Monitoring Cerebral Functional Response using sCMOS-based High Density Near Infrared Spectroscopic Imaging

Dharminder Singh Langri

Wright State University

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MONITORING CEREBRAL FUNCTIONAL RESPONSE USING SCMOS-BASED HIGH DENSITY NEAR INFRARED SPECTROSCOPIC IMAGING

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

by

DHARMINDER SINGH LANGRI
B. Tech. Instrumentation Engineering, Shree Ramanand Teerth Marathwada University, 2015

2019
Wright State University
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Dharinder Singh Langri ENTITLED Monitoring Cerebral Functional Response using sCMOS-based High Density Near Infrared Spectroscopic Imaging BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Biomedical Engineering.

Ulas Sunar, PhD.
Thesis Director.

John C. Gallagher, Ph.D.
Chair, Department of Biomedical, Industrial and Human Factors Engineering.

Committee on Final Examination

Ulas Sunar, Ph.D.

Keiichiro Susuki, Ph.D.

Tarun Goswami, Ph.D.

Barry Milligan, Ph.D.
Interim Dean of the Graduate School.
ABSTRACT

Langri, Dharminde Singh. M.S.B.M.E, Department of Biomedical, Industrial and Human Factors Engineering, Wright State University, 2019. Monitoring Cerebral Functional Response using sCMOS-based High Density Near Infrared Spectroscopic Imaging.

Neurovascular coupling is an important concept which indicates the direct link between neuronal electrical firing with the vascular hemodynamic changes. Functional Near Infrared Spectroscopy (fNIRS) can measure changes in cerebral vascular parameters of oxy-hemoglobin (Δ [HbO2]) and deoxy-hemoglobin concentrations (Δ [HHb]), and thus can provide neuronal activity through neurovascular coupling. Currently many commercial fNIRS devices are available, but they are limited by the number of channels (usually having only 8 detectors), which can limit the sensitivity, contrast and resolution of imaging. High-density imaging can improve sensitivity, contrast and resolution by providing many measurements and averaging the signals originating from the target cerebral focus area compared to background tissue.

Current commercial and research fNIRS devices mostly utilize single element detectors (photomultiplier tube or avalanche photodiode), which is limited by cost for high-density configurations due to the cost of the individual detector. Here I present a multi-channel, low-cost, high density imaging system based on scientific CMOS
(Complementary Metal-Oxide-Semiconductor) detector. The CMOS camera is fiber-coupled such that on one end fibers are focused on the pixels on the CMOS camera, which allows individual pixels (or binned sub-pixels) act as detectors. The other end of the fibers can be positioned on a wearable optical probe.

In Chapter 1, provide is the motivation and significance of high density fNIRS. Chapter 2 consists of theory behind the fNIRS method. Chapter 3 covers the instrumentation part and Chapter 4 consists of device validation part in which the developed system is tested using a series of dynamic flow phantom experiments mimicking the brain activation and finally human motor cortex experiments (finger tapping experiments). It is demonstrated that this system can obtain high density data sets with higher contrast and resolution. This wearable, high-density optical neuroimaging technology is expected to find many applications including pediatric neuroimaging at the clinics and human performance assessment in military research.
# Abbreviation’s List

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NIRS</td>
<td>Near Infrared Spectroscopy</td>
</tr>
<tr>
<td>NIR</td>
<td>Near Infrared Region</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>PET</td>
<td>Photon Emission Tomography</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalographic</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
</tr>
<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
</tr>
<tr>
<td>HbO₂</td>
<td>Oxy-hemoglobin</td>
</tr>
<tr>
<td>HHb</td>
<td>Deoxy-hemoglobin</td>
</tr>
<tr>
<td>[HbO₂]</td>
<td>Oxy-hemoglobin Concentration</td>
</tr>
<tr>
<td>[HHb]</td>
<td>Deoxy-hemoglobin Concentration</td>
</tr>
<tr>
<td>[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>μₐ</td>
<td>Absorption Coefficient</td>
</tr>
<tr>
<td>μₛ’</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>ε</td>
<td>Extinction Coefficient</td>
</tr>
<tr>
<td>BV</td>
<td>Blood Volume</td>
</tr>
<tr>
<td>StO₂</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>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>LED</td>
<td>Light Emitting Diode</td>
</tr>
<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>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>sCMOS</td>
<td>Scientific Complementary Metal – Oxide – Semiconductor.</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>ASD</td>
<td>Autism Spectrum Disorder</td>
</tr>
<tr>
<td>SD</td>
<td>Source - Detector</td>
</tr>
<tr>
<td>ICG</td>
<td>Indocyanine Green</td>
</tr>
<tr>
<td>CCD</td>
<td>Charged Coupled Device</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit.</td>
</tr>
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Dedicated to

My Father for always loving and supporting me.
Chapter 1 - Introduction

In this chapter the motivation for this thesis and the approach of the functional Near Infrared Spectroscopy (fNIRS) is introduced as the basis of this thesis.

1.1 Motivation

Even though there are advances in prenatal, perinatal and neonatal intensive care over the years, the number of long-term morbidity are still disproportionate and unmatched by decrease in neonatal mortality rate. Neurodevelopmental outcome has gathered high clinical concern among these at-risk infants. Approximately 0.5% infants suffer from neonatal encephalopathy. Up to 25% of the surviving population develop poor neurodevelopment, such as epilepsy, cerebral palsy, cognitive impairment and sensorimotor deficits. Approximately 10 to 15% of the preterm infants will develop cerebral palsy, while up to 40% infants will develop mild motor deficits. Apart from these, up to 37% of preterm infants will suffer from emotional/behavioral adjustment problem, and up to 60% suffer from cognitive deficits. It is believed that long-term outcome can be reduced by early interventions and improved therapy if these cerebral deficits are diagnosed early which can be achieved by routine monitoring of cerebral irregularities. Unfortunately, due to lack of effective cerebral monitoring during initial hospitalization, ineffective screening tools, these cerebral
deficits are often complicated and delayed in diagnose and treat and are only observed after late manifestations of many functional deficits [124].

Infants requiring neonatal intensive care are at high risk for developing brain injuries due to hypoxia – ischemia and other blood flow and oxygen flow anomalies. In initial stage after birth, there is poor balance between oxygen supply and blood flow to the brain due to adaption of hemodynamics during transitional circulation, especially in preterm infants. It is confirmed, through various studies, that both hypoxia and hyperoxia can cause irreversible brain damage, causing permeant long term impairments. Hence these conditions of the vulnerable population make bedside, non-invasive, continuous, hemodynamic monitoring so crucial. The blood oxygen saturation assessment done by NIRS studies helps to adjust medical interventions if necessary, which affects blood and oxygen supply to brain, thus preventing any unwanted brain injuries [121].

To prevent these conditions from arising various intraoperative intervention methods are used, one of them is using optical imaging. Diffuse optical imaging, one of the methods in NIRS analysis helps analyzing various parameters of human body such as tissue oxygen saturation, oxygendered hemoglobin concentration, deoxygenated hemoglobin concentration, total hemoglobin concentration, oxygen consumption/ metabolism, blood flow in different parts of bodies, concentration of different chromophores in body, etc. as explained in figure 1.1(a). These methods help in determining abnormalities, if any, in the test subject, such as formation of lumps due to accidents (one example is identifying Hematoma in brain due to traumatic blunt injuries on head, which are hard to identify using other imaging modalities) or
identifying tumors, since they have different concentration of oxy and deoxy hemoglobin as compared to control population. One of the most common optical methods for detecting tumors is fluorescence imaging, which uses the concentration of fluorescence material to determine the location of tumor for both in-vitro and in-vivo analysis. These method and devices also help in preventing other different medical conditions such as Hypoxia. These devices can be installed in medical wards to prevent such conditions in patients. As explained in the flow chart in figure 1.1 (b), first these devices can help monitor the level of interested chromophore, and then based of the desired levels, medical interventions can be done accordingly. The advantage of these devices in these conditions is it’s speed of analysis and result generation in local areas being measured. Working of NIRS devices and the principle behind it will be discussed in detail in the following chapters.

Figure 1.1 (a) Possible tests that can be done using Diffuse Optical Imaging. (b)Flow chart of medical interventions that can be done using NIRS.
The benefits of such interventions are reduced mortality rate in extremely preterm infants, improved long-term results in extremely preterm infants, reduced burden of Asphyxia, hypoxia or hyperoxia in extremely preterm infants, improved cerebral oxygenation levels in infants with congenital heart defects, etc [121]. Implementing and repeating the fNIRS studies can be advantageous as they offer the ability of monitoring localized cerebral cortical activation by measuring oxyhemoglobin and deoxyhemoglobin concentration changes, at cribside regardless of the activity state and nature of pathophysiology [122]. These functional imaging of brain can also be done using fMRI in adults, but there is always a gap in understanding such functional response in neonates using fMRI, as the subject needs to be in un-sedated state to maintain cardiorespiratory stability [122].

Although most conventional neuroimaging modalities, such as magnetic resonance imaging (MRI), head ultrasound (HUS), computed tomography (CT) provides greater sensitivity to detect anatomical injuries and greater anatomical details of brain structure but lacks the ability of bedside real-time evaluation of cerebral functional activities [124]. These modalities are not portable and are not ideal for ill term or preterm infants for frequent functional scanning or prolonged periods. Hence, ideal technology for the infants or neonatal cerebral imaging and monitoring would be non-invasive, relatively inexpensive, portable, and should provide robust and reliable cerebral hemodynamic readings. Near Infrared Spectroscopy (NIRS) have the ability to cover all these qualities, and hence shows promising results in field of neonatal functional neuroimaging.
One of many cerebral deficits discussed above is Autism Spectrum Disorder (ASD), which is the reason for impairment in social skills, causing one to perform repetitive tasks, and having difficulties in communicating verbally or non-verbally [91]. Associated symptoms and medical conditions of Autism contains communication difficulties, repetitive behaviors, gastrointestinal (GI) disorders, seizure disorders, sleep dysfunction, sensory processing problems (hypo or hypersensitivity), pica, anxiety, phobia, etc. [95, 96].

Approximately 1% of earth’s population, which is significantly high number, suffers from Autism Spectrum (CDC, 2014). In USA more than 3.5 million people suffer from this disorder (Buescher et al., 2014), and 1 in every 59 births is expected to have this disorder (CDC, 2018). The number of prevalence of autism in USA for children did rise by a significant number of 119.4 % between 2000 (1 in 150) to 2018 (1 in 59) (CDC, 2018). It increases by 6 to 15 percent every year (based on biennial numbers from the CDC), making it one of the most fast growing mental development disability [94].

Most of the times, autistic signs tend to appear between age of 2 to 3 years, in some cases, it can even be diagnosed as early as 18 months. Early evaluation and intervention can improve the outcomes in medical conditions autistic behavior of autistic person and can also reduce the costs of life long care up to 2/3 times of total cost [95]. Even if the diagnosis and intervention begin at an early stage of life for an autistic person, the benefits from it continue throughout the person’s life, since these therapies can increase skills of the person and decrease autistic behavior and symptoms [96].
Recent studies, using fNIRS, indicated weaker resting-state functional connectivity between bilateral language areas, for subjects suffering from ASD. These findings are similar to that of fMRI results. Based on this resting-state functional connectivity pattern acquired by fMRI, differentiation between ASD and non-ASD subjects achieved 72% sensitivity and 82% specificity. Whereas similar study done by fNIRS achieved 81.6% sensitivity and 94.6% specificity for differentiating between ASD and non-ASD subjects. These results indicate that fNIRS may provide more accurate differentiation between ASD and non-ASD subjects as compared to other conventional modalities [125].

1.2 Approach for Optical Imaging (functional Near Infrared Spectroscopy)

Near infrared spectroscopy (NIRS) is an optical imaging technique which uses light in 600 Nano-meter to 1000 Nano-meter wavelength, to monitor and image hemodynamic and chromosomal activities in brain. Similar concept is Functional Near Infrared Spectroscopy (fNIRS) which is used to monitor functional response in brain to different tasks or functions performed, to monitor functional activity of the brain. The reason behind using NIR wavelengths is it’s ability to penetrate deeper into tissues as compared to visible, mid-infrared and ultraviolet wavelengths. When the light is illuminated on the surface of scalp or forehead, it penetrates the surface and travels through roughly three surfaces, superficial layer of skin on head, skull and cranial layer. Different path and pattern of travel is followed by these travelling photons
within the head. Some photons get absorbed due to some of the absorbing components of the tissues, they are travelling within, called chromophores, while others get scattered and reflected back to the surface. Since we are interested in reflective mode of fNIRS here, the scattered and reflected light intensity is then used to extract the functional activity in the brain. Although there are different imaging techniques superior to fNIRS in terms of penetration depth and spatial resolution, such as computed tomography (CT), photon emission tomography (PET), functional magnetic resonance imaging (fMRI), etc., but the advantage of fNIRS over these devices are it’s portability, cost, acquisition speed, temporal resolution, bed-side monitoring, ability to combine with different modalities, use of harmless dose of light and wavelength, etc [1].

Optical spectroscopy and imaging is noninvasive, nonionizing technique particularly attractive for clinical studies. Compared to other established imaging modalities such as magnetic resonance imaging (MRI), the instrumentation is relatively low-cost, compact, portable so that it can be placed in cramped spaces such as ICUs. Source, i.e. laser of NIR wavelength, is aimed at the sample of the test subject being analyzed, then detectors such as very sensitive photon collectors such as scientific cameras, APDs, PMTs or photo diodes are used to collect the signal. These signals are then calculated in the mathematical model generated for fNIRS imaging, and then the results are generated.

Although fNIRS has less resolution compared to fMRI, there are several advantages of fNIRS for pediatric application [2] including:

1) It is cheaper than most of the current and conventional modalities being used (for
example, FMRI, CT, ultrasound, etc.).

2) NIRS is completely safe, non-invasive and its target audience is not limited. It can be used for an individual of any age.

3) No complicated arrangements and fixature, or pre-examination process needs to be done on the subject, since the sensors or the fibers can be easily placed on the desired location on subject.

4) The whole device can be mounted on a wheeled portable station and is non-interfering with other modalities or devices in intra-operative settings.

5) It is not affected by any electrical, magnetic and mechanical noise.

6) It is having high resolution and it allows real-time data acquisition, making it very suitable for bedside continuous monitoring of intercranial hemodynamic changes.

1.3 Objective of the Thesis

First objective of this study is in-vivo quantification of oxy-hemoglobin and deoxy-hemoglobin concentrations which will allow us to calculate total blood volume and oxygen saturation in the tissues being examined and will help us with functional data collection in the cortical region.

Second aim is to make this device able to image using high density arrays of sources and detectors, increasing number of channels thus increasing the spatial resolution of the device. This study also aims on making the device as compact and inexpensive, non-interfering and user friendly as possible compared to the other present
conventional modalities.

1.4 Thesis Outline

In second chapter of this thesis we will discuss the background and principle of Near Infrared Spectroscopy (NIRS), optical properties such as absorption and scattering in tissues and various other chromophores affecting them. The most important is the Beer Lambert Law, which is the principle behind the foundation of this developed device, is also discussed. Also, various types of NIRS systems are described. This chapter also walks us through the literature review, history and evolution of the NIRS methods.

Third chapter basically contains the detailed description and specifications of the components used for developing the device mentioned along with its block diagram and the setup.

Fourth chapter consists of techniques and studies used for device validation along with their results and comparison with the corresponding commercial devices.

