Multispectral Imaging of Skin Oxygenation

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Jiwei Huang

Graduate Program in Biophysics

The Ohio State University

2013

Dissertation Committee:

Ronald Xu, advisor; Kun Huang; Alper Yilmaz
Abstract

This research focuses on the investigation of a multispectral imaging technique for quantitative measurement of skin tissue oxygen saturation (StO₂).

Oxygen saturation is the percentage of oxygen carried in the blood over the total oxygen carrying capacity of blood. Skin StO₂ is an important indicator of skin tissue clinical status. Measurement of skin tissue oxygenation has been in many clinical applications such as cosmetic product development, chronic wound care and plastic surgery tissue recovery monitoring.

For decades, researchers have been investigating optical spectroscopy and imaging techniques for measuring tissue oxygen saturation. However, many existing techniques are subjective and qualitative due to background bias, tissue heterogeneity, and inter-patient variation. A technique for quantitative and reliable measurement of skin tissue oxygen saturation is necessary. Multispectral / hyperspectral imaging is a promising technique which has been proposed for quantitative measurement of skin StO₂ by capturing and analyzing skin images at multiple wavelengths.

This dissertation reports a multispectral imaging method which is capable of (1) quantitative measurement of skin oxygen saturation with minimal bias from different skin types, and (2) imaging skin oxygen saturation dynamics. There’re mainly three parts of this research.
First, theoretical study and algorithm development. A numerical model was established to simulate skin tissue reflectance of different skin conditions including oxygen saturation, blood concentration, tissue scattering and melanin concentration. From the simulation result, a parameter SDR, which can be calculated from skin reflectance images of 544, 552, 568, 576, 592, 600nm, was found to be mainly determined by oxygenation regardless of different tissue conditions. Thus, skin oxygen saturation can be measured by taking multispectral images of six wavelengths.

Second, imaging system development. A multispectral / hyperspectral imaging system was built by integrating camera, filter and light source. A graphical user interface was developed for equipment control, synchronization and image acquisition. Imaging processing algorithm was developed for generation of skin oxygen saturation map from the reflectance image of six wavelengths.

Third, benchtop experiments and human subject test. The imaging method was first verified using a phantom which was a liquid mixture of different concentrations of blood, intralipid and ink to simulate different skin conditions. StO2 measured by our method was not affected by the different concentration of phantom materials. Then, multispectral imaging of skin oxygenation was demonstrated by a human subject test in which 10 subjects with different skin types were recruited. The variation of skin StO2(%) measured by multispectral imaging is over two times smaller than the measurements from other two oximetry techniques, which indicates that skin StO2 measurement by our method is not affected by different skin types. After that, skin StO2 dynamics was measured using a postocclusive reactive hyperemia (PORH) protocol. The change of
StO$_2$ measured by our system was compatible with the oxygen measurements by other oximetry techniques. Finally, in order to test if our StO$_2$ imaging method is affected by additional background absorption, measurement was taken on the skin on which a layer of ink was painted. The averaged skin StO$_2$ measured by our system did not show a significant difference with the additional ink layer.

The major innovations of this research include (1) the method of multispectral imaging of skin StO$_2$ using the above six wavelengths is firstly reported; (2) it’s capable of imaging StO$_2$ dynamics; and (3) it has the potential for quantitative imaging of skin StO$_2$ with minimal bias from different skin conditions.

This research is a comprehensive investigation on a multispectral imaging method for quantitative measurement of skin oxygen saturation. It provides not only a new approach for spectroscopic imaging algorithm development but also a clinical tool for many applications.
Dedication

This dissertation is dedicated to

My parents

who encourage me to study abroad,

My larger families including many of my aunts, cousins and nephews,

And

My advisor Dr. Ronald Xu

for always supporting me for my development
Here I would like to express my sincere thanks to those who gave me guidance, support and help during my Ph.D. study.

I would like to thank my advisor Dr. Ronald Xu for his mentoring and guidance. There’re many stories I want to tell about how Dr. Xu has helped and cared about me during my Ph.D. life. I want to write here three things which have been so critical to my last five years and to my future. First and most importantly, I want to thank Dr. Xu for really caring about my career development. He brought me to attend many national and international conferences and to visit companies. He gave me the opportunities to develop my interpersonal skills by getting in touch and working with entrepreneurs, doctors and people from other disciplines. When I felt I wanted to develop my leadership skills by participating in student organization, he supported me. When I felt I needed to networking with other researcher by attending summer schools, he supported me. When I felt I didn’t have any teaching experience so that I wanted to take a lot of time being a TA in another department, he supported me. When I felt I needed working experience in the industry so that I decided to do internships during the summer, he supported me. When I felt I needed to explore other career possibilities by attending many classes from other departments, he supported me. Now after five years when I looked back, I found I have studied or worked in almost all the engineering and science departments of OSU, I have been to most parts of US, and I have a unique and
comprehensive resume from my five years’ of life in Dr.Xu lab. At the time I most needed to explore my career, I got the support, care and opportunity from him. I feel really grateful and lucky to be Dr.Xu’s student and I do not take his genuine support and care for granted. I will work hard and make good use of all the experience I gained from these five years. Second, I would like to thank Dr.Xu to being so tolerant and patient. I made many mistakes and had many bad habits during research (such as not taking good care of some expensive instruments). I was also distracted by other issues of life. I know we’re all human beings and we all make mistakes and have personal issues. But it’s very precious to me that my advisor was so tolerant and patient and gave me time to solve my problems to improve myself. Third, I want to thank Dr.Xu for respecting my ideas and being open-minded for my research. He always respect my opinions and ideas of research including big decision of selecting biomedical optical imaging to be my dissertation to small ideas of experiment design. There’re much more than three aspects to say about my thanks to Dr.Xu. I feel extremely grateful and I really appreciate Dr.Xu’s great mentoring, guidance, support and care for my Ph.D. life.

Then I would like to Dr. Alper Yilmaz and Dr. Kun Huang for serving in my graduate committee. It was in the last half of a year to my graduation when I invited Dr. Yilmaz to be my graduate committee member. I thank him for being so nice and flexible and giving me in-depth suggestions on my imaging research. I wished I could know him earlier so that I could learn more from him. I also want to thank Dr. Huang. I began working with Dr. Huang over four years ago on a project which is the clinical application
of the imaging system presented in this dissertation. Dr. Huang is very knowledgeable
and I learned a lot from him during all the research meetings.

I would like to thank Dr. Chandan Sen at The Ohio State University Medical
Center. As an internationally renowned researcher and the leader in the field of wound
care, he has provided me a lot of scientific insights and mentorship. His enthusiasm in
translational research is the motivation for us to develop advanced systems and
algorithms for quantitative oxygenation imaging in cutaneous tissue.

I would like to thank all my colleges including Ruogu Qin, Jeff Xu, Leilei Zhang,
Junhua Tang and Surya Gnyawali for giving me constant and tremendous help on my
Ph.D research. I also want to thank Drs Shufang Chang, Shiwu Zhang and Ting Si (who
are professors from China) for giving me insightful input to my projects.

Last but my least, I would like to thank the sponsors who supported my PhD
research projects. These sponsors include National Institute of Standards and Technology,
National Institute of Health, Department of Defense, OSU Alumni Grant, OSU Davis
Heart and Lung Research Institutes, OSU Biophysics Program, OSU Biomedical
Engineering Department, and OSU Center for Minimally Invasive Surgery.
Vita

2003..........................................................Affiliated High School of South China Normal University

2007..........................................................B.S. Physics, Sun Yat-Sen University

2012..........................................................Ph.D., Biophysics Graduate Program, The Ohio State University

Publications


3. R. Qin and J. Huang (co-1st author); J. Xu; L. Ding; S. Gnyawali; C. Sen; K. Huang; R. Xu, Dual-modal quantitative imaging of wound tissue oxygenation and perfusion, Proceedings of SPIE Photonics West Conference, 7557-16(2010)

ix
4. R. Xu, K. Huang, R. Qin, J. Huang, J. Xu, L. Ding, U. Gnyawali, G. Gordillo, S. Gnyawali and C. Sen, Dual-mode imaging of cutaneous tissue oxygenation and vascular function, JoVE, 46., 2010


Fields of Study

Major Field: Biophysics
Table of Contents

Abstract ............................................................................................................................... ii

Dedication ........................................................................................................................... v

Acknowledgments .............................................................................................................. vi

Vita ..................................................................................................................................... ix

Table of Contents ............................................................................................................... xi

List of Tables .................................................................................................................. xvii

Chapter 1: Clinical significance and goal of the research .................................................. 1

1.1. Clinical background and significance ...................................................................... 1

1.1.1. Oxygen transport in biological organisms and oxygen saturation .................... 1

1.1.2. Importance and limitations of oxygen measurement ......................................... 5

1.2. Goal and overview of the research ........................................................................... 7

Chapter 2: Technical background ....................................................................................... 9

2.1. Interaction between light and skin tissue ................................................................. 9

2.1.1. Absorption ....................................................................................................... 10

2.1.2. Scattering ......................................................................................................... 11

2.2. Skin anatomy .......................................................................................................... 13
2.2.1. Seven skin layers ................................................................. 13

2.2.2. Skin types and melanin .......................................................... 16

2.3. The optical principle of SO₂ measuring techniques ...................... 18

2.4. Current SO₂ techniques, challenges and recent new methods under investigation 22

2.4.1. Technical review of current SO₂ optical oximetry techniques .......... 22

2.4.2. Challenges and limitations ......................................................... 29

2.4.3. New multispectral/hyperspectral imaging algorithms for quantitative SO₂ imaging .......................................................... 31

2.5. Basic idea of our imaging method: apply derivative spectroscopy to imaging for quantitative SO₂ measurement .................................................. 33

Chapter 3: Algorithm development of multispectral imaging of skin oxygenation .... 41

3.1. Simulation of spectral reflectance of skin ........................................ 42

3.1.1 Two light transport models for simulation of skin reflectance ............ 42

3.1.2. Monte-Carlo simulation of light transport in seven layer skin ............ 44

(1)Monte-Carlo method and seven layer skin model ................................. 44

(2)Result: simulated skin reflectance by Monte-Carlo method ..................... 48

3.1.3. Semi-infinite tissue model ........................................................ 48

(1)Modified semi-infinite tissue model .................................................... 48
(2) Result: simulated skin reflectance by semi-infinite tissue model .................................. 54

3.1.4 Finding absorption weight factors of semi-infinite tissue model ................................ 57

(1) Optimization problem of matching skin reflectance simulated by two models ............... 57

(2) Result: optimized weight factors for different skin conditions ........................................ 67

3.2. Finding the relationship of StO2 and the second derivative ratio (SDR) of r (= μa/μs') .................................................. 74

3.2.1 Backward calculation from reflectance to the ratio of absorption versus scattering ........................................................................................................ 74

3.2.2. Second derivative ratio (SDR) of r (= μa/μs') and the analytical expression of StO2~SDR ........................................................................................................... 78

3.2.3. Screening SDR to find suitable wavelengths for StO2 measurement ...................... 89

3.2.4. Result: SDR(568, 576, 24) for skin oxygen saturation imaging ............................... 92

(1) StO2 ~ SDR(568, 576, 24) ....................................................................................... 92

(2) Reason of choosing SDR(568, 576, 24) explained by analytical spectra analysis ............................................................. 97

(3) Error analysis: influence of weight factors on StO2 ~ SDR(568, 576, 24) ............. 101

(4) SDR(542, 584, 20) – A bad example which cannot be used for oxygen imaging ........................................................................... 103

Chapter 4: Multispectral imaging system ......................................................................... 105
4.1. Imaging system: hardware ................................................................. 105
4.2. Graphical user interface (GUI) ............................................................ 107
4.3. Measurement procedures ................................................................. 109
4.4. Image processing and oxygen map generation ................................. 110

Chapter 5: Experiment: Multispectral imaging of StO2 of tissue phantom ........................ 116
5.1. Introduction to phantom and its role in optical calibration .................. 116
5.2. Design of the tissue-simulating phantom ........................................... 118
5.2.1. Finding proper concentrations for the phantom ingredients ................ 118
(1) Absorption from bovine/porcine blood ............................................. 119
(2) Absorption from ink ........................................................................... 121
(3) Scattering of intralipid ..................................................................... 126
(4) Absorption of intralipid ..................................................................... 130
5.2.2. StO2 imaging algorithm for tissue phantom ..................................... 131
5.3. Experiment protocol ......................................................................... 136
5.4. Experiment result ............................................................................. 137

6.1. Imaging skin StO2 dynamics during using postocclusive reactive hyperemia (PORH) ................................................................. 140
6.1.1. Experiment protocol ...................................................................... 140
6.1.2. Oxygen map of the arm ................................................................. 141

xiv
6.1.3. Oxygenation change during PORH ................................................................. 144

6.2. Oxygenation of in vivo skin of different skin types ........................................ 146

6.2.1. Experiment protocol .................................................................................. 146

6.2.2. Evaluation of influence of skin types on oxygen measurement using
biostatistics ........................................................................................................... 146

6.3. Measuring StO2 of in vivo skin with additional background absorption ....... 154

6.3.1 Experiment protocol ................................................................................... 154

6.3.2 Comparison of oxygenation map ............................................................... 155

6.3.3. Statistical analyses ..................................................................................... 156

7.1. Major innovations of this research ............................................................... 158

7.2. Future work .................................................................................................. 159

7.3. Conclusion .................................................................................................... 160

References ........................................................................................................... 162

Appendix A: IRB approved clinical protocol ....................................................... 166

Appendix B: Selecting an oximetry technique for oxygen measurement comparison... 170

B.1. Principle of TCM ......................................................................................... 170

B.2. Fluorescence/ phosphorescence based oxygen sensor .................................... 172

B.3. A hyperspectral imaging method suitable for quantitative comparison with our
multispectral imaging method .............................................................................. 174
List of Tables

Table 1. Physical, biological and optical properties of seven skin layers ......................... 14
Table 2. Definition, features and tanning ability of six skin phototypes [13] .................. 16
Table 3. Definition of 3 skin types [14] ........................................................................... 17
Table 4. List of oximetry techniques. ................................................................................ 23
Table 5. Comparison of two methods for skin reflectance calculation ......................... 42
Table 6. Coefficients $a_1$ and $a_2$ for $\mu_s'$ of different skin layers ......................... 47
Table 7. Different tissue conditions for simulation of skin reflectance ................. 54
Table 8. Primary weight factor for each skin layer ...................................................... 60
Table 9. Resulted converged weight factors ................................................................. 63
Table 10. Six parameters affecting weight factors ..................................................... 69
Table 11. Optimized weight factor of seven skin layers at different StO$_2$ levels .... 71
Table 12. Data table of SDR(568, 576, 24) ................................................................. 93
Table 13. Data of StO$_2$–SDR relationship ................................................................. 102
Table 14. Absorbers in in vivo skin and the corresponding absorber used in phantom . 119
Table 15. Reduced scattering coefficient of 10% intralipid from oximeter measurements and published data. (Note: scattering of intralipid is much larger than the scattering of 5% blood so that here intralipid was measured without mixing with blood) ................. 129
Table 16. Absorption coefficient of intralipid ............................................................... 130
Table 17. Comparison of SDR(568,576,24) from skin and phantom model .......... 135
Table 18. 10 subjects arranged in the order of skin darkness and grouped into three skin types ................................................................................................................................ 147

Table 19. Data of averaged skin StO2 of 10 subjects from Figure 53 ......................... 148

Table 20. Data of averaged skin StO2 of 10 subjects from Figure 54 .......................... 150

Table 21. Comparison of oxygen measurement variations from different subjects. ...... 153
1.1. Clinical background and significance

This research is to explore and investigate a multispectral imaging technique for measurement of oxygen content in skin tissue (i.e., oxygen saturation, StO2). The importance of oxygen to our lives and its role as a clinical indicator of tissue viability are described here.

1.1.1. Oxygen transport in biological organisms and oxygen saturation

Oxygen is a compound essential to all aerobic organisms for survival and to maintain cellular integrity since it takes part in all kinds of metabolic reactions. Although large quantity of oxygen is used in aerobic metabolism, tissue itself does not have a storage system for oxygen. Oxygen is transported by a respiratory and circulation system from the respiratory organ (such as lung) to the peripheral tissue so that oxygen can be continuous supplied to each cell to sustain aerobic metabolism.

The first step of oxygen transport is the uptake of oxygen by lungs or other respiratory organs. In the lung, oxygen diffuses across the alveolar membrane to pulmonary capillaries. The venous blood has a lower oxygen partial pressure (pO2) than the alveoli. Oxygen partial pressure, also called oxygen tension, is the partial pressure of oxygen...
dissolved oxygen. Since there’s about 20.9% of oxygen in air and the air pressure is 760mmHg, the oxygen partial pressure in the alveoli is close to $760 \times 20.9\% \approx 160$mmHg. Driven by the partial pressure difference, oxygen molecules diffuse into the blood and then cross the membrane of the red blood cells (erythrocytes).

The second step of oxygen transport is binding of oxygen to the hemoglobin in the red blood cells. Hemoglobin is a protein with oxygen carrying capacity. Its structure and principle of oxygen binding has been studied intensively. It is made up of four protein subunits. Each subunit consists a protein chain tightly associated with a non-protein heme group. A heme group is composed of a single charged atom of iron in the center nestled in a heterocyclic ring, known as a porphyrin. When an oxygen molecule encounters a molecule of hemoglobin, it binds to one of the four heme groups by attaching to an iron atom. Since there’re totally four heme groups in a hemoglobin molecule, four oxygen molecule can be carried by a hemoglobin molecule at most. Most oxygen transported in the blood is attached to hemoglobin (>97%) with only a small amount dissolved in the plasma. Hemoglobin bind with oxygen is called oxygenated hemoglobin (i.e. oxy-hemoglobin). It has a bright red color as in arterial blood.

The third step is the global delivery of oxygen from lungs to tissue and diffusion of oxygen from capillary to cell. Oxygen, after densely packed in red blood cells, is transported by the circulation system from the lung artilleries to the capillaries of peripheral tissue of the body. Since the oxygen partial pressure in the arterial capillaries is higher than that in the tissue, oxygen is gradually unloaded from hemoglobin. Under normal circumstances, three molecules of oxygen are unloaded at most. Hemoglobin
partially bind with oxygen or without binding with oxygen is called deoxygenated hemoglobin (i.e. deoxy-hemoglobin). It makes the blood appear dark red or purplish which indicates the blood becomes venous blood. After being released from hemoglobin, oxygen molecules diffuse from blood to the cells in the surrounding tissue and are then used in all kinds of cellular metabolic reactions and bioenergetic processes.

Due to its importance to the survival of all aerobic organisms, oxygen transport has been intensely studied clinically. Oxygen saturation is the most important parameter in the research of oxygen transport.

Oxygen saturation, \( \text{SO}_2 \), is defined as the percentage of hemoglobin binding sites in the bloodstream occupied by oxygen. It can also be defined as the percentage of oxy-hemoglobin among total hemoglobin. Oxygen saturation \( \text{SO}_2 \) can be further classified into different types. Arterial oxygen saturation \( \text{SaO}_2 \) and venous oxygen saturation \( \text{SvO}_2 \) are oxygen saturation levels in arterial and venous blood, respectively. Tissue oxygen saturation \( \text{StO}_2 \) defines the averaged oxygen saturation in a volume of tissue containing a variety of vasculatures. Pulse oxygen saturation (\( \text{SpO}_2 \)) is the peripheral oxygen saturation level measured by a pulse oximeter device.

\( \text{SO}_2 \) can be defined as the averaged concentration of oxy-hemoglobin over the concentration of total hemoglobin.

\[
\text{Equation 1: } \text{SO}_2 = \frac{c_{\text{HbO}_2}}{c_{\text{Hbt}}} = \frac{c_{\text{HbO}_2}}{c_{\text{HbO}_2} + c_{\text{Hb}}}
\]

In which \( \text{HbO}_2 \), \( \text{Hb} \) and \( \text{Hbt} \) denote oxy-hemoglobin, deoxy-hemoglobin and total hemoglobin, respectively.
As mentioned above, another important parameter about the oxygen is oxygen tension, which is the partial pressure of dissolved oxygen: pO₂. The main difference between StO₂ and pO₂ is that StO₂ a parameter providing function information of biological properties while pO₂ is a parameter describing physical property of tissue. The relationship between oxygen saturation and oxygen tension is called oxygen–hemoglobin dissociation curve, as shown in Figure 1. This relationship can be expressed as [1]:

Equation 2: \[ SO_2 = \left( \frac{a}{pO_2^3 + b \cdot pO_2} + 1 \right)^{-1} \]

In which \( a \approx 23400 \) and \( b \approx 150 \) measured from human blood at 37°C and PH=7.4[1].

Figure 1. Oxygen hemoglobin dissociation curve
1.1.2. Importance and limitations of oxygen measurement

Oxygen is considered as an indicator and predictor of tissue viability in many processes such as wound healing, plastic surgery, and tissue reattachment during emergency. In this process, unsurviable tissue or unhealed wounds have been found associated with poor oxygen and perfusion. Thus, measuring tissue oxygen has huge clinical significance, which can be shown by a good clinical example: chronic wound care.

The incidence of chronic wounds is 5-7 million per year in the U.S. and the annual cost for chronic wound management is greater than $20 billion. Chronic wound may lead to many complications including infection, contractures, depression and even limb amputation. Since oxygen is one of the most important parameters to study and measure for understanding of the mechanism of chronic wound and monitoring of wound healing process, oxygen measurement is a huge clinical need. Measuring wound tissue oxygenation can help clinicians to make better informed decisions in clinical procedures including diagnosis, staging, therapeutic assessment, healing prediction and image guided wound care (such as debridement and oxygen therapy). Measuring wound tissue oxygenation can also help developing tissue database for normal tissue, acute wounds, and chronic wounds and building an expert system for clinical wound etiology. Thus, a reliable technique measuring tissue oxygen saturation is greatly needed.

There’re already many techniques clinically available for measuring tissue oxygenation. However, they all have certain limitations. First, most of these techniques
place a sensor on the skin tissue to give a single point and contact measurement of \( \text{StO}_2 \), including phosphorescence oximeter[2], fluorescence optical sensors[3], tissue oximeter [4] and pulse oximeter [5]. This method may not satisfy the clinical need in many clinical cases such as measuring wound tissue oxygen because the sensor can only be put on the surrounding healthy tissue rather than the wound tissue so that the measurements are not real local measurement of the target. Moreover, clinicians are interested in the whole oxygen distribution of the wound tissue and the surrounding healthy tissue. Thus, an imaging technique is necessary since it allows non-contact measurement of \( \text{StO}_2 \) of a large area of skin tissue. Second, there’re some imaging techniques for tissue oxygen measuring but very few are commercially available and most of them are still under investigation. This is because (1)many of these techniques only provides relative measurement of tissue oxygen and (2) the oxygen measurement are affected by skin pigmentation, background bias, tissue heterogeneity and inter-subject variations. Finally, no imaging techniques are currently available to measure the dynamic change of tissue oxygen.

As all these clinical significance and limitations of current techniques being said, a reliable imaging technique providing non-contact and quantitative measurement of tissue oxygen is greatly needed.
1.2. Goal and overview of the research

In this study, we explored and investigated a multispectral imaging method which is capable of (1) quantitative imaging of skin tissue oxygen saturation (StO2) with minimal bias by different skin conditions and (2) imaging StO2 dynamics. First, the technical background was comprehensively reviewed (Chapter 2). A numerical model was then established to simulate skin tissue reflectance of different skin conditions including oxygen saturation, blood concentration, tissue scattering and melanin concentration (Chapter 3.1). Based on the simulation result, it was found from that a parameter called second derivative ratio (SDR), which was calculated from skin reflectance at 544, 552, 568, 576, 592, 600nm, was only determined by skin oxygenation regardless of different tissue conditions (Chapter 3.2). Thus, skin oxygenation could be quantitatively measured by taking skin images at these six wavelengths (Chapter 4). This method was first verified using a phantom by mixing different concentrations of bovine blood, intralipid and ink to simulate different skin conditions (Chapter 5). StO2 at different levels measured by our imaging method were found to be unaffected by different phantom recipes. Then, multispectral imaging of skin oxygenation was demonstrated on a human subject test using a postocclusive reactive hyperemia (PORH) protocol (Chapter 6). Skin oxygen saturation (StO2) imaged by our multispectral imaging system was compatible with oxygen measurements by other oximetry techniques (Chapter 6.1). Moreover, the reliability of our imaging method was tested by measuring StO2 of subject with different skin types (Chapter 6.2). The relative variation of skin
StO2(%) measured by our multispectral imaging was much than that of deep tissue StO2(%) measured by ISS tissue oximeter and transcutaneous oxygen tension pO2(mmHg) measured by TCM oxygen tension monitor. Moreover, to test whether our algorithm could reduce measurement bias caused by background absorption, StO2 measurement was taken on the human subject with his skin painted with ink (Chapter 6.3). StO2 was measured by multispectral imaging and compared with a commercial hyperspectral system. Our system showed small difference of StO2 between ink-painted skin and normal skin, while the commercial system showed significant difference. The above results indicate that our multispectral imaging technique can be potentially used for quantitative imaging of dynamics of skin oxygen saturation with minimal bias. Finally, discussions on major innovations and future works of this research are provided and a conclusion is drawn at the end of the thesis (Chapter 7).
Chapter 2: Technical background

2.1. Interaction between light and skin tissue

When a photon hits tissue, there’re two main interactions: absorption and scattering, as shown in Figure 2.

