HYPERSPECTRAL IMAGE ANALYSIS ALGORITHM
FOR CHARACTERIZING HUMAN TISSUE

YONAS K. WONDIM
yonasphysics@yahoo.com

Department of Biomedical Engineering
Linköping Universitet, SE-581 85 Linköping, Sweden
Linköping 2011

Examiner:  Marcus Larsson
IMT,Linköping University (marcus.larsson@liu.se)

Supervisor:  Göran Salerud
IMT,Linköping University (goran.salerud@liu.se)

LiTH-IMT/MASTER-EX--11/009—SE
# Table of Contents

Abstract ........................................................................................................................................... 4  
Acknowledgment .............................................................................................................................. 5  
1 INTRODUCTION .......................................................................................................................... 6  
1.1 Motivation ................................................................................................................................... 6  
1.2 The Aim of the thesis ................................................................................................................... 7  
2 Microcirculation and Blood .......................................................................................................... 8  
2.1 Introduction ................................................................................................................................ 8  
2.1.1 The microcirculation ............................................................................................................... 8  
2.1.2 The structure of capillary wall ............................................................................................... 8  
2.1.3 The Role of microcirculation .................................................................................................. 8  
2.1.4 The effect of absence or excess of blood .............................................................................. 9  
2.1.5 Main components of blood .................................................................................................... 9  
2.2 The Skin ..................................................................................................................................... 9  
2.2.1 Types and functions of skin .................................................................................................. 9  
2.2.2 Classification of skin ............................................................................................................ 9  
2.2.3 Vascular supply and lymphatic drainage .......................................................................... 11  
3 SPECTROSCOPY ......................................................................................................................... 12  
3.1 Introduction ............................................................................................................................... 12  
3.2 Light sources of a spectrometer ............................................................................................... 12  
3.3 Spectrometer and Measurements ............................................................................................. 12  
3.3.1 The principle of spectrometer .............................................................................................. 12  
3.3.2 Basic setup of spectroscopy ................................................................................................ 13  
3.4 Optical properties ...................................................................................................................... 13  
3.4.1 Refractive Index .................................................................................................................. 13  
3.5 Absorption ................................................................................................................................ 14  
3.5.1 Absorption coefficient (μa) ................................................................................................. 15  
3.5.2 Absorption Spectra for Biological Tissue .......................................................................... 16  
3.5.3 Beer-Lambert Law .............................................................................................................. 17  
3.6 Scattering .................................................................................................................................. 18  
3.6.1 Scattering Coefficient (μs) [cm^{-1}] .................................................................................. 18
### 3.6.2 Anisotropy [g] and Scattering Function P (Θ)

### 3.6.3 Reduced Scattering Coefficient (μs’)

### 3.7 Mie Theory

### 4 CHROMOPHORES

#### 4.1 Hemoglobin

#### 4.2 Bilirubin

#### 4.3 Cytochromes

#### 4.4 Melanin

#### 4.5 Carotenoids

#### 4.6 Lipids

#### 4.7 Water

### 5 Methods and Material

#### 5.1 Hyperspectral Imaging

#### 5.2 Set up of the HSI system

#### 5.3 Spectral Collimated Transmission (SCT)

#### 5.4 Computation of optical properties using Curve Fitting

#### 5.5 Approximate light Transport Theory

#### 5.6 GPU Implementation of Non Negative Least Square Algorithm [NNLS]

##### 5.6.1 The GPU Architectures

##### 5.6.2 CUDA Programme Structure

##### 5.6.3 Device Memories and Data Transfer

##### 5.6.4 Lawson and Hanson’s Non Negative Least Square (NNLS) Algorithm

##### 5.6.5 Experiment and Result from the GPU Implementation

### 6 Evaluation of the System

#### 6.1 UV provocation of skin

#### 6.2 Data analysis

##### 6.2.1 Erythema Development

##### 6.2.2 Melanin content and change in concentration after provocation

#### 6.3 Result from the UV provoked skin and the fitted spectra

#### 6.4 Conclusion

### 7 Table of Figures
Abstract

In the field of Biomedical Optics measurement of tissue optical properties, like absorption, scattering, and reduced scattering coefficient, has gained importance for therapeutic and diagnostic applications. Accuracy in determining the optical properties is of vital importance to quantitatively determine chromophores in tissue.

There are different techniques used to quantify tissue chromophores. Reflectance spectroscopy is one of the most common methods to rapidly and accurately characterize the blood amount and oxygen saturation in the microcirculation. With a hyper spectral imaging (HSI) device it is possible to capture images with spectral information that depends both on tissue absorption and scattering. To analyze this data software that accounts for both absorption and scattering event needs to be developed.

In this thesis work an HSI algorithm, capable of assessing tissue oxygenation while accounting for both tissue absorption and scattering, is developed. The complete imaging system comprises: a light source, a liquid crystal tunable filter (LCTF), a camera lens, a CCD camera, control units and power supply for light source and filter, and a computer.

This work also presents a Graphic processing Unit (GPU) implementation of the developed HSI algorithm, which is found computationally demanding. It is found that the GPU implementation outperforms the Matlab “lsqnonneg” function by the order of 5-7X.

At the end, the HSI system and the developed algorithm is evaluated in two experiments. In the first experiment the concentration of chromophores is assessed while occluding the finger tip. In the second experiment the skin is provoked by UV light while checking for Erythema development by analyzing the oxyhemoglobin image at different point of time. In this experiment the melanin concentration change is also checked at different point of time from exposure.

It is found that the result matches the theory in the time dependent change of oxyhemoglobin and deoxyhemoglobin. However, the result of melanin does not correspond to the theoretically expected result.
Acknowledgment

This thesis work would not have been possible without the advice and guidance of my examiner assistant Professor Marcus Larsson and my advisor Professor Göran Salerud. I would like to express my heartfelt gratitude to both of you for being patient and for offering invaluable assistance during my stay at the Department of Biomedical engineering. I owe my deepest gratitude to Muneyuki Kokudai, who took a part of this project.

I am also thankful to all members of IMT; who were there for me during my stay at the department.

My special thanks also go to my friends Mahder Alemseged; he has made available his support in a number of ways and Narasimha Reddy; the opponent of this thesis work.

I wish to express my love and Gratitude to my beloved family members and friends here in Linköping and back home; for your support and been there for me.

Finally, I would like to take this opportunity to thank all the people who have contributed in some way to this thesis work.
1 INTRODUCTION

1.1 Motivation

Our body needs oxygen for its well-being and proper functioning. To fulfill this, the circulatory system supply tissue cells with oxygen. It is clinically important to know the concentration of oxygen in a given sample of blood or tissue. Low concentration of oxygen in blood may result in tissue damage.

In the field of biomedical optics measurement of tissue optical properties like absorption, scattering, and reduced scattering coefficient has gained importance for therapeutic and diagnostic applications. Accuracy in determining optical properties has a vital importance as this directly affects the quantification of chromophores in tissue.

There are different techniques used to analyze tissue chromophores, especially oxygen saturation in biological tissue. Reflectance spectroscopy is one of the common methods to rapidly characterize the blood amount and oxygen saturation in the microcirculation.

Hyper spectral imaging (HSI) is one spectroscopic technique that is being used in many biomedical applications. HSI has high resolution spatial information when compared with single point spectroscopy. Besides, HSI has the ability to generate non-invasive measurements of reflected energy at narrow bandwidths.

In HSI an image is presented with spectral information that depends both on tissue absorption and scattering. This requires developing analysis software that accounts for both absorption and scattering event. Currently there are a few algorithms that are developed by different research groups that partly accounts for both scattering and absorption events. This type of algorithm is commonly computationally demanding.

In this work an imaging system containing a VariSpec™ Liquid Crystal Tunable Filter (LCTF) is used. This system is capable of acquiring HIS images in a few seconds. However, the system needs to be properly calibrated before this data can be analyzed, a process that is algorithm dependent and in some cases fairly complex.
1.2 The Aim of the thesis

- To develop and implement a HSI Analysis Algorithm that accounts for both absorption and scattering. The algorithm should be capable of determine the concentration of selected chromophores, like oxyhemoglobin, deoxyhemoglobin and melanin in the human skin tissue.
- Reduce the computing time of the algorithm by a parallel processing implementation using a Graphical Processing Units (GPU).
- Evaluate the performance of existing HSI system by using in vivo HSI data from normal skin and provoked human tissue.
- Calibrating the system to account for the non-white spectral response
2 Microcirculation and Blood

2.1 Introduction
In this chapter the role and structure of the microcirculation and the blood is presented first, followed by the types and functions of the skin. At the end of the chapter, the structure of the epidermis and dermis is discussed.

2.1.1 The microcirculation
The human circulatory system is composed of a complicated blood vessel which is found in our entire body. The circulation of blood in the smallest vessels, like arterioles, capillaries and small venules which are embedded with-in organs to distribute blood, is known as the microcirculation. The circulation of blood through this network starts from aorta and flow through arteries that bifurcate throughout the body. Finally it returns to the heart through venules and veins. In this way, the circulatory system is completed. The purpose of circulatory system is to distribute and regulate blood flow throughout the body to supply nutritious blood to different tissues [1] [2].

As mentioned above the microcirculation is a network of small vessels such as capillaries (φ 4-10 μm), arterioles (φ 20-50 μm), metarterioles (φ 10-15 μm), arteriovenous (AV, φ 40 μm) shunts, and venules (φ -50 μm) which are entirely dedicated to supply blood as close as possible to single cells.

2.1.2 The structure of capillary wall
The capillary wall is formed by an endothelium, its basal lamina, and a few isolated pericytes. Capillaries are 4-10μm in diameter and hundreds of microns long. Typically the wall of capillary is formed by a single endothelial cell [2].

2.1.3 The Role of microcirculation
The microcirculation has two important roles. One is to transport oxygen and nutrition to the cells and to retrieve metabolic byproducts from the cells. As a result the tissue can function properly. The blood that is collected from different part of the body cells is transported to the lung for oxygenation, and then it is returned back to the heart. Then the heart pumps this oxygenated blood to the body. The flow progresses towards the capillary network, where the nutritious bloods diffuse in to the cell and the metabolic byproducts diffuses back to the capillary network [1].