At last, fifth chapter consists of conclusion of the thesis consisting the brief discussion, conclusion and future directions related to the device developed.

The Bibliography along with references is at the end of the thesis.
Chapter 2 – Theory

In this part of the thesis I will briefly describe the theory and methods that I used to quantify the optical contrasts and the light propagation model in living tissue, namely photon diffuse model.

2.1 Propagation of Near Infrared Light in Tissue

As shown by Jobsis in late 1970s, transmittance mode of Near Infrared (NIR) light could be useful for monitoring the hemodynamic properties such as oxygenation properties if proper chromophores or metabolites are measured [11]. This kind of in-vivo near infrared spectroscopy (NIRS) study was first done and applied successfully by him on a cat’s brain [12]. Over the decades the importance and impact of optical methods in diagnosis and other applications has become clear, and several instrumentations and techniques have been implemented and optimized for particular applications including brain. In the field, researchers use “functional” in front of NIRS to indicate that what we measure are functional contrasts based on a specific functional activation of a brain for a given task. Thus, in this thesis I will use fNIRS and NIRS interchangeably.

Based on the fact that biological tissues are relatively transparent to light in the NIR region (600 – 1000 nm), the NIRS studies are founded. In this wavelength region, various chromophore in human tissue absorbs light spectra, which can be used to determine concentration of that specific chromophore in the tissue being examined. As observed at many occasions, the concentration of chromophores such as oxygenate
hemoglobin [HbO₂] and deoxygenated hemoglobin [HHb] changes accordingly to the change in tissue oxygenation and metabolism, [11]. Hence it is important to determine the concentration changes of these chromophores to extract useful tissue information.

Each and every photon of the near-infrared region (NIR) light incident on the surface of tissue follows different paths, it may either get transmitted, reflected or absorbed by the tissue due to optical properties of different chromophores present in the tissue as demonstrated in Figure 2.1. Very small number of photons gets scattered in the tissue at it’s surface and exists the surface as reflected light. A small portion of light is scattered deep in tissue and using transmittance or reflectance method, could be used to determine the amount of concentration of chromophores in the tissue based on it’s absorption parameter. Also scattering and absorption parameter are wavelength dependent [13].

Figure 2.1. Photon behavior when light is incident on the surface of tissue. Three main behaviors of photon observed. 1) Un-scattered photon travelling straight, 2) Scattering occurs at multiple points and occasions, gets forward scattered and re-emits itself in transmission mode, 3) After scattering, gets reflected back and exits surface in reflection mode.
Absorption is the process, in which some of light photons passing through the medium are completely absorbed and the input intensity of light is attenuated, and output is attenuated signal which was passed, in this method the direction of propagation of light doesn’t change.

Reflection and refraction are the phenomenon which occurs due to difference in index of refraction of two bulk media through which light is passing. These phenomena are better explained in Fresnel’s and Snell’s Law respectively [126]. These phenomena are mostly used in fiber optics to transport the light and these phenomena can be seen in all daily life examples such as color of different objects (such as sky) appearing the way they do.

Scattering is the process in which the ray of light which is being passed through medium tends to change it’s direction due to it’s collision with the scattering particles present in medium. In this interaction the direction of propagation, due to difference in index of refraction, changes. There are mainly two types of scattering namely Rayleigh and Mei scattering. These will be discussed in following chapters.

In the chapters and section below, scattering and absorption are discussed in detail as mainly these two optical interactions occur in tissues and are useful to calculate various optical properties of the tissues for optical analysis of tissues.

2.2 Wavelength Selection

It is seen and observed that larger amount of light can penetrate and travel deeper into the biological tissues if it is from NIR region, hence giving penetration depth of about
3 cm, which is larger than any other wavelength band of light. The light above wavelengths of 900 nm are highly sensitive to components of tissues and chromophores such as lipids and water, resulting in more absorption of light and restraining it from penetration deep, also light having wavelength less than 700 nm are assumed to be scattering dominant [13]. Hence, due to these reasons the light having wavelength between range 700 to 900 nm is considered to be the best optimal choice of wavelength range for optical imaging since absorbance due to both the dominant chromophores is at least optimum level in this range of wavelengths and the penetration depth greater than other wavelength windows can be achieved. The molar extinction spectra (which is related to absorbance directly) for both oxy and deoxy hemoglobin is as described in Figure 2.2.

![Molar extinction coefficient spectrum of oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (HHb).](image)

Figure 2.2 The molar extinction coefficient spectrum of oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (HHb).
Important hemodynamic information such as concentration of HHb & HbO₂, and blood oxygenation can be obtained with the help of the absorption spectrum of these chromophores and their behavior towards NIR light and the response of NIR light to the exposed tissue and results of light received due to these chromophores. It can be seen that HHb has higher absorption and is sensitive to NIR light below wavelength 808 nm and HbO₂ has higher sensitivity and absorption for NIR light above 808 nm, 808 nm being Isosbestic point for both these chromophores, since absorption due to both oxy and deoxy hemoglobin is same at that point [15]. To extract information regarding these two chromophores using Continuous Wave (CW) method, minimum two wavelengths are required. One can even use more than two wavelengths to either acquire data for more than two chromophores or to get more precise and accurate measurements for the two chromophores. Usually only two wavelengths, on either side isosbestic point in NIR region is chosen for extracting information of oxy and deoxy hemoglobin using NIRS to get better sensitivities and to avoid overlap of absorption data for any single chromophore, namely HHb and HbO₂ here.

Selecting wavelength for the study and experiments to be performed is one of the important aspects to be considered. Wavelengths should be selected in such a way that there is minimum crosstalk and good contrast between data for [HbO₂] and [HHB]. According to one of the observations done by Strangmann et al, there is high possibilities of cross talk and low separability if both the wavelengths are above 790. Also, if wavelengths are further away from isosbestic point, the absorption will be high limiting the penetration depth. Hence taking into consideration all these points
and to acquire better focal changes during hemodynamic measurements, 785 nm and 810 nm were chosen which satisfies all the given conditions above [16,17].

2.3 Human Brain

As we all know, central nervous system (CNS) is made of combination of spinal cord and brain, having four major regions namely the cerebellum, the diencephalons, the brain stem and the cerebral hemispheres. An approximate 5 mm thickness of outer layer of grey matter, called cerebral cortex, covers the cerebral hemispheres, which is consists of ridges known as sulci and deeper ridges known as fissures. Such folds help increasing the surface area of the cortex, and these fissures also helps separating hemispheres into different lobes [11].

Scalp, Skull, CSF and various membranes protects the brain [18]. Although brain only amounts 2% of the total body weight there is, it receives 15% of heart’s output. Since it is a major organ of the body, and it also receives most of heart’s output, the supply of glucose and oxygen is also higher as compared to other organs [19].

When NIR light enters the brain, only a small fraction of it’s photons are able to reach the detector and rest are either absorbed or scattered due to the scattering and absorption caused by the nature of the chromophores present in the tissues. Generally, a banana-shaped path is followed by the light that reaches the detectors, and in the same banana-shaped region the changes of concentration of hemoglobin are measured (Figure 2.3) [15]. The photons have to cross the scalp twice, as shown in the Figure 2.3, photons have to cross the superficial skin on forehead and scalp twice to reach the detector, first time as output from light source and second time while reaching the
detector as input [2].

Figure 2.3: The path of light and region swept in the tissue known as “banana shape”.

The frequency range of changes in hemodynamic measurements generally range between 0.1 Hz to 10 Hz and might get affected or overlapped by other biogenic signals sharing the same frequency band as described in table 2.1[21].

Table 2.1. Table for frequency ranges of different physiological parameters which can possibly interfere in NIRS signal being measured. Generally, “fast” signal, which detect changes in cytosolic index and in membrane, caused by neuronal activities are very weak and can’t be detected easily, also they are having higher frequency range from other hemodynamic frequency range, as shown in the table below [21].

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Response time range</th>
<th>Frequency range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodynamic response</td>
<td>~ 0.2-10 sec</td>
<td>0.1-5 Hz</td>
</tr>
<tr>
<td>Mayer waves</td>
<td>~ 10 sec</td>
<td>0.08-0.12 Hz</td>
</tr>
<tr>
<td>Heart rate</td>
<td>~ 1 sec</td>
<td>1-3 Hz</td>
</tr>
<tr>
<td>Respiration</td>
<td>~ 5 sec</td>
<td>0.2-0.3 Hz</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>“Fast” signal (intrinsic scattering)</td>
<td>~ 0.050 sec period</td>
<td>20+ Hz</td>
</tr>
</tbody>
</table>

The global concentration of hemoglobin, hence hemodynamic response, changes significantly due to change in pace of heartbeat or of breathing, hence causing interference in the measured NIRS signals. Some examples of the physiological interferences that can affect NIRS measurement are changes in blood pressure, heartbeat, change in partial pressure of CO\(_2\) and change in rate of breathing. Among all of these, heartbeat has the highest frequency of ~1.2Hz. Using sampling rate of the measured NIRS signals higher than 2.4 Hz, we can easily avoid effects caused by these interferences. Current NIRS systems have sampling frequencies of 2-250 Hz [22] and it might lower or rise depending on number of channels being used.

2.4 Optical properties of tissue

2.4.1 Light absorption

Bourger in 1729 followed by Lambert in 1760 first observed and quantified the relation of absorption of light in an absorbing and non-scattering medium based on medium’s thickness. As per them, if a light having intensity I is passed through the medium consisting of successive layers having thickness δd, a fraction dI of the intensity passed I is absorbed [11]. This is described in Figure 2.4.
Figure 2.4. Effect of absorbing medium on incident light of intensity having $I_0$, after attenuation having light intensity $I$. The distance travelled by the light through the absorbing medium is $d$.

Expression given by Lambert-Bougner is as follows.

\[
\frac{dl}{I} = -\mu_a \cdot \delta d \tag{2.1}
\]

Integrating it we get

\[
I = I_0 \cdot exp(-\mu_a \cdot d) \tag{2.2}
\]

In above expressions, $-\mu_a$ is the absorption coefficient with units cm$^{-1}$. It may be defined as the probability of photon to get absorbed per unit length travel in the medium. Also, the absorption path length, which is the ability of photon to travel without being absorbed, is inverse of absorption coefficient $\mu_a$ given as $1/\mu_a$ having units in cm.
2.4.2 Absorbing compounds in the brain

Tissues can be described as a homogenous mixture or combination of different types of biological compounds named as chromophores, having different behavior towards light at different wavelength. The overall light absorbed by these tissues then depends on the chromophores it is made up of. Each and every chromophore shows distinct behavior and unique characteristics towards absorption and hence has its unique absorption spectrum for different wavelengths. We are specifically interested in those chromophores which change absorption with oxygenation in NIR and such chromophores are studied in NIRS. But it is also observed that chromophores whose concentration is not related to variation in oxygenation also attenuates the light passed, hence it is important to have information about spectrum of both type of chromophores to get the corrected data in given figure below, the absorption spectrum of different types of chromophores is mentioned (figure 2.5).

![Absorption Coefficient vs Wavelength](image)

Figure 2.5. Various chromophores demonstrated with their absorption spectrum, present in human tissues, in NIR region, which may possibly attenuate the NIR light passed for measurement. Image taken from [117].
2.4.2.1 Water

As we all know human body consists 50-65% of water, it is safe to assume it as one of the important chromophores and it is crucial to have information about it’s absorption spectrum. Based on gender, age and tissue type the percentage of water in human body varies. A neonate’s brain consists of 90% of water by mass, whereas adult skeletal muscle consists of 74% of water [27,28]. Hence owing to this high share of human body filled with water, it would be difficult to extract any useful information about tissues or any organ of body if the optical density per cm of water is more than 1. Owing to these reasons, the wavelength cutoff of spectroscopy is 1.35 µm [29]. Extinction coefficient of water is less than 0.001/cm, hence it has no effect on shorter wavelengths ranging in ultraviolet range to 600 nm [29]. Whereas it shows higher absorption for higher wavelengths as it starts to cross 600nm, and losses due to absorption due to water starts increasing, and hence giving difficulties for light to penetrate deeper in human skin, limiting the depth for NIRS

2.4.2.2 Hemoglobin

Hemoglobin constitutes of total 40 to 50% of the total volume of blood, are present in Red Blood Cells (RBCs). Hemoglobin helps transporting and exchanging oxygen from lungs to the tissues and carbon dioxide from tissues to lungs. They give information about the brain’s functional contrast based on changes in oxygen levels also called as BOLD (blood oxygen level dependent) study [32]. Figure 2.8 illustrates the absorption spectrum of HHb and HbO₂ in the NIR window. As it can be seen the
two spectra can be distinguished significantly from one another. Based on this spectra of both the types of hemoglobins, it can be easily determined the usable wavelength range or wavelengths so that it can be transmitted through the tissue to extract information about changes in both the hemoglobin. Keeping in consideration the optical density of hemoglobin, 600 nm can be approximately determined as the shortest wavelength beyond which light can pass the tissues and penetrate deeper up to few cm [33].

2.4.3 Light scattering

Cellular level mismatching of the refractive index is one of the reasons for light scattering in most of the tissues. The variation of refractive indices between the internal cell organelles and the cell membranes causes major scattering. Red blood cells are made up of approximately 2% of solid content and hence their contribution to scattering is comparatively low [26]. Throughout the tissues, light scattering is very important phenomenon, which eventually helps the diffusion of light hence making it helpful for diffuse NIRS. Photons entering the tissues have high probability of scattering more than once before reaching the boundary, even in tissues as thin as few submillimeter [11].
Due to scattering of photons, it deviates from their original path, their path length is increased and also their probability of being absorbed increases in the tissues they are scattered (figure 2.6). In the figure given above illustrated is difference and effects of absorption and scattering on a beam or photon of light. As figure 2.6 explains effects of absorption of ray of light. Light intensity only gets attenuated due to absorbing components present in the sample, but the direction of light propagation remains unaltered and continues to travel in straight light. Figure 2.6 also shows us the effect of scattering on propagation of light. If scattering agents are present in the sample through which light is passed, there is high possibility that some photons present in the light being passed might alter their path and not follow the original path of
propagation, in this way some photons might reflect back, some may pass through unaffected, and some may change their direction. Figure 2.6 explains the effect of absorption and scattering, on light photons being passed, through ray diagram.

Factors in brain tissues that determine the scattering are: 1) Light source’s wavelength 2) gestational age and 3) oxygenation of tissue [26]. Age is one of the major factors affecting scattering in brain. The lipid content and protein content in body increases twice from birth to adulthood, whereas this lipid concentration increases up to 7 times more in white matter of brain, which is significantly high, thus increasing scattering significantly. As described above, protein content is higher in newborns, but as soon as myelination starts, the lipids starts showing higher growth rate than the [34].

Assuming a medium having only one scattering component, \( \mu_s \), the scattering coefficient gives the probability of photons scattered per unit length of travel in the scattering medium. The expression of acquired intensity of light \( I \) after passing light intensity \( I_0 \) through a scattering medium having scattering coefficient \( \mu_s \) of thickness \( d \) can be described as follows.

\[
I = I_0 \exp(-\mu_s \cdot d)
\]  

(2.3)

Scattering pathlength also defined as the probability of average distances a photon travels before it is scattered again, can be described as \( 1/\mu_s \), which is reciprocal of \( \mu_s \) [11]. In simple terms, the linear combination of absorption and scattering can approximately determine the overall path of photon. \( \mu_t \), the transport coefficient of a single scattering event can be roughly illustrated as the expression given below [35].