Figure 2. Illustration of light – tissue interaction
2.1.1. Absorption

Absorption is the light energy lose and transfer to heat to the absorbers. Beer–Lambert law shows the relationship between the light energy before \((I_0)\) and after \((I)\) passing through a material with length \(l\).

\[
\text{Equation 3: } I = I_0 e^{-\varepsilon Cl} \quad \text{(a)} \quad \text{or} \quad I = I_0 \left(10^{-\varepsilon Cl}\right) \quad \text{(b)}
\]

In which \(C\) is the concentration of the material and \(\varepsilon\) is the extinction coefficient.

Then absorption is defined as

\[
\text{Equation 4: } A = \ln\frac{I_0}{I} = \varepsilon Cl \quad \text{(a)} \quad \text{or} \quad A = \log_{10}\frac{I_0}{I} = \varepsilon Cl \quad \text{(b)}
\]

From Equation 4, one can see that the extinction coefficient \(\varepsilon\) is the absorption in unit concentration (usually in one molar) and in unit length.

Then absorption coefficient \(\mu_a\) is defined as

\[
\text{Equation 5: } \mu_a = \varepsilon C
\]

Thus, \(\mu_a\) is the absorption in unit length.

The difference between equations (a) and (b) is that (a) is used for the definition of \(\varepsilon\) and \(\mu_a\), while (b) is used in the measurement by absorption spectrometer. Thus, measurement absorption coefficients need to be transformed for theoretical calculations by

\[
\text{Equation 6: } \mu_a^{(a)} = \mu_a^{(b)} \ln(10) = 2.303 \mu_a^{(b)}
\]

Main absorbers in the skin tissue include oxy- and deoxy- hemoglobin in the blood, melanin, water and fat. Absorption of oxy- and deoxy- hemoglobin makes our tissue appear pink. Absorption of melanin makes our tissue appear dark. The extinction
coefficients of these absorbers are their intrinsic properties which will be discussed in Chapter 2.3.

2.1.2. Scattering

Scattering is the change of light direction when the light encounters a scatter in a medium. The scattering coefficient $\mu_s$ is defined as the cross-sectional area per unit volume of medium. But here we can comprehend this parameter in terms of photon ‘mean free path’ (mfp) which is the pathlength of a photon travel before redirected by a scatter (i.e. before next scattering event). Scattering coefficient $\mu_s$ is the inverse of the mfp:

Equation 7: $\text{mfp} = 1/ \mu_s$

The scattering direction in the tissue can be expressed by Henyey-Greenstein function, which is the probability of photon scattered at angle $\theta$:

Equation 8: $p(\theta) = \frac{1}{4\pi} \frac{1-g^2}{(1+g^2-2g\sin\theta)^{3/2}}$

In which $g$ is the anisotropy factor which defined as the expectation of the cosine of scattering angle $\theta$.

Equation 9: $g = \langle \cos \theta \rangle = \int_{-1}^{1} p(\cos \theta) \cdot \cos \theta \cdot d(\cos \theta)$

And

Equation 10: $\int_{-1}^{1} p(\cos \theta) \cdot d(\cos \theta) = 1$

A reduced scattering coefficient $\mu_s'$ can be defined by considering both the likelihood of scattering in the forward direction and direction of scattering:
Equation 11: \( \mu'_s = \mu_s (1 - g) \)

For skin tissue, \( g \) is about 0.9. Thus \( \mu'_s \) is about 10 times smaller than \( \mu_s \).

Now the mean free path becomes reduced mean free path:

Equation 12: Reduced mfp = \( 1 / \mu'_s \)

There’re two types of scattering based on the relationship between wavelength of light and size of scatter: Rayleigh scattering and Mie scattering. Rayleigh scattering is the scattering of light by scatters which are much smaller than the wavelength of the light. Mie scattering is the scattering of light by scatters which have sizes similar to the wavelength of the light.

Skin has many scatters with different sizes. Large scatters can have size around several micrometers such as cells and collagen fiber bundle; medium size scatters are around one micron to submicron such as lysosomes; small scatters can be around 10nm such as cell membrane and the periodic striations of collagen fibrils. Since light in visible ranges from about 400 to 700nm, light scattering in skin in this range will have contributions from both Rayleigh and Mie scattering. Rayleigh contribution is from the small scatters while Mie contribution is from the large scatters.

Bashkatov reported the scattering of in vivo skin (without hypodermis layer / fat tissue) [6] which is expressed in an empirical formula:

Equation 13: \( \mu'_s = 1.1 \times 10^{12} \lambda^{-4} + 73.7 \lambda^{-0.22} \)(cm\(^{-1}\))

The first term is Rayleigh contribution and the second term is Mie contribution.
Other light-tissue interactions include specular reflection, diffuse reflection, transmission and fluorescence, as shown in Figure 2. Specular reflection is the direction reflection of light from the skin tissue surface. Diffuse reflection is the photon transmitted out from the skin surface after zigzagging through the tissue. Transmission is photon transmitted out another side of surface after zigzagging through the tissue. Fluorescence is light absorbed by fluorophores and reemitted in a longer wavelength.

2.2. Skin anatomy

2.2.1. Seven skin layers

Figure 3 shows a Picture of skin anatomy. Skin has three main layers: epidermis, dermis and hypodermis. These three main layers can be divided into seven sub-layers. Epidermis can be divided into two layers: stratum corneum and living epidermis. Dermis can be
divided into four layers: papillary dermis, upper blood net dermis, reticular dermis, deep blood net dermis.

Table 1. Physical, biological and optical properties of seven skin layers.

<table>
<thead>
<tr>
<th>Layer</th>
<th>d (µm)</th>
<th>Cblood</th>
<th>r</th>
<th>Cwater</th>
<th>Cfat</th>
<th>Cmelanin</th>
<th>g *</th>
<th>n *</th>
<th>μs * (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum corneum</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>0.86</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>Living epidermis</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0.02(Caucasions); 0.13(Asians); 0.3(Africans)</td>
<td>0.8</td>
<td>1.34</td>
</tr>
<tr>
<td>Papillary dermis</td>
<td>160</td>
<td>0.04</td>
<td>0.1114</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>1.4</td>
<td>30</td>
</tr>
<tr>
<td>Upper blood net dermis</td>
<td>100</td>
<td>0.3</td>
<td>0.1114</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>0.95</td>
<td>1.34</td>
<td>35</td>
</tr>
<tr>
<td>Reticular dermis</td>
<td>1400</td>
<td>0.04</td>
<td>0.1114</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0.8</td>
<td>1.4</td>
<td>25</td>
</tr>
<tr>
<td>Deep blood net dermis</td>
<td>100</td>
<td>0.1</td>
<td>0.1114</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0.95</td>
<td>1.38</td>
<td>30</td>
</tr>
<tr>
<td>Hypodermis</td>
<td>28140</td>
<td>0.05</td>
<td>0.1114</td>
<td>0.7</td>
<td>0.6</td>
<td>0</td>
<td>0.75</td>
<td>1.44</td>
<td></td>
</tr>
</tbody>
</table>

* data measured in 633nm;

Table 1 lists the biological and optical properties of seven skin layers. The data were obtained and calculated from [8-10]. d is the thickness of the layer. Cblood, Cwater, Cfat and Cmelanin are the volume fraction of blood, water, fat and melanin in each layer. g, n and μs, are anisotropy factor, refractive index and scattering coefficient of each layer measured in 633nm. Finally, r is the volume fraction of hemoglobin in blood calculated as

Equation 14: r = FHb • FRBC • Ht

In which Ht (=0.45) is the hematocrit, FRBC (=0.99) is the volume fraction of erythrocytes in the total volume of all blood cells and FHb (=0.25) is the volume fraction of hemoglobin in erythrocytes.

The volume fraction of hemoglobin in blood will be used to calculate the volume fraction of other content: (1 – r • Cblood). It will be seen in the calculation in the skin optics models.
The physical, biological and optical properties of seven skin layers will be discussed briefly here.

First, epidermis is surface skin layer. It’s very thin and consists of two very special layers: stratum corneum and living epidermis. Stratum corneum does not contain blood or melanin and has very strong scattering and very weak absorption. Living epidermis does not contain blood but mainly contains melanin. Actually melanin only exists in living epidermis. Different concentration of melanin in living epidermis defines different skin types, which will be introduced in detail Chapter 2.1.2.

Dermis, which is the middle layer, consists papillary dermis, upper blood net dermis, reticular dermis and deep blood net dermis. These layers are similar layers which mainly contain certain volume of blood, which can be clearly seen in the skin anatomy in Figure 3. Thus, these layers provide the major information of hemoglobin absorption and oxygen saturation. However, these layers are below the living epidermis which is the melanin containing layer. That’s why melanin is the main factor causing bias in the measurement of oxygen saturation.

Hypodermis, which is the deepest layer, is a thick layer of fat, as shown in Figure 3. The fat occupies about 60% of the total volume of hypodermis, as shown in Table 1. This layer also contains 5% of blood.

The thickness of the top six layers (from stratum corneum to deep blood net dermis) is 1860µm (about 2mm) which is much less than the thickness of hypodermis. It has been investigated that photons detected by a camera (in visible range) can only
penetrate skin as deep as 2mm[11], indicating most of the photons can only reach to the top six layers but not hypodermis.

2.2.2. Skin types and melanin

![Figure 4. Six different skin phototypes. (Courtesy of [12])]

<table>
<thead>
<tr>
<th></th>
<th>Typical Features</th>
<th>Tanning ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Pale white skin, blue/hazel eyes, blond/red hair</td>
<td>Always burns, does not tan</td>
</tr>
<tr>
<td>II</td>
<td>Fair skin, blue eyes</td>
<td>Burns easily, tans poorly</td>
</tr>
<tr>
<td>III</td>
<td>Darker white skin</td>
<td>Tans after initial burn</td>
</tr>
<tr>
<td>IV</td>
<td>Light brown skin</td>
<td>Burns minimally, tans easily</td>
</tr>
<tr>
<td>V</td>
<td>Brown skin</td>
<td>Rarely burns, tans darkly easily</td>
</tr>
<tr>
<td>VI</td>
<td>Dark brown or black skin</td>
<td>Never burns, always tans darkly</td>
</tr>
</tbody>
</table>
There’re six skin phototypes as shown in Figure 4 [12]. Table 2 shows the definition, feature and tanning ability of these skin phototypes [13]. It can be easily seen that with the lighter colored skin, pink color appears due to the absorption of hemoglobin. But in terms of the darker colored skin, the melanin absorption dominates.

Thus, these six skin phototypes can be further grouped into three skin types based on the amount of melanin existing in the living epidermis. Melanin is a skin pigment synthesized and contain in an organelle called melanosome. Figure 5 is a cross section view of skin structure showing how the melanosomes are distributed in the living epidermis. Three skin types based on melanin amount in the living epidermis are Causatians, Asians and Africans with the definitions listed in Table 3.

<table>
<thead>
<tr>
<th>Skin types</th>
<th>Skin types in short</th>
<th>Melanin concentration in living epidermis</th>
<th>Skin phototypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light skinned Caucasians</td>
<td>Caucasians</td>
<td>1-3 % (average 2%)</td>
<td>I, II</td>
</tr>
<tr>
<td>Well-tanned Caucasians, Asians</td>
<td>Asians</td>
<td>11-16% (average 13%)</td>
<td>III, IV</td>
</tr>
<tr>
<td>Darkly pigmented Africans</td>
<td>Africans</td>
<td>18-43% (average 30%)</td>
<td>V, VI</td>
</tr>
</tbody>
</table>

Table 3. Definition of 3 skin types [14].
2.3. The optical principle of SO$_2$ measuring techniques

Optical oximetry technique is the most widely investigated and used oximetry technique due to the advantage of low cost, non-invasive, non-radiation hazard and high portability. The principle of optical oximetry technique is based on the optical property of tissue. In this section, spectral characteristics of skin absorption and scattering will be discussed and the optical principle of SO$_2$ measuring techniques will be introduced.

First, equations of absorptions of different absorbers are listed here.

(1) Absorption of oxy- and deoxy- hemoglobin

Equation 15: \( \mu^H \text{bO} = 2.303 \cdot \varepsilon^\text{HbO} \cdot StO_2 \cdot \frac{C_Hb}{M.W.Hb} \cdot C_{blood} \)
Equation 16: $\mu_a^{Hb} = 2.303 \cdot \epsilon^{Hb} \cdot (1 - StO_2) \cdot \frac{\epsilon^{Hb}}{M.W.\cdot Hb} \cdot c_{blood}$

$\epsilon^{HbO}$ and $\epsilon^{Hb}$ are extinction coefficients of oxy- and deoxy- hemoglobin, respectively. The $\epsilon^{HbO} \sim \lambda$ and $\epsilon^{Hb} \sim \lambda$ relationship are very distinctive thus no empirical formula are available to describe them. Only tabulated data are available[15].

(2) Melanin absorption

Equation 17: $\mu_a^{mel} = \epsilon^{mel} \cdot c_{mel}$

In which $\epsilon^{mel}$ is absorption coefficient pure melanin.

Equation 18: $\epsilon^{mel} = 1.7 \times 10^{12} \lambda^{-3.48} \text{ (cm}^{-1}\text{)}$.

One can see that $\epsilon^{mel} \sim \lambda$ is an exponential decay function.

(3) Water absorption

Equation 19 $\mu_a^{water} = \epsilon^{water} \cdot c_{water} \cdot (1 - r \cdot c_{blood})$

In which $\epsilon^{water}$ is water molar extinction coefficient. $\epsilon^{water}$ is very small in visible range and $\epsilon^{water} \sim \lambda$ is only available in tabulated data.

(4) Absorption of skin free of any absorbers [16]

Equation 20 $\mu_a^{base} = \epsilon^{base}(1 - c_{blood} - c_{fat} - c_{mel})(1 - r c_{blood})$

Equation 21: $\epsilon^{base} = 7.84 \times 10^8 \lambda^{-3.255} \text{ (cm}^{-1}\text{)}$.

One can see that $\epsilon^{base} \sim \lambda$, like $\epsilon^{mel} \sim \lambda$, is an exponential decay function.

(5) Fat absorption:

Equation 22: $\mu_a^{fat} = \epsilon^{fat} \cdot c_{fat} \cdot (1 - r \cdot c_{blood})$

In which $\epsilon^{fat}$ is fat molar extinction coefficient. $\epsilon^{fat}$ is very small in visible range and $\epsilon^{fat} \sim \lambda$ is only available in tabulated data[17].
The absorptions spectra of the above absorbers are plotted in Figure 6. These spectra are calculated the above equations with parameters $\text{StO}_2=50\%$, $C_{\text{blood}}=0.05$, $C_{\text{melanin}}=0.3\times80/1860=0.013$, $C_{\text{fat}}=0.6$ and $C_{\text{water}}=0.7$. The scattering spectra $\mu_s' \text{ vs } \lambda$, which was introduced in 2.1.2, is also plotted.

From Figure 6, the absorption spectra of oxygenated hemoglobin and deoxygenated hemoglobin are very distinctive. The spectra of oxy- hemoglobin has two peaks while the spectra of deoxy- hemoglobin has one peaks. Two spectra cross five times in this wavelength range. The cross points are called isosbestic points: 500, 530, 545, 575, 584nm. However, the absorption of melanin, baseline and the skin scattering
are exponential decays. Finally, the absorption of water and fat is very small compared to the absorption of other component.

The principle of SO₂ measuring techniques is to measure the concentration of oxy- and deoxy- hemoglobin by measuring the overall absorption coefficient of tissue.

The overall absorption coefficient of tissue is the sum of the absorption of all these absorbers.

Equation 23:

\[ \mu_a = \mu_a(HbO_2) + \mu_a(Hb) + \mu_a(mel) + \mu_a(fat) + \mu_a(H_2O) \]

From Figure 6, absorption of water and fat is very small compared to other absorbers. Thus if we neglect \( \mu_a(fat) \) and \( \mu_a(H_2O) \),

Equation 24:

\[ \mu_a = \mu_a(HbO_2) + \mu_a(Hb) + \mu_a(mel) \]

\[ = \varepsilon(HbO_2) \cdot C_{HbO_2} + \varepsilon(Hb) \cdot C_{Hb} + \varepsilon(mel) \cdot C_{mel} \]

Moreover, there’re two situations when the melanin absorption can be neglected. First, the sample to be measured is a thick tissue (>1cm) rather than just skin; second, the skin type is Causations or light skin Asians. In this two situations, melanin concentration in tissue is small compare to hemoglobin concentration (\( C_{mel} \ll C_{HbO2}, C_{Hb} \)). Then the melanin absorption term is neglected.

Equation 25: \( \mu_a = \varepsilon(HbO_2) \cdot C_{HbO2} + \varepsilon(Hb) \cdot C_{Hb} \)

Now absorption of tissue is only hemoglobin absorption.
Thus, if the absorption coefficient $\mu_a$ at two or more wavelengths can be measured, the concentration of oxy- and deoxy- hemoglobin ($C_{HbO2}$ and $C_{Hb}$) can be measured by solved.

Finally, $SO_2$ can be calculated according to Equation 1:

$$SO_2 = \frac{C_{HbO2}}{C_{Hbt}} = \frac{C_{HbO2}}{C_{HbO2} + C_{Hb}}$$

This is the working principle of optical $SO_2$ measuring techniques.

2.4. Current $SO_2$ techniques, challenges and recent new methods under investigation

2.4.1. Technical review of current $SO_2$ optical oximetry techniques

In the previous section, the basic principle of optical $SO_2$ measuring techniques was introduced. In this section, different kinds of techniques will be reviewed.

The methodology of measuring ‘oxygen content’ is called Oximetry. ‘oxygen content’ can be oxygen saturation $SO_2$ or oxygen tension $pO_2$ (or oxygen concentration). In this review, only those oximetry techniques measuring $SO_2$ will be discussed, since they share the same basic working principle as described in previous section. Those oximetry techniques measuring $pO_2$ will be put in the Appendix.

There are many kinds of commercial and investigational oximetry product, including both nonoptical and optical, single point measurement and imaging.

Table 4 lists some common oximetry techniques and their specifications.
Table 4. List of oximetry techniques.
(*: oximetry techniques measuring pO₂ will be reviewed in the Appendix)

<table>
<thead>
<tr>
<th>Oximetry Technique</th>
<th>Contact sensor / imaging</th>
<th>Optical/ non-optical</th>
<th>Number of wavelength measured</th>
<th>Measured oxygen parameter</th>
<th>Measure sample</th>
<th>Based principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microelectrodes *</td>
<td>Contact sensor</td>
<td>Non-optical</td>
<td>n/a</td>
<td>pO₂</td>
<td>Skin and superficial tissue</td>
<td>electrochemical</td>
</tr>
<tr>
<td>Magnetic resonance imaging *</td>
<td>imaging</td>
<td>Non-optical</td>
<td>n/a</td>
<td>pO₂ and StO₂</td>
<td>Deep tissue</td>
<td>electromagnetism</td>
</tr>
<tr>
<td>Fluorescence-based optical sensor *</td>
<td>Contact sensor</td>
<td>optical</td>
<td>1 (fluorescence)</td>
<td>pO₂</td>
<td>Skin and superficial tissue</td>
<td>Fluorescence quenching</td>
</tr>
<tr>
<td>Phosphorescence-based optical sensors *</td>
<td>Contact sensor</td>
<td>optical</td>
<td>1 (phosphorescence)</td>
<td>pO₂</td>
<td>Skin and superficial tissue</td>
<td>Phosphorescence quenching</td>
</tr>
<tr>
<td>Pulse oximeter</td>
<td>Contact sensor</td>
<td>optical</td>
<td>2 (far red to NIR)</td>
<td>SpO₂</td>
<td>Peripheral tissue such as finger</td>
<td>Absorption Beer-Lambert Law</td>
</tr>
<tr>
<td>NIR frequency domain tissue oximeter</td>
<td>Contact sensor</td>
<td>optical</td>
<td>2</td>
<td>StO₂</td>
<td>Deep tissue</td>
<td>diffusion theory of frequency modulated light</td>
</tr>
<tr>
<td>NIR tissue oximeter based on derivative spectroscopy</td>
<td>Contact sensor</td>
<td>optical</td>
<td>Multiple (&gt;4)</td>
<td>StO₂</td>
<td>Deep tissue</td>
<td>Wide gap second derivative spectroscopy</td>
</tr>
<tr>
<td>Multispectral/hyperspectral imaging</td>
<td>imaging</td>
<td>optical</td>
<td>Multiple to all spectrum</td>
<td>StO₂</td>
<td>skin</td>
<td>Spectra fitting</td>
</tr>
</tbody>
</table>
(1) Pulse oximeter

Pulse oximeter is the simplest optical technique measuring tissue oxygen saturation. It was first invented in the 1940s[18]. Now it is widely used not only in hospitals but also in daily bases due to its small size, ease to use and portability. Pulse oximeter measures absorption coefficient \( \mu_a \) by measuring the overall absorption \( A \) using Equation 4.

\[
A = \ln \frac{I_0}{I} \quad \text{and} \quad \mu_a = \frac{A}{L}
\]

\( L \) is the path length of absorption which is assumed to be the thickness of sample to be measured.

![Figure 7. Two wavelengths used in pulse oximeters.](image)

Pulse oximeter usually uses two wavelengths, one at far red (such as 660 nm or 680nm) and another at near infrared to infrared (such as 910 or 940 nm). The molar extinction coefficients of oxygenated hemoglobin and deoxygenated hemoglobin are
different at two wavelengths, as shown in Figure 7. The absorption at these two wavelengths $\mu_a (\lambda_1)$ and $\mu_a (\lambda_2)$ are measured. The concentration of oxygenated hemoglobin $C_{HbO2}$ and deoxygenated hemoglobin $C_{Hb}$ become two unknowns in a linear equation group.

Equation 26: $\mu_a (\lambda_i) = \varepsilon_{HbO2} (\lambda_i) \cdot C_{HbO2} + \varepsilon_{Hb} (\lambda_i) \cdot C_{Hb} \ (i=1,2)$

Thus, $C_{HbO2}$ and $C_{Hb}$ can be solved.

Equation 27:

$$C_{HbO2} = \frac{\varepsilon_{Hb} (\lambda_1) \cdot \mu_a (\lambda_2) - \varepsilon_{Hb} (\lambda_2) \cdot \mu_a (\lambda_1)}{\varepsilon_{HbO2} (\lambda_2) \cdot \varepsilon_{Hb} (\lambda_1) - \varepsilon_{HbO2} (\lambda_1) \cdot \varepsilon_{Hb} (\lambda_2)} , \ C_{Hb} = \frac{\varepsilon_{HbO2} (\lambda_2) \cdot \mu_a (\lambda_1) - \varepsilon_{HbO2} (\lambda_1) \cdot \mu_a (\lambda_2)}{\varepsilon_{HbO2} (\lambda_2) \cdot \varepsilon_{Hb} (\lambda_1) - \varepsilon_{HbO2} (\lambda_1) \cdot \varepsilon_{Hb} (\lambda_2)}$$

There’s one approximation in this method. The equation $A=\mu_a L$ assumes there’s minimal scattering in the sample, which is true when $L$ is comparatively small and the wavelength is far red or near infrared (NIR). Thus, the pulse oximeter usually applies to a thin part of the body such as fingers. The oxygen saturation, in this case, will be the $SpO_2$ which is the oxygen saturation of the periphery of body.