Normally a pulsatile flow is observed in the larger vessel on the arterial side, however it is not always observed at the capillary level. Mentioning the movement of erythrocytes, the general movement is identified from arterioles to venules, but because there are not physical restrictions, back-flow is observed at times. The AV-shunts has a role to bypass blood flow from arterial side to venous side [1].
2.1.4 The effect of absence or excess of blood
Absence of blood in capillaries is not unusual, especially in the areas where the nutritional demand is not high. The tissues have excess capacity up to 400% compared to normal condition. Unexpected lack of oxygen in capillary flow cause local anoxia, acidosis, and if it prolongs, necrosis can be caused. Increase of nutritional demand will increase the microcirculatory flow, and if it prolongs, a higher capillary flow, oxygen extraction and eventually angiogenesis will be caused.

2.1.5 Main components of blood
The main components of blood are plasma and erythrocytes, where the ratio of erythrocytes per volume of blood is called hematocrit. Its normal value is between 0.37-0.54 for adults, and it varies depending on the place it is measured. Erythrocyte is a biconcave disc, and in fresh preparations its diameter is 7.8 μm in average, and it decreases slightly with age [2].

2.2 The Skin

2.2.1 Types and functions of skin
Skin forms 8% of the total body mass. Its surface area varies according to height and weight. Its thickness ranges from 1.5-4.0 mm, according to its state of maturation, aging and regional specialization. The skin has some important roles, such as a barrier protecting the inner tissue, controlling of body temperature, sensing the signal from environment. The color of human skin changes depending of some factors, such as the amount of blood in the cutaneous circulation, the thickness of the cornified layer, and the activity of specialized cells involving melanin. The appearance of skin is affected by many other factors [2].

2.2.2 Classification of skin
Since skin covers all parts of a human body, according to the place where the skin is located in, there are many variations in parameters. This includes the thickness, mechanical strength, softness, flexibility, degree of keratinization (cornification), sizes and numbers of hairs, frequency and types of glands, pigmentation, vascularity and innervation. The organization of two types of skin is shown in Figure 2-1 below. The right shows thin, hairy skin which covers greater parts of the body and the left shows thick, hairless skin which forms the surfaces of the palms of the hands, soles of the feet, and flexor surfaces of the digits.
Figure 2-1 The organization of skin. Thin, hairy skin (left). Thick, hairless skin (right). The epidermis has been partially peeled back to show the interdigitating dermal and epidermal papillae [2].

2.2.2.1 Epidermis
The epidermis consists of mainly keratinocytes, pigment forming cells called melanocytes, Langerhans cells and Merkel cells. Free sensory nerve endings present sparsely within the epidermis. The epidermis can be divided into several layers. From the deepest part, basal layer (stratum basale), spinous or prickle cell layer (stratum spinosum), granular layer (stratum granulosum), clear layer (stratum lucidum) and cornified layer (stratum corneum) [2]. The thickness of epidermis varies between 48-170 μm. The stratum corneum, the outermost layer, has about 8-20 μm thickness but it changes up to 10 times thicker on the palm and soles [1].

2.2.2.2 Dermis
The dermis is an irregular, moderately dense connective tissue. It is mainly consisting of collagen, elastic fibers and extracellular matrix. Because of the collagen fibers and its elastic fibers, the dermis provides considerable strength to the skin. The proportion is different depending on the body area, age and sex. The dermis can be divided into two layers, a narrow superficial papillary layer and a deeper reticular layer where the boundary between them is indistinct. In general, the thickness of dermis is 1-4 mm and the components and those sizes are hair follicles, (φ 30-120 μm), the associated erector muscle, different nerves and nerve endings and sebaceous glands and sweat glands (φ 20-50 μm).

2.2.2.3 Hypodermis
Hypodermis is the layer lying under dermis and it is organized with loose connective tissue. That is often adipose especially between the dermis and musculature of the body wall. It mobilizes the skin, and the adipose component has the role as a thermal insulation layer, as the shock absorber
and as a store of metabolic energy. Subcutaneous nerves, vessels and lymphatic are in hypodermis and their main trunks lie in its deepest part, where adipose tissue is scant.

2.2.3 Vascular supply and lymphatic drainage
Due to the skin’s role in Thermoregulatory function, the amount of blood flowing to the skin exceeds its normal nutritional requirements.

There are three main sources for delivering blood to the skin tissue: the direct cutaneous system, the musculocutaneous system and the fasciocutaneous system. The direct cutaneous vessels, the musculocutaneous perforators and the fasciocutaneous perforators each contribute to six anastomosing horizontal reticular plexi of arterioles as shown on Figure 2-2 which have vascular connections between them and ultimately supply the blood supply for the skin.

The lymphatic’s system of the skin collects interstitial fluid and macromolecules to return them for circulation via larger vessel. The lymphatic’s of the skin also carries lymphocytes, Langerhans cells, and macrophages to regional lymph nodes [2].

Figure 2-2 Vascular supplies to the skin. A, Note the various horizontal plexuses fed by direct cutaneous, fasciocutaneous and musculocutaneous arteries. B, Higher magnification of vascular supply [15]
3 SPECTROSCOPY

3.1 Introduction
Spectroscopy is the study of the chemical structures and dynamics of a sample based on absorption, emission, and scattering of electromagnetic radiation [1]. Initially spectroscopy was used by physicists and chemists. But now optical spectroscopy is practiced by many kinds of specialists, such as biophysicists, biochemists, geophysicists and so on [3].

In this thesis, spectroscopy is used to obtain optical properties of human tissue, such as absorption coefficient and reduced scattering coefficients of samples. Therefore, the principle and its basic set up are discussed below.

3.2 Light sources of a spectrometer
Collimated transmission and diffuse reflectance spectroscopy require a light source to shine on the tissue under study to get information about the chemical composition of the tissue. Different light sources are being used for different applications and type of spectrometer. The whole electromagnetic spectrum is composed of x-rays, radio waves, UV (ultraviolet radiation), visible light, and near infrared radiation. In this work the specific focus is on visible light.

3.3 Spectrometer and Measurements
A spectrometer is an instrument that measure and records the light intensity per wavelength, or frequency [1]. There are many different types of spectrometers which are designed for different tasks. For instance, optical absorption spectrometer, luminescence spectrometer, and polarization spectrometer are few of them [1].

3.3.1 The principle of spectrometer
Most spectrometers have similar working principles. As can be seen in Figure 3-1 the incident light is guided into a spectrometer where it is directed to a dispersive element. This element splits the light into separate wavelength components which are recorded by a suitable detector.

---

![Figure 3-1 One of the Principle of Spectrometer](image)
3.3.2 Basic setup of spectroscopy

Collimated transmission and diffuse reflectance set ups consist of a light source, sample under study, and detector. The following Figure 3-2 shows the basic set up.

![Figure 3-2 Basic Set up of a spectrometer](image)

The light is radiated by the light source, delivered through an optical fiber into a sample, detected by a detector, computed and finally displayed on and stored in a computer. In this process, when the light is transported into samples, the light attenuates by the effects of absorption and scattering. For this analysis, Beer-Lamberts law whose detail is mentioned in section five of this document is used. By studying how the collimated transmitted light intensity depends on the optical path length it is possible to estimates the optical the properties of the sample [4].

There are many different types of optical techniques in use to measure the microcirculation. Some of the most frequently used techniques are: diffuse reflectance spectroscopy, optical coherence tomography (OCT), photoplethymography (PPT), laser doppler flowmetry, microscopic techniques, erythemameters and colorimetry, photon migration spectroscopy, raman spectroscopy and fluorescence spectroscopy [1]. In this thesis work diffuse reflectance spectroscopy is used.

3.4 Optical properties

In this section the tissue optical properties are explained. This includes the refractive index, absorption coefficient ($\mu_a$), scattering coefficient ($\mu_s$), reduced scattering coefficient ($\mu'_s$), and anisotropy factor (g).

3.4.1 Refractive Index

The refractive index ($n$) is a dimensionless quantity. It is defined as the ratio between the speed of light in vacuum ($c$) and the speed of light in the media ($v$).

$$n = \frac{c}{v}$$  \hspace{1cm} (1)
To explain both the effect of absorption and scattering, the refractive index needs to have the complex representation.

\[ N(\lambda) = n(\lambda) + ik(\lambda) \]  

(2)

In equation 2 the real part stands for the scattering effect and the imaginary part is related to the absorption of the tissue. The attenuation factor \( k \) is related to tissue absorption according to [1] [4].

\[ \mu_a(\lambda) = \frac{4\pi k(\lambda)}{\lambda} \]  

(3)

Where, \( \lambda \) is the wavelength. Since refractive index provides information about the tissue under study it is one of the most important optical properties in biomedical optics.

Refractive index value ranges from 1 in air (approximately) to larger values. Tissue refractive index varies depending on the wavelength used. The refractive index of stratum corneum at a wavelength range of 400-700nm is around 1.55 [1].

### 3.5 Absorption

Photon is the fundamental unit of light or electromagnetic radiation. In other words, light is a collection of discrete photons. The speed of light in vacuum is \( c = 3.0 \times 10^8 [m/s] \). The energy of a photon depends on its frequency and it is given by [1] [5] [6].

\[ E = hv \]  

(4)

where, \( h=6.62618 \times 10^{-34} [J \cdot s] \) is plank’s constant and \( v \) is the frequency of light, which is the ratio of speed of light in vacuum and wavelength. Therefore, the energy of a photon can be give as

\[ E = h \left( \frac{c}{\lambda} \right) \]  

(5)

where, photon wavelength is given in meter [m] and energy is joule[j] and frequency in per second [s\(^{-1}\)]

“The state of the molecular charge separation can change in a quantized fashion by "absorbing" the energy of a photon.” [6] For absorption or photon energy transfer to occur, the photon frequency must match with the frequency associated with molecular energy transition.

Biological molecules that absorb photons are known as chromophores. These biological chromophores can absorb light either via electronic or vibrational transition. Melanin, the “heme” groups, carotenoids and chlorophyll are examples of biological chromophores that
absorb light via electronic transition. On the other hand, water absorbs light via vibrational transition [1] [4] [6].

### 3.5.1 Absorption coefficient ($\mu_a$)

![Diagram of chromophore idealized as a sphere]

To define absorption coefficient, a chromophore is idealized as a sphere as shown in the Figure 3-3 above. The part of the sphere that blocks the light is called effective cross sectional area ($\sigma_a$) and it is proportional with absorption efficiency ($Q_a$).

$$\sigma_a = Q_a A$$  \hspace{1cm} (6)

where, $A$ is the geometrical size of the idealized chromophore.

