsilon_{\lambda 1, \text{HHb}} & \varepsilon_{\lambda 2, \text{HHb}}
\end{bmatrix}
\quad \text{and} \quad
\bar{DPF} = \begin{bmatrix}
DPF_{\lambda 1} & 0 \\
0 & DPF_{\lambda 2}
\end{bmatrix}
\] (2.29)

Considering other elements to be constant in the blood, the total hemoglobin concentration change (\( \Delta [\text{HbT}] \)) is the total blood volume change (\( \Delta BV \)):

\[
\Delta BV = \Delta [\text{HbT}] = \Delta [\text{HbO}_2] + \Delta [\text{HHb}]
\] (2.30)

Oxygen saturation is given as:

\[
StO_2(\%) = \frac{[\text{HbO}_2]}{[\text{HbO}_2] + [\text{HHb}]} \ast 100,
\] (2.31)

2.12 Types of NIRS

There are three types of NIRS instruments: continuous wave (CW), frequency modulated (FM) and time-resolved (TR) spectroscopy as shown if Figure 2.12, each having its own advantages and disadvantages [45,46].
Figure 2.12: Types of NIRS techniques. (a) Continuous wave (CW) where the light undergoes attenuation in amplitude, (b) TD, where a Pico second pulse undergoes both attenuation in amplitude and gives the temporal spread function (TPSF), (c) FD, where a sinusoidally modulated light undergoes both change in amplitude and phase. Image taken from [47]

### 2.12.1 Continuous wave (CW) systems

The CW NIRS systems (fig. 2.12(a)) are the most commonly developed technique. They are the simplest and the most inexpensive technique. In these systems, a continuous beam of light source illuminates the tissue, which is then detected by the detector after passing through the tissue either in transmission or back-reflectance geometry.
Measurements of a CW system are not sufficient to separate the effects of absorption and scattering. These systems use the MBLL to obtain the relative changes in hemoglobin concentration. The absolute value of the chromophores concentration cannot be obtained by this technique. CW systems assume that the intensity of light changes as a result of absorption and that the scattering remains constant. CW systems work similar to the oximeter systems of the 1930-1970s [15]. Also, the actual photon pathlength cannot be measured using these systems and have to be estimated from earlier studies and simulations. These also have lower penetration depths but have faster acquisition rates when compared to the FM and TR methods.

2.12.2 Time Resolved (TR) or Time Domain (TD) Systems

Time Resolved or otherwise known as Time Domain systems use laser sources that emit pulses of light of the order of picoseconds. The emerging light is detected as a function of time by a fast photon counting detector. These systems measure the time-of-flight (TOF) of photons through the tissue, giving a temporal distribution known as the Temporal Point Spread Function (TPSF) [11] and use it to measure the optical absorption, scattering and also the DPF. In 1988 D.T. Deply et al. proposed the use of time of flight of picosecond length light pulses to estimate DPF values [42]. The photon pathlength can be calculated using the mean transmit time of the scattered photons and the velocity of light (d=v.t). Absolute absorption, scattering coefficients and hence absolute hemoglobin concentrations, can be calculated from the TPSF using the light transport model in tissue [48]. TD systems are expensive and more complicated as they use expensive electronics,
specialized lasers, and fast photodetectors. Inspite of having highly sensitive detectors, these systems are slower than other techniques as they have to detect the entire TPSF.

2.12.4 Frequency modulated (FM) or Frequency Domain (FD) systems

FM NIRS systems lie in between CW and TR systems in terms of cost, complexity, SNR and resolution. In FM systems the light intensity is modulated in the RF range of 100-200MHz. The detected light will have a lower amplitude and will be phase shifted by an amount proportional to the propagation distance [15]. This detected signal is compared to the reference signal and the phase shift and the amplitude decay of the modulated wave is recorded which is used to separate the absorption and scattering parameters. These systems have faster acquisition times than TD but are limited by the finite number of modulated frequencies that can be used.

Table 2.2. Differences in the characteristics of the three types of NIRS systems [49,50]

<table>
<thead>
<tr>
<th>Properties</th>
<th>CW</th>
<th>FM</th>
<th>TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling rate (Hz)</td>
<td>Fastest (≤100)</td>
<td>Moderate (≤50)</td>
<td>Least (≤10)</td>
</tr>
<tr>
<td>SNR</td>
<td>Most</td>
<td>Moderate</td>
<td>Least</td>
</tr>
<tr>
<td>Spatial resolution (cm)</td>
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<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>Depth resolution with SDS=4 cm</td>
<td>Least</td>
<td>Moderate</td>
<td>Most</td>
</tr>
<tr>
<td>Cost</td>
<td>Wide range from low to high</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Physical Size</td>
<td>Varies from large to small wireless versions</td>
<td>Portable, Bulky</td>
<td>Large</td>
</tr>
<tr>
<td>Commercial Availability</td>
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<td>Oximeter: yes; Imaging: yes</td>
<td>Oximeter: yes; Imaging: no</td>
</tr>
<tr>
<td>Instrument stabilization</td>
<td>Not required</td>
<td>Not required</td>
<td>Required</td>
</tr>
<tr>
<td>Parameters Measured</td>
<td>Decimation between Scalp, Skull, CSF</td>
<td>[HbO₂], [HHb],[HbT]</td>
<td>Scattering and absorption coefficient and pathlength</td>
</tr>
<tr>
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<td>--------------------------------------</td>
<td>---------------------</td>
<td>---------------------------------------------------</td>
</tr>
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<td></td>
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<td>Yes, Changes</td>
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<td>Feasible</td>
<td>Yes, absolute value</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Feasible</td>
<td>Yes, absolute value</td>
<td>Yes</td>
</tr>
</tbody>
</table>
3.1 History of NIRS

Over the last two decades, the knowledge and understanding of optical processes has increased. There has been improvement and innovation of new optical technologies resulting in a rapid growth in the development and use of optical methods and devices for medical and biological applications.

The inter-relation between light and medicine goes way back to 460-370 BC when Hippocrates, a Greek physician who is considered as the father of medicine, had established “sun clinics” as it was thought that the penetration of sunlight into the body had beneficial effects [14]. During 1824-1887, Gustav Kirchhoff along with Bunsen found the science of chemical analysis by spectra. They established the basis of colorimetry and spectrophotometry with their observation that the amount of light absorbed by certain chemicals was proportional to their concentrations [14]. In 1873, Tyndall made significant contributions regarding dual-wavelength spectroscopy [51]. In 1876, Von Vierodt observed the changes in light penetration in tissue on occluding the blood circulation [10]. In the early nineteenth century, diagnostic capabilities of optical methods started being
realized as a result of increasing interest in the relative advantages of artificial light as compared to sunlight. In the first few decades of the twentieth century, visible light based instruments began to be used. However, Butler & Norris eventually brought attention to the NIR region of the spectrum by examining plant tissues and human hand. Hemoglobin was one of the first compounds that was studied by optical spectroscopy with its NIR absorption bands reported by Hartridge and Hill in 1914 [19]. In 1929, Cutler described the detection of large tumors by eye, by using light to transilluminate the breast [52].