Pulse oximeter has very wide clinical applications. It can be used in any setting where a patient's oxygenation is unstable, including intensive care, intra-operative monitoring, recovery monitoring and emergency. One example is to use pulse oximeter in wound status monitoring in the lower limb would healing process[19].

(2) NIR tissue oximeter based on diffusion theory of frequency-modulated light

A tissue oximeter based on diffusion theory of frequency modulated NIR light has become an investigational medical device and is marketed by ISS. Inc[20]. This device is
considered as one of the devices that can do absolute oxygen measurement. The principle of this tissue oximeter is based on the diffusion theory of frequency-modulated photon in tissue.

NIR light with intensity modulated at frequency \( \omega \) (110MHz) is illuminated into the tissue and detected at a site with distance \( r \) away. Due to the light-tissue interaction, the detected light will have a different DC, AC and phase, as illustrated by Figure 8.

![Figure 8. Measured parameters of the modulated light](image)

Using the diffusion theory, one can solve the equations of DC, AC and phase \( \Phi \) as functions of the distance \( r \). It’s found that the relationships (1)\( \ln(r^2 \cdot \text{DC}) \sim r \), (2) \( \ln(r^2 \cdot \text{AC}) \sim r \) and (3) \( \Phi \sim r \) are all linear. Let \( S_{\text{DC}}, S_{\text{AC}}, S_{\Phi} \) be the slopes of the above linear relationships respectively, then the absorption coefficient \( \mu_a \) and the reduced scattering coefficient \( \mu_s' \) can be calculated using the following equations.
Equation 28: \( \mu_a = \frac{\omega \cdot S_{DC}}{v \cdot S_{\Phi} \sqrt{\left(\frac{S_{\Phi}}{S_{DC}}\right)^2 + 1}} \)

Equation 29: \( \mu_{s'} = \frac{S_{DC}^2 - \mu_a^2}{3 \cdot \mu_a} \)

In the above algorithms, \( \mu_a \) and \( \mu_{s'} \) are measured using \( S_{DC} \) and \( S_{\Phi} \). \( \mu_a \) and \( \mu_{s'} \) can also be measured using \( S_{AC} \) and \( S_{\Phi} \), which is not listed here.

In order to measure the slopes, this device uses a sensor with four illumination points and one detection point with known distances between illumination and detection points so that the slopes can be measured. Similar to pulse oximetry, this device measures the absorption coefficient of two wavelengths (690nm and 834nm) to measure the concentration of oxy- and deoxy-hemoglobin.

(3) NIR tissue oximeter based on derivative spectroscopy

A tissue oximeter was developed based on the principle of NIR wide gap second derivative spectroscopy [21]. This device was marketed with the name Inspectra StO2 tissue oxygenation system by Hutchinson Technology Inc. [22] This device has been used clinically and is considered as one of the device that is capable for quantitative tissue oxygen measurement.

The idea of wide gap second derivative spectroscopy will be introduced comprehensively in Chapter 2.5. Here only the basic principle will be introduced.

The second derivative of any parameter \( x \) at wavelength \( \lambda \) is

Equation 30: \( x(\lambda)'' = \frac{[x(\lambda + \delta \lambda) + x(\lambda - \delta \lambda) - 2x(\lambda)]}{(2\delta \lambda)} \)
In the case of this oximeter, let \( x \) be absorption \( A \), let \( \lambda = 720 \) and 760nm, and let \( \delta \lambda = 40 \text{nm} \). Then we have

\[
A(720)'' = A(680) + A(760) - 2A(720)
\]
\[
A(760)'' = A(720) + A(800) - 2A(760)
\]

Then a scaled second derivative value is defined as

\[
scale \ A(720)'' = \frac{A(720)''}{A(760)''}
\]

It was found that this scale \( A(720)'' \) value was only determined by oxygen saturation but not influenced by the total hemoglobin concentration and the optical path length of light. Thus, an empirical calibration curve can be created to determine the \( StO_2 \)~ scale \( A(720)'' \) relationship which can be further used to measure \( StO_2 \).

(4) Multispectral and hyperspectral imaging

Multispectral imaging (MSI) and hyperspectral imaging (HSI) have been a focus in optical imaging research in the last couple of years. MSI and HSI both capture images at many wavelengths across the electromagnetic spectrum. The difference is that MSI captures images at multiple specific wavelengths while HSI usually captures images continuously throughout the whole spectrum range. But both imaging techniques have three major advantages (1) noncontact measurement; (2) capture 2-D image rather than single point measurement, and (3) capable of utilizing the spectral information of the images, especially the information of relationship of adjacent wavelengths.

MSI and HSI capture skin spectral reflectance image \( R \). Then the absorption \( A \) can be calculated by
According to Equation 25 (if melanin absorption is neglected)

\[
A = \mu_a L = [\mu_a(HbO_2) + \mu_a(Hb)]L = \epsilon(HbO_2) \cdot (C_{HbO_2} \cdot L) + \epsilon(Hb) \cdot (C_{Hb} \cdot L)
\]

\[
= a \cdot \epsilon(HbO_2) + b \cdot \epsilon(Hb)
\]

Then the tissue absorption spectrum is the linear combination of the extinction coefficient spectra of oxy-hemoglobin and deoxy-hemoglobin. Some linear fitting algorithms can be used to fit the overall absorption spectrum to solve the two linear coefficients a and b. Then StO2 will be \(a/(a+b)\).

Many multispectral and hyperspectral imaging methods have been investigated \[23\] \[24\] \[25\] \[26\]. A commercial hyperspectral imaging system was marketed by Hypermed Inc. \[27\] and used tissue oxygenation measurement in many clinical researches. But Hypermed Inc. filed bankruptcy in 2009. After that, to the author’s knowledge, there’s no commercial multispectral or hyperspectral imaging system launched into market.

2.4.2. Challenges and limitations

There’re two major limitations of the current SO₂ oximetry techniques.

First, a method of placing a sensor on the skin to provide contact and single-point measurement of SO₂ may not satisfy the clinical need in many clinical cases. For example, oximetry techniques are used to measure wound tissue oxygenation. Since the oxygen sensor can be only put on the healthy tissue around the wound rather than on the
wound tissue, the oxygenation measured is not a ‘local measurement’ of the point of interest. It has been found that the tissue oxygenation has a spatial distribution within and around the wound which is used as important information by the clinicians in wound care. Thus, a single point measurement does not satisfy the need of measuring oxygen saturation of a large area of tissue. In this case, an imaging technique is necessary since it allows non-contact measurement of StO$_2$ of a large area of skin tissue.

Second, many optical oximetry techniques based on tissue absorption measurement were not able to provide quantitative measurement because the measurement of skin tissue oxygenation can be affected by background bias, tissue heterogeneity and inter-subject variation.

In the previous review of current oximetry techniques, several assumptions and approximations were made in the SO$_2$ algorithm. The absorptions of melanin, fat and water have been neglected in many calculations and the influence of scattering on absorption path lengths was ignored. However, these assumptions do not meet the real situation in many cases.

It was first found that tissue oxygenation measurement was easily affected by the concentration of melanin in skin[28] since it introduces a strong absorption background to the overall absorption signal. The concentration of melanin in skin is known to be very different between light-skinned people (such as Caucasians) and dark-skinned people (such as African).

Besides the difference melanin concentration of different skin types, it’s also very different for different skin sites such as the palm and the back of the hand. Besides that,
the concentration of oxy-hemoglobin, deoxy-hemoglobin and tissue scattering were found to be very different among different skin types and skin sites by two in vivo tests [29] [30] which measured these tissue parameters on dorsal forearm, upper inner arm and palm of >15 subjects of African descent, Asians and five Caucasians.

With the above limitations, quantitative and reliable imaging of skin tissue SO2 is still a challenging problem to be addressed.

2.4.3. New multispectral/hyperspectral imaging algorithms for quantitative SO2 imaging

Many spectroscopic imaging techniques have been proposed to measure skin StO2 quantitatively by considering the effect of different tissue scattering, melanin concentration, and background absorption. Seo proposed a method [31] treating the apparent absorption of skin tissue from 520 to 585 nm as a linear combination of oxy- and deoxy- hemoglobin plus a straight line that represented a modulation by the scattering and melanin. Stamatas proposed using the absorption spectrum in 630-700nm to calculate the melanin concentration and correct the absorption spectrum which was then fitted by the absorption spectra of oxy- and deoxy- hemoglobin in 560-580nm region [32, 33]. Mansfield reported a method using fuzzy C-means clustering [34] to analyze spectrum and then perform a four-term linear regression fitting of oxyhemoglobin, deoxyhemoglobin, offset and slope terms [35, 36]. Sowa used a Orthogonal Subspace Projection for hyperspectral image classification [37, 38] to reduce the bias from melanin. Nishidate proposed a multiple regression method to find the
coefficient of oxy-, deoxy-hemoglobin and melanin assisted by an inverse Monte Carlo simulation to determine optical path length of different absorbers[39]. Jakovels proposed to fit oxy-, deoxy-hemoglobin and melanin absorption by multiple Gaussian and exponential functions, then used nonlinear least square approximation to fit the optical density spectrum from 500-700 spectrum using those Gaussian and exponential functions[26]. All of the above imaging techniques and algorithm provided new possibilities for more quantitative and reliable imaging of skin tissue oxygen saturation.

However, there was not a method which has been demonstrated to be obviously superior to the others. Moreover, many of these hyperspectral imaging methods required the acquisition of images at a wide range of wavelengths which takes a considerable time so that they’re not capable of imaging skin StO$_2$ dynamics. There is not any study which systematically tested the robustness and reliability of the imaging method of measuring skin StO$_2$ on different skin conditions including different tissue scattering, blood and melanin concentration. Also, no commercial system is clinically available for imaging skin tissue StO$_2$. Thus, we are all still seeking for a quantitative and reliable imaging method. The requirements for this imaging technique include: (1) reliable and with minimal bias caused by different skin conditions; (2) compact and fast so that it can be eventually used in a clinical application in which doctors needs to make a clinical decision based on the measurement.
2.5. Basic idea of our imaging method: apply derivative spectroscopy to imaging for quantitative SO2 measurement

The purpose of study is to develop an imaging algorithm which is capable of (1) measuring skin tissue StO2 quantitatively with minimal bias from different skin conditions and (2) imaging StO2 dynamics. The idea of the algorithm is to apply a wide gap second derivative spectroscopy method to multispectral imaging. The wide gap second derivative spectroscopy was demonstrated in [21] and used to develop a commercial oximetry product [22] as being reviewed previously. However, this product is an oxygen sensor placed on the skin to provide contact and single-point measurement of deep tissue StO2. The wide gap second derivative spectroscopy had not been used for imaging. Thus, in our research, this method was modified and applied to multispectral imaging for non-contact and quantitative imaging of skin StO2.

The principle of wide gap second derivative spectroscopy is discussed here. This idea is originated from the observation of the spectral characteristics of skin absorption and scattering. As discussed in Chapter 2.3, the absorption spectra of oxy- and deoxy-hemoglobin are very distinctive, while the melanin absorption and skin scattering follow an exponential decay shape, as shown in Figure 6. Based on this observation, one may think about utilizing the distinctive spectral shape of hemoglobin absorption to measure skin oxygen saturation. One approach is to use the derivative of the spectra.
The first and the second derivative of a term $x$ (can be any absorption or scattering) are respectively calculated as

Equation 32: $x(\lambda)' = \frac{[x(\lambda + \delta \lambda) - x(\lambda)]}{\delta \lambda}$

Equation 30: $x(\lambda)'' = \frac{x(\lambda - \delta \lambda) - 2x(\lambda) - 2x(\lambda + \delta \lambda)}{(2\delta \lambda)}$

Figure 9. First derivative of the spectra in Figure 6 (from 500 to 650nm).
Figure 10. Second derivative of the spectra in Figure 6 (from 500 to 650nm).

Figure 9 and Figure 10 show the first and second derivative of the spectra in Figure 6 (from 500 to 650nm), respectively. In this case, we let $\delta\lambda=2\text{nm}$. One can see that by taking the first derivative, the hemoglobin absorption is still very distinct, while skin scattering and other absorptions are comparatively smaller. After taking the second derivative, skin scattering and melanin absorption are all close to zero.

Beside $\delta\lambda=2\text{nm}$ in the above case, the second derivative of absorptions of different skin absorbers and skin scattering can also be calculated at other $\delta\lambda$s. Figure 11 (1) ~ (15) show these second derivative at $\delta\lambda=2$, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58nm, respectively. The second derivative spectra at large wavelength gaps are called wide gap second derivative spectra. There’re two main observations from these wide gap second derivative spectra. First, no matter what $\delta\lambda$ is, the hemoglobin absorptions are very distinctive while skin scattering and other absorptions are all close to zero.
zero. But when $\delta \lambda$ is larger, the second derivative spectra of scattering and melanin absorptions become larger, although they’re still small compared to the hemoglobin absorptions. Second, when $\delta \lambda$ is small, the spectra are not smooth and have more local maximums and minimums; when $\delta \lambda$ is large, the spectra are smoother and have less local maximums and minimums. Based on the above observations, an algorithm can be developed by utilizing the wide gap second derivative spectra of the skin absorption and scattering at a certain wavelength interval.
Figure 11. Second derivative of absorptions of different skin absorbers and skin scattering calculated at $\delta \lambda = 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58$ nm.
Figure 11 continued

(5) $\delta \lambda = 18 \text{ nm}$

(6) $\delta \lambda = 22 \text{ nm}$

(7) $\delta \lambda = 26 \text{ nm}$

(8) $\delta \lambda = 30 \text{ nm}$
Figure 11 continued

(9) $\delta\lambda = 34\text{nm}$

(10) $\delta\lambda = 38\text{nm}$

(11) $\delta\lambda = 42\text{nm}$

(12) $\delta\lambda = 46\text{nm}$
Figure 11 continued

(13) $\delta \lambda = 50 \text{nm}$

(14) $\delta \lambda = 54 \text{nm}$

(15) $\delta \lambda = 58 \text{nm}$
A multispectral imaging algorithm for skin tissue oxygen saturation is developed using two steps: forward simulation (Chapter 3.1) and backward calculation (Chapter 3.2). In the first step, physical and physiological conditions of skin are known, including skin layer structure, concentration of different absorbers (blood, melanin, fat and water), skin scattering and StO₂. Using numerical models of light transport in tissue, spectral reflectances of skin with different conditions are simulated from the known skin parameters. In the second step, skin reflectance is assumed to be the known parameter since it’s the only parameter that can be directly measured by imaging. A multispectral algorithm is developed to calculate StO₂ from skin reflectance. Several designated wavelengths are selected to calculate a parameter called second derivative ratio (SDR) so that SDR is mainly determined by StO₂ regardless of other skin conditions. Then skin StO₂ can be calculated from skin reflectance of those designated wavelengths.
3.1. Simulation of spectral reflectance of skin

3.1.1 Two light transport models for simulation of skin reflectance

The first step, ‘forward simulation’, is to use numerical models of light transport in tissue to simulate spectral reflectance of skin with different skin conditions including different blood concentration, scattering, melanin level and oxygen saturation. The purpose of this step is that the simulated skin reflectance will be used to develop and test the StO$_2$ algorithms as described in step 2 ‘backward calculation’ in Chapter 3.2. In step 2, an algorithm calculating StO$_2$ from skin reflectance will be proposed. Without doing actual experiment, this algorithm can be first tested on the simulated reflectance of skin with different skin conditions. Thus, the simulated skin reflectances are called ‘numerical phantom’.

Table 5. Comparison of two methods for skin reflectance calculation

<table>
<thead>
<tr>
<th>Model</th>
<th>Monte-Carlo (MC) simulation</th>
<th>Light transport model on semi-infinite medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle</td>
<td>Rely on repeated random sampling of photons</td>
<td>Based on light transport theory</td>
</tr>
<tr>
<td>Feature</td>
<td>Is a multi-layer model</td>
<td>Is a one layer model (semi-infinite homogenous medium); Thus, a modified model for seven-layer skin will be developed.</td>
</tr>
<tr>
<td>Whether can be used for backward calculation</td>
<td>Do not have close form expression of reflectance $R(\mu_a, \mu_s)$; Cannot be used to develop backward algorithm.</td>
<td>Has close form expression of reflectance $R(\mu_a, \mu_s)$; Can be used to develop backward algorithm.</td>
</tr>
<tr>
<td>Purpose</td>
<td>The result will be used for comparison between two models.</td>
<td>The result will be used for backward algorithm development.</td>
</tr>
</tbody>
</table>
Two light transport models will be used to simulate skin reflectance. First, a light transport model in semi-infinite homogenous turbid media; Second, the Monte-Carlo Photon Migration simulation. Table 5 is the comparison of the two methods.

Monte-Carlo photon migration simulation is a method using random numbers to determine the behavior of a photon in the skin tissue, and then using the total effect of larger numbers of photon to get the result of optical parameters such as reflectance. It’s based on the fact that the behavior of photon has a defined probabilities. Absorption and scattering are the two main photon-tissue interactions which are described by absorption coefficient \( \mu_a \) and scattering coefficient \( \mu_s \), respectively. Absorption coefficient \( \mu_a \) describes the probability of photon absorbed by tissue in unit length. The reduced scattering coefficient \( \mu_s \) can describe the mean free path of photon \( 1/ \mu_s \). The scattering direction angle \( \theta \) is described by anisotropy factor \( g \) and the Henyey-Greenstein scattering function \( p(\theta) \). Thus, we can see that both absorption and scattering can be described by well-defined probability functions. Monte-Carlo method will be used to simulate reflectance of skin with seven layers of respective absorption and scattering. The advantage of MC simulation is that (1) it can simulate reflectance very accurately as long as larger enough number of photons being simulated, and (2) it can simulate skin using multiple layers with different optical properties. However, since MC simulation does not provide any analytical expression of reflectance in terms of overall absorption and scattering of skin, this method cannot be used for ‘inverse calculation’ of skin StO2.
Thus, skin reflectance simulated using MC simulation will be used to correct the result simulated by another light transport model.

Light transport model on semi-infinite medium is based on radiant transport theory. This model provides an approximation algorithm which calculates reflectance from absorption and scattering of semi-infinite homogenous turbid media [40]. The advantage of this model is that it provides close-form analytical expression of reflectance in terms of absorption and scattering. Thus, this model can be inversely deducted and used in inverse calculation. However, this model is based on light transport in one-layer homogenous semi-infinite tissue, which is not the case for multi-layered skin. Thus, a modified semi-infinite model will be developed to calculate the equivalent overall absorption of skin by weighted averaging of absorption of seven skin layers. The simulated reflectance will be corrected by comparing the result with those simulated by MC method.

3.1.2. Monte-Carlo simulation of light transport in seven layer skin

(1) Monte-Carlo method and seven layer skin model

Five parameters (of each skin layer) are required in MC model: thickness d, absorption $\mu_a(\lambda)$, scattering $\mu_s(\lambda)$, refractive index $n$ and anisotropy factor $g$.

The absorption of $i^{th}$ layer, $\mu_{ai}$, is the sum of absorptions from different absorbers including oxygenated-hemoglobin ($\mu_{HbO}^{Hb}$), deoxygenated-hemoglobin ($\mu_{Hb}^{Hb}$), melanin
\( \mu_a^{mel} \), water \( \mu_a^{water} \), fat \( \mu_a^{fat} \) and a baseline without any absorbers \( \mu_a^{base} \). The following equation can be used for calculation for the all layers except stratum corneum.

Equation 33: 
\[
\mu_a = \mu_a^{HbO} + \mu_a^{Hb} + \mu_a^{mel} + \mu_a^{water} + \mu_a^{fat} + \mu_a^{base}
\]

In which

\[
\mu_a^{HbO} = 2.303 \cdot \varepsilon_{HbO} \cdot StO_2 \cdot \frac{C_{Hb}}{M.W.Hb} \cdot C_{blood} \quad (a)
\]

\[
\mu_a^{Hb} = 2.303 \cdot \varepsilon_{Hb} \cdot (1 - StO_2) \cdot \frac{C_{Hb}}{M.W.Hb} \cdot C_{blood} \quad (b)
\]

\[
\mu_a^{mel} = \varepsilon_{mel} \cdot C_{mel} \quad (c)
\]

\[
\mu_a^{water} = \varepsilon_{water} \cdot C_{water} \cdot (1 - r \cdot C_{blood}) \quad (d)
\]

\[
\mu_a^{fat} = \varepsilon_{fat} \cdot C_{fat} \cdot (1 - r \cdot C_{blood}) \quad (e)
\]

\[
\mu_a^{base} = \varepsilon_{base} (1 - C_{blood} - C_{fat} - C_{mel})(1 - r C_{blood}) \quad (f)
\]

\( M.W.Hb (=64458 \text{ g/mole}) \), \( C_{Hb} (=150\text{g/L}) \) and \( StO_2 \) are molecular weight of hemoglobin, concentration of hemoglobin in blood and oxygen saturation of hemoglobin, respectively. \( \varepsilon_{HbO}, \varepsilon_{Hb}, \varepsilon_{water}, \varepsilon_{fat} \) are extinction coefficients of the corresponding absorbers with data obtained from [17, 41].

\( \varepsilon_{mel} \) is absorption coefficient of melanin[10]:

Equation 34: 
\[
\varepsilon_{mel} = 1.7 \times 10^{12} \lambda^{-3.48} \text{ (cm}^{-1}).
\]

\( \varepsilon_{base} \) is absorption coefficient of skin free of any absorbers[16]:

Equation 35: 
\[
\varepsilon_{base} = 7.84 \times 10^8 \lambda^{-3.255} \text{ (cm}^{-1}).
\]

The multiplier 2.303 (=ln10) is the conversion factor from base 10 to base e. This is because the extinction coefficients \( \varepsilon \) are usually based on spectrometer measurements.
and reported as $T = 10^{\varepsilon_T}$ (T is transmission) but the absorption coefficient in tissue optics is defined by $T = e^{-\mu aL}$.

Finally, the absorption of stratum corneum is different from the other layers[8, 9]:

Equation 36: $\mu_{stratum} = (1 - 3 \times 10^{-4} \lambda + 0.125 \varepsilon_{base}) \times 0.95 + \varepsilon_{water} \times 0.05 \text{ (cm}^{-1})$

$\mu_s$, n and g are all wavelength-dependent. The value of $\mu_s$, n and g of each layer measured in 633nm are listed in Table 1.

Table 1There’re also published data of $\mu_s(\lambda)$, n(\lambda) and g(\lambda) for the overall in vivo skin. However, there’s no published data of $\mu_s(\lambda)$, n(\lambda) and g(\lambda) of each skin layer. Thus, approximation will be made to find $\mu_s(\lambda)$, n(\lambda) and g(\lambda) of each skin layer.

Reduced scattering coefficient of the top six layers will be calculated by Tseng’s[29] empirical formula.

Equation 37:

$\mu_s' (\lambda < 600nm) = a_1 \lambda^{-b_1} \text{ (cm}^{-1}) \text{ (b1=1.35~1.6)} \text{ (1)}$

$\mu_s' (\lambda > 600nm) = a_2 \lambda^{-b_2} \text{ (cm}^{-1}) \text{ (b2=1.00~1.15)} \text{ (2)}$

Fix $b_1=1.5$ and $b_2=1.07$. Then parameter $a_2$ is calculated by solving Equation 37(2) using the $\mu_s'(633nm)$ values listed in Table 1..

With the known $a_2$ and $b_2$, $\mu_s'(600nm)$ can be calculated and then used to solve Equation 37(1) to calculate $a_1$. The resulted $a_1$ and $a_2$ of each layer are listed in Table 6.
Table 6. Coefficients $a_1$ and $a_2$ for $\mu_s'$ of different skin layers.