The absorption coefficient ($\mu_a$) of a medium containing many chromophores with a volume density ($g_a$) is the cross sectional area per unit volume of the medium [5].

$$\mu_a [cm^{-1}] = g_a [cm^{-3}] \sigma_a [cm^2]$$  \hspace{1cm} (7)

The probability of a photon to survive or the probability of photon transmission is given as follows

$$T = \exp(-\mu_a L)$$  \hspace{1cm} (8)

where, $T$ is transmission and $L$ [cm] is the photon path length through the medium.
3.5.2 Absorption Spectra for Biological Tissue

As can be seen on Figure 3-4, in the ultraviolet and shorter wavelength the absorption is mainly by protein, DNA, and other molecules. In the red to near infrared (NIR) region, the absorption is very minimal. Because of this minimal absorption at this wavelength the region is called the diagnostic and therapeutic window. In the infrared region, the absorption increases with longer wavelengths due to water absorption.

As can also be seen on Figure 3-4 the red line shows absorption by whole blood. In the red to NIR region absorption by whole blood is very high. However, the fraction of blood in tissue is small. As a result the average absorption coefficient that affects light transport is moderate.

Figure 3-4 also shows absorption by melanosomes. As can be seen they are strong absorbers. The local interaction of light with them is very strong. Even though they are only confined to the thin top layer of the skin, the contribution to the average absorption coefficient may modestly affect light transport.

The absorption effect in tissue is of vital importance in biomedical optics; it is because of absorption that a light source can be used to cause a potential therapeutic effect on tissue. The chemical composition of a tissue can also be investigated by studying light absorption for
diagnostic purpose. In general absorption of light in tissue plays a dynamic role in imaging and spectroscopic applications [6] [1] [5].

3.5.3 Beer-Lambert Law

As explained above, when a photon travels in a tissue it may be scattered or absorbed by the tissue. Assuming a collimated light beam traveling in to a tissue, with absorption coefficient of $\mu_a$, non-scattering, homogenous and travelling along the x-direction. When the light has travelled a distance $dx$ a fraction $dl$ of the incident light will be absorbed according to

$$ dl = -\mu_a I \, dx $$

Equation 9 can be rewritten as

$$ \frac{1}{I} \, dl = -\mu_a \, dx $$

Integrate the above equation we get

$$ \ln(I) = -\mu_a \, x $$

This gives equation 10 below using boundary condition:

$$ I(x = 0) = I_0 \quad I(\lambda) = I_0(\lambda)e^{-\mu_a \, x} \quad (10) $$

$$ I(\lambda) = I_0(\lambda)e^{-\varepsilon(\lambda)cX} \quad (11) $$

where, $\varepsilon(\lambda)$ - is molar absorptivity in $(L/(mol \, x \, mm))$, $c$ is concentration of chromophores, $I$ is the transmitted intensity, $I_0$ is the incident light intensity, and $x$ is optical path length.

If there are multiple absorbers, then equation 11 can be modified as follows

$$ I(\lambda) = I_0(\lambda)e^{-(\varepsilon_1(\lambda)c_1+\varepsilon_2(\lambda)c_2+\cdots)x} \quad (12) $$

Equation number 10 is called beer-lambert law, describing the relationship between incident light and the light that is not absorbed by the tissue after traveling through a medium with an absorption coefficient $\mu_a$.

Transmittance, $T$ is given by

$$ T = \frac{I}{I_0} \quad (13) $$

Absorbance, $A$ is given by
Absorbance can also be written as

$$A = \log_{10} \left( \frac{I_0}{I} \right)$$

(14)

Absorbance can also be written as

$$A = \ln(10) \varepsilon(\lambda)CX = 2.3026(\sum \varepsilon_i(\lambda)CiX)$$

(15)

Where $$\mu_a(\lambda) = \ln(10)\varepsilon(\lambda)C = \ln(10)a(\lambda)C$$

$$a(\lambda)$$ -is absorptivity in \((L/(gxmm))\)

Since specific extinction coefficient is described using base 10 logarithm and absorption is described using natural logarithm the scaling factor, \(\ln(10) = 2.30259\) is used in the above equations.

### 3.6 Scattering

Biological tissue has many structures with different size including nuclei, membranes, cells, collagen fibers and etc. These different structures have different size. Tissue structures having the same size with wavelength of the photon can scatter the photon strongly; this type of scattering is described by Mie theory. If the tissue structure is much smaller than the photon wavelength, then the scattering is called Rayleigh scattering [1] [6] [4].

Mitochondrion (1-µm) is one of the structures in tissue having many folded internal lipid membrane (9-nm) surrounded by aqueous medium. The refractive index difference between the aqueous medium and the lipid membrane results in strong scattering of light. Due to the large size of the folded lipid membrane, there is a strong scattering of long wavelength of light. Collagen fibers (2-3 µm) also contribute for Mie scattering in the infrared wavelength range.

#### 3.6.1 Scattering Coefficient \((\mu_s)\) [cm\(^{-1}\)]

![Figure 3-5 Schematics of Scattering particle idealized as sphere with geometric size \(A\) and effective cross section \(\sigma_s\) [5].](image-url)
As can be seen in the Figure 3-5 above parts of the incoming photons are redirected in to another direction casting a shadow. This shows an oversimplified situation of scattering.

The effective cross section ($\sigma_s$) is related by the scattering efficiency ($Q_s$) to the geometrical size as of the scattering particle.

$$\sigma_s \ [cm^2] = Q_s \ [-] A_s \ [cm^2]$$  \hspace{1cm} (16)

The scattering coefficient $\mu_s \ (cm^{-1})$ is given by the cross sectional area per unit volume of medium times its volume density $\varrho_s$.

$$\mu_s = Q_s \sigma_s$$  \hspace{1cm} (17)

The probability of a photon to be transmitted without being redirected by scattering after a photon path length $L$ is given by

$$T = \exp(-\mu_s L)$$  \hspace{1cm} (18)

where, $T$ is transmission and $L$ is the photon path length measured in [cm]

### 3.6.2 Anisotropy [$g$] and Scattering Function $P(\theta)$

For a single scattering even shown in Figure 3-6 below, the photon deflection angle is the angle measured from the photon original trajectory. There are many possible scattering angles with which the photon may deflect to its new trajectory. The distribution of these scattering angles is described by a probability function $P(\theta)$ or scattering phase function. Therefore, $P(\theta)$ describes the angular dependence of the scattering event.

![Diagram showing photon deflection angle for single scattering event](image)

Figure 3-6 Shows photon deflection angle for single scattering event [6]
The anisotropy factor “g” is defined as the mean of the cosine of all the scattering angles, \( \langle \cos (\Theta) \rangle \) with which the photon deflects.

The calculation of the anisotropy factor g is given as follows

\[
g = \int_0^\pi p(\theta) \cos(\theta) \cdot 2 \sin(\theta) \, d\theta = \cos(\theta)
\]  \hspace{1cm} (19)

where,

\[
\int_0^\pi p(\theta)2\pi \sin(\theta) \, d\theta = 1
\]  \hspace{1cm} (20)

If the photons scatter with equal efficiency to all possible deflection angles, then this is called an isotropic scattering function. It is given as follows

\[
P(\Theta) = 1/4\pi
\]  \hspace{1cm} (21)

The value of g ranges in between -1 till 1. If g is equal to 0 it implies that the scattering is isotropic and a value near one implies highly forward scattering. The angular dependence of scattering function for Biological tissue can also be described using the Henyey and Greenstein expression [1]. In the Henyey and Greenstein function the anisotropy factor g is used to describe the scattering function \( P(\Theta) \) [6] as follows

\[
P(\Theta) = \frac{1}{4\pi} \cdot \frac{1-g}{[1+g^2-2g\cos(\theta)]^{3/2}}
\]  \hspace{1cm} (22)

where,

\[
\int_0^\pi p(\theta)2\pi \sin(\theta) \, d\theta = 1
\]

and \( \int_0^\pi p(\theta)\cos(\theta)2\pi \sin(\theta) \, d\theta = g \)

### 3.6.3 Reduced Scattering Coefficient (\( \mu_s' \))

The reduced scattering coefficient, a property depending on both scattering coefficient (\( \mu_s \)) and anisotropy (g), has a great importance to describe the diffusion of photons in scattering dominated light transport (diffusion regime) as a random walk of step size of 1/ \( \mu_s' \).

The reduced scattering coefficient (\( \mu_s' \)) is expressed using scattering coefficient (\( \mu_s \)) and anisotropy factor (g) as follows

\[
\mu_s' = \mu_s(1 - g) \hspace{0.5cm} [cm^{-1}]
\]  \hspace{1cm} (23)
Since the propagation of visible and near infrared light in biological tissue involves diffusion, the reduced scattering coefficient has a great importance in Biomedical Optics applications.

### 3.7 Mie Theory

Mie theory provides a solution for scattering of light by homogenous spherical particles having any size.

Gustav Mie in 1908 came up with a theory that uses two parameters; one is the relational refractive index between the scattering particle and the surrounding medium.

\[
(n_r) = \frac{\text{refractive index of particle } (n_p)}{\text{refractive index of medium } (n_m)}
\]  

(24)

Where, \( n_r \) is the relative magnitude of refractive index, \( n_p \) is refractive index of particle and \( n_m \) is refractive index of medium.

The other property used by Mie theory is the size parameter (\( X \)) (the size of the surface of refractive index mismatch) is given as the ratio of circumference of the sphere \( (2\pi r) \) to the wavelength of the light in the medium \( (\lambda/n_{med}) \).

\[
X = \frac{2\pi r}{\lambda/n_{med}}
\]  

(25)

Where, \( r \) is radius of the sphere assumed as a scattering particle.

One can implement Mie theory calculation to calculate scattering efficiency \( (Q_s) \) using scattering cross sectional area \( (\sigma_s) \) and the true geometrical area \( (A) \)

\[
\sigma_s = Q_s A
\]  

(26)

And using \( \sigma_s \) and scatterer number density \( (\varrho_s) \) the scattering coefficient is calculated.

\[
\mu_s = \varrho_s \sigma_s
\]  

(27)

The full version of Mie Theory can be found elsewhere [6] [5].
4 CHROMOPHORES
In this chapter Chromophores that are most important in human skin at visible and UV wavelength region are covered.

4.1 Hemoglobin
Hemoglobin is made up of protein molecules called globulin chains. It is located in Red blood cells and its role is to transport oxygen from Lung to body tissue and it also returns carbon dioxide from body tissue to the Lung. Because of its role in oxygen transport, hemoglobin is one of the most important Chromophores in human tissue. Hemoglobin concentration for adults is in the range of 120-180g/L [1].