In 1932 began the spectroscopic measurements of hemoglobin by Nicolai [26] and the first instruments were made by Matthes and Kramer in 1935. Kramer’s instrument was made of only one red wavelength and so could not compensate for changes in hemoglobin concentration whereas, Matthes resolved this by using two wavelengths, red (oxygen dependent) and green (oxygen independent) [26]. Later in 1939 he improved his measurements by modifying his instrument to use an infrared wavelength that was oxygen independent [26]. Around 1940s, Glenn Millikan invented the muscle oximeter and thus began the development of optical methods. He made an ear oximeter for aviation, which was modified by Wood and Geraci in 1949 to measure absolute saturation [26]. However, it wasn’t accurate and stable enough for clinical use and in spite of being taken up by physiologists, it wasn’t used on patients often. Later, Shaw’s contributions resulted in the Hewlett Packard Ear Oximeter in 1970. This measured attenuation at 8 wavelengths and was made such that it provided absolute calibration regardless of skin pigmentation. This instrument is not widely used because of its high cost and bulky sensor but is still considered the “gold standard” oximeter [26].
In 1977, Frans Jöbsis who is considered the creator of NIRS, reported that the NIR region allows for real-time, non-invasive detection of hemoglobin oxygenation using transillumination spectroscopy due to the high degree of transparency of the tissue to NIR light. Following Jöbsís’s observations, the use of NIR to achieve deeper penetration of tissues than that possible by the visible region, has been worked on. In 1980 Marco Ferrari started measuring changes in brain oxygenation, using prototype NIRS instruments, in animal models [53] and human adults [54]. In 1985, Jöbsis with his colleagues studied sick, newborn infants’ cerebral oxygenation using NIRS [55]. In 1984, David Delpy started developing NIRS instruments and in 1986 the first quantitative measurement of oxygenation and hemodynamic parameters i.e. changes in [HbO₂], [HHb], [HbT], CBV and and CBF in sick newborn infants were reported [56]. In 1989 the first commercial system, a single channel continuous wave NIRO-1000, was built by Hamamatsu Photonics K.K, whose basis was a four- wavelength system described by Cope and Delpy in 1988 [57]. Several NIRS systems have been designed henceforth and a large range of applications have been tested over the years [10,14,50]. Table 3.1 gives a timeline of events that have occurred hence forth.

Table 3.1 Major events in the development of NIRS systems chronologically [50].

<table>
<thead>
<tr>
<th>Year</th>
<th>Major events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>Jöbsis demonstrates the possibility to detect changes of adult cortical oxygenation during hyperventilation by near-infrared spectroscopy.</td>
</tr>
<tr>
<td>1985</td>
<td>First NIRS clinical studies on newborns and adult cerebrovascular patients (Brazy; Ferrari)</td>
</tr>
<tr>
<td>1989</td>
<td>First commercial single-channel CW clinical instrument: NIRO-1000 by Hamamatsu Photonics, Japan</td>
</tr>
<tr>
<td>1991/1992</td>
<td>First fNIRS studies carried out independently by Chance, Kato, Hoshi, and Villringer by using single-channel instruments</td>
</tr>
<tr>
<td>1993</td>
<td>Publication of the first 6 fNIRS studies</td>
</tr>
<tr>
<td>Year</td>
<td>Event</td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>1994</td>
<td>Simultaneous monitoring of different cortical areas by 5 single-channel instruments (Hoshi)</td>
</tr>
<tr>
<td></td>
<td>First application of fNIRS on subjects affected by psychiatric disorders by using a single-channel system (Okada)</td>
</tr>
<tr>
<td></td>
<td>Hitachi company (Japan) introduces a 10-channel CW system (Maki)</td>
</tr>
<tr>
<td></td>
<td>First simultaneous recording of positron emission tomography and fNIRS data (Hoshi)</td>
</tr>
<tr>
<td>1995</td>
<td>First evidence of a fast optical signal related to neuronal activity (Gratton)</td>
</tr>
<tr>
<td></td>
<td>First two-dimensional image of the adult occipital cortex activation by a frequency domain spectrometer (Gratton)</td>
</tr>
<tr>
<td>1996</td>
<td>First simultaneous recording of fMRI and fNIRS data (Kleinschmidt)</td>
</tr>
<tr>
<td></td>
<td>First simultaneous recording of fMRI and TRS fNIRS data (Obrig)</td>
</tr>
<tr>
<td>1998</td>
<td>First application of fNIRS on newborns using a commercial single-channel CW system (Meek)</td>
</tr>
<tr>
<td></td>
<td>First images of the premature infant cortex upon motor stimulation by using a CW-fNIRS prototype (Chance)</td>
</tr>
<tr>
<td></td>
<td>First application of the Hitachi 10-channel system in clinics (Watanabe)</td>
</tr>
<tr>
<td>1999</td>
<td>First introduction of a 64-channel TRS system for adult optical tomography (Eda)</td>
</tr>
<tr>
<td></td>
<td>First introduction of a 32-channel TRS system for infant optical tomography (Hebden)</td>
</tr>
<tr>
<td></td>
<td>First optical tomography TRS images of the neonatal head (Benaron)</td>
</tr>
<tr>
<td></td>
<td>Introduction of the first compact 8-channel TRS system (Cubeddu)</td>
</tr>
<tr>
<td></td>
<td>TechEn company (USA) starts to release its first fNIRS commercial system</td>
</tr>
<tr>
<td>2000</td>
<td>Hitachi company starts to release its first commercial system: (ETG-100, 24 channels)</td>
</tr>
<tr>
<td>2001</td>
<td>First fNIRS study using a single-channel CW portable instrument and telemetry (Hoshi)</td>
</tr>
<tr>
<td></td>
<td>Shimadzu company (Japan) starts to release its first commercial system: (OMM-2001, 42 channels)</td>
</tr>
<tr>
<td></td>
<td>ISS Inc.(USA) starts to release the frequency domain system: Imagent (up to 128 channels)</td>
</tr>
<tr>
<td></td>
<td>First three-dimensional CW tomographic imaging of the brain (DYNOT, NIRx Medical Technologies, US) (Bluestone)</td>
</tr>
<tr>
<td>2002</td>
<td>Hitachi company starts to release the ETG-7000 (68 channels)</td>
</tr>
<tr>
<td>2003</td>
<td>Hitachi company starts to release the ETG-4000 (52 channels)</td>
</tr>
<tr>
<td></td>
<td>Artinis company (The Netherlands) starts to release the Oxymon MkIII (up to 96 channels)</td>
</tr>
<tr>
<td>2004</td>
<td>Shimadzu company (Japan) starts to release the NIRStation (64 channels)</td>
</tr>
<tr>
<td></td>
<td>First simultaneous recording of DC-magnetoencephalography and CW fNIRS data (Mackert)</td>
</tr>
<tr>
<td>2005</td>
<td>Hitachi company starts to release the ETG-7100 (72 channels)</td>
</tr>
<tr>
<td>2007</td>
<td>Shimadzu company starts to release the FOIRE-3000 (52 channels)</td>
</tr>
<tr>
<td>2009</td>
<td>fNIR Devices company (USA) starts to release a wearable 16-channel system for adult PFC measurements</td>
</tr>
<tr>
<td></td>
<td>Hitachi company starts to release a battery operated wearable/wireless 22-channel system for adult prefrontal cortex measurements</td>
</tr>
<tr>
<td>2011</td>
<td>NIRx Medical Technologies company (USA) starts to release a battery operated wearable/wireless 256-channel system for adult frontal cortex measurements</td>
</tr>
</tbody>
</table>
3.2 NIRS Instrumentation

3.2.1 Sources

The two most common sources used in spectroscopy are laser diodes (LDs) and light emitting diodes (LEDs).