<table>
<thead>
<tr>
<th>Layer</th>
<th>$\mu_s' ,(\text{mm}^{-1})$ (633nm)</th>
<th>$a_1$</th>
<th>$a_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum corneum</td>
<td>100</td>
<td>83824.81</td>
<td>23320.74</td>
</tr>
<tr>
<td>Living epidermis</td>
<td>45</td>
<td>53887.37</td>
<td>14991.9</td>
</tr>
<tr>
<td>Papillary dermis</td>
<td>30</td>
<td>17962.46</td>
<td>4997.301</td>
</tr>
<tr>
<td>Upper blood net dermis</td>
<td>35</td>
<td>10478.1</td>
<td>2915.092</td>
</tr>
<tr>
<td>Reticular dermis</td>
<td>25</td>
<td>29937.43</td>
<td>8328.835</td>
</tr>
<tr>
<td>Deep blood net dermis</td>
<td>30</td>
<td>8981.229</td>
<td>2498.651</td>
</tr>
</tbody>
</table>

The scattering of the deepest layer hypodermis (fat layer), which is very different from other layers, is given by[6]

Equation 38: $\mu_s' = 1050.6 \cdot \lambda^{-0.68} \, (\text{cm}^{-1})$

Then let $n=1.4$ and $g=0.9$ as constant for all skin layers for approximation.

Finally, $d$, $\mu_a(\lambda)$, $\mu_s(\lambda)$, $n$ and $g$ were fed into the Monte-Carlo Photon Migration package[42]. 200K photons were used for a single simulation. Spectral reflectance $R(\lambda)$ of skin with different skin conditions were simulated.
(2) Result: simulated skin reflectance by Monte-Carlo method

Skin reflectance of different skin types/conditions were simulated. A typical result is shown in Figure 12 with skin conditions of varied melanin concentration and StO₂.

![Figure 12. Spectral reflectance simulated by Monte-Carlo photon migration method](image)

3.1.3. Semi-infinite tissue model

(1) Modified semi-infinite tissue model

Four parameters are required in this modified semi-infinite tissue model: overall skin absorption $\mu_a(\lambda)$, overall skin scattering $\mu_s(\lambda)$, averaged refractive index $n$ and
averaged anisotropy factor g. The major difference between this model and the MC method is that this model deals with the overall/averaged optical properties of skin tissue while the MC method deals with the optical properties of each skin layer. In semi-infinite model, the overall skin absorption coefficient $\mu_a$ will be the weighted average value calculated from all skin layers. Then average scattering $\mu_s$, anisotropy factor g and refractive index n are all available from published data and will be discussed in the later section.

First, overall skin absorption coefficient $\mu_a$ is calculated taking weighted average of the absorption of seven layers.

Equation 39: $\mu_a = \sum_{i=1}^{7} \mu_{ai} \cdot f_i$

where $f_i$ is weight factor ($\sum_{i=1}^{7} f_i = 1$) and $\mu_{ai}$ is the absorption of $i^{th}$ layer which is calculated using Equation 29.

Weight factor $f_i$ indicates how significant a specific layer will affect the overall absorption. It’s reasonable to infer that the weight factor should be larger for the top layer than for the bottom layer, since the deeper the tissue, the less photons can penetrate. The values of the weight factors are determined by empirically matching the skin reflectance simulated by the numerical model described in this section to the one by Monte Carlo photon migration model described Chapter 3.1.4. Actually, Monte Carlo photon migration model is used to prove the validity of the numerical model described in this section. The weight factors for seven layers were found to be 0.255, 0.299, 0.208, 0.079, 0.153, 0.001, 0.001, respectively.
Figure 13. Absorption of seven skin layers before(a) and after(b) multiplied by the weight factor. Absorption of living epidermis of three skin types (Causations, Asians and Africans) are all shown. Figure (b) is in next page.
Figure 13 shows absorption of seven skin layers including the absorption of epidermis of three skin types. (a) and (b) show the spectra before and after multiplied by the weight factors, respectively. From these two figures, we can see the shape of absorption spectra of papillary dermis, upper blood net dermis, reticular dermis, deep blood net dermis and hypodermis are characterized by the hemoglobin absorption. In comparison, the absorption of living epidermis is characterized by an exponential descending curve from melanin. The absorption of the first layer stratum corneum is very small compared to other layers. After the absorption spectra are multiplied by the weight
factors as shown in (b), the contributions of the absorptions of the top layers are enhanced while the contributions of the bottom layers are weakened.

Scattering of in vivo skin (without hypodermis layer / fat tissue) was reported by Bashkatov [6] and Tseng [29].

Bashkatov’s empirical formula is

\[ \mu'_s = 1.1 \times 10^{12} \lambda^{-4} + 73.7 \lambda^{-0.22} \text{(cm}^{-1}) \]

The first term is Rayleigh scattering and second term is Mie scattering.

Tseng’s empirical formula is

Equation 41:

\[
\begin{align*}
\mu'_s (\lambda < 600 \text{nm}) &= a_1 \lambda^{-b_1} \text{ (cm}^{-1}) \quad (b_1=1.35\sim1.6) \\
\mu'_s (\lambda > 600 \text{nm}) &= a_2 \lambda^{-b_2} \text{ (cm}^{-1}) \quad (b_2=1.00\sim1.15)
\end{align*}
\]

One can check that Bashkatov’s and Tseng’s formula are compatible. Thus, either one can be used to calculate the average scattering of skin.

Finally, anisotropy factor \( g \) and refractive index \( n \) are set to be 0.87 and 1.4, respectively.

Finally, the diffuse reflectance of skin \( R \) can be calculated using a simple and accurate algorithm from light transport model in semi-infinite turbid media reported by Scott Paul [43].

\[ R = r_i + \frac{(1-r_i)(1-s)(1-b_is)}{(1+b_is)} \]
Equation 43: \[ s = \sqrt{\frac{\mu_a}{\mu_a + \mu_s'}} \]

In the above equations, \( s \) is specular reflectance of the collimated illumination in which \( n \) is refractive index.

Equation 44: \[ r_s = \frac{(n-1)^2}{(n+1)^2} \]

\( b_1, b_2 \) are two constants as a function of \( g \) which is anisotropy factor.

Equation 45: \[ b_1 = -0.34 - 1.1g + 0.68g^2, \quad b_2 = 4.11 - 2.77g + 2.27g^2 \]

This model with \( b_1, b_2 \) calculated by Equation 45 works best when the refractive index of the media \( n \) is close to 1.4, indicating this algorithm is applied to tissue or blood.

The overall goal of the research of this dissertation is to find an algorithm which can calculate the oxygenation for all skin types. Thus, we need to simulate skin reflectance of different skin types. Totally 450 different skin conditions are considered in the simulation of skin reflectance, including different blood concentrations (\( C_{\text{blood}} = 80\%, 90\%, 100\%, 110\%, 120\% \) of normal \( C_{\text{blood}} \) as listed in Table 1), melanin concentrations (\( C_{\text{melanin}} = 0.02, 0.13 \) and 0.3 in living epidermis, corresponding to Caucasians, Asians and Africans), scattering (\( \mu_s' = 80\%, 90\%, 100\%, 110\%, 120\% \) of normal \( \mu_s' \)) and oxygen saturation (\( \text{StO}_2 = 0, 20, 40, 60, 80, 100 \% \)). These conditions are summarized in Table 7. Reflectance spectrum \( R(\lambda, C_{\text{blood}}, C_{\text{melanin}}, \mu_s', \text{StO}_2) \) were simulated in the wavelength range of 500nm to 650nm.
Table 7. Different tissue conditions for simulation of skin reflectance

<table>
<thead>
<tr>
<th>Tissue properties</th>
<th>Number of different levels</th>
<th>Different levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{blood}</td>
<td>5</td>
<td>80%, 90%, 100%, 110%, 120% of normal C\textsubscript{blood} listed in Table 1</td>
</tr>
<tr>
<td>C\textsubscript{melanin}</td>
<td>3</td>
<td>0.02 (Caucasions), 0.13 (Asians), 0.3 (Africans) (in living epidermis)</td>
</tr>
<tr>
<td>μ\textsubscript{s}'</td>
<td>5</td>
<td>80%, 90%, 100%, 110%, 120% of normal μ\textsubscript{s}'</td>
</tr>
<tr>
<td>StO\textsubscript{2}</td>
<td>21</td>
<td>0, 5,10,..., 90, 95, 100 %</td>
</tr>
</tbody>
</table>

(2)Result: simulated skin reflectance by semi-infinite tissue model

Figure 14 (a), (b) and (c) show the skin reflectance spectra of different levels of StO\textsubscript{2}, blood concentration and scattering, respectively. For each of these plots, the reflectance spectra of all three levels of melanin concentrations are shown. Two main characteristics can be seen in these plots. On one hand, the shape of the reflectance spectra is mainly determined by StO\textsubscript{2}, regardless of other three factors. On the other hand, the overall levels of the skin reflectance is determined by melanin concentration and slightly affected by scattering.
Figure 14. Skin spectral reflectance.
(a): $R(\lambda)$ of 6 StO$_2$ levels at 3 melanin concentrations (fixed blood concentration and scattering to be the values listed in Table 1);
(b): $R(\lambda)$ of 5 blood concentration levels at 3 melanin concentrations (fixed scattering to be the values listed in Table 1 and fix StO$_2$ = 60%);
(c): $R(\lambda)$ of 5 scattering levels at 3 melanin concentrations (fixed blood concentration to be the values listed in Table 1 and StO$_2$ = 60%);
Figure 14 continued

(b) Blood concentration levels

(c) Scattering levels
3.1.4 Finding absorption weight factors of semi-infinite tissue model

(1) Optimization problem of matching skin reflectance simulated by two models

Skin spectral reflectances of different skin conditions are simulated using Monte-Carlo method (Chapter 3.1.2) and semi-infinite tissue model (Chapter 3.1.3). In the semi-infinite tissue model, overall absorption of skin is approximated by the weighted average value of absorption of seven skin layers. The weight factors for seven layers will be determined by comparing the skin reflectance simulated by two models, which can be represented by a nonlinear least square optimization problem.

In this optimization problem, the reflectance simulated by semi-infinite model is matched with the reflectance simulated by MC method. The optimal weight factors are found when the square error between the reflectance of two models is minimized.

Decision variable: weight factor $f_i$ (i=1~7)

Objective function:

Equation 46: $\text{Min} \sum_\lambda (R(\mu_a(f)) - R_{MC})^2$

Constrains:

Equation 47: $\sum_i f_i = 1$ and $0 < f_i < 1$ (i=1~7)
In which $\mu_a(\lambda)$ is the averaged absorption, $R$ is the spectral reflectance calculated using light transport model, and $R_{MC}$ is the spectral reflectance simulated using Monte-Carlo method.

To solve the above nonlinear optimization problem, there’re four issues to address. First, we need to find good initial values for the decision variables. Second, there’re seven decision variables to optimize, which is too much for computation. Thus, we need to reduce the number of decision variables. Third, the constrains $0 < f_i < 1 (i=1\sim7)$ are very loose so that the optimized values may not be physically significant. Thus, a more restrictive and specific lower bound and upper bound should be given to each weight factor. Fourth, we need to modify objective function by considering difference skin spectral reflectance of difference skin conditions.

These four issues will be addressed one by one as follows.
(a) Finding good initial values for decision variables

Figure 15. Flow chart of finding initial values for weight factors

The initial values for weight factors are found using Monte-Carlo simulation on a one layer tissue model (i.e: semi-infinite model) which is different from the MC simulation performed in Chapter 3.1.2. Figure 15 shows the flow chart of steps to use MC simulation of a one-layer model to find a converged weight factor which is used as the initial value for the optimization problem.
In Step (1), the first weight factor $f_i$ is set as the ratio of thickness of $i^{th}$ skin layers ($d_i$) over the total skin thickness (layer 1 to layer 6). Set the first weight factor

\[ f_i = \frac{d_i}{\sum_{i=1}^{6} d_i} = \frac{d_i}{2000 \mu m} \quad (i=1\sim6) \quad \text{and} \quad f_7 = 0.001 \]

Table 8 shows the value of the first weight factor.

<table>
<thead>
<tr>
<th></th>
<th>Thickness ($\mu$m)</th>
<th>Primary $f_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stratum corneum</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Living epidermis</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>Papillary dermis</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>Upper blood net dermis</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Reticular dermis</td>
<td>1400</td>
</tr>
<tr>
<td>6</td>
<td>Deep blood net dermis</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>Hypodermis</td>
<td>28140</td>
</tr>
</tbody>
</table>

The weight factor for hypodermis $f_7$ is always set to 0.001. This is because of the following reasons. First, investigation has shown that during optical imaging, light (in the visible range) detected by the camera can only penetrate to 2mm deep in the tissue[11], which is the total thickness of layer 1 to layer 6. Thus, the contribution of the $7^{th}$ layer is very small. Second, a Monte-Carlo simulation is run to compare the spectral reflectance simulated using model with the top 6 layers and that using all 7 layers at three melanin levels, as shown in Figure 16. The result shows that for each melanin level, the difference between two spectra is less than 1%, which is at the same level of error caused by Monte-Carlo simulation due to finite number of photons are used. Third, the Monte-Carlo simulation also shows that the average absorption in the $7^{th}$ layer is about 0.001 of the
total photon. Thus, in order to still take into account the very minor contribution of the 7th layer, the weight factor for hypodermis f7 is set to 0.001.

![Figure 16. Skin spectral reflectance simulated by Monte-Carlo simulation using model with the top 6 layers (black curves) and that using all 7 layers (red curves)](image)

In Step (2), average absorption $\mu_a$ and anisotropy $g$ are calculated using weight factors.

$$\mu_a = \sum_{i=1}^{7} \mu_{ai} \cdot f_i, \quad g = \sum_{i=1}^{7} g_i \cdot f_i$$

$\mu_{ai}$ is the absorption of $i^{th}$ layer, which can be calculated using Equation 33.

In Step (3), Monte-Carlo simulation of a one-layer homogenous tissue model is performed. Besides $\mu_a$ and $g$ already calculated previously, parameters used in this simulation include: ① Wavelength of photon: 633nm; ② Thickness of the layer: it should
be the total thickness: \( d = \sum_{i=1}^{7} d_i = 3 \text{cm}. \) ③ Melanin volume faction in living epidermis: 0.13 (the mid-level of melanin concentration); ④ Oxygen saturation StO2: 50%; ⑤ Scattering \( \mu_s' = 47 \text{cm}^{-1} \) (the scattering at 633nm) and ⑥ all extinction coefficients \( \varepsilon \) which are set to the value at 633nm. Then 200K photons were used for a single simulation.

In Steps (4) to (6), absorption of different depth is calculated and a new weight factor is defined. Simulation in step (3) will yield the number of photons absorbed in specific depth of tissue, as shown in Figure 17. Absorption vs. depth relationship approximates an exponential decay.

![Figure 17. Absorption vs. depth of 1 layer tissue model simulated by Monte-Carlo method](image)

Let \( A_i \) be the absorption from depth \( D_i \) to \( D_i + d_{i+1} \). Here

\[
A_i = \int_{D_i}^{D_{i+1}} A(x) \, dx
\]

In which \( D_i = \sum_{j=1}^{i} d_i \) and \( A(x) \) is the absorption on depth \( x \).
A_i can be interpreted as the separated area under the curve of absorption vs. depth, as shown in Figure 17. Intuitively we can consider A_i as the ‘absorptions of i^{th} layer’. This is because by definition of Equation 49 A_i is the average absorption of the specific region of the homogenous tissue which corresponds to the i^{th} layer of the heterogeneous tissue.

Then, a new weight factor can be defined as the ratio of A_i to the total absorption:

Equation 50: \[ f_i = \frac{A_i}{\sum_{i=1}^{7} A_i} \]

In Steps (7) and (8): MC simulation with the new weight factors will be repeated until weight factors converge. The new weight factors are checked to see if they’re very close to the original ones. If not, steps (2) to (6) were repeated; if yes, the result is the converged weight factors.

Table 9. Resulted converged weight factors

<table>
<thead>
<tr>
<th></th>
<th>Thickness (um)</th>
<th>First f</th>
<th>Converged f</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stratum corneum</td>
<td>20</td>
<td>0.0108</td>
</tr>
<tr>
<td>2</td>
<td>Living epidermis</td>
<td>80</td>
<td>0.043</td>
</tr>
<tr>
<td>3</td>
<td>Papillary dermis</td>
<td>160</td>
<td>0.086</td>
</tr>
<tr>
<td>4</td>
<td>Upper blood net dermis</td>
<td>100</td>
<td>0.0537</td>
</tr>
<tr>
<td>5</td>
<td>Reticular dermis</td>
<td>1400</td>
<td>0.7527</td>
</tr>
<tr>
<td>6</td>
<td>Deep blood net dermis</td>
<td>100</td>
<td>0.0538</td>
</tr>
<tr>
<td>7</td>
<td>Hypodermis</td>
<td>28140</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The weight factor converges after 7 iterations. Table 9 shows the values of first and the converged weight factors. Figure 18 shows the absorption vs. depth in seven iterations. The curve of absorption vs. depth eventually converges to a certain profile
which is indicated by the black line. Figure 19 shows how the weight factors of different layers converge to certain values after seven iterations.

Figure 18. Absorption vs. depth of seven iterations simulated using one-layer homogenous tissue model by Monte-Carlo method

Figure 19. Convergence of weight factors after seven iterations
(b) Reducing the number of decision variables

Seven weight factors are refined to three control parameters by the following method. First, as mentioned previously, the fat layer is very deep and has very small influence on the photon. Thus, the weight factor for the 7th layer is set to constant 0.001. Second, the converged weight factor of 6th layer is very small. This layer is very deep and thin thus has very small influence on photon. Thus, the weight factor for the 7th layer is set to constant 0.001. Actually value of the weight factor of 6th and 7th layer almost will not affect the optimization result at all. Third, the 3rd, 4th and 5th layer are very similar layers with similar composition in terms of absorbers. They only differ by thickness and depth. Thus, the weight factors of these three layers are kept at a constant ratio, which is 0.34:0.13:0.25. Then three control parameters x1, x2, x3 can be defined.

Equation 51: \[ x_1 = f_1; x_2 = f_2; x_3 = (1 - x_1 - x_2 - 0.002) / (0.34 + 0.13 + 0.25) \]

Then the seven weight factors can be represented by x1, x2 and x3 as

\[ f = [x_1, x_2, 0.34 \cdot x_3, 0.13 \cdot x_3, 0.25 \cdot x_3, 0.001, 0.001] \]

(c) Set new constrains for the decision variables

In the definition of x3, the constraint \( \sum f_i = 1 \) was already used. Thus, we only need to set the lower and upper bound of x1, x2.

Since x1 is the weight factor of the 1st layer which is very thick and does not contain any absorber, let

\[ 0.01 < x_1 < 0.3 \]
Since $x_2$ is the weight factor of the 2\textsuperscript{nd} layer which is very thin but contains strong absorber melanin, let

$$0.1 < x_2 < 0.6$$

(d) Redefine objective function by considering different skin conditions

The objective function is redefined by considering the difference of spectral reflectance from different skin tissue conditions.

From Figure 14, it has been already discovered that melanin concentration is biggest factor which influences the skin spectral reflectance among all tissue conditions. Actually one can easily see from Figure 14 that the influence by melanin is much larger than blood concentration, tissue scattering and oxygen saturation. Thus, here we will consider optimize the weight factor for all three melanin level at the same time. The objective function becomes

$$\text{Min} \quad \{ \sum_{\lambda} (R_{C_{mel=1}}(x_1, x_2, x_3) - R_{MC})^2 + \sum_{\lambda} (R_{C_{mel=2}}(x_1, x_2, x_3) - R_{MC})^2 + \sum_{\lambda} (R_{C_{mel=3}}(x_1, x_2, x_3) - R_{MC})^2 \}$$

Subject to: $0.01 < x_1 < 0.3$ and $0.1 < x_2 < 0.6$

A built-in algorithm from Matlab was used to solve this optimization problem. The algorithm is ‘trust-region-reflective’ method. The optimization is performed from 500 nm to 610 nm. This is because this wavelength region is more of interest and will be used for oxygen saturation calculations.
Influence of different skin conditions on the weight factors was considered. These skin conditions include different thickness of skin layers, different blood concentration and different skin scattering.

(2) Result: optimized weight factors for different skin conditions

![Figure 20. Comparison of skin spectral reflectance simulated by Monte-Carlo method and by semi-infinite model with optimized weight factors](image)

A typical optimization result is shown in Figure 20. These are the skin spectral reflectance at three melanin levels simulated by Monte-Carlo method and by semi-infinite model with optimized weight factors. Skin tissue has a condition of StO2=80% and blood and scattering are at normal level as listed in Table 7. The optimized weight factors are $f=\{0.255, 0.299, 0.208, 0.079, 0.153, 0.001, 0.001\}$.

There’re two important points to discuss.
(1) The reasons and influence of the difference between the reflectance spectral simulated by Monte-Carlo method and by semi-infinite model;

(2) The influence of different skin conditions to the optimized weight factors.

The 1st issue to discuss is the difference between the reflectance spectral simulated by Monte-Carlo method and by semi-infinite model with optimized weight factors, as shown in Figure 20. Although the difference between two spectra has been minimized with the optimized weight factor, the difference is still obviously observable. The reason is that Monte-Carlo simulation is also an approximation to simulation skin reflectance. As mentioned in Chapter 3.1.2, several major assumptions are made in the Monte-Carlo seven layer tissue model. First, due to the lack of published data of wavelength-dependent scattering coefficient of each layer, the relationship of $\mu_s'$ vs $\lambda$ is assumed to be the same as the overall skin scattering which is described by Equation 40. Although it should still be true that the scattering should contain a Mie and a Rayleigh term, the exponential factor and the coefficients should be different. But in the Monte-Carlo model, we use the same equation to describe the scattering of each layer only multiplied with a scale factor. Second, similar to the problem of scattering, there’re no published data of wavelength dependent anisotropy factor $g$ and reflective index $n$. Thus $g$ and $n$ are considered as constant throughout the wavelength range for a specific layer, which is only a rough approximation. As a conclusion, the skin spectral reflectance simulated by Monte-Carlo method is also an approximation.
Thus, when we match the spectral reflectance of light transport model to that of Monte-Carlo method, we only match them to the same overall level, as shown in Figure 20.

The 2\textsuperscript{nd} issue to discuss is the influence of different skin conditions on weight factors. The question is whether the optimized weight factors are different for different skin tissue conditions. The following six parameters which determine different skin conditions were considered, as listed in Table 10.

Table 10. Six parameters affecting weight factors.

<table>
<thead>
<tr>
<th>parameter</th>
<th>Low level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood concentration</td>
<td>-20% of average value</td>
<td>+20% of average value</td>
</tr>
<tr>
<td>Scattering</td>
<td>-20% of average value</td>
<td>+20% of average value</td>
</tr>
<tr>
<td>Thickness of skin layers</td>
<td>Stratum corneum 10µm 30µm</td>
<td>Living epidermis 47µm 55µm</td>
</tr>
<tr>
<td></td>
<td>Dermis 0.5mm 4mm</td>
<td></td>
</tr>
<tr>
<td>StO\textsubscript{2}</td>
<td>0, 20, 40, 60, 80, 100%</td>
<td></td>
</tr>
</tbody>
</table>
Figure 21. Comparison of skin spectral reflectance simulated by semi-infinite model and by Monte-Carlo method of different StO2. (Oxygen saturation for these plots are: 0%, 20%, 40%, 60%, 80%, 100% respectively. The optimization is performed from 500nm to 610nm.)
Figure 21 shows the comparison of skin spectral reflectance simulated by Monte-Carlo method and by semi-infinite model with optimized weight factors at six StO$_2$ level. The optimal weight factors of six StO$_2$ levels are listed in Table 11.

Table 11. Optimized weight factor of seven skin layers at different StO$_2$ levels (The optimization is performed from 500nm to 610nm.)

<table>
<thead>
<tr>
<th>Skin layer</th>
<th>StO$_2$(%)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>average</th>
<th>std%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.250</td>
<td>0.249</td>
<td>0.248</td>
<td>0.251</td>
<td>0.255</td>
<td>0.257</td>
<td>0.252</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.303</td>
<td>0.305</td>
<td>0.305</td>
<td>0.305</td>
<td>0.299</td>
<td>0.284</td>
<td>0.300</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.208</td>
<td>0.208</td>
<td>0.208</td>
<td>0.207</td>
<td>0.208</td>
<td>0.214</td>
<td>0.209</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.080</td>
<td>0.080</td>
<td>0.080</td>
<td>0.079</td>
<td>0.079</td>
<td>0.082</td>
<td>0.080</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.153</td>
<td>0.153</td>
<td>0.153</td>
<td>0.152</td>
<td>0.153</td>
<td>0.157</td>
<td>0.154</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

One can see from Table 11 that the difference of weight factors among different StO$_2$ levels is less than 3%. The average weight factors for seven skin layers are:

\[ f = [0.252, 0.300, 0.209, 0.080, 0.154, 0.001, 0.001] \]

Thus, a single set of weight factor works for all StO$_2$ levels.
Figure 22. Weight factors obtained from matching skin reflectance of Monte-Carlo simulation and semi-infinite model. (a) to (e) show the result of different blood concentration, scattering, stratum corneum thickness, living epidermis thickness and dermis thickness, respectively.
Figure 22 (a) to (e) show the weight factors of seven skin layers for different skin conditions including different blood concentration, scattering, stratum corneum thickness, living epidermis thickness and dermis thickness, respectively.