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

(2.4)
But it is seen that in more practical applications as in biological tissues, the photons scatter multiple times, hence invalidating the assumptions of single scattering event given above.

Figure 2.7: Angular probability of photon scattering multiple times, shown as phase diagram. Image acquired from [11]

Assuming \( \hat{S} \) as unit vector showing the incident, photon which is scattered. As shown in figure 2.7, the angular probability of the photon being scattered in the direction of vector \( \hat{S}' \) can be stated as the normalized phase function \( f(\hat{S}, \hat{S}') \).

Probability distribution function for random soft tissue is assumed to be function of angle between scattered photons and incident photons. Hence, \( f \), the phase function can be described as a cosine function of scattering angle \( \hat{S} \cdot \hat{S}' = \cos \theta \). Anisotropy factor, \( g \), is the mean of scattering angles and is representation of phase function [11]. Hence, the reduced scattering coefficient, \( \mu'_s \), characterizes the scattering.

The reciprocal of the distance travelled by a photon before randomizes completely, or one photon random walk step, is called as reduced scattering coefficient [35]. Reduced scattering coefficient can be expressed as product of scattering coefficient with factor\((1-g)\) and expressed as follows:

\[
\mu'_s = \mu_s(1 - g) \tag{2.5}
\]
Under Ideal circumstances, the ideal tissue have $g=1$, which means that all the radiations are travelled in forward direction of incident light, and for complete isotropic scattering $g = 0$. Generally, in tissues from human, $g$ lies somewhere in the range between 0.69 to 0.99 [36]. Hence owing to this change of reduced scattering coefficient caused by scattering coefficient, the reduced transport coefficient can be expressed as:

$$\mu_t' = \mu_s' + \mu_a$$

(2.6)

### 2.5 Model of fNIRS in vivo

#### 2.5.1 Beer-Lambert Law

The process of decrease in light intensity after passing through a medium (here forehead) can be broken down in two stages. In first stage the collimated beam, which is incident on the surface, diffuses after scattering, within the first 8 mm layer of skin, and hence the rate of decrease in light intensity is very high. After that, the rate of decrease is chromophore dependent which are present in the related tissues, generally this rate of decrease of intensity is smaller here. After travelling through the first few millimeters, the intensity of travelling light drops linearly due to both absorption and scattering present due to similar tissues (Homogenous tissues), and hence the depth to which the light travels can be calculated based on intensity drop [26,41].

In trans – illumination mode, as per Beer-Lambert law [127], logarithmic value of detected light is linearly related to changes in concentration of oxyhemoglobin and
deoxy-hemoglobin [15]. In 1854, August Beer, a German Mathematician/Physicist, and Johann Lambert, a Swiss Mathematician, introduced and devised this law in one of their books named Einleitung in die höhere Optik (Introduction to the Higher Optical). Although Bougner discovered this law approximately 100 years before Beer and Lambert [15].

Let’s assume $I_0$ as the intensity of incident light and $I$ be the intensity of the light received, so according to the law, optical density (OD), which is assumed to be proportional to concentrations of the chromophores present in the tissue can be stated as:

$$OD = -\log \frac{I}{I_0}$$  \hspace{1cm} (2.7)

Relation between OD and the concentration $[C]$ can be shown as:

$$OD = \varepsilon \times [C] \times d,$$  \hspace{1cm} (2.8)

In the above expression, $\varepsilon$, known as molar extension coefficient, is a physical constant which describes, in numerical form, the light absorbing capability of the chromophore or compound. Extension coefficient differs from chromophore to chromophore based on the factors such as wavelength, chemical and physical state of chromophore or compound, pH, temperature osmolarity, etc. [15]. It is observed that the intensity decreases exponentially when light passes through any solution or compound which is related to the distance travelled in the medium and also due to presence of light absorbing compounds and is related with the absorption coefficient $\mu_a$. 

26
Hence, assuming the medium to be non-scattering, change in intensity of incident light due to presence of only absorptive compounds or chromophores, as per law, is stated as:

\[ I = I_0 e^{-\mu_a d}, \]  

(2.9)

Also, the relation between concentration of absorptive component in solution and the absorption coefficient (\(\mu_a\)) is stated as:

\[ \mu_a = \ln(10) \varepsilon_{\lambda} [C], \]  

(2.10)

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

(2.11)

In above expression \(\varepsilon_{\lambda}\) denotes the molar extinction coefficient (unit = \(L \text{ cm}^{-1} \text{ mol}^{-1}\)) of the solution or medium at wavelength \(\lambda\), \(d\) denotes the distance between source and detector (units = cm) and \([C]\) denotes the concentration of solution (units = \(\text{cm}^{-1} \text{ mol}^{-1}\)) [15].

Based on these denotations and above explanations, the Beer-Lambert law can be stated as:

\[ OD = -\log \frac{I}{I_0} = \varepsilon \star [C] \star d \]  

(2.12)

Since, any tissue has more than one chromophore present in it, thus for multiple such chromophores, optical density at wavelength \(\lambda\) (\(OD_{\lambda}\)) or the absorption (\(A\)) due to the medium can be expressed as:

\[ A = OD_{\lambda} = \sum \varepsilon_{i\lambda} [C]i d, \]  

(2.13)

The conditions discussed above, and the expression derived are for ideal case
scenario, but the light propagation in tissues in real life is far more complex, limiting the use of Beer-Lambert law because [15]:

1) The tissue or the medium is considered to be homogenous, which ideally is not the case, since biological tissues are not homogenous and sometimes consists of several layers of different optical properties.

2) Atomic effects namely stimulated emission, optical saturation, multi-photon absorption, etc., were not considered.

3) In the law discussed above, scattering phenomenon, which is very important factor in NIRS is not considered.

Due to the reasons discussed above, the modified Beer-Lambert law was introduced.

2.5.2 Modified Beer-Lambert Law (MBLL) for accounting light scattering

Both scattering and absorption causes attenuation of NIR light in a tissue. Scattering is observed to cause more effect in attenuation than absorption and is considered to be dominating parameter, since it is the reason behind approximately 80% of the total attenuation of the incident photons, while absorption causes only the remaining 20% photon attenuation from total photons attenuated [11]. Light travelling in a biological tissue or any turbid solution, causes scattering of photons of incident light multiple times in different directions causing their total path length to increase.

This effect of pathlength increase caused by scattering component of the medium has to be included in the attenuation equation along with the effect due to absorption.
This is the reason of emergence of MBLL. Attenuation of light photons or intensity due to non-homogenous tissues can be predicted by MBLL, which includes a unitless quantity known as Differential Pathlength Factor (DPF) to include the effect due to multiple scattering events, which causes increase in total path length, in the expression and is expressed as [42]:

\[
A = \text{OD} = -\log \frac{1}{I_o} = \varepsilon [C] \cdot d \cdot \text{DPF} + G, \quad (2.14)
\]

In above expression, G denotes geometry and scattering dependent terms which includes losses caused by scattering and other boundary losses. Distance between source and detector is denoted by \(d\) and differential path length factor is denoted by DPF. The product of SDS denoted by \(d\) and the DPF (\(d\cdot\text{DPF}\)) is the “differential pathlength” or the “effective optical pathlength”, \(L_\lambda\) in cm. It is difficult to measure the absolute attenuation, since G is a factor which is dependent on measurement geometry. 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]:

\[
\Delta A = \Delta \text{OD}_\lambda = \sum_i \varepsilon_i, \lambda \cdot \Delta [C]_i \cdot d \cdot \text{DPF}_\lambda, \quad (2.15)
\]

From the equation, the background and geometry effect gets nullified due to subtraction of equations after taking into considerations the changes in OD. The values of OD, DPF and extinction coefficient must be known at the wavelengths at which NIRS is being done as all of the factors mentioned are wavelength dependent [15]. Generally using NIRS, concentration changes of the two main chromophores namely, \(\text{HbO}_2\) and \(\text{HHb}\) are measured, hence for measurements, at least two wavelengths are
required. If the number of chromophores exceeds the number of wavelengths used, then the concentration of chromophores can’t be identified and quantified properly. Whereas if the number of wavelengths used exceeds the number of chromophores being measured, the accuracy of quantification of these chromophores can be improved by using multi-linear regression so that fitting of each chromophore spectrum can be done [26].

For change in OD considering the two chromophores HbO₂ and HHb, the equation at a specific wavelength \( \lambda \) can be stated as follows:

\[
\Delta OD_{\lambda} = (\varepsilon_{\lambda,HbO_2} \Delta[HbO_2] + \varepsilon_{\lambda,HHb} \Delta[HHb]) \cdot dPF_{\lambda},
\]

(2.16) The DPF is expressed as follows [43]:

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

(2.17) \( \Delta OD \), at a specific wavelength \( \lambda \), can be expressed as follows:

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

(2.18)

\[
\Delta OD_{\lambda} = \log \frac{I_0}{I_2} - \log \frac{I_0}{I_1},
\]

(2.19)

\[
\Delta OD_{\lambda} = \log \frac{I_0}{I_2} \cdot \frac{I_0}{I_1},
\]

(2.20)

\[
\Delta OD_{\lambda} = \log \frac{I_1}{I_2},
\]

(2.21)

In above expressions, detected light intensities at times \( t_1 \) and \( t_2 \) are denoted by \( I_1 \) and \( I_2 \).
and I₂ respectively [15]. Using Equation.2.16, at two different wavelengths λ₁ and λ₂, the change in OD at those wavelengths can be calculated and solving those linear equations, we can calculate the changes in the concentration of oxy- hemoglobin and deoxy- hemoglobin.

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

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

\[
\Delta [HbO_2] = \frac{\varepsilon_{\lambda_1,HHb} \frac{\Delta OD_{\lambda_2}}{dPF_{\lambda_2}} - \varepsilon_{\lambda_2,HHb} \frac{\Delta OD_{\lambda_1}}{dPF_{\lambda_1}}}{(\varepsilon_{\lambda_1,HHb} \varepsilon_{\lambda_2,HbO_2} - \varepsilon_{\lambda_2,HHb} \varepsilon_{\lambda_1,HbO_2}) \cdot d^2}.
\]

\[
\Delta [HHb] = \frac{\varepsilon_{\lambda_2,HHb} \frac{\Delta OD_{\lambda_1}}{dPF_{\lambda_1}} - \varepsilon_{\lambda_1,HHb} \frac{\Delta OD_{\lambda_2}}{dPF_{\lambda_2}}}{(\varepsilon_{\lambda_1,HHb} \varepsilon_{\lambda_2,HbO_2} - \varepsilon_{\lambda_2,HHb} \varepsilon_{\lambda_1,HbO_2}) \cdot d^2}.
\]

Studies done by Y. Fatmehsari [22], Kim et al [44], demonstrated the use of these equations. Studies done by Bozkurt et al [1], M. Papademetriou [11] arranged and solved the equations in matrix form after simplifying them:

\[
\begin{bmatrix}
\Delta [HbO_2] \\
\Delta [HHb]
\end{bmatrix} = \overline{M}^{-1} \begin{bmatrix}
\Delta OD_{\lambda_1} \\
\Delta OD_{\lambda_2}
\end{bmatrix},
\]

Where \(\overline{M}\) denotes a constant matrix as follows:

\[
\overline{M} = d \cdot (\overline{\varepsilon} \ast \overline{PF})^T,
\]

Also, equations for \(\overline{PF}\) and \(\overline{\varepsilon}\) are defined as:
\[
\bar{\varepsilon} = \begin{bmatrix}
\varepsilon_{\lambda_1, HbO_2} & \varepsilon_{\lambda_2, HbO_2} \\
\varepsilon_{\lambda_1, HHb} & \varepsilon_{\lambda_2, HHb}
\end{bmatrix}
\]
and
\[
\overrightarrow{DPF} = \begin{bmatrix}
DPF_{\lambda_1} & 0 \\
0 & DPF_{\lambda_2}
\end{bmatrix}
(2.28)
\]

Since blood is majorly constituted of oxy and deoxy hemoglobin, the total change in blood volume (ΔBV) can also be stated as change in total hemoglobin concentration (Δ[HbT]) and can be expressed as combination of change in concentration of oxy and deoxy hemoglobin as follows:

\[
\Delta BV = \Delta [HbT] = \Delta [HbO_2] + \Delta [HHb]
(2.29)
\]

Based on concentrations of oxy and deoxy hemoglobin,

Oxygen saturation can be calculated as:

\[
StO_2(\%) = \frac{[HbO_2]}{[HbO_2] + [HHb]} \times 100
, (2.30)
\]

### 2.5.3 Differential Pathlength Factor (DPF)

Due to scattering present in tissues, path travelled by the photons is larger than actual source-detector separation. This factor is taken in consideration in modeling by multiplying the total distance (SD Separation) \(d\) by a differential pathlength factor (DPF) which is dependent on various other factors like wavelength used, type of tissue, subject age, etc.
Since from equation (2.14), we know the expression for absorption, hence light transportation through tissue, using MBBL can be expressed as,

\[ I(\lambda) = I_0(\lambda)e^{\mu_a(\lambda)d DPF(\lambda) + G(\lambda)}, \]  

(2.31)

where \( I(\lambda) \) is measured reflected intensity of light at wavelength \( \lambda \), \( I_0(\lambda) \) is incident intensity of light, \( \mu_a \) is absorption coefficient of the tissue, \( d \) is source-detector distance, DPF is differential path length factor and \( G(\lambda) \) is geometry dependent constant at wavelength \( \lambda \). The term DPF means the scaling factor which indicates by how many times farther than Source-detector distance (\( d \)) the detected light has traveled.

According to the diffusion equation for modeling light transport in semi-infinite homogenous medium, it can be shown that DPF is dependent on \( \mu_a(\lambda) \) (absorption coefficient), \( \mu'_s(\lambda) \) (reduced scattering coefficient) and \( d \). The equation is as follows,

\[
DPF(\lambda) = \frac{1}{2} \left( \frac{3\mu'_s(\lambda)}{\mu_a(\lambda)} \right)^{\frac{1}{2}} \left[ 1 - \frac{1}{\left( 1 + d(\mu_a(\lambda)\mu'_s(\lambda))^{\frac{1}{2}} \right) \left[ 1 + d(\mu_a(\lambda)\mu'_s(\lambda))^{\frac{1}{2}} \right]^{\frac{1}{2}}} \right] \] (from 2.17)

\[
DPF(\lambda) \approx \frac{1}{2} \left( \frac{3\mu'_s(\lambda)}{\mu_a(\lambda)} \right)^{\frac{1}{2}} \] (2.32)

As per above equations, DPF increases with increase in scattering and decreases with increase in absorption. DPF is dependent on \( d \) for only small values of \( d \), for \( d > 2.5 \) cm DPF are virtually independent of \( d \). According to mathematical point of view, if the inequality \( d\sqrt{3\mu_a(\lambda)\mu'_s(\lambda)} \gg 1 \) holds, than DPF is independent of \( d \). Since these equations are derived from homogenous semi-infinite medium, it is only valid for those, brains are inhomogeneous, hence equation only gives a rough approximation of the real situation in human brain. But the dependency of DPF on scattering and absorption coefficient remains unhindered [123].
2.6 Different Types of NIRS Techniques

Based on the technique of how signal is transmitted, received, signal modulation and analysis, NIRS studies are classified in three major types known as Time-resolved (TR) also known as Time domain (TD) spectroscopy, Frequency-Modulated (FM) also known as Frequency domain (FD) spectroscopy and Continuous wave (CW) Spectroscopy as illustrated and explained in Figure 2.8, with each having its pros and cons [45,46]. The working of CW and TD NIRS techniques is as described in the following paragraphs.