If hemoglobin is saturated with oxygen, then it is called Oxyhemoglobin, if not it is called Deoxyhemoglobin. Depending on the saturation of oxygen, the absorption spectra of Hemoglobin is different at different wavelength. The following Figure 4-1 shows how the absorption spectra vary for Oxyhemoglobin and Deoxyhemoglobin depending on the oxygen saturation level of the hemoglobin.

![Absorption Spectra of Oxy and Deoxyhemoglobin](image)

Figure 4-1 shows the absorption spectra of Oxy and Deoxyhemoglobin [5].

As can be seen in Figure 4-1 above, when the hemoglobin has bound with oxygen, the spectrum has two peaks in the wavelength range of 540nm till 580nm. The figure also shows the absorption peak for oxyhemoglobin at a wavelength of 415nm [7] [8].

For the case of deoxyhemoglobin, the spectrum shows us that it has absorption peak at a wavelength of around 555nm and a slightly shifted peak at a wavelength of around 415nm to 430nm [1] [4].
When hemoglobin binds with carbon dioxide it is called carboxyhemoglobin. As a result the absorption spectra show a shift. Oxidation of iron also results in another chromophore called methemoglobin, which disables the oxygen binding and releasing ability of hemoglobin.

4.2 Bilirubin

Bilirubin is produced as a byproduct of hemoglobin breakdown. In the normal case it is absorbed in the liver. When this process fails due to a liver disease, the excess bilirubin start to be visible in skin and changes the skin color to yellowish. Excess bilirubin is also seen in jaundice infants.

4.3 Cytochromes

Cytochromes are hemoprotein groups located in mitochondrial membrane, endoplasmic reticulum, eukaryotes and etc. Cytochromes carry out electron transfer.

Cytochromes are classified in to many groups depending on their haem type, iron coordination or sequence similarity. In this thesis we only focus on cytochromes that are involved in electron transport chain. These are aa3 also called cytochrome C oxides, cytochrome b, and cytochrome a. Absorption spectra of cytochromes change after oxidation and reduction [1].

4.4 Melanin

Melanin, produced by melanocytes in the epidermis, is one of the most important chromophores in skin optics. Once melanin is produced in melanocytes, it diffuses to keratinocytes. It is found in skin, hair and eye. Melanin concentration in skin and hair changes the skin and hair color.

The two types of melanin are eumelanin and pheomelanin. As can be seen in

Figure 4-2 these two melanin types have different absorption spectra. Eumelanin is present both in caucasian and dark skin types but pheomelanin is mainly present in caucasian skin types.

UV radiation stimulates the production of melanin. Melanin filters further UV radiation and as a result the skin looks sunburned. Depending on the concentration and depth of produced melanin the sunburn color varies [1] [9]. Dark skin types have higher melanin concentration than white skin types. Melanosome absorption depends both on melanin absorption coefficient and its volume fraction in the epidermis.
The melanin granules are assumed to be responsible for melanin extinction due to Rayleigh scattering behavior. Melanin extinction is assumed to follow an extinction pattern as a function of $\lambda^{-4}$ [1].

### 4.5 Carotenoids

Carotenoids are Chromophores found in skin, blood streams, and aorta of peoples eating carotenoid rich foods like carrots. This Chromophore is a good absorber in the wavelength region of UV and blue wavelength

### 4.6 Lipids

Lipids or fats are located almost everywhere in our body. Lipids are considered both as scattering substances and an absorber with its own characteristics of absorption.

Lipids or body fats are found in adipose tissue, subcutaneous fat layer and epicardial fat. The concentration varies from one tissue to the other.

The absorption graph for fat is given in Figure 4-3 below.
Figure 4-3 Absorption coefficient corrected for scattering contribution versus wavelength. The error bars represent the standard deviation over the 2 temperatures i.e. 37, 60°C [9] [11]

4.7 Water

Water is a good absorber in the wavelength region of UV and near infrared. But in the visible wavelength its absorption is minimal. The concentration of water in human body varies from one tissue type to the other. For instance it is around 92% in the plasma and it may go down up to 11% in adipose tissue [1] [12].
5 Methods and Material
In this chapter the Hyperspectral Imaging system used for this study is explained. In addition, the HSI analysis algorithm that includes both scattering and absorption and its GPU implementation of the analysis algorithm are discussed at the end of the chapter.

5.1 Hyperspectral Imaging

Hyperspectral Imaging (HSI) is imaging techniques that acquire two dimensional (2D) spectroscopic images at several wavelengths resulting in Hyperspectral image cube (see Figure 5-1) [6]. Due to the system’s ability to produce a noninvasive measurement of reflected light at a narrow bandwidth, the imaging system is being widely used in many biomedical applications [1]. Besides, HSI systems can give a large number of spectra within a short time when compared with the ordinary single point spectroscopy.

Figure 5-1 Shows a three dimensional (two spatial dimension and one spectral dimension) Hyperspectral image cube [13].

For the spectral cube shown above, each pixel has a reflectance spectrum. This reflectance spectrum $R(\lambda)$ is different at different wavelengths due to difference in Chromophores absorption spectra at different wavelength [6].

In this study hyperspectral imaging is used to asses chromophores in the microcirculation of human skin. HSI systems are also being used in biomedical optics for characterization of wounds, for investigation of cancerous tissue and many other applications. Hyperspectral imaging systems are also used to diagnose arteriosclerosis and cancer. This system is being used by medical doctors to perform diagnosis without the need for sample excision.
5.2 Set up of the HSI system

The HSI system used on this study for spectral acquisition includes a charged coupled device (CCD) camera (Dolphin F-145B, Allied Vision Technologies GmbH, Germany), an optical lens (Zoom 7000, Navitar Inc., USA) and a liquid crystal tunable filter (LCTF, VariSpec™, Cambridge research & Instrumentation, Inc., USA), covering the wavelength range of 400 to 720nm. A white-LED light source (ILP ACIS-100/100, Volpi AG, Switzerland) is used to illuminate the tissue under study through a semi-transparent mirror. The camera and the LCTF are computer controlled (FireWire, IEEE 1394) [14].

The spatial resolution of the system is limited by the systems optical zoom, the CCD array and each sensor element size (1040x1390 elements with a size of 6.45x6.45µm). The spectral resolution is described as the full width at half maximum of the LCTF (10nm) [14] [1].

The following Figure 5-2 is the overview of the system used.

![Figure 5-2 set up of HSI system [14]](image)

Our set up gives an image of size 520x696 pixel (vertical and horizontal binning of camera sensor elements) with rectangular field of view 38x29mm. In addition, the camera has variable sampling time and possibility of binning the pixels.

The system set up has a graphical user interface which is implemented in Matlab (Mathworks Incorporated, USA) which controls both the tunable LCTF and the camera. In addition, the graphical user interface allows the visualization of the images during data collection.
5.3 Spectral Collimated Transmission (SCT)

Absorption coefficient, scattering coefficient and reduced scattering coefficient are called optical properties. The parameters describe the optical characteristics of a tissue. The Spectral collimated transmission set up shown below is used to compute optical properties of different phantoms which are compounded from ink, milk, and water. In this experiment two different phantoms were made, one from blue ink (Blå HUSHÅLLSFÄRG, Drr. Oetker Sverige AB) and water the other from milk and water. The collimated transmission set up shown on Figure 5-3 is used to calculate absorption coefficient and scattering coefficient, where blue ink is used as pure absorber and milk is used as pure scattering solution.

Collimated transmission is a widely used and accepted method for measuring the optical properties, such as scattering coefficient, absorption coefficient and the total attenuation coefficient of optical phantoms. The method is based on the Beer-Lambert law which describes the relation between the intensity of incident light, transmitted light, optical properties and optical path length through a medium. A setup of the Spectral Collimated Transmission (SCT) is shown in Figure 5-3 above. The two fiber collimators (Edmund Optics Inc., UK) were connected to a broadband tungsten halogen lamp (HL-2000, Ocean Optics Inc., USA) and a spectrometer (400-900 nm AvaSpec 2048-5-RM, Avantes BV, The Netherlands, grating: VB 600 lines/mm), respectively, using glass fibers with core/cladding sizes of 200/230 μm and a numerical aperture (NA) of 0.37. The two collimators where aligned and placed 250 mm apart facing each other to ensure a uniform and collimated illumination and detection of the light passing through the sample cuvette. The divergence of the collimators was 0.37 degrees. A high resolution translational micro stage was used to adjust the different sample thicknesses.

In this process we used the setup as follows. At first we prepared the phantoms. The proportion of each solution was: - (blue ink) :- (water) = 1: 100 and, (milk) : (water) = 1: 5. Next, we filled the cuvette to approximately 3/4 with the solution. At last, the intensity for each wavelength in the range 387.3 to 925.9nm was recorded at multiple optical path lengths using an incremental increase of 0.5mm. The distance was increased until 10mm for ink and 2.5mm for milk.
5.4 Computation of optical properties using Curve Fitting

The data acquired from the collimated spectral transmission setup was analyzed and optical properties of both milk and ink was calculated using the Beer Lambert law and using the polynomial approximation in MATLAB.

“Beer Lambert law” shows the relation between the optical path length in a medium and the intensity of transmitted light. On one hand, in the medium where absorption is dominant, the transmittance $I$ is described as \[ I = I_0 e^{-\mu_a L} \] (28)

Where, $I_0$ is the intensity of incident light and $L$ is the optical path length. On the other hand, in the medium where scattering is dominant the transmittance $I$ is described as \[ I = I_0 e^{-\mu_s L} \] (29)

Where, $I_0$ is the intensity of incident light, $\mu_s$ scattering coefficient of the medium and $L$ is the optical path length.

Equation 28 and 29 can be written as follows

\[
\ln\left(\frac{I}{I_0}\right) = -\mu_a L \tag{30}
\]

\[
\ln\left(\frac{I}{I_0}\right) = -\mu_s L \tag{31}
\]

Therefore, computing the slope of the graph of distance against transmittance for each solution gives the absorption coefficient and the scattering coefficient respectively. Both optical properties are computed according to the equation 30 and 31 using polynomial approximation on MATLAB, besides, the coefficients are filtered using order 2 low pass digital Butterworth filter with normalized cutoff frequency 0.1.

Figure 5-4 shows the absorption coefficient of ink
Figure 5-5 shows the reduced scattering coefficient of milk.

During the least square fitting process, spectral signals with wavelength under 520nm are not used due to too much noise. As a result those wavelength intervals are not used during the fitting process to get a good fit. The following Figure 5-6 and Figure 5-7 shows how the signals behave at different wavelength intervals during the fitting process.

Figure 5-6 the curve fitting at 630nm showing a good fit
Figure 5-7 the curve fitting at 500nm it shows the noisy range.