From figure (a), when the blood concentration is high, the weight factors for the first layer was smaller and weight factors for the 3rd, 4th and 5th layer were larger. This is because the 3rd to 6th layers are blood containing layers while the 1st layer does not contain blood.

From figure (b), the weight factor for the first layer is significantly higher when the skin scattering is higher. This is because the 1st layer has a much higher scattering coefficient than other layers and does not contain any absorbers. Thus, this layer can be considered as a main contribution of skin scattering.

From figure (c), the weight factor for stratum corneum is larger when this layer is thicker. Similarly, from figure (d), the weight factor for living epidermis is larger when this layer is thicker. This is because when the layer is thicker, there’s more contribution of absorption of this layer to the overall absorption. However, from figure (e), the weight factors are the same for both dermis thicknesses. This is because the hypodermis, which is the fat-containing layer below dermis, has also 5% of blood which is similar to the blood concentration of dermis. Thus, the absorption by blood of both dermis and hypodermis are similar.

As a conclusion, the optimized weight factors are not affected by StO2 and dermis thickness but affected by blood concentration, skin scattering, stratum corneum thickness
and epidermis thickness. Thus, one set of weight factors can be used for different StO$_2$ and dermis thickness. Since three melanin levels are optimized at the same time, one set of weight factors are used for all there melanin levels. But different weight factors should be used for different blood concentration, skin scattering, stratum corneum thickness and epidermis thickness. However, the different weight factors will not affect the algorithm development, which will be described in the next section.

3.2. Finding the relationship of StO$_2$ and the second derivative ratio (SDR) of $r (= \mu_a/\mu_s')$

3.2.1 Backward calculation from reflectance to the ratio of absorption versus scattering

The first step in the backward calculation is to consider the effect of optical filter. Since the multispectral and hyperspectral images are taken using optical filter which has certain bandwidth, we need to consider this effect in our modeling. The transmission of the filter with a bandwidth FWHM is assumed to be a Gaussian shape:

\[ G(\lambda_0 - \lambda) = \frac{1}{\sigma\sqrt{2\pi}} \cdot e^{-\frac{(\lambda-\lambda_0)^2}{2\sigma^2}} \]

The full width of half maximum (FWHM) of a Gaussian distribution is $FWHM = 2\sqrt{2\ln2}\sigma$. Since the optical filter we used is liquid crystal tunable filter (LCTF) which has a bandwidth of 7nm, the variance is

\[ \sigma = \frac{2\sqrt{2\ln2}}{FWHM} = 3\text{nm} \]
Thus,

\[ E \left( \lambda_0 - \lambda \right) = \frac{2\ln2/\pi}{FWHM} \cdot e^{-4\ln2 \cdot \left( \frac{\lambda - \lambda_0}{FWHM} \right)^2} \]

Then the reflectance spectrum \( R(\lambda_0) \) measured using this filter will be the convolution of \( E(\lambda_0 - \lambda) \) and the original reflectance spectrum \( R(\lambda) \).

\[ R(\lambda_0) = R(\lambda) \ast G(\lambda_0 - \lambda) = \int_{\lambda_0-3\sigma}^{\lambda_0+3\sigma} R(\lambda) \cdot G(\lambda_0 - \lambda) d\lambda \]

To do numerical integration:

\[ R(\lambda_0) = \int_{\lambda_0-9}^{\lambda_0+9} R(\lambda) \cdot G(\lambda_0 - \lambda) \Delta \lambda = \int_{\lambda_0-9}^{\lambda_0+9} R(\lambda) \cdot \frac{2\ln2/\pi}{FWHM} \cdot e^{-4\ln2 \cdot \left( \frac{\lambda - \lambda_0}{FWHM} \right)^2} \Delta \lambda \]

We have the spectral data every 2nm, thus \( \Delta \lambda = 2 \text{nm} \).
Figure 23. Skin tissue spectral reflectance before (a) and after (b) optical filters
Figure 24. Skin tissue spectral reflectance of StO2 = 0% (a) and 100% (b) before and after optical filters.
Figure 23 shows the original spectral reflectance of the skin tissue $R(\lambda)$ (a) and the spectral reflectance measured by the imaging system with optical filters $R(\lambda_0)$ (b). Figure 24 plots the skin spectral reflectance before and after filter within the same plot. (a) and (b) are the cases of StO2 = 0% and 100%, respectively. The largest difference of reflectance is 1% and 3% StO2 = 0% and 100%, respectively. The largest difference happens at the point when the spectrum has sharp turns such as the local maximums and minimums in Figure 24(b). Our modeling will be based on the spectral reflectance calculated after the optical filters.

Once skin reflectance $R(\lambda)$ is measured from the multispectral images of skin tissue, then the ratio of absorption to scattering coefficient ($r = \mu_a/\mu_s'$) can be solved using the Equation 42 backwardly.

First $s$ can be solved by

Equation 57: $b_1 \cdot s^2 - (b_1 + R_s \cdot b_2 + 1) \cdot s + (1 - R_s) = 0$ in which $R_1 = \frac{R-r_s}{1-r_s}$

Then $r$ can be solved by

Equation 58: $r = \frac{\mu_a}{\mu_s'} = \frac{s^2}{1-s^2}$

3.2.2. Second derivative ratio (SDR) of $r (= \mu_a/\mu_s')$ and the analytical expression of StO2~SDR

A parameter called wide gap second derivative ratio (SDR) is defined here, which is the single most important parameter for the whole research. This parameter shows the basic idea of our multispectral imaging algorithm for skin tissue oxygen saturation StO2.
Define $\delta \lambda$ as the wavelength gap. The second derivative of $r (= \mu_a/\mu_s)$ at wavelength $\lambda$ can be numerically approximated as

Equation 59: $r(\lambda)'' = [r(\lambda + \delta \lambda) + r(\lambda - \delta \lambda) - 2r(\lambda)]/2\delta \lambda$.

Define

Equation 60:

\[ \lambda_1^- = \lambda_1 - \delta \lambda; \ (a) \]
\[ \lambda_1^+ = \lambda_1 + \delta \lambda; \ (b) \]
\[ \lambda_2^- = \lambda_2 - \delta \lambda; \ (c) \]
\[ \lambda_2^+ = \lambda_2 + \delta \lambda; \ (d) \]

Then, the wide-gap second derivative value of $r$ of the 1st wavelength $\lambda_1$ is:

Equation 61: $r(\lambda_1)'' = [r(\lambda_1^+) + r(\lambda_1^-) - 2r(\lambda_1)]/2\delta \lambda$

The wide-gap second derivative value of $r$ of the 2nd wavelength $\lambda_2$ is:

Equation 62: $r(\lambda_2)'' = [r(\lambda_2^+) + r(\lambda_2^-) - 2r(\lambda_2)]/2\delta \lambda$

The ratio between $r(\lambda_1)''$ and $r(\lambda_2)''$ is called the second derivative ratio (SDR).

Equation 63:

\[
SDR(\lambda_1, \lambda_2, \delta \lambda) = \frac{r(\lambda_1)''}{r(\lambda_2)''} = \frac{[r(\lambda_1^+) + r(\lambda_1^-) - 2r(\lambda_1)]/2\delta \lambda}{[r(\lambda_2^+) + r(\lambda_2^-) - 2r(\lambda_2)]/2\delta \lambda} = \frac{r(\lambda_1 + \delta \lambda) + r(\lambda_1 - \delta \lambda) - 2r(\lambda_1)}{r(\lambda_2 + \delta \lambda) + r(\lambda_2 - \delta \lambda) - 2r(\lambda_2)}
\]

From the above equation, SDR of any $(\lambda_1, \lambda_2, \delta \lambda)$ is calculated from $r$ at six wavelengths.
The SDR has a very special relationship with oxygen saturation StO$_2$, which is the parameter we try to measure. This relationship can be shown by the analytical expression of SDR, which is calculated in 3 steps.

**Step 1: Expressing SDR as a function of absorption and scattering**

Since $r = \frac{\mu_a}{\mu_s}$,

Equation 64: $\frac{dr}{d\lambda} = \frac{d\mu_a}{d\lambda} + \frac{d\mu_s}{d\lambda} \frac{d\mu_s}{d\lambda} - \frac{\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda}$

Equation 65: $\frac{d^2r}{d\lambda^2} = \frac{1}{\mu_s^4} \left[ \mu_s^2 \frac{d^2\mu_a}{d\lambda^2} - \mu_s \frac{d\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda} - 2\mu_s \frac{d\mu_s}{d\lambda} \frac{d\mu_a}{d\lambda} \right]$ 

In which

Equation 66

$$\frac{d}{d\lambda} \left( \frac{d\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda} - \mu_a \frac{d\mu_s}{d\lambda} \right) = \mu_s \frac{d^2\mu_a}{d\lambda^2} + \frac{d\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda} - \frac{d\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda} - \mu_a \frac{d^2\mu_a}{d\lambda^2} = \mu_s \frac{d^2\mu_a}{d\lambda^2} - \mu_a \frac{d^2\mu_a}{d\lambda^2}$$

Thus

$$\frac{d^2r}{d\lambda^2} = \frac{1}{\mu_s^4} \left[ \mu_s^2 \frac{d^2\mu_a}{d\lambda^2} - \mu_s \frac{d\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda} - 2\mu_s \frac{d\mu_s}{d\lambda} \frac{d\mu_a}{d\lambda} + 2\mu_a \mu_s \frac{d\mu_s}{d\lambda} \frac{d\mu_s}{d\lambda} \right]$$

$$\frac{d^2r}{d\lambda^2} = \frac{1}{\mu_s^4} \left[ \mu_s^2 \frac{d^2\mu_a}{d\lambda^2} - \mu_s \frac{d\mu_a}{d\lambda} \frac{d\mu_s}{d\lambda} - 2\mu_s \frac{d\mu_s}{d\lambda} \frac{d\mu_a}{d\lambda} - \mu_s \frac{d^2\mu_s}{d\lambda^2} \frac{d\mu_a}{d\lambda} + 2\mu_s \left( \frac{d\mu_s}{d\lambda} \right)^2 \frac{d\mu_a}{d\lambda} \right]$$

Equation 67:

$$\frac{d^2r}{d\lambda^2} = A \frac{d^2\mu_a}{d\lambda^2} + B \frac{d\mu_a}{d\lambda} + C \cdot \mu_a$$

$A = \frac{1}{\mu_s^2}$; $B = -\frac{2}{\mu_s^2} \frac{d\mu_s}{d\lambda}$; $C = \frac{1}{\mu_s^2} \left[ \frac{2}{\mu_s^2} \left( \frac{d\mu_s}{d\lambda} \right)^2 - \frac{d^2\mu_s}{d\lambda^2} \right]$
Since $\mu'_s = a\lambda^{-4} + b\lambda^{-0.22}$ ($a=1.1\times10^{12}; b=73.7$)

Thus,

Equation 68: $\frac{d\mu'_s}{d\lambda} = -4a\lambda^{-5} - 0.22b\lambda^{-1.22}$

Equation 69: $\frac{d^2\mu'_s}{d\lambda^2} = 20a\lambda^{-5} + 0.22 \cdot 1.22b\lambda^{-2.22}$
Figure 25. Comparison of 3 coefficients (a) and 3 terms (b) in the expression of $\frac{d^2 r}{d\lambda^2}$
From Figure 25, it was found that \( C < B < A \) and also \( C \cdot \mu_a < B \cdot \frac{d\mu_a}{d\lambda} < A \cdot \frac{d^2\mu_a}{d\lambda^2} \).

Thus,

\[
\text{Equation 70:} \quad \frac{d^2 r}{d\lambda^2} \approx \frac{1}{\mu_{s'}} \cdot \frac{d^2 \mu_a}{d\lambda^2}
\]

Step 2: calculate \( \frac{d^2 \mu_a}{d\lambda^2} \)

From Equation 33:

\[
\mu_{a_i} = \mu_{a_i}^{HbO} + \mu_{a_i}^{Hb} + \mu_{a_i}^{mel} + \mu_{a_i}^{water} + \mu_{a_i}^{fat} + \mu_{a_i}^{base}
\]

Equation 33 and Equation 39,

\[
\mu_a = \sum_{i=1}^{7} \mu_{a_i} \cdot f_i
\]

\[
\mu_{a_i} = \mu_{a_i}^{HbO} + \mu_{a_i}^{Hb} + \mu_{a_i}^{mel} + \mu_{a_i}^{water} + \mu_{a_i}^{fat} + \mu_{a_i}^{base}
\]

Thus,

**Equation 71**

\[
\mu_a = \sum_{i=1}^{7} \mu_{a_i}^{HbO} \cdot f_i + \sum_{i=1}^{7} \mu_{a_i}^{Hb} \cdot f_i + \sum_{i=1}^{7} \mu_{a_i}^{mel} \cdot f_i + \sum_{i=1}^{7} \mu_{a_i}^{water} \cdot f_i + \sum_{i=1}^{7} \mu_{a_i}^{fat} \cdot f_i + \sum_{i=1}^{7} \mu_{a_i}^{base} \cdot f_i
\]

Now calculate the absorption of each absorber.

(1) Oxyhemoglobin absorption:

**Equation 72**

\[
\sum_{i=1}^{7} \mu_{a_i}^{HbO} \cdot f_i = \varepsilon^{HbO} \cdot StO_2 \left( 2.303 \frac{c_{Hb}}{M.W_{Hb}} \sum_{i=1}^{7} c_{i}^{blood} \cdot f_i \right) = F^{Hbt} \cdot \varepsilon^{HbO} \cdot StO_2
\]
(2) Deoxyhomoglobin absorption:

Equation 73

\[
\sum_{i=1}^{7} \mu_{\text{ai}}^{\text{Hb}} \cdot f_i = \varepsilon^{\text{Hb}} \cdot (1 - StO_2) \left( 2.303 \frac{C_{\text{Hb}}}{M \cdot W_{\text{Hb}}} \sum_{i=1}^{7} C_{i}^{\text{blood}} \cdot f_i \right) \\
= F^{\text{Hbt}} \cdot \varepsilon^{\text{Hb}} \cdot (1 - StO_2)
\]

(3) Melanin absorption:

Equation 74

\[
\sum_{i=1}^{7} \mu_{\text{ai}}^{\text{mel}} \cdot f_i = \varepsilon^{\text{mel}} \cdot \sum_{i=1}^{7} C_{i}^{\text{mel}} \cdot f_i = F^{\text{mel}} \cdot \varepsilon^{\text{mel}}
\]

(4) Water absorption:

Equation 75

\[
\sum_{i=1}^{7} \mu_{\text{ai}}^{\text{water}} \cdot f_i = \varepsilon^{\text{water}} \cdot \sum_{i=1}^{7} C_{i}^{\text{water}} \cdot (1 - r_i \cdot C_{i}^{\text{blood}}) \cdot f_i = F^{\text{water}} \cdot \varepsilon^{\text{water}}
\]

(here \( r \) is the volume fraction of hemoglobin in blood which is 0 or 0.1114)

(5) Fat absorption:

Equation 76

\[
\sum_{i=1}^{7} \mu_{\text{ai}}^{\text{fat}} \cdot f_i = \varepsilon^{\text{fat}} \sum_{i=1}^{7} C_{i}^{\text{fat}} \cdot (1 - r_i \cdot C_{i}^{\text{blood}}) \cdot f_i = F^{\text{fat}} \cdot \varepsilon^{\text{fat}}
\]

(6) Baseline absorption (of skin without any absorbers):

Equation 77

\[
\sum_{i=1}^{7} \mu_{\text{ai}}^{\text{base}} \cdot f_i = \varepsilon^{\text{base}} \sum_{i=1}^{7} (1 - C_{i}^{\text{blood}} - C_{i}^{\text{mel}} - C_{i}^{\text{fat}}) \cdot (1 - r_i \cdot C_{i}^{\text{blood}}) \cdot f_i \\
= F^{\text{base}} \cdot \varepsilon^{\text{base}}
\]

Thus,
Equation 78

\[ \mu_a = F^{Hbt} \cdot \varepsilon^{HbO} \cdot StO_2 + F^{Hbt} \cdot \varepsilon^{Hb} \cdot (1 - StO_2) + F^{mel} \cdot \varepsilon^{mel} + F^{water} \cdot \varepsilon^{water} + F^{fat} \cdot \varepsilon^{fat} + F^{base} \cdot \varepsilon^{base} \]

In which

\[ F^{Hbt} = \left(2.303 \frac{C_{Hb}}{M.W. Hb} \sum_{i=1}^{7} C_i^{blood} \cdot f_i\right) \quad (a) \]

\[ F^{mel} = \sum_{i=1}^{7} C_i^{mel} \cdot f_i \quad (b) \]

\[ F^{water} = \sum_{i=1}^{7} C_i^{water} \cdot (1 - \eta_i C_i^{blood}) \cdot f_i \quad (c) \]

\[ F^{fat} = \sum_{i=1}^{7} C_i^{fat} \cdot (1 - \eta_i C_i^{blood}) \cdot f_i \quad (d) \]

\[ F^{base} \sum_{i=1}^{7} (1 - C_i^{blood} - C_i^{mel} - C_i^{fat})(1 - \eta_i C_i^{blood}) \cdot f_i \quad (e) \]

Although the coefficient F may vary because of different weight factor f_i of different skin conditions, the absorption of water and fat are much smaller than others because \( \varepsilon^{fat}, \varepsilon^{water} \ll \varepsilon^{HbO}, \varepsilon^{Hb}, \varepsilon^{mel}, \varepsilon^{base} \). This can be seen from Figure 26.

85
Figure 26. The absorption of water and fat are much smaller than that of other absorbers.

Thus, the fat and water absorption is ignored.

Equation 79

\[ \mu_a = F^{Hbt} \cdot \varepsilon^{HbO} \cdot StO_2 + F^{Hbt} \cdot \varepsilon^{Hb} \cdot (1 - StO_2) + F^{mel} \cdot \varepsilon^{mel} + F^{base} \cdot \varepsilon^{base} \]

Thus,

Equation 80

\[ \frac{d^2 \mu_a}{d\lambda^2} = F^{Hbt} \frac{d^2 \varepsilon^{HbO}}{d\lambda^2} \cdot StO_2 + F^{Hbt} \frac{d^2 \varepsilon^{Hb}}{d\lambda^2} \cdot (1 - StO_2) + F^{mel} \frac{d^2 \varepsilon^{mel}}{d\lambda^2} + F^{base} \frac{d^2 \varepsilon^{base}}{d\lambda^2} \]
Figure 27. Comparison of four terms in $\frac{d^2\mu_a}{d\lambda^2}$.

Figure 27 shows the comparison of four terms in Equation 80. As shown in the figure, $\frac{d^2\epsilon_{\text{mel}}}{d\lambda^2}, \frac{d^2\epsilon_{\text{base}}}{d\lambda^2} \ll \frac{d^2\epsilon_{HbO}}{d\lambda^2}, \frac{d^2\epsilon_{Hb}}{d\lambda^2}$, thus,

Equation 81

$$\frac{d^2\mu_a}{d\lambda^2} = F_{Hbt} \frac{d^2\epsilon_{HbO}}{d\lambda^2} \cdot S_{O_2} + F_{Hbt} \frac{d^2\epsilon_{Hb}}{d\lambda^2} \cdot (1 - S_{O_2})$$

Step 3: analytical expression of $\frac{d^2r}{d\lambda^2}$

From Equation 70 and Equation 81,

Equation 82:
\[
\frac{d^2 r}{d\lambda^2} \approx \frac{1}{\mu_s'} \frac{d^2 \mu_a}{d\lambda^2} = \frac{F_{HbT}}{\mu_s'} \left[ \frac{d^2 \varepsilon_{HbO}}{d\lambda^2} \cdot StO_2 + \frac{d^2 \varepsilon_{Hb}}{d\lambda^2} \cdot (1 - StO_2) \right]
\]

Thus, from Equation 63, the second derivative ratio SDR is:

\[
SDR = \frac{r''(\lambda_1)}{r''(\lambda_2)} = \frac{\mu_s'(\lambda_2)}{\mu_s'(\lambda_1)} \cdot \frac{(\varepsilon_{HbO})''_{\lambda_1} StO_2 + (\varepsilon_{Hb})''_{\lambda_1} (1 - StO_2)}{(\varepsilon_{HbO})''_{\lambda_2} StO_2 + (\varepsilon_{Hb})''_{\lambda_2} (1 - StO_2)}
\]

Equation 83:

\[
SDR = \frac{\mu_s'(\lambda_2)}{\mu_s'(\lambda_1)} \cdot \frac{[(\varepsilon_{HbO})''_{\lambda_1} - (\varepsilon_{Hb})''_{\lambda_1}] StO_2 + (\varepsilon_{Hb})''_{\lambda_1}}{[(\varepsilon_{HbO})''_{\lambda_2} - (\varepsilon_{Hb})''_{\lambda_2}] StO_2 + (\varepsilon_{Hb})''_{\lambda_2}}
\]

This is the approximated analytical expression of SDR for any \(\lambda_1\) and \(\lambda_2\).

Equation 83 shows a very special relationship between SDR and StO2.

First, the SDR \(\sim\) StO2 relationship is not associated with the coefficient F (which contains the weight factor f). Thus, the same SDR will be found from all different weight factors used.

Second, the SDR \(\sim\) StO2 relationship is associated with scattering. In order to find a SDR only associated with StO2, the condition should be \(\mu_s'(\lambda_1) \approx \mu_s'(\lambda_2)\), which requires (1) \(\lambda_1\) and \(\lambda_2\) are wavelengths as long as possible and (2) \(\lambda_1\) and \(\lambda_2\) are wavelengths as close as possible. Moreover, this means if the skin sample to be measured has a larger difference between \(\mu_s'(\lambda_1)\) and \(\mu_s'(\lambda_2)\), the measurement will not be accurate.

Third, this SDR \(\sim\) StO2 relationship in analytical form will be compared with the SDR \(\sim\) StO2 relationship from numerical simulation result, which will be discussed later.
**3.2.3. Screening SDR to find suitable wavelengths for StO$_2$ measurement**

Based on the analytical expression of SDR ~ StO$_2$ relationship shown by Equation 83, it’s expected that if $\lambda_1$ and $\lambda_2$ (and also $\delta \lambda$) are selected appropriately, the value of SDR is only determined by StO$_2$ regardless of different blood concentration, melanin concentration and scattering. In order to find out a suitable $\lambda_1$, $\lambda_2$ and $\delta \lambda$, SDR of all the possible combination of $\lambda_1$, $\lambda_2$ and $\delta \lambda$ were calculated. The range of $\lambda_1$, $\lambda_2$ is from 500nm to 650nm and the range of $\delta \lambda$ is from 8nm to 40nm (since the bandwidth of the filter is 7nm). Then, a screening method will be used to select the optimal $\lambda_1$, $\lambda_2$ and $\delta \lambda$.

There’re two criteria for the optimal $\lambda_1$, $\lambda_2$ and $\delta \lambda$.

1) The selected $\lambda_1$, $\lambda_2$ and $\delta \lambda$ should give SDR values that are only related to StO$_2$ but not related to blood concentration $C_{\text{blood}}$, melanin concentration $C_{\text{mel}}$ and scattering $\mu_s$.

2) The SDR value should have a one-to-one relationship with StO$_2$. In this way, once the relationship of SDR vs. StO$_2$ is determined theoretically, one can measure StO$_2$ experimentally by measuring SDR.