![Figure 2.8: Three types of NIRS techniques. (a) Continuous wave (CW) spectroscopy in which the different parameters and content concentrations are measured using the level of attenuation in light, (b) Time domain (TD) spectroscopy, in which a short Pico second impulse is sent into medium which undergoes amplitude attenuation and also gives the temporal spread function (TPSF).](image-url)
Before looking into different techniques of fNIRS, knowing about the penetration depth of laser in tissue based on SD separation is important, which will provide basic idea about working of different techniques and their drawbacks based on penetration depth and SD separation.

Generally, while doing NIRS measurements on head, reflective mode is used, in which detectors and light source both are on the surface of head separated by distance in few centimeters, since the penetration depth for this type of measurement is limited and hence is limited to acquire information from only superficial layers of brain. This penetration depth can be increased to some extent by increasing the distance between source and detector pair called Source Detector Separations (SDS), thus enabling the system to get information from comparatively deeper layers of brain. This increase in SDS, however requires increase in the intensity of light being transmitted through the source, which is also limited to maximum permissible power of laser according to ANSI standards. Also, the spatial resolution degrades with increase in SDS, thus establishing a trade-off between penetration depth and SDS. Patil et al, after conducting some phantom experiments observed the penetration depth to be 0.5, 1.3, 1.5 cm for the source detector separations of 1, 3, 4 cm respectively as described in figure 2.8 [116]. Also, as reported by Gervain et al, a for SDS of 3 cm, penetration depth of 3-5 mm into the cortex of adults and 10-15 mm into the cortex of newborns was observed [40].
Figure 2.9: Variation of penetration depth with respect to variation of source detector separation. Greater the of source detector separation, larger the penetration depth acquired. [116]

Photons incident of the head, for NIRS application, needs to travel through scalp skull, CSF and dura in order to reach brain. The scalp, which is composed of fats, skin and muscle, is 5 to 7 mm in thickness and the skull, which is made up of calcium, is 7 to 8 mm thick for adult humans, thus making 12 to 15 mm of thickness before reaching to brain [15]. Owing to this reason, surface measurements are difficult for studying brain function. Whereas the thickness of scalp and skull combined for neonates is around 5 mm, hence making it convenient for measurements. According to the studies done by Abdo et al. [37] observed is the penetration depth of NIR light, using a GaAs PIN photodiode and 830 nm wavelength laser source, into the rat brain cortex and peripheral nerve. It is found that the penetration depth of gray matter was 0.41 ± 0.029 mm, of rat sciatic nerve was 0.35 ± 0.023 mm and of white matter was 0.35 ± 0.026 mm. In this experiment, the received intensity had to be 37% photons of the incident photons for
a specific SDS, for that penetration depth. According to the studies done on human gray matter, a penetration depth of around 1.6 mm was observed for NIR light that has at 850 nm light source [38], which is comparatively larger than that in the rat. This may conclude that the attenuation due to brain cortex and peripheral cortex and nerves in rats for NIR light is larger than that in humans.

Penetration depth is also dependent on the type of wavelength being used and the tissue it is being used on based on their scattering and absorption spectrum. As seen in humans, the penetration depth in human brain starts decreasing with maturity due to the variation in lipids and proteins in the brain which affects the scattering and also it decreases with wavelength due to increase in absorption coefficient of hemoglobin at lower wavelengths [41]. Also, it is observed that infant’s brains are more transparent as compared to adults due to high penetration depth which may get affected with age owing to myelination which cause increase in scattering [26]. It is also observed that, owing to skin melanin content, the penetration depth for darker skin is lower than that of lighter skin due to lesser absorption.

Since we acquired a basic instinct about penetration depth and signal strength based on SD separation, below explained are two different types of fNIRS techniques which uses different SD separations and their limitations based on penetration depth explained.
2.6.1 Continuous wave (CW) systems

CW is the most common and simple technique in NIRS systems and is the most commonly developed (fig. 2.8(a)). Some of the advantages of this technique is it’s simplicity, absence of complex instrument and algorithms, and it’s inexpensive instrument specifications and requirements. In this technique the beam of light source is constantly illuminated continuously on the tissue being examined. These light intensities, after being attenuated, gets collected through detector, in back-reflectance mode or transmittance mode, after travelling through the tissues. In this technique, number of minimum wavelength of light depends on the number of chromophores being analyzed, number of wavelengths used is the same of number of chromophores being analyzed.

The individual scattering and absorptive effect can’t be analyzed and separated using this technique, only combined effective response is seen. Only relative changes in concentration of hemodynamics can be obtained using this technique making use of MBBL, absolute quantification of chromophore concentration can’t be determined using CW technique. In this technique, attenuation of light intensity by the tissue is assumed to be due to combined effect of scattering and absorption. Oximetry also follows the same principle as CW [15]. Earlier studies and the simulations done are referred to get the actual photon path length and DPF, as this technique can’t measure the actual photon path length. For CW measurements, DPF values affects the magnitude calculation for concentration changes in hemodynamics in the tissues. For CW-NIRS, the magnitude of change in concentration of hemodynamics are mostly
determined based on consecutive measurements or initial measurements of I(λ). Using wrong DPF(λ) may lead to wrong calculations of magnitude changes. CW-NIRS can’t measure actual wavelength dependent DPF and hence must always be estimated using the tabulated values which are previously calculated. This technique is also limited due to penetration depth as compared to other techniques such as TD and FD, but it is least expensive and has lesser acquisition time making it faster than the other two methods.

2.10.2 Time domain (TD) systems

In this technique named as Time domain (TD) spectroscopy, a small impulse of light source is transmitted through the medium being observed and then the impulse response at that impulse is analyzed, in short a small pulse in range of few picoseconds is applied and then the data, in form of photons, from the medium is collected by a fast gated detector, along with time stamps, for several seconds at detector side. The detectors used are special kind of fast photon counting detectors which counts each and every photon as function of time. A function named as Temporal Point Spread Function (TPSF) [11] is observed and analyzed by measuring the Time-Of-Flight (TOF) of the photons detected through the tissues, and the same TPSF is used to quantify the optical properties of the medium such as absorption, scattering and DPF. This time of flight in order of picoseconds can be used to analyze and estimate DPF values as proposed by D.T. Deply et al. in 1988 [42]. Using the time of flight “t” the photon pathlength i.e., distance travelled by a single photon, can be calculated by making use of the velocity of light “v” such that d = v.t. Making use of light transport
model in tissues [48], the absolute values of optical properties of the tissues such as
absorption, scattering and the absolute concentrations of hemoglobin can be calculated
from the observed TPSF. This technique uses hardware and instruments which are
more expensive and complicated as compared to other techniques. This technique uses
high speed photoreactors and photon counters, fast specialized lasers and high-speed
shutters or detectors having high speed shutters. Even though this technique uses high
speed detectors, the system needs to analyses TPSF for up to several seconds
sometimes, making this technique slower than other techniques available.

2.7 History of NIRS

Improvement and new innovations in the field of optical technology, since last
two decades, are leading to better understanding of knowledge in this field and caused
the growth and development of these methods in medical, scientific and biological
fields.

Frans Jöbsis, in 1977, expressed that non-invasive and real time detection of
oxygenated hemoglobin using trans illuminated spectroscopy is possible in NIR
region. Based on this theory, work in direction of measurement of changes in
oxygenation in brain for animal models and human adults, with the help of NIRS
prototype instrument was done by Marco Ferrari in 1980 [53] [54]. Following this
development, Jobsis and his colleagues were able to study newborn infants’ cerebral oxygenation
using NIRS, in 1985 [55]. First quantitative hemodynamics measurements, in newborn
infants, were done and reported in 1986 [56]. Hamamatsu Photonics K.K, built the first
commercial one channel continuous wave spectroscopy system named a NIRO-1000, which was based on idea of four-wavelength system as explained by Cope and Delpy in 1988 [57]. Afterwards many NIRS systems were developed for variety of applications over many years [10,14,50]. Table 2.3 describes these developments in chronological order.

Table 2.2 Chronologically arranged main events in history of NIRS systems development [50] [114].

<table>
<thead>
<tr>
<th>Year</th>
<th>Main events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>Possibility of using NIRS during hyperventilation to detect changes in adult cortical oxygenation was shown by Jobsis.</td>
</tr>
<tr>
<td>1985</td>
<td>Brazy and Ferrari did first NIRS clinical studies on newborns and adult cerebrovascular patients.</td>
</tr>
<tr>
<td>1989</td>
<td>Hamamatsu Photonics developed NIRO-1000, first commercial single-channel CW clinical instrument.</td>
</tr>
<tr>
<td>1993</td>
<td>First 6 fNIRS studies published. Using 5 single – channel instruments, simultaneous monitoring of different cortical areas (Hoshi).</td>
</tr>
<tr>
<td>1994</td>
<td>With use of single – channel NIRS system, it's first application on fNIRS was on subjects affected by psychiatric disorder (Okada). A 10 channel CW system was developed by Hitachi company (Maki). PET and fNIRS were recorded simultaneously for first time (Hoshi).</td>
</tr>
<tr>
<td>1995</td>
<td>Fast optical signal acquisition using NIRS to observe neuronal activity was proposed (Gratton). Frequency domain spectrometer used for getting first 2-D image of activated adult occipital cortex (Gratton).</td>
</tr>
<tr>
<td>1996</td>
<td>CW fNIRS data and fMRI first recorded simultaneously (Kleinschmidt). TRS fNIRS data and fMRI first recorded simultaneously (Obrig).</td>
</tr>
<tr>
<td>1998</td>
<td>Commercial single-channel CW system for fNIRS was used on newborns for first time (Meek). CW-fNIRS prototype used for first time to image motor cortex of an infant upon motor stimulation (Chance). Hitachi 10-channel system used in clinics for first time (Watanabe).</td>
</tr>
<tr>
<td>1999</td>
<td>First 64-channel TRS system introduced for adult optical tomography (Eda). First 32-channel TRS system introduced for infant optical tomography (Hebden). First optical tomography TRS images of the neonatal head (Benaron). First compact 8-channel TRS system developed (Cubbeddu). First fNIRS commercial system by TechEn company (USA) was commercialized and released.</td>
</tr>
<tr>
<td>2000</td>
<td>A 24 – channel system named ETG-100, was first commercial system and was released by Hitachi.</td>
</tr>
<tr>
<td>2001</td>
<td>A single-channel CW portable instrument and telemetry were used first time to do fNIRS study (Hoshi). First 42 channel NIRS system named OMM-2001 was released by Shimadzu company (Japan).</td>
</tr>
<tr>
<td>Year</td>
<td>Event Description</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
</tr>
<tr>
<td>2002</td>
<td>First 68 channel NIRS system named ETG-7000 was released by Hitachi company.</td>
</tr>
<tr>
<td>2002</td>
<td>First 3-D CW tomographic imaging of the brain was released (DYNOT, NIRx Medical Technologies, US) (Bluestone)</td>
</tr>
<tr>
<td>2003</td>
<td>First 52 channel NIRS system named ETG-4000 was released by Hitachi company.</td>
</tr>
<tr>
<td>2003</td>
<td>First 96 channel NIRS system named Oxymon MkIII was released by Artinis company (The Netherlands).</td>
</tr>
<tr>
<td>2004</td>
<td>First 64 channel NIRS system named NIRStation was released by Shimadzu company (Japan). CW fNIRS data and DC-magnetoencephalography were recorded simultaneously for first time (Mackert)</td>
</tr>
<tr>
<td>2005</td>
<td>First 72 channel NIRS system named ETG-7100 was released by Hitachi company.</td>
</tr>
<tr>
<td>2007</td>
<td>First 52 channel NIRS system by Shimadzu company named FOIRE-3000 was released.</td>
</tr>
<tr>
<td>2009</td>
<td>For adult pre-frontal cortex measurements, fNIR Devices company (USA) started to release a wearable 16-channel system.</td>
</tr>
<tr>
<td>2009</td>
<td>For adult pre-frontal cortex measurements, a battery-operated wearable/wireless 22-channel system was released by Hitachi company.</td>
</tr>
<tr>
<td>2011</td>
<td>For adult pre-frontal cortex measurements, a battery-operated wearable/wireless 256-channel system was released by NIRx Medical Technologies company (USA).</td>
</tr>
</tbody>
</table>
Chapter 3 – Instrumentation

Aim of this chapter is to describe the components and instruments used to develop the Fiber-Camera setup for high density multi-channel CW-NIRS system. The specifications, design and working of various parts to make the whole system, and their implementation is discussed below.

3.1 Block Diagram

As illustrated in Figure 3.1(a), is the basic 3D block diagram of the fiber-camera setup of multichannel CW-NIRS system and figure 3.1(b) shows the actual setup of the multichannel CW-NIRS device which is built. There are various components involved in the hardware namely, two lasers one for each wavelength, sCMOS camera, beam splitters and beam samplers, focusing lenses, NI DAQ card, 1 Dimensional Galvo, optical density filters, 3D printed coupling probes, optical fibers and computer. These components and their working will be discussed in the following sections.
Figure 3.1: (a) Block diagram of the multichannel CW-NIRS device setup illustration various components and their interconnections. (b) Actual setup of multichannel CW-NIRS device
3.2 Light source unit

3.2.1 Light sources

The light source used by us in our setup are laser diodes from Laserland company. The modules are named Laserland 3380 are 800 mW in power and are continuous wave laser diodes. Since we need two wavelengths, we used 785 and 808 nm lasers, reasons for these wavelengths selection are their ease of availability and since they are on two different sides of isosbestic points, they provide better quantification for change in concentration of oxygenated and deoxygenated hemoglobin. The modules used for our setup are named 808MD – 0.8W, and 785MD – 0.8W, for 808 and 785 nm respectively. One of the lasers 808MD – 0.8W is as shown in Fig. 3.2, both the lasers looks same.

These infrared laser diodes are industrial components and are mostly used in industrial tests, biomedical applications, Labs, DIY, etc. These lasers can’t work for
longer time without temperature controllers, hence fans are attached to the back of laser heads to the Heat Sink to dissipate the heat and maintain temperature of lasers under 30°C. It is powered through a separate module which has output of 12 VDC to the laser head. TTL of this module takes 3 to 5 V as On and 0V as off, if the user wants to operate it with some other triggering hardware. This laser draws current of 3 A, have divergence of 5 mrad, and have dimensions of 33*33*80 mm.

### 3.2.2 Beam splitter

To focus both the lasers at a single spot, a 50:50 beam splitter is needed which transmits 50% of light intensity and reflects remaining at an angle of 90 degree. Hence in this way as illustrated in Figure 3.1, both the wavelengths, which are at 90 degrees to each other, are diverted to the common direction with a loss of half of the optical power of both the lasers. The beam splitter being used is CCM1-BS014 by Thorlabs. It has an operation range of wavelengths from 700 nm to 1100 nm. Figure 3.3(a) shows the beam splitter used by us and figure 3.3(b). shows the illustration of working of beam splitter in form of ray diagram, which will help us understand how the intensity of incident is divided into transmitted and reflected light. After being diverged due to this beam splitter, the original power output of the lasers, which was around 800 mW, reduces to 350 mW (due to reflectance losses at beam splitter and 50% power loss by beam splitter).
3.2.3 Focusing lens

The original spot of illumination created by the lasers is elliptical in shape and has a radius of 1.5 mm, which is very big to be focused on a single optical fiber for proper transmission.
coupling. To counter this problem, a plano-convex focusing lens of focal length 250 mm was used to converge the focal spot of the lasers from 1.5 mm radius to 0.25 mm radius, so that it could couple with the fibers having radius 0.5 mm without much coupling losses.