Therefore, the scattering and absorption coefficients of the phantoms are calculated only for wavelength interval of 520 till 650nm.

5.5 Approximate light Transport Theory

Since one of the purposes of this master’s thesis work is to determine the concentration of skin chromophores, it is important to approximate how light travels in skin. The amount of diffusely back scattered light which is detected depends both on scattering and absorption. It is therefore, important to find an approximate model that encompasses both scattering and absorption processes.

There are different spectroscopic models that are being used to determine the concentration of chromophores in skin. Beer-Lambert model is one of the spectroscopic models that are valid for the spatial and temporal difference spectra in tissue based on the assumption of constant scattering and bulk absorption in tissue over time and space [1]. This model also assumes a constant optical path length, which is not always true. A model based on Beer-lambert approximation can only be used just to know the absence or presence of chromophores.

According to Beer-lambert model, the absorbance at the selected wavelength interval is given as follows

\[ A = \Delta C \mu_a(\lambda)L \]  \hspace{1cm} (32)
where, $\Delta C$ is change in chromophore concentration and $L$ is the optical path length.

In this model intensity loss due to scattering is not included, which made the application of this model very limited only for determining the presence of chromophores. Therefore, to include the effect of scattering a modification of the Beer-Lambert model is required.

A modified Beer-Lambert model which combines the intensity loses due to scattering and absorption when light propagates through skin has been proposed by Delpy et al, and revised by Kocsis et al. [1] [4].

The modified Beer-lambert law is given by

$$A(\lambda) = \mu_a(\lambda)\bar{L} + G$$  \hspace{1cm} (33)

where, $\bar{L}$ is the total mean optical path length of the detected photons, and $G$ is a geometry-dependent factor representing intensity loss by scattering.

There is also a model based on the modifications to the Beer-Lamberts law, which includes the effect of simultaneous changes in both absorption coefficient and scattering coefficient, by Jacques in 2003. In this model, the light transport is described by $T$, which is the intensity as a function of $\mu'_s$ and $\mu_a$, and the light transport is expressed with polynomials in $\mu'_s$, and an exponential dependence on $\mu_a$

$$T(\mu'_s, \mu_a) = Ke^{-\mu_aL}$$  \hspace{1cm} (34)

where, $K$ is the intensity lose due to scattering given by

$$K = a + b\mu'_s + c\mu'_s^2$$  \hspace{1cm} (35)

and $L$ is the change in path length due to scattering given as

$$L = d + e\mu'_s + f\mu'_s^2$$  \hspace{1cm} (36)

In the above equations, $a$ to $f$ are numerical constants, which can be determined by performing calibration measurements on multiple optical phantoms. The reduced scattering coefficient is modeled by

$$\mu'_s = \alpha \left(\frac{\lambda}{700nm}\right)^{-\beta}$$  \hspace{1cm} (37)

where, $\alpha$ and $\beta$ are free fitting parameters.

Taking the natural logarithm of equation 34,

$$\ln(T(\mu'_s, \mu_a)) = \ln(K) - \mu_aL$$  \hspace{1cm} (38)
In equation 38, the term \( \ln(K) \) represents the intensity loss due to scattering. This term is modeled as a polynomial function in this work. This is similar to the term \( G \) in equation 33 above. The term \( \mu_a L \) represents the intensity loss due to absorption [5] [11].

In this thesis work the modified Beer-lambert model is used to model the light propagation in skin. In this entire work pure milk phantom is used to model the intensity lose due to scattering by assuming that milk and skin has similar scattering property.

The model for term \( G \) or \( \ln(K) \), was evaluated by analyzing measurements from a few pure milk phantoms prepared as follows.

At first five phantoms were made each with absorption coefficient value of zero and reduced scattering coefficient of 0.5, 1.0, 1.5, 2.0, and 2.5, assuming a milk anisotropy value of 0.74 (see Table 1).

Table 1 Reduced scattering coefficient of samples and its component ratio.

<table>
<thead>
<tr>
<th>( \mu_s' ) [mm(^{-1})]</th>
<th>Component</th>
<th>Amount [mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Milk</td>
<td>5.85</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>94.15</td>
</tr>
<tr>
<td>1.0</td>
<td>Milk</td>
<td>11.70</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>88.30</td>
</tr>
<tr>
<td>1.5</td>
<td>Milk</td>
<td>17.56</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>82.44</td>
</tr>
<tr>
<td>2.0</td>
<td>Milk</td>
<td>23.41</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>76.59</td>
</tr>
<tr>
<td>2.5</td>
<td>Milk</td>
<td>29.26</td>
</tr>
<tr>
<td></td>
<td>water</td>
<td>70.74</td>
</tr>
</tbody>
</table>

For calibration purposes, white calibration and black calibration were implemented. White calibration was for obtaining how the system itself affects the detected color \( B_a SO_4 \), and black calibration is for subtracting offset effect caused by the surrounding environment and the setup.

Using the image cubes of the samples, corresponding image cube of white reference and the image cubes of black reference, we calibrated every image cubes of each sample pixel-wise using the following equation [1] [4] [5].

\[
A_{i,j} = -\ln \left( \frac{S_{i,j} - B_{i,j}}{S_{o,i,j} - B_{o,i,j}} \right)
\]  (39)
Where,

\[ i, j = \text{Denote the pixel position in the image cube} \]
\[ A_{i,j} \text{ Denotes the Apparent Absorbance Spectrum (AAS)} \]
\[ S_{i,j} \text{ Denotes the measured reflection spectrum} \]
\[ S_{o,i,j} \text{ Denotes the reflection spectrum of white reference} \]
\[ B_{i,j} \text{ Denotes the measured background spectrum for sample} \]
\[ B_{o,i,j} \text{ Denotes the measured background spectrum for white reference} \]

The intensity data collected by the HSI system at a wavelength interval of 520 nm till 720nm with a step wavelength of 7nm is used to find the relationship between the reduced scattering coefficients of milk, wavelength and the intensity collected using HSI using polynomial fitting. The fitting resulted in a polynomial function of power two as a function of wavelength (see Figure 5-8).

![wavelength vs. ln(reflectance)](image)

Figure 5-8 the fitting result of wavelength vs. intensity for four different values of \(\mu_s' [\text{mm}^{-1}]\)

\[ G = \ln(I) = a\lambda^2 + b\lambda + c \] (40)

After comparing the mean residual for 5th, 4th, 3rd and 2nd degree polynomials it is found that the mean residual for the 2nd degree polynomial is small when compared form the other polynomials. Therefore, equation 40 is used to model the intensity lose due to scattering and the final absorbance equation can be re written as

\[ A = a\lambda^2 + b\lambda + c - \mu aL \] (41)
where, $\mu_a$ for ink and milk sample is given by

$$\mu_a(\lambda) = C_{\text{ink}}\mu_{\text{ink}}(\lambda)$$  \hspace{1cm} (42)

For human skin it is given by

$$\mu_a(\lambda) = (C_{\text{OH}}\mu_{\text{aOH}}(\lambda) + C_{\text{DOH}}\mu_{\text{aDOH}}(\lambda) + C_{\text{melanin}}\mu_{\text{a melanin}}(\lambda) + C_{\text{water}}\mu_{\text{a water}}(\lambda))$$  \hspace{1cm} (43)

In order to extract information about tissue oxygenation and melanin content, a prediction model of the skin’s total absorption and scattering was used according to equation 44.

$$A_{i,j} = (C_{\text{OH}}\mu_{\text{aOH}} + C_{\text{DOH}}\mu_{\text{aDOH}} + C_{\text{melanin}}\mu_{\text{a melanin}} + C_{\text{water}}\mu_{\text{a water}})\bar{L} + \sum_{k=0}^{2}a_k(\lambda/625)^k$$  \hspace{1cm} (44)

Where $C_{\text{OH}}$ and $C_{\text{DOH}}$ denote reference absorption spectra for oxyhemoglobin (OH) and deoxyhemoglobin (DOH) respectively, $\mu_{\text{a melanin}}$ denotes a reference eumelanin absorption spectrum, $\mu_{\text{a water}}$ denotes reference absorption spectra for water, $\bar{L}$ denotes the mean optical path length and $a_k$ ($k = 0, 1, 2$) are the coefficients of scattering factor.

The prediction model, equation 44, was then fitted to each measured tissue reflection spectrum by solving the least-squares problem with non-negativity constraints:

$$\min_{p \geq 0} \frac{1}{2} \left\| A_{i,j} - \text{measured tissue spectrum}_{i,j} \right\|^2_2$$  \hspace{1cm} (45)

This computation yields every unknown coefficient in equation 44 as the respective content values at each pixel location.

Since we acquired the coefficients by solving the least-squares problem with non-negativity constraints, all the computed values are positive. However, the coefficients describing scattering effects can be either positive or negative. This can cause less accuracy of our prediction. We solved this problem by multiplying minus one to the coefficients which we expected to be negative. When not knowing which coefficients that was negative, we predicted the best combination of sign comparing the mean residual value over field of view (FOV). In our case, where the scattering effect is described by a two degree polynomial function, there are three coefficients to evaluate. We compared eight patterns, which are calculated from two to the power of three, of different combination of sign. The Tukey test as statistical method was used to compute the significant difference of each combination, where eight data-cubes from sample1 and sample2 - skin type V were used for the calculation (see Table 2).
Table 2 Tukey test, (what do not share a letter (A to D) are significantly different.)

<table>
<thead>
<tr>
<th>Sign combination</th>
<th>N</th>
<th>Mean residual</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>nnn</td>
<td>8</td>
<td>0.27882</td>
<td>A</td>
</tr>
<tr>
<td>pnn</td>
<td>8</td>
<td>0.21601</td>
<td>A B</td>
</tr>
<tr>
<td>ppn</td>
<td>8</td>
<td>0.20754</td>
<td>B C</td>
</tr>
<tr>
<td>ppp</td>
<td>8</td>
<td>0.19983</td>
<td>B C</td>
</tr>
<tr>
<td>pnp</td>
<td>8</td>
<td>0.16333</td>
<td>B C D</td>
</tr>
<tr>
<td>npn</td>
<td>8</td>
<td>0.15592</td>
<td>B C D</td>
</tr>
<tr>
<td>npp</td>
<td>8</td>
<td>0.14631</td>
<td>C D</td>
</tr>
<tr>
<td>npn</td>
<td>8</td>
<td>0.13338</td>
<td>D</td>
</tr>
</tbody>
</table>

According to this analysis, the combination of sign (n,p,n), where n stands for negative and p stands for positive, is significantly better than the combination (p,p,p),(p,p,n),(p,n,n) and (n,n,n) at the 5% significant level, and the comparison of mean value shows (n,p,n) is better than the other combinations. Therefore we used the combination, (n,p,n), in our model.