Laser Diodes

CW laser diodes are the most commonly used sources as they are efficient, linear, powerful, compact, give high flux density and have a narrow spectral bandwidth. Laser diodes have very high temporal response and can be easily modulated. Care must be taken to never overdrive these or it can lead to permanent damage [21]. CW laser diodes come in two basic modes: single mode and multimode. Horizontal confinement is achieved in single mode lasers through similar index-guiding mechanism whereas multimode lasers are gain-guided. Multimode lasers are preferred in Diffuse Optical Spectroscopy (DOS) applications due to their improved temporal and spectral stability [21]. Both types of lasers have emitting areas of only a few µm, hence the beam is spatially coherent and fiber coupling is relatively easy. Issues related to beam quality such as ellipticity, uniformity, astigmatism (results in distorted images) are rare since the core diameters of source fibers are usually large exceeding 200 µm [21]. Its disadvantage is that it takes some time to heat up and achieve stability (even 1 hour for some LDs) [14].

Pulsed solid state lasers can also be used as sources. They have high repetition rates (100MHz) and produce pulses even faster than LDs (100ps-100fs). But they are too bulky and expensive compared to LDs and LEDs. These are used for time resolved spectroscopy [10,58].
**LEDS**

LEDS are efficient, inexpensive, rugged and are available in a wide range of wavelengths. They also have low power consumption, easy circuitry and are usually not harmful to the eyes. However, they have a wide spectral bandwidth (~20nm to 40nm), nearly 10 times larger than LDs, which could complicate measurements and calculations [21]. Fiber coupling LEDs is complicated and inefficient due to their large and diffused emitting area. In the telecommunication industry fiber- coupling techniques are aimed for few mW light at 1330nm or 1550nm and are not commonly available for wavelengths below 800nm. Therefore, the best and simplest way to use LED sources for spectroscopy measurements is to place them directly on the probe assembly without fiber coupling [21]. LEDs have high electrical biasing tolerances as they are more electrically and thermally robust [21].

Table 3.2 Comparison between various parameters of LEDs and LDs [50].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LED</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Inexpensive (&lt;$10)</td>
<td>Expensive (&gt;1000)</td>
</tr>
<tr>
<td>Fiber coupling</td>
<td>Inefficient and not needed</td>
<td>Easy and needed</td>
</tr>
<tr>
<td>Safety</td>
<td>Usually not harmful</td>
<td>Heating issues</td>
</tr>
<tr>
<td>Spectral Bandwidth</td>
<td>Large (~40nm)</td>
<td>Narrow (~5nm)</td>
</tr>
<tr>
<td>Penetration depth</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Circuitry</td>
<td>Simple</td>
<td>Complex</td>
</tr>
<tr>
<td>Size</td>
<td>Small/ Compact</td>
<td>Bulky</td>
</tr>
<tr>
<td>Power</td>
<td>Low (mw)</td>
<td>High (even 10W)</td>
</tr>
</tbody>
</table>
As discussed in chapter 2 section 2.9.2.3, melanin attenuates NIR light causing the signal to be lower in dark skin people. Taking this into consideration, Bozkurt et al included in their system an adjustable LED driver that provides constant current proportional to the pigmentation of the person’s skin [1].

3.2.2 Detectors

The three most common detectors used in spectroscopy measurements are: silicon PIN photodiodes, silicon avalanche photodiodes (APDs) and photomultiplier tubes (PMTs). Spectrometer, CCD and multispectral cameras are also used in certain types of spectroscopic measurements where information regarding the entire spectrum is of interest.

Usually in reflection mode, light from the tissue can be detected by detectors such as photo-multiplier tube (PMT) or avalanche photo-diode (APD), whereas in transmission geometry, light can be detected by a CCD camera system as well as PMT and APD [59].

PIN photodiodes

These are inexpensive (~$5-$10), very linear with a large dynamic range. These lack having an internal gain which makes them less sensitive in spite of having high quantum efficiency specially over 600nm to 900nm spectral band. This makes them more suited for small source detector separations (SDS), where penetration depth and sensitivity are not a problem. CW instruments applied on small animals and neonates have the best use of these photodiodes as the SDS are usually small and signal levels are relatively large in such applications [21]. A very popular silicon photodiode detector used by many groups in a wide range of applications is the Burr-Brown OPT101 Photodiode/Preamplifier Module.
Fong et al [1, 22, 60] used silicon photodiode (SiPD) from Hamamatsu along with a programmable gain amplifier to increase its sensitivity [61].

**Avalanche Photodiodes**

APDs are more sensitive than PIN photodiodes and also a much higher gain-bandwidth product, but are much more expensive (> $500) with lesser dynamic range. Similar to PIN photodiodes, these too have high quantum efficiency over 600nm to 900nm range and due to their higher sensitivity. They can be used in instruments applied on adult humans with larger SDS [21]. A popular example of an APD module is the Hamamatsu C5460 series.

**Photomultiplier Tubes**

PMTs are very sensitive below 800nm but also expensive as the APDs. They can provide much higher gain than the APDs but are very sensitive to supply voltage and stray magnetic fields and can be damaged easily. They also have poor linearity [21]. An example of a compact PMT module is the Hamamatsu H5783-20.

### 3.2.3 Spatial distribution of source-detector

The spatial distribution of source and detectors is important and should be fixed or else it would lead to noisy signals, which could otherwise be interpreted as changes in the signal due to activity. The source separation distance (SDS) and the optodes’ configuration is contentious as there are many possible configurations and varies based on the application and also the subject. For example, the SDS is smaller when the subject is a neonate or an infant and can be larger for adults. Haensee et al used a SDS of 2.5 cm for functional studies.
of the brain in neonates and adults [62]. Yurtsever et al’ design had a SDS of 2.5 cm [60]. Fong et al took multi-distance measurements by having SDS of 0.64-1.39 cm and 1.25-2.0 cm for neonates [61]. Yurtsever et al used 10 photodetectors and four LEDs with a SDS of 2.5 cm [63]. Their optode configurations are as shown in Figure 3.1. Macnab et al performed fetal oxygenation measurements by taking SDS as 3, 3.5, 4 cm [64]. Bozkurt et al [1] performed spectroscopy measurements on newborn’s brain and used 2 cm SDS. Liu et al [65] and Chen et al [66] used 3 cm which would give 1 cm penetration depth. According to Kassab, the conventional SDS is cited to be 3±0.5 cm [67]. The commercial NIRS systems mostly have adjustable SDS ranging from 1.5-4 cm as stated by Scholkmann et al [10].