The focusing lens being used is LA1461-B-M by Thorlabs. It has an operation range of wavelengths from 650 nm to 1050 nm. Figure 3.4.1(a) shows the focusing lens being used by us. Figure 3.4.1(b) shows the working of plano-convex lens, in form of ray diagram, of how the incident light is being converged and focused at a single spot called focal point. This focusing lens transmits approximately 90% of the optical power for both the lasers, making power output close to 310 mW for both the lasers.

Also, there is another focusing lenses used at the camera side as an objective piece to collect the data, in form of received light from sample, from fibers and to focus it on the

![Figure 3.4.2. (a) Compound lens '85-355' by Edmund optics, being used as objective piece for the sCMOS being used as detector. (b) Ray diagram of compound lens to project image from a point source to the CMOS chip on camera (Image taken from [113].)](image)

sensor of the sCMOS camera. For this application a special kind of compact and compound lenses is used, named as ‘85-355’ from Edmund optics (Figure 3.4.2(a)).
This lens has a focal length of 25 mm and has a C-mount camera montage, so it can be easily used with the camera we have. This compact lens is designed specifically and used for volume integration and are very suitable for applications such as analytical medical devices. The specifications of this lens are as following table 3.1.

Table 3.1 Lens ‘85-355’ specifications

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal length</td>
<td>25 mm</td>
<td>Aperture (f/#)</td>
<td>f/1.4</td>
</tr>
<tr>
<td>Minimum working distance</td>
<td>100 mm</td>
<td>Magnification range</td>
<td>0x – 0.25x</td>
</tr>
<tr>
<td>Focus range</td>
<td>100 mm onwards</td>
<td>Camera mount</td>
<td>C - Mount</td>
</tr>
</tbody>
</table>

The ray diagram of such compound lens to project image at a certain distance is as illustrated in figure 3.4.2(b) in which the light needs to be focused on the CMOS chip of camera which is at 25 mm from lens.

3.2.4 Optical density (OD) filters / Neutral density (ND) filters

According to ANSI standards, the maximum permissible power output for exposure of skin to laser radiation is 2.4 to 4 mW/mm². The power output we are getting for 1mm diameter fiber is 310 mW, which converts to 395 mW/mm² approximately. This power output is comparatively very high according to these standards, hence to reduce this power output to get it in the expected range, we need some intensity attenuators. In order to attenuate the intensity, we used NE20A-B by Thorlabs, the OD filters of OD 2. According to the equations as stated below, where
T is the transmittance, we will get intensity closer to 100 times less than the original intensity using OD filters of magnitude OD 2.

\[
OD = \log_{10}\left(\frac{1}{T}\right), \text{ or } T = 10^{-OD}
\] (3.1)

So according to the equations, power drops to 3.1 mW, and we get power close to 3.947 mW/mm², which is well within the permissible range according to ANSI standards.

Figure 3.5 (a) Optical density or Neutral density filter of order 2 which helps getting power range of lasers well within permissible range for skin exposure according to ANSI standards. NE20A-B, OD 2 filter, by Thorlabs. (b) Ray diagram of working of OD filters with approximate illustration of attenuation in incident light intensity to give transmitted light intensity.

Figure 3.5(a) shows the OD 2 Filter. This OD filter shows only 0.25 % power losses for the lasers being used. Figure 3.5(b) illustrates the role of using OD filter in reduction of incident light and providing the transmitted light with comparatively less intensity as shown in form of ray diagram.
3.2.5 Galvo scanner system (1 dimensional)

As illustrated in figure 3.1., a Galvo scanner is needed to focus the beam of light on different points on the laser coupling probe to get multiple source points from single laser source. A 1 dimensional Galvo scanning system consists of main three components, as explained in the block diagram of 1 dimensional Galvo scanning system (figure 3.6.a), namely (a) 1 axis Galvo motor with a mirror attached to it (figure 3.6.c), (b) Servo controller board also known as Galvo driver card (figure 3.6.c) and (c) A linear power supply (figure 3.6.d).

Figure 3.6: Galvo scanner system. (a) Block diagram of 1 dimensional Galvo scanning system, (b) Heat sink for driver card, (c) 1 dimensional Galvo mirror scanner along with Galvo driver card, (d) Galvo system linear power supply.
The 1-dimensional Galvo scanner being used is GVS311 by Thorlabs. It has an operation range of wavelengths from 532 nm to 1064 nm. A Galvo scanner is basically a servo motor with its shaft attached to a reflective mirror to deflect the incident light at a particular angle, this deflection is controlled by the rotation of shaft which can be controlled with high resolution using a Galvo driver card. Galvo scanner can scan ±20 degrees as full range for an output voltage of ±10V respectively, although maximum optical scanning angle is +40° and -16°, but is not recommended. It has an angular resolution of 0.0008° and maximum scanning frequency of 1 kHz. The system needs 0.5V/°, means every 0.5V input provided, it can deflect to one degree. The maximum operable input it can take is ±15V at 3A.

One of the most important and complex part of Galvo scanning system is the Galvo driver card. This component is used to set various parameters for the servo motor used,
such as the scanning angles resolution, step size, external or internal input, the sequence for driving the servo motor, etc. It is divided into various parts as described in figure 3.7.a. All of these connections are as described in the manual provided by Thorlabs for GVS311. We are interested in giving external input to the driver card to synchronize it with the external controlling unit. To do so, JP4 section of driver card needs to be identified on the card. All the sections on the driver card is as illustrated in figure 3.7.a. The pin 2 and pin 3 of JP4, as shown in figure 3.7.c., needs to be connected via a jumper. After that to enable the external input, J7 connector needs to be identified. Pin 4 of the J7 needs to be applied with 5V signal to get the driver card into ‘enable external’ mode (figure 3.7.b.). After doing that the Driver card can be used by external control unit to control the servo motor. The pin 1 and 2 of J7 are +ve and -ve command inputs respectively for the driver card which will be used to control the servo motor. Pin 3 is not connected, pin 5 and 6 are +ve and -ve 12V output respectively for the servo motor, and pin 7 and 8 are ground. Operating temperature range of this driver card is +40°C, hence to maintain it’s temperature below the specified temperature, a heat sink is used for this card as shown in figure 3.6.b., which helps dissipating the heat to the surrounding, maintaining the temperature of driver
card lower than 40°C or close to room temperature.

Another component for the Galvo scanner system is the linear power supply. The output from this power supply is connected in J10 section of the driver card (figure 3.7.a.). The input voltage of this power supply 115 V or 230 V at 47 to 63 Hz AC. This power supply steps down the voltage and provides the driver card with ±15 V and 3A DC. The main reasons of using this power supply is to convert AC to DC and supplying a regulated power supply.

### 3.2.6 DAQ

To act as interface between the controlling unit (computer), laser sources and Galvo, to control, by computer, the switching of laser sources and setting the positions for Galvo, an interfacing medium was required. The DAQ modules by National Instruments helped to fulfill this purpose. Two NI modules were used, one to connect to computer and other to connect to the peripheral devices namely cDAQ 9171 (Figure 3.8.a.) and NI 9263 (Figure 3.8.b.) respectively.

![Figure 3.8: NI DAQ modules. (a) cDAQ 9171, (b) NI 9263 with pinout.](image)
The NI module named as cDAQ 9171 is a compact chassis which used to connect over +60 different interface modules, to the computer, based on the peripherals and the connection type. In the device developed, this module is used to connect computer with NI 9263, which in turns connect to the other peripheral devices to be controlled. It uses a USB 2.0 bus connectivity protocol and has only one slot. This module has power consumption of 5V and 500Ma.

The second module NI 9263 is a module which converts the digital signals from computer to analog signals which is to be transmitted to the peripheral devices. It is basically a 4-channel input output device, as it can be seen in pinout in figure 3.8.b. This module has an output range of ±10V, is 16 bits, meaning minimum resolution of $3.5 \times 10^{-4}$ V, and operates at speed of 100 Kilo samples/sec/channel. It has screw terminal connectivity which is well suited for our peripherals as they have only single stranded wires as input. Since we have only three peripheral devices namely 808 nm laser, 785 nm laser and Galvo scanner, this module with 4 channels is enough for interfacing the whole device. Since the TTL of lasers takes only +3 to +5 V as ON state, and Galvo has range of ±10 V as input, the output range for this module is also well suited for the application. Using this module, the lasers are switched and the Galvo scanner sweeps the laser pointer over well-defined spots, which is well explained in coming sections.

NI DAQs have an Application Programming Interface (API) with a variety of development options including LabVIEW, DAQExpress, C, C#, Python, MATLAB and others. This is like a source code file which can be downloaded, from National Instrument website, in computer to operate the hardware using different programming
3.2.7 Optical fiber

Optical fibers are a transparent flexible fiber made by materials such as Plastic or glass. It has diameter ranges from several millimeters to few micrometers. It is used to transport light using the principle known as total internal reflection, which can be better explained by Snell’s law. Since light is the used to transmit the data through these optical fibers, losses are very less, and speed of data transmission is very high, which is favorable for our application, since we need both transportation of light and high-speed data to get higher frequency of data. There are different types of fibers used and they are classified based on the material of composition, their diameter, etc., for example, some of them are single mode fibers having diameter less than 50 micrometers, multimode fibers having diameter more than 50 micrometers, plastic fibers having core made of plastic and shows fiber losses more than glass fibers whose core are made up of glass but are less flexible and more fragile as compared to plastic fibers.

For our application, we need multiple source and detector locations for multichannel data collection. Some of our application may be based on hairy region of head. Owing to above points the issues are flexibility of the fiber to place inside probes on head, and the signal attenuation in hairy part of head. Hence to counter this
problem, we decided to use plastic fibers to counter the flexibility of fiber problem, and to check the low signal problem, we decided to go with multimode fiber having greater diameter, hence allowing better signal collection.

Figure 3.9: Optical fiber SH4001-1.3. (a) Optical fiber SH4001-1.3, (b) Cross section of optical fiber, (c) Many optical fibers bundled together to form a multichannel source detector probe.

The fibers being used in the device is named SH4001-1.3 (figure 3.9.a), manufactured by Mitsubishi Rayon Co. LTD. It has small transmission losses close to 2 dB per meter, based on the transmission loss spectra provided by the manufacturing company, for wavelengths we are using. Since we are using fiber length of 3 meters, optical losses will be close to 6 dB. For this optical fiber the specifications are as described in table 3.2. Also, these fibers are light weight, hence for multichannel
measurements for high density applications on brain scanning and other applications, this fiber is suitable. Due to all these unique characteristics these fibers are ideal for multichannel imaging for NIRS application, and a bundle of optical fibers (figure 3.9(c)) can be used for this application.

Table 3.2 Optical Fiber SH4001-1.3 Specifications

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical fiber</td>
<td></td>
</tr>
<tr>
<td>Core refractive index</td>
<td>1.49</td>
</tr>
<tr>
<td>Core diameter</td>
<td>980 micrometers</td>
</tr>
<tr>
<td>Cladding diameter</td>
<td>1000 micrometers</td>
</tr>
<tr>
<td>Numerical aperture</td>
<td>0.5</td>
</tr>
<tr>
<td>Refractive index profile</td>
<td>Step Index</td>
</tr>
<tr>
<td>Jacket</td>
<td></td>
</tr>
<tr>
<td>Diameter</td>
<td>1300 micrometers</td>
</tr>
<tr>
<td>Approximate weight</td>
<td>1.5 gram/meter</td>
</tr>
</tbody>
</table>

3.2.8 Coupling probes

Since we are using fibers as the mode of transport of light as signals, we need something to hold these fibers at proper place for both coupling of laser into fiber faces and also to be at a fixed place at the camera lens so that each fiber has an allotted location while imaging this grid of fibers via the camera.
Figure 3.10: 3-D printed coupling probes (a) Camera coupling probe (on left) and Sample analysis probe (on right), (b) Laser source coupling probe

There are two main fiber coupling probes and one sample analysis probe which has been used in the setup. The two coupling probes are laser source coupling probe and camera coupling probe. The sample analysis probe (Figure 3.10.a) is designed according to the application, and changed on various occasions, but the two coupling probes (figure 3.10.a and figure 3.10.b) are kept constant always.

These probes are 3-D printed using a flexible material known as “Ninja Flex” and are printed with a printer named as ‘CraftBot Plus’. These coupling probes are solid cylindrical structures with holes printed in it to hold the fibers at proper place. These probes are cylinders with diameter 30 mm and height 10 mm. It has holes printed with diameter 1.2 mm and each hole is at least 3 mm away from each other. As described by the color coding in figure 3.10.a, red circles are used to hold short separation (SS) detector fibers, on the camera coupling probes, after placing the fibers properly in the SS slots, the fiber faces are covered with a polarizer film to attenuate the intensity of these fibers so that the dynamic range of the detector camera can accommodate both
the SS and long separation (LS) signals. Since LS signals are less in intensity, blue circles on camera coupling probe, they are not covered with polarizer film. This camera coupling probe is placed right before the objective piece (compound lens) in front of camera such that the objective piece is in middle of camera coupling probe and camera. By the design of the camera coupling probe, we can see we have 9 SS detectors, 16 LS detectors and 1 reference fiber.

The laser source coupling probe (figure 3.10.b) is also a cylindrical structure with diameter of 30 mm and height of 10 mm, we are using nine fibers on the laser coupling probe, so that we can have nine sources. This laser coupling probe is kept in front of Galvo scanner, so that Galvo can deflect the laser beam on the specified location on probe and we can get many number of sources as Galvo deflects laser sources on each fiber face at different times (mostly after couple of milliseconds, so that we get enough signal and the speed required for scanning). Since the material of probe is black in color and has a rough surface, even if laser beam between two source fibers, it won’t give any overlapping signal as most of the light will be absorbed by the material of probe.

3.3 sCMOS Camera (Detector)

Since, the aim of this study and device development is to create a cheaper alternative to the current brain imaging techniques, we did certain changes in the current conventional optical imaging devices. First of all, we used scientific cameras, especially for this purpose, which can image many detectors instead of just single detector. Now these kinds of setups were also used in past, but with help of a CCD
which are more expensive and less efficient than sCMOS (Scientific CMOS) cameras which is used in the setup designed. Let’s discuss the suitability of each camera in details as follows [115].

While selecting a camera, special care should be taken based on its application. For optical imaging purpose, CCD technology were widely used due to its high resolution and sensitivity, especially in low light applications. However, a less expensive alternative was developed called CMOS cameras which even allowed the user to take data at a faster rate, or faster reading capability. For a long time, CMOS cameras were inferior to CCD cameras in terms of quality, but in recent years sCMOS sensors were developed which overcame this obstruction and helped CMOS cameras to replace CCD in terms of efficiency, Signal to Noise Ratio (SNR), quantum efficiency, dark noise, etc. [115].