When we compute each coefficient of our model, we computed them pixel by pixel. However we assumed that each pixel includes errors, we compensated the errors taking average of the values from those skin regions which are under same physical condition. The area surrounded a square in Figure 5-9 shows the provoked skin area where we take the average of concentration as an example.

![Figure 5-9 Concentration of oxyhemoglobin](image)

From the curve fitting we got the product of concentration of each chromophores and the optical path length (CL). It might be possible to estimate optical path length-L by using Monte Carlo simulation.
5.6 GPU Implementation of Non Negative Least Square Algorithm [NNLS]

One of the aims of this thesis work is to speed up the computation of non-negative least square analysis, which is used to compute the concentration of chromophores, by implementing the NNLS algorithm on GPU to gain speed. To address this aim the NNLS fitting algorithm is implemented on a GPU by writing a separate CUDA code.

5.6.1 The GPU Architectures

A graphics processing unit or GPU is highly parallel, multithreaded, many core processor with tremendous computational horsepower and very high memory bandwidth [15] [16]. GPUs are composed of multiprocessors, each multiprocessor containing a smaller set of scalar processors (SP) with Single-Instruction-Multiple-Data (SIMD) architecture. As a result, GPUs are mainly used for algorithms that map to a single instruction multiple thread architecture or problems that can be expressed as parallel computation [17].

The following Figure 5-10 shows how GPUs have evolved much faster in the past few years in comparison with CPUs

![Figure 5-10](image)

Figure 5-10 Shows how fast GPU calculation power has improved exponentially and much faster than that of the CPU [18]

5.6.2 CUDA Programme Structure

CUDA (Compute Unified Device Architecture) is a general purpose parallel computing architecture that allows programmers to use C as high level programming interface. CUDA is used as a computing engine in GPUs that are developed by NVidia and it also support various
programming languages or application programming interfaces. One possible application of CUDA is through MATLAB and MATLAB MEX functions. In this thesis work MEX functions, MATLAB executable functions, have been developed to interface MATLAB with the GPU and perform the computation on the GPU.

In CUDA programming the GPU is viewed as a compute device that works as a coprocessor with the CPU as host. The GPU or the Device has its own DRAM (device memory) and it runs many threads in parallel. As shown in Figure 3-9 a CUDA code is a single source code consisting of both the host and device code. The part of a code that exhibits little or no data parallelism is executed in the host or CPU; on the other hand the part that exhibits rich amount of data parallelism is executed by the NVIDIA C Compiler (NVCC) in the device or GPU.

Kernels are C function defined by the programmer that are executed N times in parallel by N different CUDA threads [16]. A kernel is defined by adding the extension __global__ to declare the function as a kernel. The number of CUDA threads that execute the kernel for a given kernel call are declared by using a new <<<dimGrid, dimBlock>>> execution configuration syntax. Each thread has a unique thread ID that is accessible within the kernel through the built in threadIdx variable.

As an illustration, the following sample code adds two vectors A and B of size N and stores the result in to vector C (see Table 3)

Table 3 Sample CUDA code [16]

```
// Kernel definition
__global__ void VecAdd(float *A, float *B, float *C)
{
    int i = threadIdx.x;
    C[i] = A[i] + B[i];
}

Int main ( )
{
...
// kernel invocation with N threads
VecAdd<<<1, N>>> (A, B, C);
```

Here, each of the N threads that execute VecAdd( ) performs one pair wise addition.
As shown in Figure 5-11 the execution of a CUDA programme starts by executing the host code and then kernel invocation follows. Once the kernel function is invoked the execution moves to the device, where a large number of threads are generated to take advantage of abundant data parallelism. Figure 5-11 shows the execution of one Grid, a collection of thread generated by a kernel during invocation.

As can be seen in Figure 5-12 it is also possible to have more than one grid in kernel execution.
A Kernel is executed as a grid of thread-blocks and a thread-block is a batch of threads that can cooperate with each other by synchronizing their execution and also for by sharing data through a low latency shared memory. In Figure 5-12 there are two grids and in grid 1 there are six block of thread and each block have 15 threads.

Figure 5-12 also shows threads and Blocks with unique IDs so that each thread can be assigned what data to work on.

5.6.3 Device Memories and Data Transfer

In CUDA programming the Device and Host have their own separate memories. Each GPU device comes with its own DRAM (device memory), where kernels operate. The GPU used for this particular application is GeForce 310M with a total memory of 1,024MB and with 16384 bytes of total amount of shared memory per block and 16 cores [21].

In order to execute a kernel on the device, one has to allocate memory on the device and transfer the date to work with from the host memory to the device memory. Once the kernel is executed on the device the output data has to be transferred from the device memory to the host memory and finally all the allocated memory on the host and device has to be freed. In order to perform all this memory allocation on the device and data transfer to and from the device, the CUDA runtime system provides Application Programming Interfaces (API) function calls.

![Figure 5-13 CUDA Device Memory Model [21]](image-url)
Figure 5-13 shows the model of device memory and as can be seen at the bottom of the figure there are Global and constant memories where the Host code can write to (W) and read (R) from. We can also see that device code can R/W per-thread Registers, R/W per-thread local memory, R/W per-block shared memory, and R/W per-grid global memory.

GPUs have hierarchical memory model with significantly different access times to each level. CUDA threads can access data from multiple memory spaces during their execution as shown in Figure 5-13. All threads have private local memory and each thread-block has shared memory visible to all threads in the block and also all threads have access to global memory. Shared memories are the most efficient means for threads to cooperate by sharing the results of their work.

Global memory is a common space where data’s from the host are generally copied and stored and it is also the slowest memory to access data. On the same level to the global memory, we have texture memory on the device but can only be written to from the host. However, access from texture memory is faster than global memory when access patterns are spatially local [17].

As explained on 5.6.2 above CUDA organizes threads in to thread-blocks and thread-blocks are arranged in to 2-D grids. A thread-block can have a 1-D or 2-D threads that are executed on a single multiprocessor. All threads in thread-block can share data through shared memory and these threads in a thread-block efficiently synchronize their instructions and pass data through the shared memory. Synchronization in a thread-block helps to ensure that the thread-block waits for all its threads to complete its last instruction.

As an illustration, the following median filtering code of kernel size 7x7 is given in Table 4

Table 4 median filtering code of kernel size 7x7

```
#include <cuda.h>
#include "mex.h"
#include "cuda_runtime.h"

#define BLOCK_W 8
#define BLOCK_H 8

/* Kernel that executes on the CUDA device*/
__global__ void Med_filt_cuda_mex( float *F, mwSize DATA_W, mwSize DATA_H)
{
  __shared__ float window[BLOCK_W*BLOCK_H][49];

  int x=blockIdx.x*blockDim.x+threadIdx.x;
  int y=blockIdx.y*blockDim.y+threadIdx.y;
  int tid=threadIdx.y*blockDim.y+threadIdx.x;
```
if (x >= DATA_W && y >= DATA_H)
    return;

window[tid][0] = (y < 3 || x < 3) ? 0.0f : F[(y - 3) * DATA_W + x - 3];
window[tid][1] = (y < 3 || x < 2) ? 0.0f : F[(y - 3) * DATA_W + x - 2];
window[tid][2] = (y < 3 || x < 1) ? 0.0f : F[(y - 3) * DATA_W + x - 1];
window[tid][3] = (y < 3) ? 0.0f : F[(y - 3) * DATA_W + x];
window[tid][4] = (x > DATA_W - 2) ? 0.0f : F[(y - 3) * DATA_W + x + 1];
window[tid][5] = (x > DATA_W - 3) ? 0.0f : F[(y - 3) * DATA_W + x + 2];
window[tid][6] = (x > DATA_W - 4) ? 0.0f : F[(y - 3) * DATA_W + x + 3];
window[tid][7] = (y < 2 || x < 3) ? 0.0f : F[(y - 2) * DATA_W + x - 3];
window[tid][8] = (y < 2 || x < 2) ? 0.0f : F[(y - 2) * DATA_W + x - 2];
window[tid][9] = (y < 2 || x < 1) ? 0.0f : F[(y - 2) * DATA_W + x - 1];
window[tid][10] = (y < 2) ? 0.0f : F[(y - 1) * DATA_W + x];
window[tid][11] = (x < DATA_W - 2) ? 0.0f : F[(y - 2) * DATA_W + x + 1];
window[tid][12] = (x < DATA_W - 3) ? 0.0f : F[(y - 2) * DATA_W + x + 2];
window[tid][13] = (x < DATA_W - 4) ? 0.0f : F[(y - 2) * DATA_W + x + 3];
window[tid][14] = (y < 1 || x < 3) ? 0.0f : F[(y - 1) * DATA_W + x - 3];
window[tid][15] = (y < 1 || x < 2) ? 0.0f : F[(y - 1) * DATA_W + x - 2];
window[tid][16] = (y < 1 || x < 1) ? 0.0f : F[(y - 1) * DATA_W + x - 1];
window[tid][17] = (y < 1) ? 0.0f : F[(y - 1) * DATA_W + x];
window[tid][18] = (x < DATA_W - 2) ? 0.0f : F[(y - 1) * DATA_W + x + 1];
window[tid][19] = (x < DATA_W - 3) ? 0.0f : F[(y - 1) * DATA_W + x + 2];
window[tid][20] = (x < DATA_W - 4) ? 0.0f : F[(y - 1) * DATA_W + x + 3];
window[tid][21] = (y == 0 || x < 3) ? 0.0f : F[(y) * DATA_W + x - 3];
window[tid][22] = (y == 0 || x < 2) ? 0.0f : F[(y) * DATA_W + x - 2];
window[tid][23] = (y == 0 || x < 1) ? 0.0f : F[(y) * DATA_W + x - 1];
window[tid][24] = (y == 0) ? 0.0f : F[(y) * DATA_W + x];
window[tid][25] = (x > DATA_W - 2) ? 0.0f : F[(y) * DATA_W + x + 1];
window[tid][26] = (x > DATA_W - 3) ? 0.0f : F[(y) * DATA_W + x + 2];
window[tid][27] = (x > DATA_W - 4) ? 0.0f : F[(y) * DATA_W + x + 3];
window[tid][28] = (y > DATA_H - 3 || x < 3) ? 0.0f : F[(y + 1) * DATA_W + x - 3];
window[tid][29] = (y > DATA_H - 2 || x < 2) ? 0.0f : F[(y + 1) * DATA_W + x - 2];
window[tid][30] = (y > DATA_H - 1 || x < 1) ? 0.0f : F[(y + 1) * DATA_W + x - 1];
window[tid][31] = (y > DATA_H) ? 0.0f : F[(y + 1) * DATA_W + x];
window[tid][32] = (x > DATA_W - 2) ? 0.0f : F[(y + 1) * DATA_W + x + 1];
window[tid][33] = (x > DATA_W - 3) ? 0.0f : F[(y + 1) * DATA_W + x + 2];
window[tid][34] = (x > DATA_W - 4) ? 0.0f : F[(y + 1) * DATA_W + x + 3];
window[tid][35] = (y > DATA_H - 3 || x < 3) ? 0.0f : F[(y + 2) * DATA_W + x - 3];
window[tid][36] = (y > DATA_H - 2 || x < 2) ? 0.0f : F[(y + 2) * DATA_W + x - 2];
window[tid][37] = (y > DATA_H - 1 || x < 1) ? 0.0f : F[(y + 2) * DATA_W + x - 1];
window[tid][38] = (y > DATA_H) ? 0.0f : F[(y + 2) * DATA_W + x];
window[tid][39] = (x < DATA_W - 2) ? 0.0f : F[(y + 2) * DATA_W + x + 1];
window[tid][40] = (x < DATA_W - 3) ? 0.0f : F[(y + 2) * DATA_W + x + 2];
window[tid][41] = (x < DATA_W - 4) ? 0.0f : F[(y + 2) * DATA_W + x + 3];
window[tid][42] = (y > DATA_H - 3 || x < 3) ? 0.0f : F[(y + 3) * DATA_W + x - 3];
window[tid][43] = (y > DATA_H - 2 || x < 2) ? 0.0f : F[(y + 3) * DATA_W + x - 2];
window[tid][44] = (y > DATA_H - 1 || x < 1) ? 0.0f : F[(y + 3) * DATA_W + x - 1];
window[tid][45] = (y > DATA_H) ? 0.0f : F[(y + 3) * DATA_W + x];
window[tid][46] = (x > DATA_W - 2) ? 0.0f : F[(y + 3) * DATA_W + x + 1];
window[tid][47] = (x > DATA_W - 3) ? 0.0f : F[(y + 3) * DATA_W + x + 2];
window[tid][48] = (x > DATA_W - 4) ? 0.0f : F[(y + 3) * DATA_W + x + 3];
// Order elements (only half of them)
for ( int j=0; j<25; ++j)
{
  // Find position of minimum element
  int min=j;
  for ( int l=j+1; l<49; ++l)
    if (window[tid][l] < window[tid][min])
      min=l;