Figure 3.1: Different optode configurations from literature. (a) Has 2 detectors and 4 LEDs arranged such that the system had 8 channels with varying SDS, (b) has 4 LEDs and 10 detectors with SDS of 2.5 cm, (c) has one LED and 2 detectors with a fixed SDS [1], and (d) has one LED and four detectors for multi-distance measurements [68].
3.2.4 Optode housing

In NIRS systems, the housing of the source and detectors is an important factor and we have to ensure their stability during analysis which depends on the housing method used. As in most cases, the surface to be measured is curved, the use of flexible circuit boards helps in better coupling of the probe to the surface. Figure 3.2 shows three different variations of head caps used in NIRS [69, 70].

![Figure 3.2: Different configurations of Head caps. (a) Stretchable cap design to hold the optodes in place [70] (b) Head band design for the prefrontal cortex assessment [69]. (c) Probe placed inside the baby’s hat for better coupling and comfort [1].](image-url)
Bozkurt et al’s probe design included a flexible circuit board which was covered with a black medically graded foam with the source and detectors (optodes) embedded in it. The foam is to prevent the sharp edges of the optodes from touching the subject and also to block the ambient light from saturating the detector. Stable coupling was provided by placing the foam under a baby hat [1]. This can be seen in Figure 3.2(c). Yurtsever et al used a flexible probe made of two parts: a reusable flexible circuit board that housed the optodes and a disposable cushioning material for better coupling [60]. Incorporating the optodes in a head cap is also a way to maintain the SDS and configuration [67].

### 3.3 Wireless Systems

The first NIRS systems used to be large, bulky and difficult to move around. With the development of electronics and technology these systems have become more compact and user-friendly over time. Wireless systems have evolved using Bluetooth and Wi-Fi to transfer data and provide Graphical interface remotely. These systems are very helpful when accessing subject that involve movement and activity such as infants, athletes, etc.

Wireless option in the NIRS systems can be Wi-Fi, Bluetooth, ZigBee, wiMAX. ZigBee is more commonly known as the Institute of Electrical and Electronics Engineers (IEEE) 802.15.4 and is used to design a related architecture [71]. Depending on the application, Wi-Fi and Bluetooth are mainly used. Wi-Fi has the advantages of flexibility, larger connectivity ranges (50-600m), high data rates but the signal tends to get noisy [71]. Bluetooth has the advantage of low power consumption and low price but has smaller
connectivity ranges (~100m) and lower data rates [71]. Some studies that involve the use of wireless systems are: Yurtsever et al [63], Fong et al [61], Chen et al [66].

Table 3.3 Commercially available fNIRS systems. Table taken from Ferrari et al [50]

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Technique</th>
<th>Year of release</th>
<th>No. of channels</th>
<th>Company</th>
<th>Web site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynot Compact</td>
<td>CW</td>
<td>2004</td>
<td>288–2049</td>
<td>NIRx, USA</td>
<td><a href="http://www.nirx.net">www.nirx.net</a></td>
</tr>
<tr>
<td>ETG-4000</td>
<td>CW</td>
<td>2003</td>
<td>52</td>
<td>Hitachi, Japan</td>
<td><a href="http://www.hitachimed.com">www.hitachimed.com</a></td>
</tr>
<tr>
<td>ETG-7100</td>
<td>CW</td>
<td>2007</td>
<td>72–120</td>
<td>Hitachi, Japan</td>
<td><a href="http://www.hitachimed.com">www.hitachimed.com</a></td>
</tr>
<tr>
<td>OXYMON MkIII</td>
<td>CW</td>
<td>2003</td>
<td>Up to 96</td>
<td>Artinis, The Netherlands</td>
<td><a href="http://www.artinis.com">www.artinis.com</a></td>
</tr>
<tr>
<td>NIRO-200</td>
<td>CW</td>
<td>2008</td>
<td>10</td>
<td>Hamamatsu, Japan</td>
<td><a href="http://www.hamamatsu.com">www.hamamatsu.com</a></td>
</tr>
<tr>
<td>NIRS2 CE</td>
<td>CW</td>
<td>2007</td>
<td>16</td>
<td>TechEn, Inc., USA</td>
<td><a href="http://www.nirsoptix.com">www.nirsoptix.com</a></td>
</tr>
<tr>
<td>CW6</td>
<td>CW</td>
<td>2009</td>
<td>20–1024</td>
<td>TechEn, Inc, USA</td>
<td><a href="http://www.nirsoptix.com">www.nirsoptix.com</a></td>
</tr>
<tr>
<td>FOIRE-3000</td>
<td>CW</td>
<td>2007</td>
<td>52</td>
<td>Shimadzu, Japan</td>
<td><a href="http://www.med.shimadzu.co.jp">www.med.shimadzu.co.jp</a></td>
</tr>
<tr>
<td>NIRScout</td>
<td>CW</td>
<td>2008</td>
<td>128–1536</td>
<td>NIRx, USA</td>
<td><a href="http://www.nirx.net">www.nirx.net</a></td>
</tr>
<tr>
<td>HD-NI</td>
<td>CW</td>
<td>2009</td>
<td>Over 200</td>
<td>Cephalogics</td>
<td><a href="http://www.alliedminds.com">www.alliedminds.com</a></td>
</tr>
<tr>
<td>Imagent</td>
<td>FD</td>
<td>2001</td>
<td>Up to 128</td>
<td>ISS, USA</td>
<td><a href="http://www.iss.com">www.iss.com</a></td>
</tr>
</tbody>
</table>

Table 3.4 Commercially available wireless/wearable CW fNIRS systems. Table from [50]

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Year of release</th>
<th>Wireless</th>
<th>No. of channels</th>
<th>Company</th>
<th>Web site</th>
</tr>
</thead>
<tbody>
<tr>
<td>fNIR 1100</td>
<td>2009</td>
<td>No</td>
<td>16</td>
<td>fNIR Devices, USA</td>
<td><a href="http://www.fnirdevices.com">www.fnirdevices.com</a></td>
</tr>
<tr>
<td>fNIR 1100w*</td>
<td>2011</td>
<td>Yes</td>
<td>2 or 4</td>
<td>fNIR Devices, USA</td>
<td><a href="http://www.fnirdevices.com">www.fnirdevices.com</a></td>
</tr>
<tr>
<td>HOT 121B</td>
<td>2011</td>
<td>Yes</td>
<td>2</td>
<td>Hitachi, Japan</td>
<td><a href="http://www.hitachimed.com">www.hitachimed.com</a></td>
</tr>
<tr>
<td>NIRSport</td>
<td>2011</td>
<td>Yes</td>
<td>Up to 256</td>
<td>NIRx, USA</td>
<td><a href="http://www.nirx.net">www.nirx.net</a></td>
</tr>
<tr>
<td>OEG-16</td>
<td>2009</td>
<td>No</td>
<td>16</td>
<td>Spectratech, Japan</td>
<td><a href="http://www.spectratech.co.jp">www.spectratech.co.jp</a></td>
</tr>
</tbody>
</table>
3.4 Types of NIRS system validation methods