Working of CCD & CMOS sensors is described as follows. CCD sensors and CMOS sensors have different ways and methods of handling charges generated due to exposure time of photosensitive cells, as illustrated in figure 3.11. In CMOS sensors, each pixel has its own electronic circuit and hence, electrical signal generated due to signal (photons) can be read simultaneously from all of them, thus improving the readout time of signals. Whereas in CCD the electrons generated due to signals are collected in pixels and then shifted throughout the row or column, using electrical field, and then read by A/D converter. This is the main cause of higher read time, causes excess electrical consumption, more heating and gets subjected to effects of blooming and smearing. [115].
Now to compare CCD and CMOS cameras, specific examples are taken, and their specifications are compared in a table. These data are EMVA1288 data and are collected according to standardized European Machine Vision Association specifications to make it feasible for different cameras to be compared based on their performance data. Now the cameras being compared are ICX285 and ICX274, high quality CCD sensors, and ICX625, medium quality CCD sensor being compared with IMX174 a high-quality CMOS sensor and IMX250, a medium quality CMOS sensor. As shown in table 3.3, according to their specifications such as quantum efficiency, temporal dark noise, saturation capacity, dynamic range and SNR, comparisons are done. As we can see in the table below, all the specifications are showing better quality in CMOS as compared to CCD sensors. [115].
Table 3.3  EMVA1288 data comparison of various Sony CCD and CMOS sensors. Taken from [115].

<table>
<thead>
<tr>
<th>Technology</th>
<th>CCD</th>
<th>CCD</th>
<th>CMOS</th>
<th>CCD</th>
<th>CMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensor</td>
<td>ICX285</td>
<td>ICX274</td>
<td>IMX174</td>
<td>ICX625</td>
<td>IMX250</td>
</tr>
<tr>
<td>Resolution (Mega pixels)</td>
<td>1.4</td>
<td>2.0</td>
<td>2.3</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Pixel size (Micro pixels)</td>
<td>6.45</td>
<td>4.4</td>
<td>5.86</td>
<td>3.45</td>
<td>3.45</td>
</tr>
<tr>
<td>Frame rate (per second)</td>
<td>17</td>
<td>14</td>
<td>155</td>
<td>17</td>
<td>75</td>
</tr>
</tbody>
</table>

EMVA Data

<table>
<thead>
<tr>
<th></th>
<th>CCD</th>
<th>CCD</th>
<th>CMOS</th>
<th>CCD</th>
<th>CMOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantum efficiency (545 nm)</td>
<td>58%</td>
<td>51%</td>
<td>70%</td>
<td>47%</td>
<td>67%</td>
</tr>
<tr>
<td>Temporal dark noise (e-)</td>
<td>7.8</td>
<td>8.0</td>
<td>6.8</td>
<td>12.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Saturation capacity (e-)</td>
<td>19,000</td>
<td>9,000</td>
<td>32,700</td>
<td>7,000</td>
<td>10,700</td>
</tr>
<tr>
<td>Dynamic range (dB)</td>
<td>67.7</td>
<td>61.0</td>
<td>73.6</td>
<td>54.8</td>
<td>73.0</td>
</tr>
<tr>
<td>SNR max (dB)</td>
<td>42.8</td>
<td>39.5</td>
<td>45.1</td>
<td>38.5</td>
<td>40.3</td>
</tr>
</tbody>
</table>

To simplify the comparison for one example of both sensor type with same quality and identical pixel type, based on SNR and dynamic range, a SNR graph is produced for wavelength 545 nm. Figure 3.12 shows the diagram of SNR with respect to the response the sensor gives to the amount of light striking the sensor for both 5 mega pixel camera sensors. The higher value of SNR means higher precision and capability of camera to convert incoming light photons to electrical signal. As shown in figure 3.12, yellow line is the SNR of an ideal camera (assuming the camera don’t have any noise and have 100 % quantum efficiency) which represents an optimized signal. The closer the camera’s SNR curve runs toward the yellow line, the better the signal quality.
of camera and low noise. The more camera moves towards right, higher is the saturation capacity of camera, meaning it can collect and process more photons of light in single exposure. Also, camera have a term called ‘shot noise’ which is also known as photon noise and is the lowest point of SNR on graph where SNR is 0 Db, which implies that signal and noise are same in camera image at that particular intensity of photons. This is also the minimum amount of light required to get an image or signal. The difference between the right most and the left most point on SNR graph, i.e. difference between the lowest photon count a camera is required to work and highest photon counts a photon can process and accommodate, is known as dynamic range of that camera. Higher the dynamic range of camera greater the ability of camera to differentiate between dark and light signals and better the resolution. [115].

Figure 3.12. Signal to noise ratio diagram of CCD and CMOS cameras (Ideal camera (Yellow), CCD (ICX625 red) and CMOS (IMX250 Green)). Images taken from [115].
The point in the SNR diagram where SNR reaches 0dB, also represents temporal
dark noise in the camera, which occurs in darkness with exposure time of 0 s (meaning
this noise is having greater effect in low light conditions). The electronics and their
reading process plays an important role in this condition. The influence of temporal
dark noise on SNR is very high in low light conditions. As illustrated in figure 3.13,
CCD and CMOS sensors were compared (keeping variables such as exposure time,
gain, sensor temperature constant

Figure 3.13. Noise behavior comparison (Temporal Dark Noise) of CCD and CMOS
sensors with 10 milliseconds exposure time. Images taken from [115].

constant and same for both the sensors). As seen in figure above, difference between
ICX625 (CCD) and IMX250 (CMOS) can be seen clearly. Both sensors have
resolution of 5 mega pixels. As we can see, CCD is showing higher noise (lines and
text can’t be seen clearly) as compared to the CMOS sensor. Now testing
ICX285(CCD) with IMX250 (CMOS), the CMOS was tested with binning 2x2 to compensate for the bigger pixel size of CCD. For both the binning cases CCD is showing higher noise as compared to CMOS. The unbinned image is also shown which shows lower noise than the binned image since resolution increased four times.

[115].

There are even more parameters influencing then image quality for low light applications. Some of them are quantum efficiency, pixel size and dark current noise. The dark current gets more pronounced as the exposure time of camera increases, since it is generated in sensor of camera e.g. with means of leakage current. This noise is also dependent on the temperature of the sensor, higher the exposure time higher the temperature and hence giving rise to more dark current. To compare the dark current noise in both the sensors they were again compared, this time at exposure time of 4 seconds. As in previous example both the cameras were compared (keeping the influencing variables constant) and keeping in mind their sensor size, and resolution. As it can be seen as illustrated in figure 3.14, both CCD and CMOS sensors were compared. As seen in figure 3.14, difference between ICX625 (CCD) and IMX250 (CMOS) can be seen clearly, since they have same resolution and pixel size, the difference in noise behavior is more clear. As we can see, CCD is showing higher Hot pixels (pixels that respond disproportionately strong to the incoming photons and hence seem bright) as compared to the CMOS sensor. Now testing ICX285(CCD) with IMX250 (CMOS), the CMOS was tested with binning 2x2 to compensate for the bigger pixel size of CCD. For both the binning cases CCD is showing higher noise
and more hot pixels as compared to CMOS. The unbinned image is also shown which shows higher hot pixels than the binned image but it also has resolution increased four times. [115].

**Figure 3.14.** Noise behavior comparison (Dark current noise) of CCD and CMOS sensors with 4 seconds exposure time. Images taken from [115].

Many people have done studies using similar setups, but they used one detector specially dedicated to a single detector fiber, these detectors ranged from simple photo diodes to highly advanced Avalanche photo diodes (APD), making several detectors
for multiple channel studies, making the setup complex, bulky and expensive. The reason behind using a camera for detector is to use each of it’s pixels (here 6553600 pixels for zyla 5.5) as an individual detector. This gives us, theoretically speaking, 6553600 separate detectors (in case of this particular camera), thus removing all the extra detectors required, for each detector location, making the setup more affordable, less space consuming, simple, fast for algorithm and peripheral interfacing, etc. thus giving us many advantages.

Figure 3.15. (a). ZYLA 5.5 sCMOS camera being used in our setup. (b) Pixel sensor array of CMOS sensor. c) Parts of CMOS sensor. Images taken from [110] and [112].
As described in figure 3.15(c), a CMOS camera sensor consists of some basic parts such as color filter, pixel array, digital control and ADC. These are as described in following paragraphs.

**Color filter:**

Color filter is basically an array of tiny color filters placed over pixel sensor array to capture color information. These filters allow only a certain band of wavelengths to transmit through to pixel sensor, by filtering out other unwanted wavelengths, because array of pixel sensors can only detect light intensity but not wavelength, hence a color filter is needed [Zumdahl, 2008]. These color filters can be designed so that it can permit transmittance of any desired single color (band of wavelengths) [112].

**Pixel array:**

Pixel array is made up of millions of active sensors in form of pixels. Each of these pixels captures the light intensity falling on them and converts these intensity into voltages and then transmits these voltages to other part of chip (ADC) as shown in figure 3.15(b). The conversion of voltages from light intensity is based on a principle called photoelectric effect, in which it is stated that when energy in form of electromagnetic radiation (of certain wavelength) is absorbed by or bombarded on a certain metal, they emit electrons thus causing potential difference to rise causing voltage generation. The energy being absorbed by the metal or being bombarded on the metal is directly proportional to the intensity of photons, and this is directly proportional to the electrons being generated, hence voltage generated (electrons emitted) is directly proportional to the intensity of photons incident on the pixel of CMOS chip [Fowler, 1997]. These voltage signals from each pixel is then combined
and outputted as single signal [112].

Digital control:

Digital controller consists of various components such as oscillators, clock generator, etc. to make sure of synchronization of each and every pixel in an array. This helps controlling the initialization of capturing of light, exposure time etc. This circuit basically controls the pixels in the array of CMOS chip [112].

Analog to digital converter (ADC):

As stated by the name itself, this part is responsible for converting the analog voltages from the array of pixels to it’s corresponding digital values, which will be further available for post processing to different parts of the cameras, which is not CMOS sensor, or to the end user [112].

Theory of operation

After receiving signal from digital controller, the pixel sensors in pixel array starts capturing the intensity of light which is color filtered, and then provides the corresponding analog voltage values. The analog voltages are then converted into the corresponding digital values with the help of ADC so that these digital values are ready for further post processing by other components of the device or by end user [112].

The camera used for detection of the light intensity is a scientific grade Complementary Metal–Oxide–Semiconductor (CMOS) camera. We are using a sCMOS camera named ‘ZYLA 5.5 sCMOS’ by ANDOR, as shown in figure 3.15 (a). It is a 5.5 megapixel camera having very fast frame rate of magnitude of 100 fps with camera link with rolling shutter and 49 fps with global shutter for an array size of
2560x2160 pixels. The speed of the camera depends on the type of shutter being used, exposure time, type of connectivity, number of pixels being used, binning, etc. Table 3.4 shows the rated speed of camera with respect to connection type, shutter type and region of interest (ROI) array size of camera being used. The quantum efficiency of the camera, at the wavelengths we are using, is in range of 30% - 35%.

Table 3.4 Frame rate of Zyla 5.5 across different ROI sizes and connectivity: 12 (16) bit mode

<table>
<thead>
<tr>
<th>Array size</th>
<th>Zyla 5.5 USB 3.0</th>
<th>Zyla 5.5 Camera Link</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rolling shutter</td>
<td>Global shutter</td>
</tr>
<tr>
<td>2560 x 2160</td>
<td>40(30)</td>
<td>40(30)</td>
</tr>
<tr>
<td>2048 x 2048</td>
<td>53(40)</td>
<td>52(39)</td>
</tr>
<tr>
<td>1920 x 1080</td>
<td>107(80)</td>
<td>98(80)</td>
</tr>
<tr>
<td>512 x 512</td>
<td>419(419)</td>
<td>201(201)</td>
</tr>
<tr>
<td>128 x 128</td>
<td>1639(1639)</td>
<td>716(716)</td>
</tr>
</tbody>
</table>

This camera has a highly advanced parallel readout architecture so that the fps could be increased. All the 2560 columns of the sensor have their own built in amplifiers and Analogue to digital converters (ADC), at both the ends, meaning at top and bottom of all the columns, ensuring that all the columns read in parallel and to increase speed further, readout direction of these columns is center spliced so that the signals can be read out from top and bottom halves simultaneously.

Since this camera is 16 bit, it has high dynamic range, making it suitable for multichannel data acquisition setup. This camera has a sensor size of 16.6 x 14.0 mm, with each pixel of 6.5 µm size (width x height). It has maximum pixel readout rate of 560 Hz, and quantum efficiency of 60% maximum. It has maximum dynamic range of 33,000 :1 for a data range of 16 bit. Minimum ROI possible for this camera is 4 x 8
This camera is highly linear with linearity of 99.8% for full light range. It has limited binning options, for better read out of data and to improve speed of acquisition, such as 2x2, 3x3, 4x4 and 8x8. This camera also has various types of trigger mode such as Internal, external, software, etc. increasing the versatility this camera.

Andor have different software packages and command libraries to enable user to use this camera on different programming platforms. These software packages are named as Solis Spectroscopy, Andor SDK3, GPU Express, etc. Among these softwares we used Andor SDK3, which enables user to use this camera with various programming platforms such as C, C++, LabView, Matlab, etc. These softwares are available on the company website.

### 3.4 User- interface

In order to receive the data continuously from the camera, switch the lasers, operate Galvo to set it to certain locations and to synchronize all these devices with computer, a user interface platform is necessary. As it is mentioned in previous sections, interfacing can be done using various softwares and different programming platforms, for our application, we interfaced these devices using MATLAB. The counts for different phantoms, location of fibers, performance of different fibers, minimum exposure time required, etc., could also be checked using the software specially developed for the camera being used, the software is called ‘Andor Solis’.
3.4.1 Andor Solis

Andor Solis is a software specially developed to control and analyze the output of Andor’s cameras. There are different packages and versions of this software namely time resolved Solis, spectroscopy Solis, etc. This software is generally used for data acquisition, real time analysis and processing of the signals coming from the camera, automate storage of the files generated, communicate and control eternal peripheral devices, manipulate the data and macros building to automate the experiments.

We generally used this software for preliminary data testing and signal strength analysis for different combinations of phantoms and SD separations. This software provides the signal strength for all it’s pixels in real time analysis, thus giving clear idea of the scenario. Since each pixel is quantified in terms of signal strength, it makes it easy to figure out the problem with the setup in developing phase. This software also provides tabs for binning, Imaging dimensions, exposure time, shutter type, etc., which makes it easy to try different combinations to get optimum result.