  // Put found minimum element in its place
  const float temp=window[tid][j];
  window[tid][j]=window[tid][min];
  window[tid][min]=temp;
  __syncthreads();
}

F[y*DATA_W + x]=window[tid][24];

/*====================================================================
 Gateway function */
void mexFunction(int nlhs, mxArray *plhs[],
int nrhs, const mxArray *prhs[])
{
/* Find the dimensions of the input matrix */
mwSize DATA_H = mxGetM(prhs[0]);
mwSize DATA_W = mxGetN(prhs[0]);
mwSize size = DATA_H*DATA_H*sizeof(float);

float *F;

/*GPU Memory allocation to hold input and output data*/
if(cudaMalloc((void**)&F, size)!= cudaSuccess)
{
  mexErrMsgTxt("Memory allocating failure on the GPU.");
}

/*Copy data To GPU*/
cudAcp(F, (float*)mxGetData(prhs[0]), size, cudaMemcpyHostToDevice);

/*Excute Kernel*/
// Define the block and grid size - this will have 512 threads per
// thread block and a sufficient grid so that there is a GPU thread per
// element of the input data.
dim3 BlockSize(BLOCK_W,BLOCK_H);

//dim3 Blocks=4;
dim3 Blocks=4;
Medfilt_cuda_mex<<<<Blocks, BlockSize>>>(F, DATA_W, DATA_H);
5.6.4 Lawson and Hanson’s Non Negative Least Square (NNLS) Algorithm

Non Negative least squares (NNLS) problem is a constrained least-squared regression problem where the variables are only allowed to take non negative values.

Lawson and Hanson observed that, if one has M linear equations in N unknowns that are constrained to be non-negative, let the active set Z be the subset of variables which violate the non-negative constraint or are zero and the passive set P be the variables with positive values. According to Lawson and Hanson’s algorithm, only a small subset of variables remains in the candidate active set Z at the solution. If the true active set Z is known, then the NNLS problem is solved by an unconstrained least squares problem using the variables from the passive set [17].

In this thesis work the following Lawson and Hanson’s NNLS algorithm (see Table 5) is adopted and used to calculate the Chromophore concentration at each pixel in the hyperspectral image.

Table 5 Lawson and Hanson’s NNLS Algorithm

| Require: | A ∈ R^{MxN}, x = 0 ∈ R^N, b ∈ R^N, set Z = \{1, 2, …… N\}, P = \ø |
| Ensure: | solution \ x \geq 0 s.t. \ x = \arg \min_{x} \frac{1}{2} \|Ax - b\|^2 |
| 1. While true do |
| 2. Compute negative Gradient \ w = A^T(\ b - Ax ) |
| 3. If Z ≠ \ø, and \ \max_{i \in Z} (w_i) > 0 then |
| 4. Let \ j = \arg \max_{i \in Z} (w_i) |
| 5. Move j from set Z to P |
| 6. While true do |
| 7. Let matrix A^p ∈ R^{Mx*} s.t A^p = \{ columns A_i s.t i ∈ P \} |
| 8. Compute least squares solution y for A^p \ y = b |
| 9. If \ \min_{y_i} (y_i) \leq 0 then |
| 10. Let \ \alpha = -\min_{i \in P} \left( \frac{x_i}{x_i - y_j} \right) s.t \ (column j \in A^p) = (column i \in A) |
| 11. Update feasibility vector \ x = x + \alpha (y - x) |
| 12. Move from P to Z, all i ∈ P s.t. \ x_i = 0 |
In this particular work the NNLS algorithm given above (see Table 5) is implemented at each pixel position of the hypercube. The $A$ matrix contains the reference absorption spectrum of oxyhemoglobin, deoxyhemoglobin, melanin, and water. The $b$ vector contains the measured spectrum of the tissue under investigation.

As can be seen in line 3 in the algorithm the termination condition checks if the gradient is strictly positive or if the residual can no longer be minimized. And in line 5 of the NNLS algorithm, the active set $Z$ is updated by first moving the largest positive component variable in the negative gradient $w$ to the positive set; as a result the residual 2-norm is reduced.

Line 7 and 8 of the algorithm variables move from set $Z$ to set $P$, the variables that move from set $Z$ to set $P$ form a candidate linear least squares system $A_P y = b$, where matrix $A_P$ contains the column vectors in matrix $A$ that corresponds to indices in the positive set. At line 11 of the algorithm the feasibility vector $x$ moves towards the solution vector $y$ while preserving non negativity.

The inside loop, line 6 till 17, involves solving the unconstrained least square problem. This in turn involves QR factorization. The QR factorization method used in this implementation is Modified Gram Schmidt algorithm, which is easy to implement on the GPU.

### 5.6.5 Experiment and Result from the GPU Implementation

Once Lawson and Hanson’s NNLS algorithm is implemented on the GPU with full QR factorization using Modified Gram Schmidt algorithm, an experiment was conducted to compare the speed achieved over Matlab non negative least square function (lsqnonneg).

It is found that the GPU implementation outperforms the Matlab lsqnonneg function by the order of 5-7X faster. It is also found that as the number of linear systems increases or the number of pixels analyzed in the image increases the GPU outperforms Matlab even more. Therefore, to get the maximum speed it is recommended to use as many multiprocessors as possible.

In addition to the speed test, the GPU implementation result is compared with Matlab result in order to determine if it gives a reasonable result. UV provoked skin image shown in
Figure 5-14 is used to see if the GPU implementation can fit with the measured spectrum of the tissue and the following acceptable result is found.

Figure 5-14 UV provoked skin image

Figure 5-15 Comparison between data from the measured spectrum and fitted spectrum

Figure 5-16 Comparison between data from measured spectrum and fitted data from GPU Implementation
As can be seen in Figure 5-15, Figure 5-16, and Figure 5-17 the CPU gets a better fit than the GPU implementation. The GPU fitting result can possibly be improved by changing the QR factorization method used.
6 Evaluation of the System
The Hyperspectral Imaging system and the developed algorithm is evaluated by conducting two experiments. One is to occlude the flow of blood in the tip of the finger to obtain a difference in concentration of chromophores. The other is by provoking the skin using a UV light source and checked for erythema development by analyzing the oxyhemoglobin image at different point in time. The melanin concentration change is also checked at different point of time from exposure. At the end of this chapter the discussion and result part is presented.

6.1 Change of the blood flow in the tip of fingers
In this experiment, we obtained four different image cubes under different conditions. One is obtained under a general condition, no provocation is done. The second is obtained under a low or no blood condition. This was achieved by rolling a tightly tied-up rubber band from the tip of a finger to the direction of the palm. The third one is obtained from occluded finger; the tip of the finger is tied up tightly using rubber band and waited for two minutes till the oxygen level in the finger tips is used up. The last is obtained from a re-perfused finger, where oxygenated blood flows in to a previously occluded fingertip. The image is taken immediately after releasing the occlusion of the finger tip. All image cubes are calibrated by taking white reference and black reference image cubes for the calibration.

In this experiment three different skin type subjects, caucasian, dark, and yellow skins are used to see the consistency of the result.

![Figure 6-1 Tip of finger under occlusion. Left panel: concentration of OH and Right panel: concentration of DOH](image)

In Figure 6-1, the left panel shows the concentration of oxyhemoglobin. This panel shows low concentration of oxyhemoglobin in the finger tip, and comparatively to this area, high concentration of oxyhemoglobin is observed in the area where blood is not occluded. In contrast,
the right panel shows the concentration of deoxyhemoglobin. This panel shows high concentration of deoxyhemoglobin at the fingertip and low concentration of oxyhemoglobin in the area where blood is not occluded. Under the condition of occlusion, where the blood supply is limited at the finger tip, the oxygenation of hemoglobin in the area decreases.