3.4.1 Tissue simulating phantom

This testing technique involves using a tissue simulating phantom. In this a large volume of tissue simulating intralipid solution (~1L) is taken in a beaker which is maintained at a constant temperature (~32-37 °C) and stirred continuously. On the further end of the beaker a pipe is inserted from the oxygen tank. Its oxygen supply pipe has to be placed as far from the detector as possible in order to avoid increase in the noise due to the bubbling oxygen. Baseline reading is taken for about 3 min followed by the addition of blood, either human blood, bovine hemoglobin or mice blood. This causes the oxy-hemoglobin concentration \([HbO_2]\) to increase and deoxy-hemoglobin concentration \([HHb]\) to decrease gradually. Once the detector output intensity reaches equilibrium (steady state), dry yeast is added (~4g for 1L). This results in the reduction of \([HbO_2]\) and increase in \([HHb]\) due to the deoxygenation caused by yeast action. After the reading reaches a steady state (~15-20 min) oxygen is pumped from the tank to reverse the process and return the \([HbO_2]\) and \([HHb]\) to their initial state. The result of this dynamic phantom experiment is as shown in the Figure 3.3.
This validation technique aims at observing two things; 1) To observe the effect of addition of HbO₂ and HHb to the intralipid solution; 2) To observe the deoxygenation due to addition of yeast. Some studies that used this validation technique are: Bozkurt et al [1], Yurtsever et al [63], Shao et al [72].

![Figure 3.3: NIRS during tissue simulating phantom testing and the expected result. Red shows the oxy-hemoglobin and blue is the deoxy-hemoglobin. (a) The increase in both oxy and deoxy hemoglobin on addition of blood to the phantom. (b) the increase in deoxy and decrease in oxy hemoglobin on addition of yeast to the phantom. Image taken from Bozkurt et al [1].](image)

### 3.4.2 Arterial Occlusion

This is the most common validation method for NIRS systems. In this technique, the probe is placed on the left forearm of the subject and a pressure cuff is placed above it on the upper forearm. Measurement is taken for around 30 seconds to a minute as a baseline followed by 3 minutes of readings on applying a pressure of ~200-250 mmHg. This induces arterial occlusion. The pressure is then released causing the blood flow to go back to
normal. On occluding the blood flow, [HHb] increases and [HbO₂] decreases gradually. On releasing the pressure, the opposite happens but almost instantaneously. This effect is as shown in Figure 3.4. Studies that have used this validation technique include: Zimmermann et al [73], Haensse et al [62], Muehlemann et al [74], Rue et al [75], J. Safaie et al [68].

Figure 3.4: NIRS during the occlusion validation method and its expected results. The occlusion periods are marked during which the oxy-hemoglobin (red) is observed to decrease and deoxy-hemoglobin (blue) increases. Image taken from Muehlemann et al [74].

### 3.4.3 Finger tapping

This experiment involves the placement of the optodes on the cortex and readings are taken for ~20 sec. The subject is then asked to tap their thumb/finger for about 20 sec. the expected result is the increase in concentration of oxy hemoglobin [HbO₂] during
stimulation. Studies that involved this type of testing include: Haensse et al [62], Muehlemann et al [74], J. Safaie et al [68].

![Figure 3.5](image.png)

Figure 3.5: Average hemodynamic response obtained for four source detector positions by finger tapping stimulation of the prefrontal cortex. Image taken from Muehlemann et al [74]

### 3.4.4 Breath Holding

J. Safaie et al demonstrated the influence of apnea the hemodynamics [68]. In this, the subject was relaxed and seated comfortably while 30 s of baseline readings were taken following which they were asked to hold their breath for 30 s and then 60s of relaxation data was acquired [68].

### 3.4.5 Climbing stairs

Safaie et al also described the device testing by climbing up and down the stairs. The subject was asked to stand upright and baseline readings were acquired for 60 s. then the subject had to climb 10 stairs at the rate of 1 stair per s. After 50 s of recovery period they
were asked to go back down the stairs at the same rate. The results indicated a reduction in both oxy and deoxy-hemoglobin concentrations [68].
Chapter 4 – Instrumentation

This chapter aims at describing the instrumentation of the compact CW-NIRS system. The various modules and parts used in the design of the device are mentioned and their implementation is discussed.

4.1 Block Diagram

The basic block diagram is as shown in the Figure 4.1. Various components involved in the hardware of the system such as the dual wavelength LED, constant current LED driver design, photodetector, LED switching mechanism and the optode configuration will be discussed.

Figure 4.1: Block Diagram of the NIRS device built showing various modules and their interconnections.
4.2 Light Source Unit

4.2.1 Light Source

The light source used in our design was a dual wavelength LED (L760-840-05A2) (Fig. 4.2(a)) by Epitex. It consists of AlGaAs LEDs on a lead frame in a clear epoxy lens. It emits 2 different wavelengths, 760 and 840nm, in a single package.

Figure 4.2: LED light source. (a) Image of the LED from epitex, (b) the pinout of the LED [76], (c) the current-voltage characteristics of the LED, (d) wavelength spectrum of the 760nm Diode [77].
This Bi-color LED has a typical total radiated power of 15mW and 18mW at a forward current of 50mA for 760 and 840nm respectively [76]. Fig. 4.2(b) shows the pinout of this LED. It has a common anode configuration [76].

4.2.2 Constant Current LED Driver

To drive the LED, simply connecting it to the required power supply is not enough as this would definitely run the LED but will not guarantee a stable output. As no source is an ideal source, fluctuations in the supply voltage given to the LED can lead to fluctuations in the LED’s output power. These in turn can cause changes in the detected signal over time, which may not be due to the hemodynamics of the tissue, misleading our readings. Therefore, it is essential to have a constant current LED driver that blocks the supply fluctuations and helps in providing constant power from the LED. The constant current driver was designed using a voltage regulator LM317 from Texas Instruments [78]. This regulator has a constant voltage of 1.25V between its pins 1 and 2. This fact was used to create our constant current driver. By placing a resistor between these pins of a resistance value given by Equation 4.1, a constant current is achieved at the output pin of the regulator. The pin configuration of the LM317 and the circuit designed for the constant current driver are shown in Figure 4.3.

\[
R \ (k\Omega) = \frac{1.25 \ V \ (fixed)}{I \ (mA) (required \ constant \ current)}, \quad (4.1)
\]
4.2.3 LED wavelength switching

It was needed to switch between the two wavelengths in order to acquire data at both 760 and 840nm. Having a single package helped in maintaining almost the same position and having a fast switching time would approximately provide the data at the same time (few ms to 1 sec difference) for the two wavelengths. Initially a multiplexer Integrated Circuit (IC) chip, CD54HC4052, from Texas Instruments [79] was used for this purpose. This chip consists of 2 sets of 1*4 multiplexers. The pin out and its truth table are shown in the Figure 4.4. The multiplexer worked well when switching between the two wavelengths but when connected with the constant current source, due to its relatively
higher $R_{ON}$ resistance, it caused a huge voltage drop. This lead to insufficient voltage supply to the LED, preventing it from turning on.

![Multiplexer Diagram](image)

**Figure 4.4:** Multiplexer for LED wavelength multiplexing. (a) Pin out of the IC chip, (b) Truth table for the control of the channels [79].