Some of the benefits of this softwares are it’s real time data display which is well suited for improving alignment of the setup, real time quantification and charting capability useful for optimizing the experiments, real time data calculation and processing such as background correction, minimizing memory and increasing fps using simple variations in binning, exposure time and array controls, and user selectable triggering options. This software is compatible with most of the operating systems.
3.4.2 MATLAB

While Andor Solis was helpful in development phase of the setup, MATLAB was used to interface and synchronize all the peripherals such as Lasers, Galvo through DAQ, and also the initialization, triggering, data acquisition, parameters settings, and shutting down the camera were controlled using MATLAB. All of these steps were performed by MATLAB. After collecting the images from the camera, the images were processed to identify the location of each and every fiber location, then data for each fiber from each image was collected and then processed simultaneously to give us the hemodynamic responses and contrast information, as will be shown in coming sections along with the experiments performed. The data is collected serially and then stored. After acquisition is done, the data is processed to give results in form of linear graphs or 2D images. This whole process is batch wise process rather than continuous process, at this moment. To make this process continuous, algorithm change is required, which is a task to be done in future.
Chapter 4 - Device validation and results

4.1 Testing for laser stability

Output stability of the Laser is an 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 Laser output fluctuates over the course of the measurements, these fluctuations will result in changes in the detected NIRS output, which might then be considered falsely as tissue hemodynamic changes. Therefore, the Laser stability was measured over a span of 10 minutes to measure its standard deviation after normalizing it’s output with the initial value. A liquid phantom made from intralipid and Indian ink, having optical properties of absorption parameter 0.1cm⁻¹ and scattering parameters of 10.0 cm⁻¹ at wavelengths 795 nm respectively, was used. Shown in Figure 4.1. are the normalized results. For the same input current and output power rating for both the Lasers, the standard deviation obtained for the 785nm Laser and that of the 808nm Laser was approximately same. The standard deviation for both the wavelengths is as shown in Table 4.1. The standard deviation is low enough to not have a significant effect on intensity change calculations or on the chromophore concentrations changes calculation. The % change in intensity of laser in cortical region is assumed to be less
than 5 %, hence the laser should have stability of standard deviation less than 5 %.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>808nm</th>
<th>785nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard deviation</td>
<td>0.0004</td>
<td>0.0006</td>
</tr>
<tr>
<td>% Standard deviation</td>
<td>0.04</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 4.1 The results of the laser stability testing showing standard deviation and percentage standard deviation of the two wavelengths.

Figure 4.1: Plots of the results of the laser power stability testing over 10 minutes.
4.2 Signal – to – noise ratio (SNR) testing

To quantify the measurement sensitivity of developed sCMOS based NIRS- DOT imaging system, a liquid phantom with absorption coefficient 0.1 cm\(^{-1}\) and scattering coefficient 10 cm\(^{-1}\) was used (made using Indian Ink and Intralipid). The distance between source – detector fibers ranged from 1 to 4 cm. The integration time for sCMOS was set for 2 ms. The signal to noise ratio calculated for both the wavelengths. The actual signal acquired was average to get signal intensity. Now to get noise, standard deviation of the background noise was taken when lasers were off. Now the ratio of signal to noise was calculated.

The intensity measurements for wavelengths 785 and 808 nm for the fiber camera setup is presented as in fig.4.2.1. We can see that the SNR is decreasing exponentially as function of distance for both the wavelengths. But the SNR can be considered quite well even at 4 cm S-D separation since it is close to 200, it is better than most of electronic photodiodes which has SNR of 60 at SD separation at 2 cm, for both the wavelengths. SNR for both the wavelengths at 1 cm to 4 cm for sCMOS camera setup is close to PMTs as compared from other papers. Hence this setup works quite well [88].
SNR of camera is also calculated by the formula as follows:

\[
SNR = \frac{QE \times S}{\sqrt{F_n^2 \times QE \times (S + I_b) + (Nr/M)^2}}
\]

This equation gives the relation of SNR with respect to the signal or counts, where,

- \( QE \) = Quantum efficiency (0.35 for 785 nm and 0.3 for 808 nm),
- \( F_n \) = Noise factor (1.44 in our sCMOS),
- \( I_b \) = Background counts,
- \( S \) = Input signals,
- \( Nr \) = Readout noise (2.4 for this case for global shutter (at 560 MHz readout rate)),
- \( M \) = Electron multiplying (EM) gain (1 for sCMOS),
Nd = Dark noise (assumed to be zero) [110, 111].

Quantum efficiency is the ability of camera to convert the incident photons into photoelectrons. This is dependent on wavelength. SNR is directly related to QE.

Readout noise is the measure of fluctuations of the photoelectrons which converts the charge of photoelectrons from each pixel to it’s related digital number expressing it’s intensity.

Dark noise is the noise generated due to thermally generated electrons which are results of heat generated by the camera. This noise is time and temperature dependent. Since most of the cameras are equipped with cooling fans and they operate at very low exposure time, this noise is usually negligible.

Signals here are the average of the signal counts acquired over a time of 10 seconds. Similarly, background signal is also averaged. Sometimes the background signals make signals look higher increasing SNR which is not actually the case, hence it is important to take the background signals into consideration [111].

All the values above are taken from datasheet of Andor zyla 5.5 sCMOS camera [110].

For ideal camera, QE = Fn = 1; N_r = I_b = 0. Therefore the equation for SNR becomes as follows,

\[
SNR = \frac{1 \ast S}{\sqrt{1^2 \ast 1 \ast (S + 0) + (0/M)^2}}
\]
Which in turns becomes

\[
SNR = \frac{S}{\sqrt{S}} = \sqrt{S}
\]

Calculating and comparing the SNR data of ideal and actual camera, we get results as illustrated in figure 4.2.2

![SNR vs Counts](image)

Figure 4.2.2: SNR with respect to counts for both ideal camera and sCMOS.

### 4.3 Ink titration linearity testing

Now to follow up on systems ability, sensitivity and accuracy in detecting small changes in the absorption parameter, and to quantify the concentration and absorption changes properly, the Ink titration experiment (in reflectance configuration) was performed. In this experiment, a tissue mimicking liquid phantom was made with absorption coefficient 0.1 cm\(^{-1}\) and scattering coefficient 10 cm\(^{-1}\) with the help of intralipid (Intralipid 20% by Fresenius Kabi) and Indian Ink. After a regular interval, 0.1ml of Indian ink was added so that absorption coefficient of the phantom should
increase linearly. The scattering parameter of the phantom was kept constant by keeping the quantity of intralipid constant in the liquid phantom. As the concentration of ink increased the intensity of the light decreased and the absorption coefficient of the phantom increased. The output as calculated theoretically and observed by the device is as demonstrated in the following Figure 4.3.

Using MBBL and the detected output Photon counts, the experimental values of absorption coefficient were obtained. As we knew the exact amount of the ink we were adding to the phantom, we were able to calculate the theoretical values of the absorption coefficient in the phantom. The figure given below (fig 4.3) shows actual absorption coefficient calculated by camera and it’s linear equation and it’s projection on the map. It has $R^2$ value of 0.9989, which indicates that the linear equation and the actual data plotted are highly co-linear, hence it proves that the system works perfectly and quantifies data accurately. The exposure time for sCMOS was 1 ms here and S-D separation was 3 cm. This shows that data can be measured and quantified appropriately and concisely using this setup since it follows the linearity trend of result (results for rise in absorption coefficient as there is rise in concentration of ink) for ink titration experiment in the figure below, solid line is observed absorption coefficient and dotted line is fitted theoretical absorption coefficient.
Figure 4.3: Ink titration experiment results. Comparing observed and theoretical values of absorption coefficients.

4.4 ICG bolus experiment

Now in order to mimic the dynamic nature of hemodynamic changes in human brain, we made a Liquid phantom mimicking human brain. The phantom consisted of a 3D printed cylinder with a hollow tube running across it, as shown in fig.4.4. The cylinder was filled with a liquid solution with absorption coefficient 0.1 cm\(^{-1}\) and scattering coefficient 10 cm\(^{-1}\). The hollow tube was connected to a variable flow pump. Initially the pump was pumping normal solution of liquid phantom, having properties absorption coefficient 0.1 cm\(^{-1}\) and scattering coefficient 10 cm\(^{-1}\), through the tube, for 7 minutes (tap A open and tap B closed). This data was collected using the setup and was considered baseline. Now to mimic the induced perturbation, the tube was pumped with a solution containing ICG (tap B open and tap A closed). The whole
solution had 1µM of ICG. Now due to the properties of ICG it absorbs one wavelength more than the other (808nm is absorbed more than 785nm) hence mimicking the perfect perturbation effect. Now this solution was pumped for 7 minutes followed by normal solution pumping for 7 minutes (tap A open and tap B closed). The exposure time for sCMOS was 1 ms here and S-D separation was 3 cm.

Figure 4.4: (a) Experimental setup showing the dynamic phantom made with help of 3D printed cylinder, hollow tubes and pump. (b) Top view of container and SD configuration.

The bolus experiment described above was tested on a commercial Frequency domain device namely ISS Oxiplex TS, and it’s results are then compared with the fiber-camera setup described. The results from both the devices as shown in fig. 4.5.1 and figure 4.5.2, shows similar trend with same magnitude of concentration changes. This shows that the fiber-camera setup described works similar to the commercial device which is far more expensive than this setup described and has limited number of sources and detector combination where as there is no theoretical limitation to S-D combination in the fiber-camera setup. The results show 7 minutes baseline, then rise in both the mimicked results of oxy hemoglobin in both the system and slightly rise in mimicked results of deoxy hemoglobin, for 7 minutes (Here ICG
is used as a perturbation and the extension coefficients of oxy and deoxy hemoglobin are used to show the results in term of chromophore concentration changes, these are mimicked concentration changes). Then both the results from both the system go back to baseline again for 7 minutes. This shows that the temporal resolution for both the system is similar as well as their ability to quantify the results. fig. 4.5.1 is ISS results and fig 4.5.2 is sCMOS camera setup results.

Figure 4.5.1: Mimicked oxy and deoxy concentrations of ICG bolus experiment using the commercial frequency domain device named ISS Oxiplex TS.

Figure 4.5.2: Mimicked oxy and deoxy concentrations of ICG bolus experiment using the sCMOS-camera setup which was developed.
4.5 Arm occlusion experiment

Now instead of phantoms, the device was tested on human arm. A standard arm occlusion test was performed. A pair of source and detector with S-D separation 3 cm was strapped to a human arm and the pressure cuff was attached to upper arm of subject. The exposure time for sCMOS was 2 ms here. First 3 minutes no pressure or perturbation was applied (baseline was observed). Then a pressure of 220 mmHg was applied on the arm occluding the blood flow. This would prevent the venous and arterial blood flow from and to the forearm. In this process, expected is a gradual drop in oxy hemoglobin and a gradual rise in deoxy-hemoglobin. This data was then collected for 3 min. Then the pressure was released, and the data was collected for 3 minutes. In this process the oxygenated blood rushes in the arm and the deoxygenated blood rushes out of arm. Here the expected trend is sudden rise in oxy hemoglobin and sudden drop in deoxy hemoglobin. The setup is as shown in fig. 4.6.

Figure 4.6: Experimental setup of arm occlusion experiment.
This experiment was performed to check if the device is able to quantify and detect the hemodynamic changes properly and accurately to observe and record the expected trend in similar well-established occlusion protocol for in-vivo cases [68,62,73,74,75].

The arm occlusion experiment described above shows the following results as described in figure 4.7, below. It follows a baseline for 3 min when there was no pressure applied. After 3 min the oxy hemoglobin starts decreasing and deoxy hemoglobin starts increasing for 3 min as arm was occluded and the blood flow was obstructed. After 3 min as pressure was released, there is sudden rise in oxy hemoglobin concentration change, and decrease in deoxy hemoglobin concentration change.

![Figure 4.7: Results obtained for arm occlusion test.](image)
The arm occlusion experiment described above shows the following results as described in figure above. It follows a baseline for 3 min when there was no pressure applied. After 3 min the oxy hemoglobin starts decreasing and deoxy hemoglobin starts increasing for 3 min as arm was occluded and the blood flow was obstructed. After 3 min as pressure was released, there is sudden rise in oxy hemoglobin concentration change, and decrease in deoxy hemoglobin concentration change.

Now in order to check the repeatability of the device to calculate the trend and hemodynamic results for the same experiment performed in repetitive blocks, the occlusion and the rest blocks were performed for three times each for 2 minutes after a baseline of 2.5 minutes.

![Oxy & De - Oxy 3 cm detector](image)

Figure 4.8: Results obtained for arm occlusion test multiple times.

As it can be seen in figure above (fig. 4.8), while the arm is being occluded, the pulses can’t be seen (the spikes in measurement) and deoxy hemoglobin increases in
concentration and oxy hemoglobin decreases in concentration. While at rest when the pressure is released, the pulses can be seen (in form of spikes in measurement) and the oxy hemoglobin increases suddenly, and the deoxy hemoglobin decreases suddenly.

4.6 **Ink perturbation bolus experiment - 2D image reconstruction**

To find, study, examine and to demonstrate difference between the studies done with sparse fNIRS and high density fNIRS, an experiment was performed. To get better visual prospectus of the difference between effect of studies of sparse fNIRS and high density fNIRS, 2D image reconstruction was done.

4.6.1 **Probe design**

To do image reconstruction for the data, there was need acquisition of multiple data from different area of the surface of interest. The probe was 3D printed using a flexible material called “NinjaFlex” to accommodate surface of the surface of interest.

![Probe design](image)

Figure 4.9: Probe design.
As stated in figure 4.9 above, the probe designed has 4 number of sources and 9 detectors, making total 36 channels, over an area of 3*3 cm² area. The SS detectors are short separation detectors with SD (source detector) separation 1 cm, LS-1 detectors are long separation detectors with SD (source detector) separation 2.3 cm, LS-2 detectors are long separation detectors with SD (source detector) separation 3 cm. There are 16 SS channels, 16 LS-1 Channels and 4 LS-2 Channels. In our stated high density fNIRS imaging, we will be using all LS-1 and LS-2 channels, making it 20 channels. In sparse fNIRS imaging, only all 4 of of LS-2 channels will be used.

4.6.2 Single cylindrical perturbation

In this experiment a single transparent cylindrical bolus was inserted in a 3D printed container. There was a hollow pipe running across the width of container which was attached to the cylindrical bolus which was of higher diameter. The cylinder was arranged in the container in such a way that it was 1.5 cm away from the top (where the fNIRS probe was placed for scanning) and was in the middle of the container running across the width of container. The hollow tube was connected to the variable flow pump. The setup diagram is as shown below (fig 4.10).

In the described setup, the container was filled with a liquid solution with absorption coefficient 0.1 cm⁻¹ and scattering coefficient 10 cm⁻¹. The hollow tube was connected to a variable flow pump. Initially the pump was pumping normal solution of liquid phantom, having properties absorption coefficient 0.1 cm⁻¹ and scattering coefficient 10 cm⁻¹, through the tube, for 7 minutes (tap A open and tap B closed). This data was collected using the setup and was considered baseline. Now to mimic
the induced perturbation in brain, the tube was pumped with higher absorption solution containing having properties absorption coefficient $0.4 \text{ cm}^{-1}$ and scattering coefficient $10 \text{ cm}^{-1}$ (tap B open and tap A closed). This solution was pumped for 7 minutes followed by normal solution pumping for 7 minutes (tap A open and tap B closed). The exposure time for sCMOS was 2 ms here.

![Diagram of Single cylindrical bolus Perturbation setup](image)

**Figure 4.10:** (a) Single cylindrical bolus Perturbation setup. (b) Top view of container and source detector configuration in the Single cylindrical bolus perturbation setup.

Now to see the contrast between the perturbation and baseline, the intensity data while perturbation was subtracted from the baseline intensity data and this data was plotted on a 2D plane. In figure 4.11, you can see the difference between the result of reconstructed images using Sparse fNIRS imaging and high definition fNIRS.
Figure 4.11: Single cylindrical perturbation image reconstruction. Sparse fNIRS imaging on left side and high definition fNIRS imaging on right side.

As we can see in the images above, Sparse fNIRS image was unable to demonstrate the geometrical shape of the perturbation induced, we can only see an approximate spherical perturbation there, whereas in the high definition fNIRS image, we can guess that the perturbation induced is cylindrical in shape which is actually the case.