Since SDR is a function of $\lambda_1$, $\lambda_2$, $\delta \lambda$, $C_{\text{blood}}$, $C_{\text{mel}}$, $\mu_s$ and StO$_2$, first denote a SDR with a specific ($\lambda_1$, $\lambda_2$ and $\delta \lambda$) to be SDR($i$, $j$), in which $i=1:75$ denotes any combination of ($C_{\text{blood}}$, $C_{\text{mel}}$, $\mu_s$) and $j=1:21$ denotes different StO$_2$ levels. The definition of different skin conditions are found in Table 7.

Then two criteria are expressed arithmetically as follows.
(1) Arithmetic expression for first criteria

The largest and smallest SDR at certain StO$_2$ are: $\max_i\{SDR(i,j)\}$ and $\min_i\{SDR(i,j)\}$ respectively.

The difference between them is:

Equation 84: $dSDR(j) = \max_i\{SDR(i,j)\} - \min_i\{SDR(i,j)\}$

The averaged value of SDR at certain StO$_2$ is:

Equation 85: $\overline{SDR}(j) = \frac{1}{75}\sum_{i=1}^{75} SDR(i,j)$

The difference between SDR of j level of StO$_2$ and (j+1) level of StO$_2$ is

Equation 86: $d\overline{SDR}(j) = \overline{SDR}(j) - \overline{SDR}(j + 1)$

If a SDR of a wavelength combination ($\lambda_1$, $\lambda_2$ and $\delta\lambda$) is only related to StO$_2$ but not related to $C_{\text{blood}}$, $C_{\text{mcl}}$ and $\mu_s$

Equation 87: $dSDR(j) \ll d\overline{SDR}(j)$

Thus, define the measurement error using a wavelength combination ($\lambda_1$, $\lambda_2$ and $\delta\lambda$) at a StO$_2$ level as

Equation 88: $error(j) = \left(\frac{100}{20}\right) \times \frac{dSDR(j)}{d\overline{SDR}(j)} \times 100\%$

Here 100/20=5(%) is the StO$_2$ difference between adjacent StO$_2$ levels.

Then, the averaged and maximum error of all StO$_2$ levels are the averaged $\overline{error}$ and $\max_j error$, respectively.

Thus, the arithmetic definition for the first criteria is

Equation 89: $\overline{error} < 2.5(\%)$
Using Equation 89, all SDR(\(\lambda_1, \lambda_2, \delta\lambda\)) are screened to find out those with small error (< 2.5%). We considered these SDR(\(\lambda_1, \lambda_2, \delta\lambda\)) are only related to StO\(_2\), but not related to \(C_{\text{blood}}\), \(C_{\text{mel}}\), \(\mu_s\). These SDRs will be used for the screening under the second criteria.

(2) Arithmetic expression for second criteria

The second criteria is that SDR(\(\lambda_1, \lambda_2, \delta\lambda\)) has a one-to-one relationship with StO\(_2\). The simplest way to check whether a SDR(\(\lambda_1, \lambda_2, \delta\lambda\)) meets this criteria is to see whether SDR\(\sim\) StO\(_2\) is monotone. This means the derivative of SDR to StO\(_2\) is always positive or always negative.

Equation 90: \(\frac{d\text{SDR}}{d\text{StO}_2}\) always > 0 or \(\frac{d\text{SDR}}{d\text{StO}_2}\) always < 0

Or

Equation 91:

\[
\Delta\text{SDR} = \text{SDR}(\text{StO}_2(i)) - \text{SDR}(\text{StO}_2(j))\] always > 0 or \(\Delta\text{SDR}\) always < 0

Finally, a SDR(\(\lambda_1, \lambda_2, \delta\lambda\)) with largest sensitivity to StO\(_2\) is selected (which means the difference between SDR(StO\(_2\)=0\%) and SDR(StO\(_2\)=100\%) is largest).
3.2.4. Result: $SDR(568, 576, 24)$ for skin oxygen saturation imaging

(1) $StO_2 \sim SDR(568, 576, 24)$

The selected wavelength combination using the screening method is

$\lambda_1=568\text{nm}, \lambda_2=576\text{nm}, \text{and } \delta\lambda=24\text{nm}.$

Thus:

$\lambda^+ = \lambda_1 + \delta\lambda = 592\text{nm};$ (b)

$\lambda^- = \lambda_2 - \delta\lambda = 552\text{nm};$ (c)

$\lambda^+_2 = \lambda_2 + \delta\lambda = 600\text{nm};$ (d)

Equation 92:

$$SDR(568, 576, 24) = \frac{r(\lambda^+_1+r(\lambda^-_1)-2r(\lambda_1))}{r(\lambda^+_2+r(\lambda^-_2)-2r(\lambda_2))} = \frac{r(592)+r(544)-2r(568)}{r(600)+r(552)-2r(576)}$$

Totally 6 wavelengths will be used in the calculation of SDR: 544, 552, 568, 576, 592, 600nm.
Table 12. Data table of SDR(568, 576, 24)

<table>
<thead>
<tr>
<th>blood (%)</th>
<th>melanin (%)</th>
<th>scattering (%)</th>
<th>StO₂ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.2</td>
<td>0.02</td>
<td>-0.2</td>
<td>1.495</td>
</tr>
<tr>
<td>0</td>
<td>0.02</td>
<td>0</td>
<td>1.495</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.02</td>
<td>0.2</td>
<td>1.495</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.13</td>
<td>-0.2</td>
<td>1.507</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.13</td>
<td>0</td>
<td>1.507</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.13</td>
<td>0.2</td>
<td>1.506</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.3</td>
<td>-0.2</td>
<td>1.529</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.3</td>
<td>0</td>
<td>1.529</td>
</tr>
<tr>
<td>-0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>1.529</td>
</tr>
<tr>
<td>0</td>
<td>0.02</td>
<td>-0.2</td>
<td>1.495</td>
</tr>
<tr>
<td>0</td>
<td>0.02</td>
<td>0</td>
<td>1.495</td>
</tr>
<tr>
<td>0</td>
<td>0.02</td>
<td>0.2</td>
<td>1.495</td>
</tr>
<tr>
<td>0</td>
<td>0.13</td>
<td>-0.2</td>
<td>1.504</td>
</tr>
<tr>
<td>0</td>
<td>0.13</td>
<td>0</td>
<td>1.503</td>
</tr>
<tr>
<td>0</td>
<td>0.13</td>
<td>0.2</td>
<td>1.503</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>-0.2</td>
<td>1.520</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>1.520</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
<td>0.2</td>
<td>1.520</td>
</tr>
<tr>
<td>0.2</td>
<td>0.02</td>
<td>-0.2</td>
<td>1.495</td>
</tr>
<tr>
<td>0.2</td>
<td>0.02</td>
<td>0</td>
<td>1.495</td>
</tr>
<tr>
<td>0.2</td>
<td>0.02</td>
<td>0.2</td>
<td>1.494</td>
</tr>
<tr>
<td>0.2</td>
<td>0.13</td>
<td>-0.2</td>
<td>1.502</td>
</tr>
<tr>
<td>0.2</td>
<td>0.13</td>
<td>0</td>
<td>1.501</td>
</tr>
<tr>
<td>0.2</td>
<td>0.13</td>
<td>0.2</td>
<td>1.501</td>
</tr>
<tr>
<td>0.2</td>
<td>0.3</td>
<td>-0.2</td>
<td>1.515</td>
</tr>
<tr>
<td>0.2</td>
<td>0.3</td>
<td>0</td>
<td>1.515</td>
</tr>
<tr>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>1.515</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td></td>
<td>1.507</td>
</tr>
<tr>
<td>%std</td>
<td></td>
<td></td>
<td>0.777</td>
</tr>
</tbody>
</table>
Table 12 shows the data of SDR(568, 576, 24) of 162 (=3×3×3×6) representative skin tissue conditions from all 1575 conditions as listed in Table 7. These 162 conditions include 3 blood concentrations (80%, 100% and 120% of normal blood concentration listed in Table 1), 3 melanin concentration, 3 scattering level (80%, 100% and 120% of normal skin tissue scattering) and 6 StO2 level. Once can easily see that SDR(568, 576, 24) is the same with the same StO2. For example, SDR of 20% StO2 is about 0.87 regardless of blood concentration, melanin concentration and scattering. Similarly, SDR of 80% StO2 is about 0.39 for all conditions. As listed at the end of
Table 12, SDR of a StO$_2$ are averaged over all other 27 tissue conditions and the percent standard deviation (%std) is also calculated. We can first see that SDR$\sim$ StO$_2$ is a decreasing function. Also the %std is very small (all less than 1%). Thus, we can say that SDR(568, 576, 24) has a one to one relationship with StO$_2$.

![Figure 28. Oxygenation StO$_2$ vs. SDR(568, 576, 24).](image)

The overall relationship between SDR and StO$_2$ is plotted in Figure 28, which shows a monotone function approximated by

Equation 93:  
\[ StO_2(\%) = 100(-1.4SDR^3 + 4.82SDR^2 - 5.66SDR + 2.38) \]

The error bar in the plot indicates the variation due to different blood concentration, melanin concentration and scattering of skin.
Based on the above relation between StO$_2$ and SDR, skin StO$_2$ can be measured by taking the skin reflectance image at 544, 552, 568, 576, 592, 600nm and calculating SDR(568, 576, 24) using Equation 93.

This SDR ~ StO$_2$ relationship is also compared with the SDR ~ StO$_2$ relationship in analytical form.

Equation 83 shows that:

$$SDR = \frac{\mu_s'(\lambda_2)}{\mu_s'(\lambda_1)} \cdot \left[ \frac{(\varepsilon_{HbO}''\lambda_1 - (\varepsilon_{Hb}''\lambda_1) StO_2 + (\varepsilon_{Hb}''\lambda_1)}{\varepsilon_{HbO}''\lambda_2 - (\varepsilon_{Hb}''\lambda_2) StO_2 + (\varepsilon_{Hb}''\lambda_2)} \right]$$

One can find that (1) $\mu_s'(568)=28.83\text{cm}^{-1} \approx \mu_s'(576)=28.2\text{cm}^{-1}$ and (2) $(\varepsilon_{HbO}'')_{568} \approx (\varepsilon_{Hb}'')_{576}$

Thus,

$$SDR(568,576,24) = \frac{\mu_s'(576)}{\mu_s'(568)} \cdot \left[ \frac{(\varepsilon_{HbO}'')_{568} - (\varepsilon_{Hb}'')_{568} StO_2 + (\varepsilon_{Hb}'')_{568}}{\varepsilon_{HbO}'')_{576} - (\varepsilon_{Hb}'')_{576} StO_2 + (\varepsilon_{Hb}'')_{576}} \right]$$

Equation 94

$$SDR(568,576,24) = \frac{(\varepsilon_{Hb}'')_{568}}{\varepsilon_{HbO}'')_{576} - (\varepsilon_{Hb}'')_{576} StO_2 + (\varepsilon_{Hb}'')_{576}}$$

This is the approximated analytical expression of SDR(568, 576, 24).

Thus

Equation 95

$$StO_2 = \frac{(\varepsilon_{Hb}'')_{568}/SDR - (\varepsilon_{Hb}'')_{576}}{(\varepsilon_{HbO}'')_{576} - (\varepsilon_{Hb}'')_{576}}$$
This StO₂ ~ SDR relationship is compared with that from numerical simulation result (Equation 93). As shown in Figure 29, black and red curves represent the StO₂ ~ SDR relationship from approximated analytical result and numerical simulation result, respectively. These two curves are close to each other. The difference of two results is because during the deduction of SDR analytical expression, certain approximations have been made and many small terms have been neglected. Thus, the numerical result will be used for oxygen map reconstruction, while the analytical expression can be used for explanation of the principle of the algorithm and the choice of the wavelengths.

Figure 29. Comparison between the StO₂~SDR(568, 576, 24) relationship from numerical simulation and analytical deduction.

(2) Reason of choosing SDR(568, 576, 24) explained by analytical spectra analysis
The analytical expression of StO$_2$–SDR can also explain why the above wavelengths are chosen. From Equation 83,

$$ SDR = \frac{\mu_s'(\lambda_2) \cdot \left[ (e^{\text{HbO}})^{''} (\lambda_1) - (e^{\text{Hb}})^{''} (\lambda_1) \right] StO_2 + (e^{\text{Hb}})^{''} (\lambda_1)}{\mu_s'(\lambda_1) \cdot \left[ (e^{\text{HbO}})^{''} (\lambda_2) - (e^{\text{Hb}})^{''} (\lambda_2) \right] StO_2 + (e^{\text{Hb}})^{''} (\lambda_2)} $$

As discussed before, the first criterion is to have $\lambda_1$ to be close to $\lambda_2$ and both to be longer wavelengths so that $\mu_s'(\lambda_1) \approx \mu_s'(\lambda_2)$. Then, SDR is only determined by StO$_2$.

Equation 96: $SDR = \frac{\left[ (e^{\text{HbO}})^{''} (\lambda_1) - (e^{\text{Hb}})^{''} (\lambda_1) \right] StO_2 + (e^{\text{Hb}})^{''} (\lambda_1)}{\left[ (e^{\text{HbO}})^{''} (\lambda_2) - (e^{\text{Hb}})^{''} (\lambda_2) \right] StO_2 + (e^{\text{Hb}})^{''} (\lambda_2)}$

$e^{\text{HbO}}$ and $e^{\text{Hb}}$ are the extinction coefficient of oxy- and deoxy- hemoglobin, respectively. $(e^{\text{HbO}})^{''}$ and $(e^{\text{Hb}})^{''}$ are the wide gap second derivative of the extinction coefficient of oxy- and deoxy- hemoglobin at certain wavelength.

If StO$_2$ = 100%,

Equation 97: $SDR = \frac{(e^{\text{HbO}})^{''} (\lambda_1)}{(e^{\text{HbO}})^{''} (\lambda_2)}$

If StO$_2$ = 0%,

Equation 98: $SDR = \frac{(e^{\text{Hb}})^{''} (\lambda_1)}{(e^{\text{Hb}})^{''} (\lambda_2)}$

This means when StO$_2$ drops from 100% to 0%, SDR (which is the parameter to be measured) will shift from the ratio of $(e^{\text{Hb}})^{''}$ at $\lambda_1$ versus $\lambda_2$ to the ratio of $(e^{\text{HbO}})^{''}$ at $\lambda_1$ versus $\lambda_2$. This can be explain graphically using the second derivative spectra of extinction coefficient of oxy-hemoglobin $(e^{\text{HbO}})^{''}$ and deoxy-hemoglobin $(e^{\text{Hb}})^{''}$, as shown in Figure 30. The black dot line indicate two wavelengths $\lambda_1$ and $\lambda_2$ selected for
SDR($\lambda_1, \lambda_2, \delta\lambda$) and three green dash lines indicate the value of SDR when StO$_2$ = 100, 50 and 0%, respectively. The value of SDR is the ratio between two end points of the green line. When StO$_2$ = 100%, the green line connects the two points where the curve of ($\varepsilon^{HbO}$)$''$ cross two black vertical lines. When StO$_2$ = 0%, the green line connects the two points where the curve of ($\varepsilon^{Hb}$)$''$ cross two black lines. When StO$_2$ is 50%, the green line connects two middle points of two black line segments.

Figure 30. Second derivative spectra of extinction coefficient of oxy-hemoglobin $\varepsilon(HbO)''$ and deoxy-hemoglobin $\varepsilon(Hb)'$ (wavelength gap $\delta\lambda$ = 24nm). Black vertical line indicate two wavelengths $\lambda_1$ and $\lambda_2$ selected for SDR($\lambda_1, \lambda_2, \delta\lambda$); green dash lines indicate the value of SDR when StO$_2$ = 100, 50 and 0%.

In order to maximize the sensitivity of measurement, one should select $\lambda_1$ and $\lambda_2$ so that the difference between ($\varepsilon^{HbO}$)$''/\lambda_1$/$($\varepsilon^{HbO}$)$''/\lambda_2$ and ($\varepsilon^{Hb}$)$''/\lambda_1$/$($\varepsilon^{Hb}$)$''/\lambda_2$ is maximized. One should look for the part of spectra where (1) two curves are both very
steep and (2) the slopes of two curves are opposite. This is the second criterion for choosing the right wavelengths.

The third criterion is that the change of SDR from \( \left( \varepsilon_{\text{HbO}}'' \right)_{\lambda_1} / \left( \varepsilon_{\text{HbO}}'' \right)_{\lambda_2} \) to \( \left( \varepsilon_{\text{Hb}}'' \right)_{\lambda_1} / \left( \varepsilon_{\text{Hb}}'' \right)_{\lambda_2} \) should be monotone when StO₂ changes from 100% to 0%, otherwise SDR will not have a one-to-one relationship with StO₂.

Figure 31. Second derivative spectra of extinction coefficient of oxy-hemoglobin \( \varepsilon(\text{HbO})'' \) and deoxy-hemoglobin \( \varepsilon(\text{Hb})'' \) (wavelength gap \( \delta \lambda = 24\text{nm} \)). Dash lines indicate two selected central wavelengths 568nm and 576nm.

Figure 31 shows the spectra of \( \varepsilon(\text{HbO})'' \) and \( \varepsilon(\text{Hb})'' \) and the selected wavelengths after screening as indicated by the red dash lines. Two selected wavelengths are 568nm and 576nm. From the figure, one can see that these two selected wavelengths satisfy all
three criteria. 568nm and 576nm are both comparatively long wavelengths and have 8nm difference. The spectra of \( \varepsilon^{\text{HbO}} \) and \( \varepsilon^{\text{Hb}} \) between 568nm and 576nm change dramatically with opposite slope. SDR(568, 576, 24) changes from 0.34 when StO₂=100% to 1.5 when StO₂=0%, as shown in Figure 28.

Finally, using this spectral analysis together with screening method in Chapter 3.2.3, one may find other wavelengths which give a SDR only related to the oxygenation regardless of different skin tissue conditions. Actually the algorithm described in this Chapter Chapter 3 is a general approach to find a parameter which is only related to the oxygenation. This method may not be limited to the six wavelengths reported. Other wavelengths may be used in the future to test the feasibility of measuring oxygenation.

(3) Error analysis: influence of weight factors on StO₂~SDR(568, 576, 24)

As mentioned in Chapter 3.1.4, weight factors can be different for different skin conditions. Thus, the resulted relationship of StO₂~SDR may be different if different weight factors are used. Thus, in this section, the influence of different skin conditions on the relationship of StO₂~SDR is investigated.

From Chapter 3.1.4, different weight factors were found from different skin conditions by solving the optimization problem of matching two skin spectral reflectances simulated by MC method and semi-infinite model. Then, the skin spectral reflectance simulated using the semi-infinite model with these optimized weight factors are used in the backward calculations. After that, the relationship between skin oxygen StO₂ and the
second derivative ratio SDR(568, 576, 24) are found and plotted in Figure 32 and listed in Table 13.

Figure 32. Relationship of StO₂~SDR obtained from semi-infinite model and second derivative algorithm. Error bar shows the variation due to different weight factors.

Table 13. Data of StO₂~SDR relationship.

<table>
<thead>
<tr>
<th>SDR</th>
<th>StO₂ (%)</th>
<th>error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.504519</td>
<td>0</td>
<td>1.022743</td>
</tr>
<tr>
<td>0.873155</td>
<td>20</td>
<td>0.458367</td>
</tr>
<tr>
<td>0.620306</td>
<td>40</td>
<td>0.954662</td>
</tr>
<tr>
<td>0.483195</td>
<td>60</td>
<td>1.098808</td>
</tr>
<tr>
<td>0.396569</td>
<td>80</td>
<td>1.031473</td>
</tr>
<tr>
<td>0.336464</td>
<td>100</td>
<td>0.810575</td>
</tr>
</tbody>
</table>

From Figure 32 and Table 13, we can see that the error of StO₂~SDR relationship due to different weight factors used was very small (~ 1%). This indicates that the StO₂~SDR relationship is not influenced by different skin conditions and weight factors used during the simulation of skin reflectance using semi-infinite tissue model. This means we can use a well-determined StO₂~SDR relationship to calculate skin oxygen saturation during our measurement. The reason that the StO₂~SDR relationship is not
affected by different weight factors can be explained by the analytical expression of StO₂–SDR which is shown in Equation 95.

\[
StO_2 = \frac{(\varepsilon_{Hb})''_{568}/SDR - (\varepsilon_{Hb})''_{576}}{(\varepsilon_{HbO})''_{576} - (\varepsilon_{Hb})''_{576}}
\]

This StO₂–SDR expression does not contain any parameters associated with weight factors or different skin conditions.

(4) SDR(542, 584, 20) – A bad example which cannot be used for oxygen imaging

The above SDR(568, 576, 24) is a good example showing SDR is only related to StO₂ regardless of any level of blood concentration, melanin and scattering. Here shows a poor example.

![Graph showing StO₂ vs. SDR(542, 584, 20).](image)

Figure 33. Oxygenation StO₂ vs. SDR(542, 584, 20).
Figure 33 shows $\text{SDR}(542, 584, 20)$ vs $\text{StO}_2$ under 15 tissue conditions including 5 level of blood concentration and 3 levels of melanin concentration as defined in Table 7. Scattering is fixed in normal tissue scattering here because the plot will be too distracted if more tissue conditions are presented.

One can see from Figure 33 that $\text{SDR}(542, 584, 20)$ does not have a one-to-one relationship with $\text{StO}_2$ because $\text{SDR}(542, 584, 20)$ are different for different $C_{\text{blood}}$ and $C_{\text{mel}}$, which is indicated by the non-overlapped curves. This combination of $\lambda_1$, $\lambda_2$, and $\delta\lambda$ is not suitable for imaging measurement because many $\text{StO}_2$ level will give the same SDR.
Chapter 4: Multispectral imaging system

4.1. Imaging system: hardware

A hyperspectral/multispectral imaging system was built for non-invasive, non-contact and near real-time imaging of cutaneous tissue oxygenation. A Hamamatsu ORCA ER deep cooling CCD camera (Hamamatsu, Bridgewater, NJ) was used to capture hyperspectral images. A set of camera lens (Edmund optics, Barrington, NJ) with different focal distance and field of view were mounted in front of the camera to acquire images of sample with different size and distance. A Varispec VIS liquid crystal tunable filter (Cambridge Research Inc., Cambridge, MA) with wavelength range of 400-720nm and a bandwidth of 7nm was mounted in front of the camera lens to enable imaging at designated wavelengths. An OSL1 fiber light source (Thorlabs, Newton, NJ) was used to illuminate the imaged object. The whole system was installed on a moving table with its height adjustable to increase portability. Figure 34 and Figure 35 show the Solidworks model and a photo of the hyperspectral/multispectral imaging system, respectively.
Figure 34. Solidworks model of hyperspectral/multispectral imaging system.

Figure 35. Photo of hyperspectral/multispectral imaging system
4.2. Graphical user interface (GUI)

A GUI was programmed in Labview for equipment control, synchronization and image acquisition. The GUI has three main modules.

(1) Real time module: users can view the real-time monochromatic image at any selected wavelength and adjust the exposure time of the image;

(2) Wavelength selection module

Uses can choose to do a multispectral imaging or hyperspectral imaging. For multispectral imaging, users can define the wavelengths for image acquisition by simply typing 4 to 6 wavelengths used for wide gap second derivative spectroscopy. For hyperspectral imaging, users can define the wavelengths for image acquisition by simply typing in the beginning wavelength $\lambda_1$, the ending wavelength $\lambda_2$ and the wavelength interval $\Delta \lambda$ (which is the spectral resolution for hyperspectral imaging).

(3) Auto-exposure module: this module is literally the most important module in the GUI. After the wavelengths for image acquisition have been defined, the module will completely automatically set up the exposure time for each wavelength so that every monochromatic image will have the optimal reflectance intensity.

(3) Image acquisition module: this module is used to take hyperspectral/multispectral images. There’re two options: taking image by repeat times, or taking image by total time. With the first option, one group of hyperspectral images of the selected wavelengths will be taken, then the next group of images will be taken and
this procedure will be repeated as many times as defined. With the second option, the above procedure will be repeated until it’s time out.

Figure 36 and Figure 37 show the overview of the front and back panel of the GUI for hyperspectral/multispectral imaging system. Some detail description will be provided in Appendix C.