To overcome this, a CMOS single pole single throw (SPST) analog switch (DG413) by Vishay was used. This has an advantage of low on-resistance $R_{ON} = 25\Omega$ and a fast switching time $t_{ON}=110\text{ ns}$. Also it consumes less power (0.35 $\mu\text{W}$), which is advantageous when making portable and compact devices. Each IC chip has 4 analog switches in it. The pinout and truth table are shown in Figure 4.5 [80].
Of the four switches, two switches are by default OFF while the other two are ON. On applying a digital high voltage signal to the digital input of the corresponding switch, its default state can be reversed. These switches are conductive in both the directions implying the input data can be given to either \( D_1 \) or \( S_1 \) and the output can be seen at \( S_1 \) or \( D_1 \) respectively [80].

Precision automatic test equipment, precision data acquisition, battery powered systems, computer peripherals, etc. are few applications, where these switches could be used [80].

### 4.4 Detector

The detector used was Burr Brown OPT 101. It is a monolithic single supply photodiode with a transimpedence amplifier built in the chip. Some features that make OPT101 a good choice as a detector for this instrument are [81]:

- Works with single supply ranging between 2.7 to 36V
- Small size: 2.29mm* 2.29mm
- 1MΩ internal feedback resistance
- Highest responsivity around 700- 900 nm - ~0.6 A/W
- Low Quiescent Current: 120 µA
- 8-pin PDIP clear plastic packaging

This photodiode has a variety of applications such as medical and laboratory instrumentation, position sensors, barcode scanners, smoke detectors, etc. [81]. Its features of single supply operation and small size make it ideal for compact and battery operated devices. Problems such as current leakage, noise pick-up and effects of stray capacitances that occur due to discrete designs are eliminated due to the amplifier being integrated with the photodiode on a single chip [81]. The photodiode operates in the photoconductive mode which helps in achieving good linearity between voltage and light intensity and also low dark current. The pin out is shown in the Figure 4.6 and the block diagram and spectral response of the OPT101 is shown in Figure 4.7. [81].

<table>
<thead>
<tr>
<th>Pin NO.</th>
<th>Pin Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$V_s$</td>
<td>Positive supply</td>
</tr>
<tr>
<td>2</td>
<td>-In</td>
<td>Negative input, NC</td>
</tr>
<tr>
<td>3</td>
<td>-V</td>
<td>Negative supply/GND</td>
</tr>
<tr>
<td>4</td>
<td>1MΩ F/B</td>
<td>Connect to pin 5</td>
</tr>
<tr>
<td>5</td>
<td>Output</td>
<td>Output</td>
</tr>
<tr>
<td>6</td>
<td>Common</td>
<td>Ground</td>
</tr>
</tbody>
</table>

Figure 4.6: Photodiode detector OPT 101. (a) Pin out of the detector, (b) Pin description [81]
The internal feedback resistance of 1MΩ is what provides an amplification with a gain of $10^6$ V/A. This gain can also be increased by connecting an external resistance in series with the internal resistor. Gains up to $51 \times 10^6$ V/A can also be achieved but at the expense of lower bandwidth, as there exists a tradeoff between the two [81].

4.5 Control Unit

The light intensity detected by the detector is given as a voltage output by the photodiode. This output has to be converted into a digital signal before further processing. Also, for a wireless device, transmission (TX) and reception (RX) circuitry is needed and the various parts of the instrument have to be powered and controlled. Hence a control unit is needed. For this design, Arduino UNO was selected as the control unit. It has a microcontroller Atmel-Atmega328P, which works as our Data Acquisition Card (DAQ
card), analog and digital pins, on board power supplies and also TX and RX pins all in one board. Moreover, programming with the Arduino is much easier and time saving than it is for a microcontroller (MC) alone. Arduino’s software, Arduino 1.6.9, was used to write the program for controlling the digital pins for LED switching as well as for obtaining the detector output and transmitting it to the PC/Mobile. DAQ cards from Texas Instrument could also be used, in fact one was used for the initial prototype. The disadvantage of using a DAQ card is that they are expensive, larger in size, and wired. Hence, to make the device cost – efficient, compact and wireless, the DAQ card was dropped and the Arduino was adopted. The analog to digital conversion (ADC) resolution of the Arduino was 5Mv, which was better than the low cost USB type DAQ card that was initially being used. Few desirable features of the Arduino UNO are [83].

- 14 digital input/output pins: used for LED switching
- 6 analog inputs: connected to the output of the detector
- 16MHz quartz crystal: ADC resolution depends on this.
- USB connection
- Operating Voltage: 5 V
- On board power supplies: 5 V and 3.3 V
- Relatively small size: 68.6mm*53.4mm
- Weight: 25g
- Reset button
- Flash memory:32 KB
- SRAM: 2KB and EEPROM :1 KB
Figure 4.8: The control unit, Arduino UNO and its pin out configuration. [83,84]
4.6 Bluetooth module

The TX and RX pins of the Arduino were made use of to turn the system into a wireless one using Bluetooth. The Bluetooth module used was HC-06. The module has a built-in 2.4 GHz digital wireless antenna/transceiver. It has a small size, is inexpensive and has low power consumption [85]. Some features include [85]:

- Supply Voltage: 3.3-5V
- Baud Rate: 1200-38400
- Size: 27mm*13mm*2mm
- Cost: $3-6
- Simple circuitry

Figure 4.9: Bluetooth module HC-06 having dimensions comparable to the size of a quarter.

The Bluetooth module, HC-06 can send and receive up to 3.3 V only on its TX and RX pins. The Arduino’s TX and RX pins can send and receive up to 5 V. Therefore, for the transmission to reception connection from the Bluetooth to the Arduino respectively, there is no problem and they can be connected directly. But the vice versa is not as simple. Hence, the TX connection has to be voltage divided and brought down to 3.3 V before
being connected to the RX pin of the module. The design of the voltage divider network, its simulation and implementation are shown in the Figure 4.10.


Data transmission and signal acquisition over Bluetooth are time-separated and so the Bluetooth device doesn’t interfere with the measurements. Due to the way the Bluetooth protocol is set up, other Bluetooth or IEEE 802.11 devices, sources of electromagnetic radiation such as laptops, computers and wireless routers do not interfere nor disturb the signal. Hence, Bluetooth can act as an apt cable replacement device and disturbance of medical devices by Bluetooth or vice versa have not been reported [74, 86].

4.7 Optode Configuration

The LED and the detectors were housed in a 3D printed probe. The probe was black and flexible such that it would eliminate any LED leakages from being detected and also adhere to the curved surface of the head easily providing better coupling. The optode
configuration was as shown in Figure 4.11(a). The current design involves two detectors and a dual wavelength led, but this can be multiplexed to incorporate more detectors and LEDs. The microcontroller controls the switching between the LEDs and also the detector data transmission. The way this was designed to work is, the MC would turn ON the 760 nm LED for 100 ms and turns it OFF for 200 ms. It then turns ON the 840 nm LED for 100ms and then OFF for 200ms as shown in Figure 4.11(b). Meanwhile the detectors are always ON and the detected values are transmitted continuously to the receiving unit. LED is composed of a semiconductor junction that can get heated upon operation. Also, the detector’s reading is composed of both the reflected light from the tissue and the ambient light of the surrounding. Therefore, the OFF time between the LEDs can be used for the correction of the offsets caused due to ambient light, LED heating/leakages and electronic components. As discussed in Chapter 3, Section 3.2.3, the optimal source detector separation in NIRS is 3 cm for adults. For infants, the surface area is smaller and also the penetration depth is higher for a given SDS when compared to adults as their scalp is thinner and tissues are under development. Therefore, the source detector separation (SDS) was set to be 2 cm. This configuration allowed for monitoring two different locations at the same time.