To explain this point even better we performed the two-cylinder perturbation experiment as explained below.
4.6.3 Two-cylinder perturbation

In this experiment two transparent cylindrical bolus were inserted and joined side by side in a 3D printed container. There was a hollow pipe running across the width of container which was attached to both of these cylindrical boluses which were of higher diameter. The cylinders were arranged in the container in such a way that it was 1.5 cm away from the top (where the fNIRS probe was placed for scanning) and were in the middle of the container running across the width of container. The hollow tube was connected to the variable flow pump. The setup diagram is as shown below (fig 4.12).
In the above setup, the cylinders were filled with a liquid solution with absorption coefficient 0.1 cm$^{-1}$ and scattering coefficient 10 cm$^{-1}$. The hollow tube was connected to a variable flow pump. Initially the pump was pumping normal solution of liquid phantom, having properties absorption coefficient 0.1 cm$^{-1}$ and scattering coefficient 10 cm$^{-1}$, through the tube, for 7 minutes (tap A open and tap B closed). This data was collected using the setup and was considered baseline. Now to mimic the induced perturbation in brain, the tube was pumped with higher absorption solution containing having properties absorption coefficient 0.4 cm$^{-1}$ and scattering coefficient 10 cm$^{-1}$ (tap B open and tap A closed). This solution was pumped for 7 minutes followed by normal solution pumping for 7 minutes (tap A open and tap B closed). The exposure time for sCMOS was 2 ms here.

Now to see the contrast between the perturbation and baseline, the intensity data while perturbation was subtracted from the baseline intensity data and this data was

Figure 4.12: (a) Two-cylinder bolus perturbation setup. (b) Top view of container and source detector configuration in the Two-cylindrical bolus perturbation setup.
plotted on a 2D plane. In figure 4.13, you can see the difference between the result of reconstructed images using Sparse fNIRS imaging and high definition fNIRS.

![Image](image.png)

Figure 4.13: Single cylindrical perturbation image reconstruction. Sparse fNIRS imaging on left side and high definition fNIRS imaging on right side.

As we can see in the images above, sparse fNIRS image was unable to demonstrate the geometrical shape of the perturbation induced, we can only see an approximate spherical perturbation there, it’s spatial resolution is very low and the results are scattered over large area of the image, whereas in the high definition fNIRS image, we can guess that the perturbation induced is cylindrical in shape and have two objects but not one which is actually the case.
4.7 High density imaging for blood occlusion experiment

Again, a standard arm occlusion test was performed. The high density fNIRS probe (as described above) was strapped to a human arm and the pressure cuff was attached to upper arm of subject. The exposure time for sCMOS was 50 ms here. First 2 minutes no pressure or perturbation was applied (Baseline was observed). Then a pressure of 220 mmHg was applied on the arm occluding the blood flow. This would prevent the venous and arterial blood flow from and to the forearm. In this process, expected is a gradual drop in oxy hemoglobin and a gradual rise in deoxy-hemoglobin. This data was then collected for 3 min. Then the pressure was released, and the data was collected for 2 minutes. In this process the oxygenated blood rushes in the arm and the deoxygenated blood rushes out of arm. Here the expected trend is sudden rise in oxy hemoglobin and sudden drop in deoxy hemoglobin. The setup is as shown in fig. 4.14.

Figure 4.14: (a) Experimental setup of arm occlusion experiment with high density probe. (b) The layout of source and detector, along with their numbering to detect the channel and it’s effect on the corresponding area, on wrist.

This experiment was performed to check if the device is able to quantify and
detect the hemodynamic changes properly and accurately to observe and record the expected trend in similar well-established occlusion protocol for \textit{in-vivo} cases [68,62,73,74,75].

The arm occlusion experiment described above shows the following results as described in figure 4.15, below for a single channel. It follows a baseline for 2 min when there was no pressure applied. After 2 min the oxy hemoglobin starts decreasing and deoxy hemoglobin starts increasing for 3 min as arm was occluded and the blood flow was obstructed. After 3 min as pressure was released, there is sudden rise in oxy hemoglobin concentration change, and decrease in deoxy hemoglobin concentration change.

![Figure 4.15: Results obtained for arm occlusion test for single channel (channel 26 for source 2 and detector 7 which is diagonal across the wrist).](image)

The arm occlusion experiment described above shows the following results as described in figure above. It follows a baseline for 2 min when there was no pressure applied. After 2 min the oxy hemoglobin starts decreasing and deoxy hemoglobin
starts increasing for 3 min as arm was occluded and the blood flow was obstructed. After 3 min as pressure was released, there is sudden rise in oxy hemoglobin concentration change, and decrease in deoxy hemoglobin concentration change.

Now 2-D image reconstruction is done for all the channels for the experiment mentioned above. Image for oxy and deoxy hemoglobin concentration changes at baseline, while occlusion and after occlusion is as illustrated in figure 4.16.
Figure 4.16: 2D images for change in hemoglobin concentration for arm occlusion experiment, using HD probe for imaging for both high density and low-density imaging. (a) 2-D images for oxygenated hemoglobin concentration changes in high density imaging (top) and low-density imaging (bottom), along with plot of oxygenated hemoglobin for channel 26, for both the images. (b) 2-D images for deoxygenated hemoglobin concentration changes in high density imaging (top) and low-density imaging (bottom), along with plot of deoxygenated hemoglobin for channel 26, for both the images.

As it can be seen in figure above (fig. 4.16), while the arm is being occluded, there is rise in deoxy hemoglobin and fall in oxy hemoglobin, while it follows opposite trend while the pressure on cuff is released. Figure above shows the difference in resolution of high density imaging versus low density imaging. It can be clearly seen that resolution is higher in high density imaging, for both oxy and deoxy hemoglobin concentrations. There are some vessels like structures which can be seen in high density imaging, while this is not the case in low density imaging, where there is a
4.8 Motor cortex signal analysis

A standard finger tapping experiment was performed, and the left motor cortex region was analyzed. A different setup of fNIRS probe was designed and strapped to the left motor cortex of a test subject, using a commercial easy cap used with commercial system called NIRX, as described in the figure 4.17. As described in the optode mesh in fig 4.17, there are three source detector separations used, SS, LS – 1, and LS – 2 having SD separations 2.5, 3.5 and 5.5 cm, with 6, 8 and 4 channels respectively, making total of 18 channels in a scanning area of 5.5 x 5.5 cm².

In this experiment a finger tapping task was performed to activate motor cortex region of brain and to quantify the concentration change of oxy and deoxy hemoglobin in that part of brain. Two devices were used to analyze these results, the Fiber–Camera setup which is developed, and the commercial NIRX system, to compare the results.
The protocol followed for this finger tapping experiment is as follows. First the optodes were secured on left motor cortex of the subject, using an Easy cap, of size 56 (adults), which were already marked with 10-20 EEG probe placement for ease of recognition of the area and places to use as optodes both source and detectors. The area was identified, and 3 sources and 6 detectors were placed as explained in fig 4.17. Then for finger tapping experiment, since we are scanning left motor cortex, the subject was instructed to touch alternate fingers of right hand using the thumb of right hand as a task, to activate the left motor cortex. The subject was in rest condition for first 3 minutes of experiment to see baseline. Then the subject was asked to do the finger tapping task for 20 seconds followed by a rest of 20 seconds. This rest and task consisted of a single block, the subject was asked to do 5 such blocks in a row. After 5 blocks, subject was asked to rest for 3 min to get the baseline for next 3 minutes. The
sampling frequency for this experiment was 0.8334Hz, exposure time was 200 milli
seconds, binning was 8 x 8, and framerate of camera was 4.762.

Figure 4.18: (a) Finger tapping data from Fiber-Camera device. (b) Finger tapping
data from a commercial device named NIRX.

As described in above figure fig 4.18, both the diagrams are output of
concentration changes of oxy and deoxy hemoglobin while doing the finger tapping
experiment. The raw data was taken and processed for better presentation, the raw data from both the devices were processed with moving average and detrending for understanding the signal better. Only LS–1 and LS–2 data were analyzed, and most of these channels gave similar readings. Figure 4.18. a. is the results of the Fiber-Camera device developed. As we can see in the results, for baseline part, both oxy and deoxy hemoglobin concentration changes are close to 1 micromolar change, and in blocks numbered from 1 to 5 where the task and rest were performed, the changes for oxy hemoglobin concentration changes are peaking up to 2.25 micromolar, and deoxy hemoglobin up to -1.25 micromolar. Similarly, in the results obtained by the commercial NIRX device, the results for oxy hemoglobin concentration changes is fluctuating and not giving any conclusive data, but the deoxy hemoglobin which is close to 0 at baseline, gives a fluctuation of 0.6 micromolar changes within blocks of rest and task, as illustrated in fig 4.18. b.

To get better understanding of this part, the results are processed, and the part where task was performed was block averaged, since it has 5 blocks, to get data which can be easily be interpreted. The block averaged data is as described in figure 4.19.
As seen in above images fig 4.19, it can be clearly seen that there is baseline for 10 seconds at initial stage, then the task i.e., finger tapping task starts for 20 seconds followed by rest for 20 seconds. It can be clearly seen in both the cases that at baseline the change in concentration of both oxy and deoxy hemoglobin is close to 0. As soon as the task starts, oxy hemoglobin starts to rise, and deoxy hemoglobin starts to fall. In fiber-camera setup, the results show rise of oxy hemoglobin up to 1.75 micromolar which is same in NIRX output. For deoxy hemoglobin the fall in concentration change in this case is close to 1 micromolar in fiber-camera setup, but in NIRX setup, it is close to 0.2 micromolar. Also, as the task ends, and rest starts, both the chromophore’s concentration changes starts to converge to baseline i.e. 0 micromolar in both the devices.
Hence, it is shown that the Fiber-Camera setup works similar to current commercial NIRX devices which are comparatively 20 times more expensive than the developed device and are limited to source and detector numbers. Although there is difference in amplitude in change of concentration of deoxy hemoglobin, but this can be countered with proper selection of wavelengths, since the wavelengths used by us are very close to each other in terms of wavelength and it is observed that the wavelengths close to each other gives similar symmetric response to changes in oxy and deoxy hemoglobin concentration conversions.
Chapter 5 - Conclusions and Future works

In this chapter, with a brief discussion of the developed-device and it’s setup, the experiments performed along with its results, the project will be concluded. Work that needs to be done in future will also be discussed.

5.1 Conclusions

The high density functional Near Infrared Spectroscopy device was developed, and the device’s testing was performed, and different experiments were done. The complete instrument did cost approximately $10,000, as described in table 5.1, which is comparatively affordable and cheaper when compared to the commercial NIRS devices which range from $50,000-$100,000 and don’t even have many source detector combination, required for whole head imaging and added sensitivity and higher resolution.

The device can be mounted on a portable station to move around to desirable spaces. This is one of the advantages in high density whole head imaging as it can give functional response of brain and is portable as compared to traditional techniques namely PET, FMRI, etc. This technique is also very affordable for single scanning reports as compared to the same traditional techniques. The advantages for this technique and devise are increased portability, occupies less space, in-vivo technique,
doesn’t require any drug for imaging, has no harmful ionizing radiations involved, etc.

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<tr>
<td>NI DAQ unit (NI 9263)</td>
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<td>Beam Splitter (CCM1-BS014)</td>
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<tr>
<td>Compound Lens (85-355 by Edmund Optics)</td>
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<tr>
<td>Optical Density Filters (NE20A-B)</td>
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<tr>
<td>Optical Fibers (SH4001-1.3.)</td>
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<td>3D Printed probes</td>
<td>100 $</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9,731</strong></td>
</tr>
</tbody>
</table>

Table 5.1 Parts used in custom developed device and their approximate pricing.

This device uses fiber optic cables to transmit the laser and to receive the affected intensity from the surface of interest. Since there are no metal parts on the data collecting probe, this device can also be used in MRI machines if one decides to study both of these modalities at same time. Since light is the medium being used to detect and analyses the functional response, the external noise due to high magnetic and electric field won’t have any effect on this device and this device wouldn’t interfere with any other devices either. This wearable, high-density optical neuroimaging technology gives us be deployed as a wearable whole head imaging technique for continuous monitoring of athletes, soldiers, and is expected to find many applications including assessing autism spectrum disorder in pediatric population at the clinics to
human performance assessment in military research.

This device can be very useful for the aid of old and weak patients as a bedside monitoring unit, as well as can be used on neonates in ICU to monitor the functional response. This device is comparatively cheap, hence it can be used in under developed countries which cannot afford the expensive devices for such measurements, since it is also portable, this device can also be moved and shared over different institutions as it may need to utilize it’s use to the maximum limit. This device demonstrates high SNR (200 dB) for SD separation as large as 4 cm. This SNR is quite impressive as compared to other electronic devices which have low SNR (< 90 dB) for small SD separation as 2 cm. The SNR could be exponentially increased by reducing the SD separation and by increasing the power of the laser.

This device can successfully and effectively notice and detect even the smallest hemodynamic concentration changes in the dynamic phantom as well as adult human brain. It can be said that, based on the experiments performed above and the results obtained due to them, that the device is highly sensitive and have high resolution. The inclusion of high density probe and imaging techniques has increased the sensitivity and resolution of the device exponentially. By making proper usage of MBLL, the physiologically relevant parameters such as changes in hemoglobin concentrations and oxygenation were observed and obtained. For calculation and analysis of the results of the experiments performed, the values of the extinction coefficient of oxy-hemoglobin (HbO₂) and deoxy-hemoglobin (HHb) and the differential pathlength factor (DPF) at the two wavelengths 785nm and 808nm were taken from literature as described in tables 5.2 and 5.3 respectively. As described in the above experiments all the
hypothesized results and patterns for change in oxy and deoxy hemoglobin concentrations were observed and identified properly.

Thus, we can say that the device developed is a unique combination of high resolution, low cost, portable and sensitive fNIRS technique, which could be very fruitful and useful for various important applications such as studying different brain diseases, monitoring patients and neonates alike in ICUs, for evaluating and analyzing mental and physiological performance and it’s effect in hemodynamic response in brain for military purpose and other human resources and human factors analysis, at low cost and less risk in real time.

5.2 Future works

As discussed in above section, the devise demonstrates high SNR (200 dB) at good SD separations (4 cm) even in hairy region of head, hence it can be safely said that it can readily be used as an analysis tool for functional response in adult humans.

Instead of a 1D galvo which is used for scanning the source array, an optical switch with large number of input to output channels can be used to reduce light coupling losses in fiber and laser source interface and moving parts can be removed this way.

Since this device is used to obtain whole head imaging, a better head gear needs to be designed so that even if the head gear is wore by persons of different head size, SD separation and position of the optodes should be exact every time for proper and accurate scanning and imaging of functional response [102].
Another possibility is making the device wireless. An autonomous power source could be attached to the device and there are different available wireless transmission and reception modules available which can be used to transmit the data and receive the commands to be executed, making the whole device completely wireless. This would be helpful to make the device more compact and then it can be carried around even in a standard backpack giving it better portability and increasing it’s field of usage even outdoors such as field activities, exercises, etc.

Also, instead of 2D imaging, this device can also be used for making, observing and analyzing the topography and tomography for functional response of brain with proper programming, which would be very helpful for depth-resolved images which would be more useful for identifying different brain regions and analyzing them while performing different task-based activations, resting state analysis for studying functional connectivity, etc.
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