Figure 36. Front panel of the GUI for hyperspectral/multispectral imaging system
Figure 37. Back panel of the GUI for hyperspectral/multispectral imaging system

4.3. Measurement procedures

A measurement procedure was developed for multispectral imaging of oxygenation $\text{StO}_2$ of either skin or tissue-simulating phantom using wavelengths 544, 552, 568, 576, 592, 600nm. First, a 99% reflected flat diffuser (National Standard and Technology, Gaithersburg, MD) was place under the camera within the field of view. The
height of the diffuser was adjusted so that it’s the same height of the sample to be measured. Then auto-exposure function was run to set up the optimal exposure time $t_{\text{TR}}$ of each wavelength. Then monochromatic images of each wavelength were taken and stored. After that, sample was placed within the camera field of view.

Now, if the exposure time (or shutter speed) of the camera could be set to absolute (i.e. in the unit of millisecond), then auto-exposure function was run to set up the optimal exposure time (for the sample image) $t_{\text{I}}$ at each wavelength. After that monochromatic images of each wavelength were taken and stored.

If the exposure time (or shutter speed) of the camera could only be set to relative (i.e. in the scale of 0 to some integer), then monochromatic images of the sample were taken at the same exposure time as the diffuse (i.e. $t_{\text{TR}}$) at each wavelength.

Multispectral images and the exposure time for each wavelength were stored for further processing.

4.4. Image processing and oxygen map generation

All the images were first processed with a low-pass filter (either moving average or Gaussian) to filter out the noise. Then let $I$ and $TR$ denote the intensity of images of sample and 99% reflected diffuser.

In the case of absolute exposure time, reflectance was calculated as

$$R = \frac{(I-I_{\text{dark}})/t_{\text{I}}}{(TR-I_{\text{dark}})/t_{\text{TR}}}$$
In which $I_{\text{dark}}$ is the image intensity in the totally dark environment (the dark current of the CCD chip).

In the case of relative exposure time, reflectance was calculated as

$$R = \frac{(I - I_{\text{dark}})}{(TR - I_{\text{dark}})}$$

Then we would have reflectance of each pixel at designated wavelengths $R(x, y, \lambda)$. The data of each pixel was processed separately. $SDR(x, y, \lambda)$ was calculated from $R(x, y, \lambda)$ using the following three steps:

1. Solve $s(x, y, \lambda)$ from equation

$$b_1 \cdot s^2 - (b_1 + R_1 \cdot b_2 + 1) \cdot s + (1 - R_1) = 0 \text{ in which } R_1 = \frac{R - r_s}{1 - r_s}$$

2. solve $r(x, y, \lambda)$ using equation:

$$r = \frac{\mu_a}{\mu_s} = \frac{s^2}{1 - s^2}$$

3. calculate $SDR(568, 576, 24)$ of each pixel using

$$SDR(568, 576, 24) = \frac{r(\lambda_1^+) + r(\lambda_1^-) - 2r(\lambda_1)}{r(\lambda_2^+) + r(\lambda_2^-) - 2r(\lambda_2)} = \frac{r(592) + r(544) - 2r(568)}{r(600) + r(552) - 2r(576)}$$

Finally, $StO_2$ of each pixel was calculated by

$$StO_2(\%) = 100(-1.45SDR^3 + 4.82SDR^2 - 5.66SDR + 2.38)$$

A preliminary oxygenation map was generated by assigning a false-color to each $StO_2$ value, as shown in Figure 38. One can see that the resulted oxygenation value is very unreasonable in those areas which is (1) not the skin, or (2) outside the illumination range, or (3) outside the area of the 99% reflected diffuser. Thus, further polishing had to be made.
During measurement, a piece of black cloth was place under the biological sample (in this case the arm). Then in the multispectral image the area without biological sample or without sufficient illumination would appear dark, as shown in Figure 39 (a). Then a threshold was set and any pixel with intensity below this threshold was considered belong
to area to be polished. Then black color was applied to these pixels within this area by assigning the RGB all to 0. The resulted oxygenation map is shown in Figure 39(b).

The next step was to polish the area outside the 99% reflected diffuser. From Figure 40(a) we can see that the area outside the diffuser appears dark. Thus the area to be polished was found by setting a threshold and screening any pixel with intensity below this threshold. Then black color was applied to these pixels within this area by assigning the RGB all to 0. The resulted oxygenation map is shown in Figure 40(b). Now the oxygenation map appears nice.

![Figure 40 Polishing the area outside the 99% reflected diffuser](image)

During the image processing, it was found that the noise is from two main sources.

(1) Roughness of the skin. Since the skin has a ridged or corrugated surface, some spots reflect light strongly and some weakly. Thus, the image is very bright in some pixels (compared to the near surrounding) and very dim in some pixels. This can be seen
in Figure 41. Figure 41(a) shows a monochromatic image of the arm and Figure 41(b) shows the image intensity profile along the dash line in (a). The signal is very noisy.

![Figure 41](image.png)

(a) (b)

Figure 41. Roughness of the skin introduces major noise in image processing.

There’re several possible solutions. First, use a camera which is less sensitive to the light intensity difference caused by skin roughness. Figure 42 (a) shows a monochromatic image of the same arm of the same subject taken by a camera with less sensitivity and Figure 42 (b) shows the image intensity profile along the dash line in (a). Compared to Figure 41 (b), although the color-depth of Figure 42(b) is much lower (8-bit vs. 16-bit), the noise is much smaller and the skin appears much smoother in Figure 42(a). Second, reduce the contrast and increase the dynamic range of the camera so that the image will have less relative noise although it appears not as ‘sharp’ as the one with high contrast. Third, make the image a little out of focus. It’s also found that the oxygenation map is smoother for the smoother skin. For example, oxygen map of a female subject is smoother than male; oxygen map of inner arm is smoother than dorsal.
115

arm; and oxygen map produced in summer is better than that produced in winter (since the skin is more moisture in summer and dried in winter).

![Image](image.jpg)

(a) (b)

Figure 42. Less noise from skin roughness when using a less sensitive camera

(2) Motion-artifact. The oxygenation measurement is a little sensitive to motion-artifact, mainly due to the reason that it’s sensitive to the roughness of skin.

Solution is to reduce the image exposure time. Possible methods to achieve this solution are: (1) increase the light intensity of illumination; (2) use a faster operating platform for GUI (such as Visual C++); and (3) use glass filter rather than liquid crystal tunable filter.
Chapter 5: Experiment: Multispectral imaging of StO₂ of tissue phantom

5.1. Introduction to phantom and its role in optical calibration

Biomedical optical imaging and spectroscopy (BOIS) uses light to reveal structural, functional, and molecular characteristics of biological tissue for screening, diagnosis, treatment, and therapeutic assessment of clinical anomalies. Hyperspectral/multispectral imaging is one of the emerging BOIS techniques. Major advantages of BOIS include low cost, portability, no radiation hazard, molecular sensitivity, and real-time non-invasive measurements of multiple tissue parameters. However, compared with other imaging modalities, such as positron emission tomography (PET), computed tomography (CT), ultrasound (US), and magnetic resonance imaging (MRI), BOIS has limited clinical exposure. The translation of BOIS techniques from the benchtop to the bedside is hampered by multiple limitations such as the lack of calibration standards, the lack of validation methods, and the trade-off between measurement depth and resolution [44].

Moreover, it is important to develop tissue simulating phantoms to validate physical models and simulations, to test and optimize instrument performance, to carry out routine quality control and calibration tasks, and to standardize inter-laboratory studies and multi-site clinical trials [45]. In this context, developing phantoms that
simulate tissue structural, functional, and molecular properties are important for reliable performance and successful translation of BOIS techniques.

In order for successful translation of BOIS techniques from benchtop to bedside, An ideal tissue-simulating phantom system should meet the following criteria: (1) controllable optical properties simulating different tissue types and physiologic conditions; (2) simulating mechanical and other physical properties of biological tissue; (3) ease to fabricate at low cost with reproducibility; (4) low shelf time and ease to transport.

To meet the above criteria, there’re many kinds of phantom fabrication techniques reported in the past decades.

First, in order to simulate absorption of blood, animal blood such as bovine blood or porcine blood[46] may be mixed into the phantom. Hemoglobin solution extracted from the animal blood or from dry hemoglobin powder are also good candidates.

Second, to simulate the scattering properties of tissue, possible materials include intralipid, milk[46], and titanium dioxide (TiO2) powder.

Third, to simulate the background color of tissue such as skin darkness originated from melanin absorption, one may introduce ink or liquid candle dyes for absorption control.

Fourth, different materials are available to serve as the host of the phantom. Phosphate buffered saline (PBS) or just distilled water can be used to make liquid phantom[47]. Agar-agar gel[48], gelatin gel[49], gel wax[50-53] can be used to fabricate gel-like phantom.
Finally, a digital phantom [46] may be used instead of a physical phantom.

In our experiment, one phantom design was used, as described in the next section. But during this study, many kinds of tissue-simulating phantoms have been explored. Different designs of tissue-simulating phantom are discussed in Appendix D.

5.2. Design of the tissue-simulating phantom

5.2.1. Finding proper concentrations for the phantom ingredients

The phantom we used was a liquid mixture of blood, ink, intralipid, and phosphate buffered saline (PBS). Blood, ink and intralipid were used to simulate the blood absorption, melanin absorption and skin scattering, respectively. Table 14 lists the absorbers in in vivo skin and the corresponding absorber simulating certain tissue properties.

In order to simulate in vivo tissue, the concentration of each of these ingredients should be adjusted so that the absorption and scattering of the phantom match with those of in vivo tissue.
Table 14. Absorbers in in vivo skin and the corresponding absorber used in phantom

<table>
<thead>
<tr>
<th>Absorber in in vivo skin</th>
<th>Corresponding absorber in phantom</th>
<th>Tissue property to simulate</th>
<th>Tissue property to match</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>Bovine blood/porcine blood</td>
<td>Absorption from blood</td>
<td>$\mu_a(630\text{nm}) = 0.15$–$0.2 \text{ cm}^{-1}$ (in vivo tissue measured by ISS tissue oximeter) $\mu_a(630\text{nm}) = 0.5$–$0.7 \text{ cm}^{-1}$ (Tseng et al: in vivo skin, type Caucasian)</td>
</tr>
<tr>
<td>Melanin</td>
<td>ink</td>
<td>Skin color</td>
<td>Absorption spectrum of melanosome vs ink</td>
</tr>
<tr>
<td>scattering</td>
<td>Intralipid</td>
<td>Skin scattering</td>
<td>$\mu_s'(630\text{nm}) = 22 \text{ cm}^{-1}$ (Tseng et al: in vivo; Bashkatov et al: in vitro)</td>
</tr>
<tr>
<td>water</td>
<td>PBS solution</td>
<td>water</td>
<td></td>
</tr>
</tbody>
</table>

(1) Absorption from bovine/porcine blood

The absorption of blood in _in vivo_ skin should be known in order to calculate how much animal blood we need to simulate the blood absorption. Tseng [29, 30] published two papers investigating _in vivo_ skin absorption of different skin types, as shown in Figure 43(a) and (b) respectively. It can be seen that from either paper, the absorption of skin of skin type I,II or Caucasian is $\mu_a(630\text{nm}) = 0.05$–$0.07 \text{ mm}^{-1}$ (i.e. $0.5$–$0.7 \text{ cm}^{-1}$). However, if we measure the skin absorption using a Oxiplex tissue oximeter (ISS Inc, Champaign, IL), we usually get $\mu_a(630\text{nm}) = 0.15$–$0.2 \text{ cm}^{-1}$. So here we will include the range of both cases and consider the absorption of skin without melanin as $\mu_a(630\text{nm}) = 0.15$–$0.7 \text{ cm}^{-1}$. This is what we need to match using animal blood.
The absorption of animal blood is from oxygenated hemoglobin and deoxygenated hemoglobin. The absorption coefficients are expressed as

Equation 101: \[ \mu_a^{Hbo} = \varepsilon^{Hbo} \cdot StO_2 \cdot \frac{c_{Hb}^{Hb}}{M.W_{Hb}} \cdot c_{blood} \]

Equation 102: \[ \mu_a^{Hb} = \varepsilon^{Hb} \cdot (1 - StO_2) \cdot \frac{c_{Hb}^{Hb}}{M.W_{Hb}} \cdot c_{blood} \]

Molar extinction coefficients \( \varepsilon^{Hbo} \) and \( \varepsilon^{Hb} \) and molecular weight of hemoglobin \( M.W_{Hb} \) should be the same values for both animal blood and human blood. But concentration of hemoglobin in blood \( c_{Hb} \) can be different from batch to batch as we collected animal blood from slaughter house or pig lab. In order to match the absorption of in vivo human skin, the concentration of animal blood will be predicted using theoretical analysis first.

In the numerical phantom we can calculate the average blood concentration

Equation 103: \[ c_{blood} = \sum_{i=1}^{7} c_{blood_i} \cdot f_i = 4.8\% . \]
The average hemoglobin concentration value in in vivo human blood is $C_{\text{Hb}} = 150\text{g/L}$. Also let $\text{StO}_2= 50\%$, $\lambda=632\text{nm}$. We have blood absorption coefficient to be

$$
\mu_a^{\text{blood}} = (\varepsilon_{632}^{\text{Hbo}} \cdot 0.5 + \varepsilon_{632}^{\text{Hb}} \cdot 0.5) \cdot \frac{150}{64458} \cdot 0.48 = (561 \cdot 0.5 + 4931 \cdot 0.5) \cdot \frac{150}{64458} \cdot 0.48 = 0.31\text{cm}^{-1}
$$

which is in the middle of the range 0.15~0.7 cm$^{-1}$.

Thus, 4.8% of blood concentration may be used.

Then experimentally we can add 4.8% of blood in the phantom and then adjust $C_{\text{blood}}$ manually so that $\mu_a(632\text{nm})$ of the phantom (without ink) is in the middle of the range 0.15~0.7 cm$^{-1}$.

(2) Absorption from ink

Ink is one of the best candidates to use to simulate absorption of melanin because they both appear black in color and have similar absorption spectra. But there’s certain difference between the extinction coefficients of ink $\varepsilon(\lambda)$ and that of melanin. Thus some adjustments have to made to let ink simulate melanin absorption best.

Let’s see the property of in vivo skin that we need to match using ink. We first make a reasonable but important assumption that the difference of absorption between African skin (skin type V, VI) and Caucasian skin (skin type I, II) is the highest possible absorption of melanin in in vivo skin, which is the African skin (skin type V, VI). This assumption should be a very reasonable since the Caucasian skin is almost free of melanin[17].

From Figure 43(a) we can calculate the melanin absorption coefficient as

$$
\mu_a^{\text{melanin}} = \mu_a^{\text{African}} - \mu_a^{\text{Caucasian}} \approx 0.35-0.075=0.275\text{mm}^{-1}=2.75\text{cm}^{-1}
$$
However, from Figure 43(b) we get

\[
\mu_{a650nm}^{melanin} \approx \mu_{a650nm}^{African} - \mu_{a650nm}^{Caucasian} \approx 0.08-0.06 = 0.02 \text{cm}^{-1} = 0.2 \text{cm}^{-1}
\]

These results are very controversial, although they were from the same research group. Here we use the larger difference. This is because if our algorithm can work for skin with very different melanin concentration levels, it should work for skin with less different melanin concentration levels. Thus,

\[
\mu_{a}^{melanin} \text{ (African, 650nm)} = 2.75 \text{ cm}^{-1};
\]

We also know the volume fraction of melanosome in living epidermis \(f_{\text{mel}}\) is 0.02, 0.13 and 0.3 for Caucasian, Asian and African skin types. Thus,

\[
\mu_{a}^{melanin} \text{ (Asian, 650nm)} = 2.75 \times 0.13 / 0.3 = 1.2 \text{cm}^{-1} \\
\mu_{a}^{melanin} \text{ (Caucasian, 650nm)} = 2.75 \times 0.02 / 0.3 = 0.18 \text{cm}^{-1}
\]

Now we need to adjust the ink concentration so that the absorption matches \(\mu_{a}^{melanin}\).

The extinction coefficient of melanin is defined as the absorption coefficient of melanoma, which is acquired from [17].

Equation 104: \(\mu_{a}^{melanosome} = 1.7 \times 10^{12} \lambda^{-3.48} \text{ (cm}^{-1})\)

The extinction coefficient of ink is measured experimentally using absorption spectrometer (i.e.: 100% ink absorption coefficient).

Equation 105: \(\mu_{a}^{ink} = 6.04 \times 10^{7} \lambda^{-1.13} \text{ (cm}^{-1})\)

By comparing the above two equations, one can see that although the absorption spectrum of both ink and melanosome are of the form of exponential, the exponential factor is different. Melanosome absorption spectra is steeper than that of ink, as shown in
Figure 44. Thus, it’s impossible to match two absorptions in the whole wavelength range. The solution is to have two absorption spectra cross at some point so that two spectra are closest in the wavelength range of interested. One possible way is to let $\mu^\text{ink} (650) = \mu^\text{melanin} (650)$. But the absorption of ink in the visible range will be much lower than that of melanin, as shown in Figure 44 (a). To do a better matching between ink and melanin, we match two spectra at 550nm, i.e. let $\mu^\text{ink} (550) = \mu^\text{melanin} (550)$. In this way, two absorption spectra are closest in the range of 500 ~ 600nm.

![Figure 44. Matching ink absorption to melanin absorption.](image)

Melanosome absorption at 650nm and 550nm can be calculated using Equation 34

$$
\varepsilon^\text{melanin} (650) = \mu^\text{melanosome} = 1.7 \times 10^{12} \lambda^{-3.48} = 1.7 \times 10^{12} \times 650^{-3.48} = 276 \text{ cm}^{-1}
$$

$$
\varepsilon^\text{melanin} (550) = \mu^\text{melanosome} = 1.7 \times 10^{12} \lambda^{-3.48} = 1.7 \times 10^{12} \times 550^{-3.48} = 494 \text{ cm}^{-1}
$$
Volume fraction of melanosome is
\[ f_V = \frac{\mu^\text{melanin} (650)}{\varepsilon^\text{melanin} (650)} = \frac{2.75 \text{ cm}^{-1}}{276 \text{ cm}^{-1}} = 0.01 \]

Melanin absorption at 550nm is
\[ \mu^\text{melanin} (550) = \varepsilon^\text{melanin} (550) \times f_V = 494 \times 0.01 = 4.94 \text{ cm}^{-1} \]

Now let
\[ \mu^\text{ink} (550) = \mu^\text{melanin} (550) = 4.94 \text{ cm}^{-1} \]

Extinction coefficient of ink at 550nm can be calculated
\[ \mu^\text{ink} = 6.04 \times 10^7 \lambda^{-1.13} = 4.83 \times 10^4 \text{ cm}^{-1} \]

Thus the ink concentration should be
\[ C^\text{ink} (\text{African}) = \frac{\mu^\text{ink} (550)}{\varepsilon^\text{ink} (550)} = \frac{4.94}{4.83 \times 10^4} = 10^{-4} = 0.01\% \]
\[ C^\text{ink} (\text{Asian}) = 10^{-4} \times 0.13 / 0.3 = 4.3 \times 10^{-5} \]
\[ C^\text{ink} (\text{Caucasian}) = 10^{-4} \times 0.02 / 0.3 = 6.7 \times 10^{-6} \]

The matching result is shown in Figure 44 (b).

Thus, ink with concentration of $10^{-4}$, $4.3 \times 10^{-5}$ and $6.7 \times 10^{-6}$ best simulates the melanin absorption effect of Caucasions, Asians and Africans, respectively. For a 200mL phantom, 1.3, 8.6 and 20µL of ink should be added into the host solution.
Figure 45. Second derivative absorption spectra of melanin and ink with concentration what will melanin absorption.

After these calculations, the second derivative absorption spectra of 1% melanin and 0.01% ink were calculated and plotted in Figure 45. Although the absorption of ink has been matched to that of melanin, the second derivative value of ink absorption is less than that of melanin. Actually this is expected since the exponential factor of ink (-1.33) is larger than that of melanin (-3.48), as shown in Equation 104 and Equation 105. Thus, the second derivative spectroscopic algorithm should be more effective for ink.

Then the argument can be why we are not matching the second derivative of ink absorption to that of melanin. If the second derivative of ink absorption is matched to that of melanin (Figure 46), the ink concentration needed will be 0.065 which is six times more than when ink absorption is matched to that of melanin. In this case, ink absorption will be six times more than melanin absorption over the whole wavelength range, as shown in Figure 46(a). Then in the phantom, most of photons will be absorbed by the ink.
The hemoglobin absorption will be totally overwhelmed by the ink absorption. We will not be able to measure enough signal from the hemoglobin. Thus, we should match ink absorption to the melanin absorption rather than matching the second derivative of two absorbers.

![Figure 46. Matching second derivative of ink absorption to that of melanin](image)

(3) Scattering of Intralipid

Intralipid is used to simulate the scattering property of skin tissue. The available commercial products are in 10% or 20% concentration. Intralipid™ has become a widely used standard material so that there’re experiment data published. Both Flock[54] and Van[55] published the data of scattering coefficient $\mu_s$ vs. $\lambda$ and anisotropy factor $g$ vs. $\lambda$.

Flock data:

Equation 106: $\mu_s = 1.17 \times 10^9 \lambda^{-2.33}$, $g = 2.25 \lambda^{-0.155}$

Van data:
Equation 107: \( \mu_s = 2.54 \times 10^9 \lambda^{-2.4} \), \( g = 1.1 - 0.58 \times 10^{-3} \lambda \)

However, there’s significant difference between these two data, as shown in Figure 47 [56]. The reason is that the intralipid is different from batch to batch.
Figure 47. Scattering coefficient (a), anisotropy factor (b) and reduced scattering coefficient (c) of 10% intralipid™ (Courtesy of [56]).
Thus we had to find out which data is more close to the intralipid we used. Reduced scattering coefficient of intralipid at 690nm and 834nm were measured using Oxiplex tissue oximeter (ISS Inc, Champaign, IL). Table 15 lists the data of $\mu_s'(\text{cm}^{-1})$ of 10% intralipid in 690nm and 834nm from Flock, Van and the oximeter measurement.

Table 15. Reduced scattering coefficient of 10% intralipid from oximeter measurements and published data. (Note: scattering of intralipid is much larger than the scattering of 5% blood so that here intralipid was measured without mixing with blood).

<table>
<thead>
<tr>
<th>$\lambda$(nm)</th>
<th>$\mu_s'(\text{cm}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flock [54]</td>
</tr>
<tr>
<td>690</td>
<td>52.04649</td>
</tr>
<tr>
<td>834</td>
<td>37.78734</td>
</tr>
</tbody>
</table>

This shows that the scattering property of our intralipid is more close to scattering data published by Van. One may argue that there’s still considerable difference. But in our numerical phantom simulation, we will change the scattering of the phantom based on this set of data, then to see if the algorithm of oxygen saturation works for very different scattering. It will cover a large range of scattering. Thus the difference between scattering of the intralipid we used and that from Van’s data does not matter.

Now, we need to adjust intralipid concentration to simulate skin scattering. The reduced scattering coefficient of skin tissue can be calculate using either Bashkatov[6] or Tseng’s [30] empirical formula.

$$\mu_s'(690) = 1.1 \times 10^{12}\lambda^{-4} + 73.7\lambda^{-0.22} = 22.35 \text{ cm}^{-1}$$

This means the corresponding intralipid concentration should be approximately
\[ C_{intralipid} = \frac{\mu'_s(skin)}{\mu'_s(10\% \text{ lipid})} \times 10\% = \frac{22.35}{117} \times 10\% \approx 1.9\% \]

Since this is just theoretical prediction, the intralipid concentration will be further adjusted experimentally to let phantom reduced scattering coefficient to be \( \mu_s'(690\text{nm}) = 22.35 \text{ cm}^{-1} \).

**(4) Absorption of intralipid**

The absorption of intralipid is very small compared to that of blood and ink. But it will still be included in the simulation for accuracy reason.

The data of intralipid absorption is published by Flock\[54\]. \( \mu_a \) vs. \( \lambda \) is a straight line in the log scale. But the data is only available in 3 wavelengths, as listed in Table 16.

<table>
<thead>
<tr>
<th>( \lambda (\text{nm}) )</th>
<th>( \mu_a (\text{cm}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>460</td>
<td>0.015</td>
</tr>
<tr>
<td>633</td>
<td>0.0029</td>
</tr>
<tr>
<td>690</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Thus, we will fit these three point using the equation '\( \log_{10}(\mu_a) = a\lambda + b \)' to get the absorption coefficient the intralipid in the whole wavelength range:

\[ \mu_a = 2.81 \times 10^{-3/205} \text{ (cm}^{-1}) \]
5.2.2. StO$_2$ imaging algorithm for tissue phantom

In the previous few sections, the experiment protocol for tissue phantom has been described. Since phantom is different from skin, some modifications have to make for the multispectral algorithm to simulation the reflectance of phantom.