Figure 4.11: Optode configuration. (a) LED and detectors configuration, (b) LED switching scheme.
4.8 User-Interface

In order to receive and monitor the incoming data from the Bluetooth module, a serial terminal emulator is needed. Any device with Bluetooth connectivity can be used as a user interface. Both, computer and mobile phone was used as a receiving unit.

4.8.1 Computer

Lots of serial terminal emulator software are available online. Some are free and some have to be purchased. Tera Term is one such serial port terminal that is available for download for free online and is very easy to use. This software was used to establish a 2-way communication via Bluetooth between the computer and the device. The connection setup window of the Tera Term is shown in Fig. 4.12 below.

![Figure 4.12: Tera Term connection window.](image)

A graphical user interface (GUI) was also made in LabVIEW to set the parameters and retrieve the data from the detector via the Bluetooth. The advantage of the LabVIEW interface over Tera-Term was that it could take it one step further and plot the incoming
data points such that the relative changes could be seen real-time. Whereas, in Tera-Term, only the values of the received data could be seen.

![Image of LabVIEW graphical user interface (GUI) designed.]

**Figure 4.13:** LabVIEW graphical user interface (GUI) designed.

### 4.8.2 Mobile

Like the computer, a mobile device with Bluetooth connectivity could also be used as a user interface. For this purpose, a serial monitor application would be needed like the Tera Term. On the android platform, there are many serial monitor applications already available for free. One such application, the Bluetooth Serial Controller, was used to initialize the instrument and acquire the detected outputs. The incoming data was saved as a text file that could be used for further processing. The mobile application used was as shown in the Figure 4.14.
Figure 4.14: Mobile Android Application-Bluetooth Serial Controller window. It allows the user to enter the LED switching times and outputs the detector values.

4.9 Power supply

Since the device was to be made compact and wireless, the power supply could not be a wall unit. The device was therefore, battery operated. Rechargeable Lithium Ion battery from Adafruit (Fig. 4.15) was used. This was a 3.7 V battery, but the Arduino UNO needed a 5V power supply to function. Therefore, two of such batteries were coupled together in series to provide sufficient power to the control unit. The remaining components were powered by the on-board 3.3V and 5V power supply of the Arduino UNO.

Figure 4.15: Li-ion battery of 3.7 V (left) and its charger (right).
Chapter 5 - Device Validation and Results

5.1 Led stability testing

Power stability of the LED is a very important factor to be considered in the NIRS instrumentation. As the hemodynamic changes being observed are small the device needs to be sensitive enough to detect these changes. But if the LED power fluctuates over the course of the measurements, these fluctuations will result in changes in the detected output, which might then be considered falsely as tissue hemodynamic changes. Therefore, the LED stability was measured over a span of 30 minutes to measure its mean power and standard deviation. A solid silicon phantom from ISS (no. 75019), having optical properties of absorption parameter 0.153 and 0.149 cm$^{-1}$ and scattering parameters of 5.0 and 4.2 cm$^{-1}$ at wavelengths 690nm and 830 nm respectively, was used. The results are as shown in Figure 5.1. For the same input current and output power rating for both the LEDs, the mean obtained for the 840nm LED was lower than that of the 760nm LED. The mean output and standard deviation for each wavelength is shown in Table 5.1. The standard deviation was low enough to not have a significant effect on small changes of the chromophore concentrations.
Table 5.1 The results of the LED stability testing showing the mean and standard deviation of the two wavelengths.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>760nm</th>
<th>840nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1594.8 mV</td>
<td>1502.6 mV</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.89 mV</td>
<td>0.76 mV</td>
</tr>
<tr>
<td>SNR</td>
<td>65.0 dB</td>
<td>65.9 dB</td>
</tr>
</tbody>
</table>

Figure 5.1: Plots of the results of the LED power stability testing over 30 minutes.
5.2 Linearity testing

In order to test the device’s sensitivity and accuracy in detecting small changes in the absorption parameter, a phantom study was performed. A liquid phantom was created using Intralipid (Intralipid 20% by Fresenius Kabi), having an optical parameter $\mu_s^\prime=10 \text{ cm}^{-1}$ but no added absorption due to ink initially. The baseline output intensity was noted. Then, Indian ink was added to increase the absorption parameter of the phantom, in increasing steps of 10 $\mu$L starting with 48 $\mu$L, while the scattering parameter was kept constant. Increasing concentration of ink increased the absorption of the phantom and hence the output intensity decreased gradually, as expected. This observation is summarized in Figure 5.2.

![Figure 5.2: Results of titrating ink in increasing concentrations, seeing corresponding decrease in output intensity.](image)

Using the MBLL and the detected output intensities, the experimental values of absorption coefficients were calculated (obtained $\mu_a$: 0.053, 0.065, 0.077, 0.0884, 0.1011, 0.116, 0.136, 0.160, 0.192, 0.236, 0.287, 0.349, 0.422, 0.509)
0.1178, 0.1284). Since the concentration of ink added was known, the exact theoretical values of the absorption coefficient was known (expected \( \mu_a : 0.054, 0.065, 0.0773, 0.0887, 0.1, 0.1114, 0.01227 \)) These two were plotted against each other to determine the accuracy of the device in quantifying the absorption changes, shown in Figure 5.3. The results indicate that there is a strong correlation between the two sets of data \((R^2 = 0.997)\).  

![Figure 5.3: Results of titrating ink in increasing concentrations, comparing the absorption coefficient calculated from the data obtained with the expected values.](image)

### 5.3 Phantom experiment

A dynamic blood-yeast phantom experiment was performed to test the device’s ability to differentiate between the two chromophores accurately. The tissue simulating phantom was prepared using Intralipid 20% (Fresenius Kabi) as the scattering medium added to water to obtain a reduced scattering coefficient of 8 cm\(^{-1}\) at 730nm. The solution was poured into a beaker and was placed on a magnetic stirrer and heater, which helped in
stirring the solution continuously and maintain the temperature around 33 °C. The solution had to be kept warm and the temperature had to be maintained constant in order to keep the added yeast active. The probe was attached to the side of the beaker, and a syringe was connected to the air pump, was adhered to the inner wall of the beaker as far away from the probe as possible to minimize the noise due to air bubbling.

Figure 5.4: Experimental setup showing the beaker containing the tissue simulating intralipid phantom placed on a magnetic stirrer.

The Intralipid baseline was measured for 2 minutes after which 1.5% by volume of mice blood was added. The blood contributed to the absorption property of the phantom which reduced the detected signal output almost instantaneously after being added. After taking the Intralipid-blood baseline for about 5 minutes, 800 mg of yeast was added. It took about 3 minutes for the yeast action to start, after which deoxygenation was observed. The detector output was continuously recorded for 30-40 minutes. Deoxygenation was observed gradually until the output reached a steady state. After reaching the steady state, re-oxygenation was initiated by an air pump.
Two sets of measurements were obtained during this experimental procedure. The first was to observe the effect of the addition of blood to the Intralipid solution (Figure 5.5). The second measurement was to observe the deoxygenation of blood due to the addition of yeast.