Figure 6-2 Top left panel: concentration of OH at the tip of a finger; Top right panel: concentration of DOH at a tip of a finger. Bottom left panel: Absorbance relating to wavelength in the normal area; bottom right panel: Absorbance relating to wavelength in the occluded area. In Figure 6-2 yellow color represents high value.

In Figure 6-2, different curve shape is observed. There is low blood remaining in the occluded area and there is a significant peak at 555nm which is known as a peak of absorbance of deoxyhemoglobin and there are two peaks at 540nm and 580nm which are known as peaks of absorbance of oxyhemoglobin compared to the normal area. The difference of color in Figure 6-2 shows that the occluded area is less oxygenated than the normal skin area.
6.1 UV provocation of skin

In this experiment a UV-B diagnostic device (TH-1 Skintest Kit, Philips PL-12 lamp, Cosmedico Medizintechnik GmbH, Villingen-Schwenningen, Germany) produced a homogeneous broadband irradiance field with a constant value of 3.2 mW/cm². The device has a grid pattern which may deliver up to 6 different irradiance values simultaneously, but was modified in order to produce only one homogenous irradiance field at a time, with the same square area sizes from one exposure site to another.

The provocation is conducted at two different skin type subjects, dark, and Asian skin types, to evaluate the skin type dependency on erythema development. The skin type of each subject is type V, type IV respectively (Fitzpatrick’s skin type classification [22]). Each subject is exposed with UV light for 37 and 25 seconds at four sites on the hand as shown on Figure 6-3, with a dose in the exposed area of 118 mj/cm² and 135 mj/cm² respectively.

![Figure 6-3 shows the exposed place of the hand from the dark skin subject](image)

All skin sites were evaluated before provocation (0h) as well as at 2,3,4,5,6,24 and 48 hours after exposure. Table 6 summarizes all the measurements.
Table 6 Summery of the measurements and data analysis performed. Roman numerals refer to test subject and skin phenotype according to Fitzpatrick.

<table>
<thead>
<tr>
<th>wavelength region</th>
<th>time of assessment</th>
<th>reactions assessed</th>
<th>number of measurements</th>
<th>performed analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>450–720 nm</td>
<td>0</td>
<td>IV, V</td>
<td>8</td>
<td>baseline determination</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>Initial erythema development</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td></td>
<td>melanin production</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.2 Data analysis

The data analysis performed aimed at demonstrating the system’s ability to evaluate various aspects of the UV responses. The analysis of erythema development, i.e. the change in melanin concentration was done by using the images obtained from the fitting process of the provoked area based on the discussed above. The images obtained are called OH, DOH and melanin images and all of them are filtered using median filter in order to reduce noise before further analysis was conducted.

6.2.1 Erythema Development

Research teams, trying to describe the onset mechanisms of UV-B erythema by the action of UV produced mediators, have previously shown that the onset of the response starts very early, in some cases almost immediately after irradiation. The reaction is then expected to peak at some time between 6 and 24 hours after provocation. In our results subject1 (measurement at 0, 2, 3, 4, 5, 6, 24 hours with different exposure time) showed an increase in Oxyhemoglobin within the irradiated area and a decrease in Deoxyhemoglobin during the first 6 hours (Figure 6-4) and the change in melanin concentration is also evaluated during the same time interval and the analysis is given below. [14]

The erythema development was found as an increase in the response magnitude of the fitted OH concentration in the provoked area during the first 6 hours for skin type V Figure 6-4. In this result, faster increase is observed in higher dose during the first four hours. Since a lower dose is associated with a slower erythematous onset [14], this result follows this statement.
Figure 6-4 Change in Concentration of Oxyhemoglobin and Deoxyhemoglobin with different dose.

We can see on Figure 6-5 a faster response in subject 1. This corresponds to the result that after 2 hours from the provocation of the skin of both subjects, the provoked area of subject 1 reacted faster. And finally, the concentration of Oxyhemoglobin and Deoxyhemoglobin reached stable value.

Figure 6-5 Concentration of Oxyhemoglobin and Deoxyhemoglobin with different skin type.
6.2.2 Melanin content and change in concentration after provocation
Increase in melanin concentration is known to occur the next few days after UV-B irradiation; 72 hours post provocation is a commonly reported time for detectable enhancement of epidermal melanin [14].

Figure 6-6 Concentration of melanin with different skin type
Since the skin of subject1 is darker than subject2, the initial concentration (0 hour) of melanin of subject1 is higher than the one of subject2. Though increase of melanin concentration was expected after 96 hours from the provocation, the concentration decreased in both of subjects. For accurate determination of melanin concentration the analysis also requires the study of the scattering coefficients.
Since the dose is different from each other, the more provoked skin region, which is the area exposed 37 seconds, was expected to get high melanogenesis after 96 hours from the provocation. However significant difference was not observed between these. In addition, though the increase of melanin concentration was expected, the concentration decreased in both of the subjects.

6.3 Result from the UV provoked skin and the fitted spectra

Figure 6-8 Example of oxygenated hemoglobin (OH) image as derived from the analysis
Figure 6-8 Example of oxygenated hemoglobin (OH) image as derived from the analysis. (subject V, exposure time: 37 sec, 6 hours after provocation). Number 1 indicates the place which is not provoked. Number 2 indicates the place which is provoked.

Figure 6-9 The left panel shows the absorbance spectra taken from Figure 6-9 and the right panel shows the absorbance spectra taken from the concentration image of deoxyhemoglobin.

### 6.4 Conclusion

Accuracy in determining optical properties has a vital importance to quantitatively determine Chromophores in tissue. Accurate determination of tissue Chromophores requires an analysis algorithm that considers the effect of both scattering and absorption during light tissue interaction.

In this thesis work the effect of scattering in tissue is added in the prediction model by assuming that human skin has approximately similar scattering event as milk and the milk scattering model is adopted and included in this analysis algorithm. The findings shows that our HSI system gives similar results that have been reported by other researchers. This algorithm has given very good fitting result for the measurements done at 7nm wavelength step.

We can also see that the result matches the theory in the time dependent change of oxyhemoglobin and deoxyhemoglobin. However, the result of melanin does not correspond to the theoretically expected result.

It is found that the GPU implementation outperforms the Matlab lsqnonneg function by the order of 5-7X faster than Matlab. It is also found that as the number of linear systems increases or the number of pixels analyzed in the image increases the GPU implementation displays an even larger performance increase compared to the Matlab implementation. Therefore, to get the maximum speed it is recommended to use as many multiprocessors as possible.

In addition to the speed test, the GPU implementation result is compared with Matlab result and found that the CPU resulted in a better fit than the GPU. The GPU implementation can be
improved by changing the full QR factorization into partial decomposition or by full QR decomposition via given’s rotation and Householder’s reflection also helps to improve the fitting result.
7 Table of Figures

Figure 2-1 The organization of skin. Thin, hairy skin (left). Thick, hairless skin (right). The epidermis has been partially peeled back to show the interdigitating dermal and epidermal papillae [2]................................................................. 10

Figure 2-2 Vascular supplies to the skin. A, Note the various horizontal plexuses fed by direct cutaneous, fasciocutaneous and musculocutaneous arteries. B, Higher magnification of vascular supply [15]................................................................. 11

Figure 3-1 One of the Principle of Spectrometer................................................................. 12

Figure 3-2 Basic Set Up of a Spectrometer ........................................................................ 13

Figure 3-3 Schematic diagram of Chromophore idealized as a sphere [5]............................ 15

Figure 3-4 Primary absorption spectra of biological Tissues [6]........................................ 16

Figure 3-5 Schematics of Scattering particle idealized as sphere with geometric size A and effective Cross section δs [5].................................................................................. 18

Figure 3-6 Shows photon deflection angle for single scattering event [6]............................. 19

Figure 4-1 shows the absorption spectra of Oxy and Deoxyhemoglobin [5]......................... 22

Figure 4-2 Extinction coefficient vs. wavelength for eumelanin and pheomelanin [10]......... 24

Figure 4-3 Absorption coefficient corrected for scattering contribution versus wavelength. The error bars represent the standard deviation over the 2 temperatures i.e. 37, 60°C [9] [11]......... 25

Figure 5-1 Shows a three dimensional (two spatial dimension and one spectral dimension) Hyperspectral image cube [13].................................................................................... 26

Figure 5-2 set up of HSI system [14].................................................................................. 27

Figure 5-3 the collimated transmission set up [4]............................................................... 28

Figure 5-4 shows the absorption coefficient of ink ............................................................. 29

Figure 5-5 shows the reduced scattering coefficient of milk. ........................................... 30

Figure 5-6 the curve fitting at 630nm showing a good fit ................................................ 30

Figure 5-7 the curve fitting at 500nm it shows the noisy range .......................................... 31

Figure 5-8 the fitting result of wavelength vs. intensity for four different values of μ_s\textsuperscript{\textit{s}} [mm\textsuperscript{-1}] .................................................................................. 34

Figure 5-9 Concentration of Oxyhemoglogin.................................................................... 36

Figure 5-10 Shows how fast GPU calculation power has improved exponentially and much faster than that of the CPU [18]........................................................................... 37

Figure 5-11 Execution of a CUDA Programme [19]......................................................... 39

Figure 5-12Thread Batching in a Kernel: Grids and Blocks [20]........................................ 39

Figure 5-13 CUDA Device Memory Model [21]................................................................... 40

Figure 5-14 UV provoked skin image .................................................................................. 46

Figure 5-15 Comparison between data from the measured spectrum and fitted spectrum...... 46

Figure 5-16 Comparison between data from measured spectrum and fitted data from GPU Implementation ................................................................. 46

Figure 5-17 Comparison between measured spectrum, the fitted spectrum from Matlab, and GPU ........................................................................................................ 47
Figure 6-1 Tip of finger under occlusion. Left panel: concentration of OH and Right panel: concentration of DOH. 

Figure 6-2 Top left panel: concentration of OH at the tip of a finger; Top right panel: concentration of DOH at a tip of a finger. 

Figure 6-3 shows the exposed place of the hand from the dark skin subject. 

Figure 6-4 Change in Concentration of Oxyhemoglobin and Deoxyhemoglobin with different dose. 

Figure 6-5 Concentration of Oxyhemoglobin and Deoxyhemoglobin with different skin type. 

Figure 6-6 Concentration of melanin with different skin type. 

Figure 6-7 Change in Concentration of melanin with different dose. 

Figure 6-9 Example of oxygenated hemoglobin (OH) image as derived from the analysis. 

Figure 6-10 The left panel shows the absorbance spectra taken from Figure 6-9 and the right panel shows the absorbance spectra taken from the concentration image of deoxyhemoglobin.
8 Bibliography