For the analysis and computation of the results, the values of the extinction coefficient of oxy-hemoglobin ($\text{HbO}_2$) and deoxy-hemoglobin (HHb) and the differential pathlength factor (DPF) at the two wavelengths chosen for this device (760nm and 840nm) were taken from literature as shown in tables 5.2 and 5.3 respectively.

Table 5.2 Extinction Coefficients used for the analysis of data adapted from [87].

<table>
<thead>
<tr>
<th>Wavelengths</th>
<th>$\varepsilon_{\text{Hb}} (m.M^{-1}.cm^{-1})$</th>
<th>$\varepsilon_{\text{HbO}_2} (m.M^{-1}.cm^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>760 nm</td>
<td>0.4205</td>
<td>0.1494</td>
</tr>
<tr>
<td>840 nm</td>
<td>0.1954</td>
<td>0.2768</td>
</tr>
</tbody>
</table>

Table 5.3 Absorption and scattering coefficient, differential pathlength factor (DPF) at the two wavelengths chosen derived from [88,89]

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>$\mu_a (cm^{-1})$</th>
<th>$\mu_s' (cm^{-1})$</th>
<th>DPF (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>760 nm</td>
<td>0.10</td>
<td>5.8</td>
<td>4.78</td>
</tr>
<tr>
<td>840 nm</td>
<td>0.16</td>
<td>6.8</td>
<td>4.42</td>
</tr>
</tbody>
</table>
Using the $\mu_a$ and $\mu'_s$ from literature, the DPF obtained was in correspondence to that in literature as shown in [90]. Changes in oxy and deoxy hemoglobin due to yeast action are as shown in Figure 5.6.

Figure 5.5: Plot showing the result of the addition of blood to the Intralipid phantom. The output was in the form of rectangular pulses due to the LED switching. Blood corresponds to absorption component resulting in reduction in the output intensity of the detector.

Figure 5.6: Changes in the output of the spectrometer sensor due to yeast action in blood.
Figure 5.6: Results of the dynamic phantom experiment. (a) Changes in output intensity of the detector with the addition of yeast in terms of the wavelengths, (b) Changes in oxy-deoxy hemoglobin obtained by applying the MBLL to (a).

5.4 Occlusion Experiment

This experiment was performed to check if the device was able to detect the hemodynamic changes in-vivo and to observe the expected trend in a well-established occlusion protocol [68,62,73,74,75].

The probe was fastened to the forearm of the subject. Baseline readings were taken for 60 seconds after which a pressure cuff attached to the upper arm of the subject was inflated to approximately 200mmHg of pressure for 180 seconds. This would prevent the venous and arterial blood flow from and to the forearm. The instrument monitored the oxygenation changes continuously, where the oxy-hemoglobin is expected to deoxygenate. After the occlusion period, the cuff was released allowing the blood to flow back into the arm for a
resting period of 180 sec. This was repeated two times. The values of the optical parameters, $\mu_a$, $\mu'_s$ and the DPF and extinction coefficients used were as shown in Tables 5.5 and 5.3. The experimental setup and the results obtained are as shown in Figure 5.7.

Figure 5.7: The experimental setup (left) and the results obtained from the occlusion test (right).
Chapter 6 - Conclusion

This chapter will conclude the project with a brief discussion of the developed-device and the experiments performed along with its results. Future directions will also be discussed.

6.1 Discussion

The Near Infrared Spectroscopy device was developed and the device testing was performed. The cost of the complete instrument was less than $100 which is very affordable when compared to the commercial NIRS devices which range from $10,000-$100,000.

The device is wireless. This is advantageous as it reduced the weight of the device, increased portability, occupied less space, avoids tripping hazards, etc. Since it is wireless, it is possible to obtain measurements using any device having Bluetooth facility. It can also operate by using an open-source software for data acquisition, which also contributes to being low-cost.

The developed device is also very compact of the size 5cm X 5cm X 5cm which is comparable to the size of an adult palm. This could have many advantages in the intra-operative settings as it could save a lot of space and would not interfere with the other
devices present in the Intensive Care Unit (ICU). This could also open the possibilities and opportunities to be deployed as a wearable biosensor for continuous monitoring of athletes and soldiers.

Being wireless and highly portable makes the device very useful for the aid of old and weak patients who have difficulty moving to the hospitals for diagnoses. Another possible advantage of portable device is that it could be used in underdeveloped countries, which cannot afford expensive devices for diagnoses. So, this device could be moved from one place to another depending upon its need. This device provided a high signal to noise ratio (SNR, 65 dB) for source-detector separation of 2 cm. The SNR can further be increased by optimizing the source-detector separation and using more efficient light source and detector components.

The device could effectively detect small changes in oxy- and deoxy-hemoglobin concentrations in the dynamic phantom experiment. With the addition of blood to Intralipid, an increase in absorption and decrease in the output intensity was observed. By implementing the modified Beer-Lambert’s Law, the physiologically relevant parameters such as changes in hemoglobin concentrations and oxygenation were obtained. For analysis and computation of the results of the testing experiments, the values of the extinction coefficient of oxy-hemoglobin (HbO₂) and deoxy-hemoglobin (HHb) and the differential pathlength factor (DPF) at the two wavelengths (760nm and 840nm) were taken from literature as shown in tables 5.2 and 5.3 respectively. Addition of yeast resulted in an increase in deoxy- and a decrease in oxy-hemoglobin. This was then applied for measuring changes in oxy- and deoxy-hemoglobin in an arm in the occlusion experiment. The
expected trend of increase in deoxy-hemoglobin and decrease in oxy-hemoglobin was observed.

Thus, the developed device is unique mix of being compact, wireless and low-cost. This unique combination could be useful for several applications and could be particularly important in several physiological monitoring such as pediatric brain at the intensive care units, for evaluating mental and physiological loads in military units, and possibly monitoring of brain function in low-resource settings.

6.2 Future work

The developed device had a high signal to ratio for a source detector separation (SDS) of 2cm, which is sufficient for neonates. To obtain even better SNR, the detector used for this device, which is a photodiode, can be replaced with a more sensitive detector like Silicon photomultipliers. Thus our next step would be to use these detectors to obtain high signal and higher SNR for 3 cm or larger source detector separations (for more that 2 cm of light penetration depth). Adding of these types of detector would slightly increase the cost of the instrument (~$500). Thus, there always exists a tradeoff between device cost and SNR ratio. For applications on infants with low SDS, this prototype works well.

Another possibility with the device would be the increase in the number of light sources and detectors. Since the device is highly modular, adding more sources and detectors is straightforward. Moreover, increasing the number of LEDs and detectors would not affect the cost of the device significantly because the cost of each LED and detector is than $10. By increasing the number of source and detectors, topographic (image-
based) and tomographic (depth-resolved imaging) measurements can be performed, where the heterogeneity of the focal brain activation with respect to lateral (x-y) or axial (z, depth) can be obtained.
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