Here all the formulas will be summed up to give an overall view of the numerical model which is used to simulate the spectral reflectance of tissue phantom.

Absorption of phantom:

$$\mu_a = \mu_a^{HbO} + \mu_a^{Hb} + \mu_a^{ink} + \mu_a^{lipid} + \mu_a^{water}$$

Absorption of blood (oxygenated and deoxygenated hemoglobin) are

Equation 101: $\mu_a^{HbO} = \varepsilon^{HbO} \cdot StO_2 \cdot \frac{C_{Hb}}{M.W_{Hb}} \cdot C_{blood}$

Equation 102: $\mu_a^{Hb} = \varepsilon^{Hb} \cdot (1 - StO_2) \cdot \frac{C_{Hb}}{M.W_{Hb}} \cdot C_{blood}$

In which normal blood concentration is $C_{blood} = 4.8\%$.

Ink absorption

Equation 108: $\mu_a^{ink} = C^{ink} \times 6.04 \times 10^7 \lambda^{-1.13}$ (cm$^{-1}$)

In which ink concentration $C^{ink}$ are $10^{-4}$, $4.3 \times 10^{-5}$ and $6.7 \times 10^{-6}$ for Causations, Asian and African, respectively.

Intraplipid absorption:

Equation 109: $\mu_a = 2.81 \times 10^{\lambda/205}$ (cm$^{-1}$)

Water absorption:

Equation 110: $\mu_a^{water} = \varepsilon^{water} \cdot C^{water}$
Scattering of phantom is the same as the intralipid scattering:

\[ \mu_s^{\text{phantom}} = \frac{C_{\text{lipid}}}{10\%} \times \mu_s^{10\% \text{lipid}} \times g^{10\% \text{lipid}} \]

\[ \mu_s^{10\% \text{lipid}} = 2.54 \times 10^9 \lambda^{-2.4}, \quad g^{10\% \text{lipid}} = 1.1 - 0.58 \times 10^{-3} \lambda \]

in which \( C_{\text{lipid}} = 1.9\% \) for normal skin tissue scattering.

Then the spectral reflectance of the phantom is simulated using Equation 42 to Equation 45 in Chapter 3.1.
Figure 48. Spectral reflectance $R(\lambda)$ of skin (a,c,e) and tissue phantom (b,d,f) simulated using semi-infinite model as described in Chapter 3.

(a,b): $R(\lambda)$ of 6 $\text{StO}_2$ levels at 3 melanin concentrations (fixed blood concentration and scattering to be the values listed in Table 1);

(c,d): $R(\lambda)$ of 5 blood concentration levels at 3 melanin concentrations (fixed scattering to be the values listed in Table 1 and fix $\text{StO}_2 = 60\%$);

(e,f): $R(\lambda)$ of 5 scattering levels at 3 melanin concentrations (fixed blood concentration to be the values listed in Table 1 and $\text{StO}_2 = 60\%$);
Figure 48(a), (c) and (e) show the skin reflectance spectra of different levels of StO2, blood concentration and scattering, respectively. For each of these plots, the reflectance spectra of all three levels of melanin concentrations are shown. Two main characteristics can be seen in these plots. On one hand, melanin concentration - the factor determining the skin darkness - has largest effect on the skin reflectance. On the other hand, the shape of the reflectance spectra is mainly determined by StO2, regardless of other three factors. Thus, it indicates that spectroscopy algorithm should be used to measure StO2 to get rid of the bias from other three skin factors.

Figure 48(b), (d) and (f) show the reflectance of tissue phantom simulating the corresponding skin tissue in (a), (c) and (e), respectively. It can be seen that the reflectance spectra of the phantom matched the corresponding skin spectra very well. The difference between these two spectra can be explained as follows. In the phantom, ink and intralipid are used to simulate the melanin absorption and skin scattering, respectively. The absorption spectra $\mu_a(\lambda)$ of melanin and ink are very similar and can expressed as power functions with negative powers. But the power of ink absorption spectrum (~-1.13) is larger than that of melanin(~-3.48) [10]. Similarly, the scattering spectra $\mu_s'(\lambda)$ of skin tissue and intralipid can both expressed as power functions. But the power for intralipid scattering spectrum (~ -2.3)[54, 55] is larger than that of skin scattering (~-4)[6]. Thus, using ink and intralipid in the phantom can closely simulate the spectroscopic behavior of in vivo skin but not exactly the same.

Then, SDR(568,576,24) vs StO2 relationship was found using the calculation method in Chapter 3.2.
Table 17 lists the data of SDR(568,576,24) vs StO₂ from skin and phantom model. The relationship of SDR(568,576,24) ~ StO₂ is plotted in Figure 49. Both shows the SDR values are almost the same from skin and phantom model, indicating (1) the tissue phantom can closely simulate tissue optical properties; and (2) the wavelengths used in this SDR (which are \(\lambda_1=568\text{nm}, \lambda_2=576\text{nm}, \delta\lambda=24\text{nm}\)) are a good selection suitable for StO₂ imaging. Thus, same data (SDR~ StO₂) may be used to calculate oxygenation for both skin and phantom if we measure SDR(568,576,24).

Table 17. Comparison of SDR(568,576,24) from skin and phantom model

<table>
<thead>
<tr>
<th>StO₂ (%)</th>
<th>SDR from skin model</th>
<th>SDR from phantom model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>std%</td>
</tr>
<tr>
<td>0</td>
<td>1.51</td>
<td>0.75</td>
</tr>
<tr>
<td>10</td>
<td>1.10</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>0.87</td>
<td>0.41</td>
</tr>
<tr>
<td>30</td>
<td>0.72</td>
<td>0.65</td>
</tr>
<tr>
<td>40</td>
<td>0.62</td>
<td>0.79</td>
</tr>
<tr>
<td>50</td>
<td>0.54</td>
<td>0.87</td>
</tr>
<tr>
<td>60</td>
<td>0.48</td>
<td>0.90</td>
</tr>
<tr>
<td>70</td>
<td>0.43</td>
<td>0.88</td>
</tr>
<tr>
<td>80</td>
<td>0.40</td>
<td>0.82</td>
</tr>
<tr>
<td>90</td>
<td>0.36</td>
<td>0.73</td>
</tr>
<tr>
<td>100</td>
<td>0.34</td>
<td>0.61</td>
</tr>
</tbody>
</table>
5.3. Experiment protocol

The StO₂ algorithm was verified using a liquid blood phantom with a total volume of 200 mL. The phantom was prepared by mixing fresh bovine blood, India ink (Sanford, Bellwood, IL), and 10% intralipid (Henry Schein Inc., Melville, NY) in phosphate buffered saline (Fisher Scientific, Newton, NJ). Different recipes were used to simulate different skin conditions. First, a normal skin condition was simulated using 2.5mL blood, 4mL intralipid and 30µL ink in totally 200mL PBS solution. Then skin conditions of different blood concentration, scattering and darkness were simulated using 20% more
blood, 20% more intralipid and 100% more Indian ink, respectively. The pH level of the phantom was adjusted to 7.4 by adding NaOH and HCl.

StO$_2$ of phantom was measured using the multispectral imaging method described in Chapter 4. To eliminate the measurement artifact caused by oxygen in ambient air, we placed the blood phantom in a cardboard container and ventilated it with argon gas. During the test, 8 oxygenation plateaus ranging from nearly 100% to nearly 0% were achieved by dropwise addition of 0.1 g/mL sodium hydrosulfite. At each oxygenation plateau, the blood phantom was mixed to homogeneity by a stir bar. Images at 544, 552, 568, 576, 592, 600nm were taken to measure StO$_2$ using our algorithm as described previously.

5.4. Experiment result

The StO$_2$ algorithm was verified using a tissue-simulating phantom which was prepared following the experimental protocol as described previously. At each oxygenation level of each recipe, the reflectance of the phantom was averaged over 3 regions of interest (ROIs). The oxygenation levels StO$_2$ were first reconstructed from multispectral images of 544, 552, 568, 576, 592, 600nm using our algorithm. Then StO$_2$ were reconstructed using whole reflectance spectra from hyperspectral images using the algorithm reported in [31]. In the hyperspectral algorithm, the apparent absorption spectrum from 506 to 592 nm was treated as a linear combination of oxy- and deoxy-hemoglobin plus a straight line representing the effect of scattering and melanin which is
treated as a lumped parameter that linearly alters the spectrum. This method is one of the methods for quantitative measurement of skin StO₂ by considering the effect of scattering and melanin. The detail of this algorithm is described in Appendix B.3. The oxygenation levels measured by our algorithm from six multispectral images were compared with those measured by hyperspectral imaging and algorithm. Three regions of interest were selected randomly from the phantom images and the StO₂ were calculated using both algorithms and averaged from three ROIs.

Figure 50. Phantom StO₂ measured by multispectral algorithm versus that measured by hyperspectral imaging using algorithm in Appendix B.3. [31]
Figure 50 shows the StO₂ of phantom measured by multispectral algorithm versus that measured by hyperspectral imaging. According to the figure, the multispectral measurements are all linearly correlated with the hyperspectral measurements. Moreover, measurements of all four recipes simulating different skin conditions fall on the diagonal line, indicating the StO₂ measurement by our algorithm is not affected by different skin conditions including blood concentration, scattering and melanin level. However, the average standard deviation of StO₂ measurement from six multispectral images is 2.96%, which is similar to that of human subject test which will be discussed in Chapter 6.2.2. This is slightly higher than the average standard deviation of StO₂ measurement from hyperspectral imaging (1.52%), as indicated by the error bars. This may be because the hyperspectral imaging uses all the spectral images (43 images in our algorithm) to calculate StO₂ so that it’s less susceptible to the noise of a few images. In comparison, the multispectral imaging only uses six wavelengths so that it can be more sensitive to the noise of any one of these six images. However, because only using 6 wavelengths, the multispectral imaging method has the capability of imaging fast change of StO₂, which is not possible for hyperspectral imaging. One may consider this is the trade-off between temporal resolution and measurement variation.
Chapter 6. Human subject test

6.1. Imaging skin StO2 dynamics during using postocclusive reactive hyperemia (PORH)

6.1.1. Experiment protocol

The multispectral imaging method of measuring StO2 was then verified by a human subject test under a clinical protocol approved by the OSU IRB (protocol No: 2010H0017) as attached in Appendix A. The test measured the cutaneous tissue oxygenation responses to vascular occlusion on healthy volunteers following a protocol of post-occlusive reactive hyperemia (PORH)[57]. Before the PORH test, the subject's systolic and diastolic blood pressures were measured by a pressure cuff placed on the left upper arm. The PORH test consisted of a pre-occlusive baseline period (no pressure applied to the arm) of two minutes, a suprasystolic occlusion (systolic + 50 mm Hg) period of two minutes, and a reactive hyperemia period (pressure released) of two minutes. During the PORH test, multispectral images were taken to measure skin StO2. At the meantime, deep tissue oxygen saturation and cutaneous tissue oxygen tension on the same arm were measured by an OxiplexTS tissue oximeter (ISS Inc., Urbana Champaign, IL) and a TCM transcutaneous oxygen monitor (Radiometer, Denmark), respectively. The StO2 measurement by multispectral imaging was compared with the result of two other devices. The rationale of selecting the OxiplexTS tissue oximeter and
TCM transcutaneous oxygen monitor for measurement comparison is discussed in Appendix B.

6.1.2. Oxygen map of the arm

Monochromatic image of the arm (a) and 99% reflected diffuser (b); skin oxygenation map before vascular occlusion (c), during occlusion (d), at the end of occlusion (e), during hyperemia (f, g), at the end of hyperemia (h), after hyperemia (i, j, k) and at the end of the test (l).

Figure continue on next page
Figure 51 continued
Oxygenation of in vivo skin was measured by the multispectral imaging method in a human subject experiment following a protocol of post-occlusive reactive hyperemia (PORH). During the PORH test, vascular occlusion was applied to the arm of a human subject and multispectral images of 544, 552, 568, 576, 592, 600nm were continuously taken before, during and after vascular occlusion. After the test, oxygen saturation maps were reconstructed from the multispectral images. Figure 51 shows a monochromatic image of the arm (a) and 99% reflected diffuser (b) and skin oxygenation map before, during and after vascular occlusion (c-l). The color map for StO₂ map is also shown below. Significant changes in cutaneous tissue oxygenation were observed before, during, and after vascular occlusion. Since the 99% reflected diffuser didn’t cover the whole field of view (as shown in b), we can only show the oxygen map within the area.
covered by the diffuser. The oxygen map for the baseline period (c) appears as cyan and yellow which corresponds to 40-50% of StO2 level, which is the skin oxygen saturation under normal condition[58]. Skin oxygen began to drop during occlusion (d) reach the lowest point at the end of occlusion (e). The oxygen map at this time appears blue, indicating the skin StO2 dropped to below 10% due to vascular occlusion (systolic pressure + 50mmHg). After the pressure was released, the StO2 quickly increased due to hyperemia (f, g) and reach the maximum which is above 60%, as indicated by the red color (h). After this point, StO2 gradually decreased (i, j, k) and at the end of the test recovered to normal level (l). This change of oxygen saturation during PORH is consistent with the result reported by [59].

6.1.3 Oxygenation change during PORH

StO2 measurement by multispectral imaging was also compared with deep tissue oxygenation and cutaneous tissue oxygen tension. Figure 52 shows the above three tissue oxygen parameters continuously monitored during a PORH test. Figure 52(a) shows the test protocol including a period of pre-occlusion baseline, a suprasystolic occlusion period, a reactive hyperemia period. The history of cutaneous tissue oxygenation is plotted in (b), which was obtained by averaging 10 selected regions of interest (ROIs) in the oxygenation map shown in Figure 52. The deep tissue oxygenation by a tissue oximeter (c) and the transcutaneous tissue oxygen tension (d) were measured simultaneously during the PORH procedure. It can be seen that, during the PORH
procedure, the change of cutaneous tissue oxygenation coincides well with that of deep tissue oxygenation and that of cutaneous oxygen tension.

Figure 52. Oxygenation change during PORH
PORH test protocol (a), and the history of cutaneous tissue oxygenation (b), deep tissue oxygenation(c) and transcutaneous tissue oxygen tension (d) measured by multispectral imaging, tissue oximeter and TCM device, respectively.
6.2. Oxygenation of in vivo skin of different skin types

6.2.1. Experiment protocol

In order to test the influence of skin types on skin oxygenation measurement, totally 10 subjects of different skin types were recruited for measurement of skin tissue oxygenation using PORH protocol. The test procedure of each subject was the same as described in Chapter 6.1.1. Influence of skin types on skin oxygenation measurement by multispectral imaging system was evaluated statistically and compared with the result of OxiplexTS tissue oximeter and TCM transcutaneous oxygen monitor.

6.2.2. Evaluation of influence of skin types on oxygen measurement using biostatistics

After the experiment, 10 subjects were arranged in the order of skin darkness based on the calculation of skin reflectance at 544nm. Number 1 (highest reflectance) represents the lightest skin and number 10 (lowest reflectance) represents the darkest skin. Then, in order for further statistical analysis, 10 subjects were grouped into three skin types based on the level of skin reflectance, as shown in Table 19. Skin types 1 (reflectance >0.538) are Caucasians and light tanned Hispanos who has lowest melanin level. Skin type 2 (reflectance = 0.45-0.53) are Asians with medium melanin levels. Skin type 3 (reflectance < 0.45) are dark pigmented Asians with highest melanin.
Table 18. 10 subjects arranged in the order of skin darkness and grouped into three skin types

<table>
<thead>
<tr>
<th>subject number</th>
<th>reflectance at 544nm</th>
<th>Skin type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.609</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.579</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0.551</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0.546</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0.538</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0.510</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0.506</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>0.464</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>0.406</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>0.356</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 53 shows the averaged skin StO$_2$ of 10 subjects under normal condition (no pressure was applied) measured by multispectral imaging with our new algorithm. Detail data corresponding to Figure 53 is listed in Table 19. For each subject, 10 oxygen saturation maps from 10 measurements were generated. Then, 10 regions of interest (ROIs) were randomly selected from the area of skin without hair from each oxygenation map. Average StO$_2$ was calculated from totally 100 ROIs of 10 oxygen saturation maps. The lowest and highest StO$_2$ measured are 37.55% (subject 6) and 46.79% (subject 10). In order to statistically evaluate the influence of skin darkness on StO$_2$ measurement, an ANOVA test was performed. The p-value is 0.29 indicating skin type does not significantly affect StO$_2$ measurement.
Figure 53. Averaged skin StO$_2$ of 10 subjects under normal condition measured by multispectral imaging with our new algorithm. The subjects were arranged in the order of skin melanin levels with 1 being the lowest to 10 being the highest.

Table 19. Data of averaged skin StO$_2$ of 10 subjects from Figure 53

<table>
<thead>
<tr>
<th>subject number</th>
<th>StO$_2$(%)</th>
<th>std</th>
<th>Skin type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41.97</td>
<td>1.40</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>40.61</td>
<td>0.79</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>39.86</td>
<td>2.92</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>40.13</td>
<td>1.14</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>40.82</td>
<td>0.81</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>37.55</td>
<td>1.48</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>41.99</td>
<td>1.72</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>44.42</td>
<td>1.60</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>41.49</td>
<td>1.18</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>46.79</td>
<td>1.18</td>
<td>3</td>
</tr>
</tbody>
</table>
As a comparison, skin StO$_2$ of 10 subjects were also calculated from reflectance images of 544nm and 576nm using an algorithm commonly used in many tissue oximeters (Equation 27), as shown in Figure 54. Detail data corresponding to Figure 54 is listed in Table 20. The lowest StO$_2$ measured is 71.37% from subject 10 who has darkest skin color and the highest StO$_2$ measured is 86.17% from subject 1 who has lightest skin color. The same ANOVA test was performed to statistically evaluate the influence of skin darkness on StO$_2$ measurement. 10 subjects were grouped into three groups as described previously. The p-value is 0.05 indicating StO$_2$ measurement using 2-wavelength algorithm is very likely affected by skin types. Moreover, the range of StO$_2$ calculated using this method (71-86%) is much higher than the result using multispectral algorithm (37-46%). The skin StO$_2$ calculated using multispectral algorithm is more consistent with reported values in previous studies [58, 59]. The 2-wavelength method is a very simplified method which does not consider the influence of melanin absorption, background absorption and scattering. Thus, skin StO$_2$ calculated using this method may be significantly biased by these factors, causing the whole range of StO$_2$ being much higher than actual values.
Figure 54. Skin StO2(%) of 10 subjects under normal condition calculated from reflectance image at 552nm and 576nm using Equation 27.

<table>
<thead>
<tr>
<th>subject number</th>
<th>StO2(%)</th>
<th>std</th>
<th>Skin type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.18</td>
<td>1.17</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>83.21</td>
<td>0.57</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>78.58</td>
<td>0.53</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>79.98</td>
<td>1.55</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>74.84</td>
<td>0.94</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>72.52</td>
<td>0.63</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>75.38</td>
<td>0.61</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>76.34</td>
<td>0.95</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>74.08</td>
<td>2.75</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>71.37</td>
<td>0.45</td>
<td>3</td>
</tr>
</tbody>
</table>

The average skin StO2(%), deep tissue StO2(%) and transcutaneous oxygen tension pO2(mmHg) of different subjects during PORH are shown in Figure 55, Figure 56 and Figure 57, respectively. The error bars indicate the variation among different subjects.
Subjects. During the baseline (first 2 minutes), the variation of measurement results are only caused by skin types. The standard deviation of skin \( \text{StO2} \) during baseline measured by multispectral imaging is 2.76 while the standard deviation of deep tissue \( \text{StO2} \) measured by ISS tissue oximeter is 7.46. This indicates that the \( \text{StO2} \) measurement by our multispectral imaging is much less affected by different skin types. At the end of occlusion (at 4 minute), the \( \text{StO2} \) dropped to the lowest value which was varied from subject to subject since it’s related to the subjects’ blood perfusion and vascular function. Thus, the variation of \( \text{StO2} \) at this point is not only caused by skin types. But the standard deviation of \( \text{StO2} \) measured by multispectral imaging (6.5) is also much smaller than that measured by tissue oximeter (13), which indicates it’s very likely that \( \text{StO2} \) measured by multispectral imaging is less affected by skin types.

![Figure 55. Average skin \( \text{StO2} \) of different subjects during PORH measured by multispectral imaging](image-url)
Figure 56. Average deep tissue $\text{StO}_2$ of 10 subjects during PORH measured by tissue oximeter.

Figure 57. Average transcutaneous oxygen partial pressure $\text{pO}_2$ of 10 subjects during PORH measured by TCM oxygen tension monitor.
The comparison of oxygen measurements by three devices are summarized in Table 21. The absolute variation of skin StO$_2$(%) measured by multispectral imaging is three times smaller than that of deep tissue StO$_2$(%) measured by ISS tissue oximeter. The relative variation of skin StO$_2$(%) measured by multispectral imaging is two times smaller than that of deep tissue StO$_2$(%) measured by ISS tissue oximeter and three times smaller than that of transcutaneous oxygen tension pO$_2$(mmHg) measured by TCM oxygen tension monitor. This result indicates that our multispectral imaging system can provide skin StO$_2$ measurement with much less bias caused by different skin types.

Finally, as discussed in Chapter 5.4, the average variation of StO$_2$(%) of phantom measured by our multispectral imaging method is 2.96%, which is similar to the average variation of StO$_2$(%) of skin listed in Table 21 (2.76%).

<table>
<thead>
<tr>
<th>Device</th>
<th>Modality/principle</th>
<th>Parameter measured</th>
<th>Average value</th>
<th>Variation from different subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multispectral imaging</td>
<td>Optical</td>
<td>Skin StO$_2$ (%)</td>
<td>42.5</td>
<td>2.76</td>
</tr>
<tr>
<td>ISS oximeter</td>
<td>Optical</td>
<td>Deep tissue StO$_2$ (%)</td>
<td>55</td>
<td>7.46</td>
</tr>
<tr>
<td>TCM</td>
<td>Electrochemical</td>
<td>Transcutaneous pO$_2$ (mmHg)</td>
<td>62</td>
<td>12.21</td>
</tr>
</tbody>
</table>

Table 21. Comparison of oxygen measurement variations from different subjects.
6.3. Measuring StO₂ of in vivo skin with additional background absorption

6.3.1 Experiment protocol

To test whether our algorithm can reduce measurement bias caused by background absorption, StO₂ measurement was taken on the human subject with his skin painted with ink. A portion of the subject’s forearm within the field on view was painted with 1% India ink dissolved in ethanol to simulate darker skin. Multispectral images of the arm of both within and outside the ink-painted area were acquired simultaneously, and an oxygenation map was reconstructed. The same experiment was repeated at the same skin location on the same subject using a Hypermed OxyVu hyperspectral imaging system (Hypermed, Inc) which also measured skin StO₂. The reconstructed oxygenation maps by two systems were compared.
6.3.2 Comparison of oxygenation map

Figure 58. Oxygenation of skin partially painted with ink
(a, c) picture of the arm with a portion of skin painted with ink; (b) oxygenation map generated by our multispectral imaging system; (d) oxygenation map generated by a commercialized hyperspectral imaging system

Oxygenation of skin with additional absorption background was measured by the multispectral imaging algorithm to test the reliability of the imaging algorithm. A portion of the subject’s forearm was painted with ink to add additional absorption background. StO2 was measured both within and outside the ink-painted area and oxygenation map was reconstructed. Figure 58(a) shows the picture of the skin tissue with a portion painted with ink and (b) shows the corresponding oxygen saturation map. Two square-shaped regions of interest (ROIs) are selected within and outside the ink-painted area. The averaged cutaneous tissue oxygenation is 41.6% for the ROI within the ink-painted area and is 38.1% for the ROI outside the ink-painted area. A difference of 3.5 % is observed. In comparison, the same test on the same subject was performed using an OxyVu
hyperspectral imaging system. Figure 58(c) shows the picture of the skin tissue with a portion painted with ink and (d) shows the corresponding oxygen saturation map generated by the OxyVu hyperspectral imaging system. The averaged cutaneous tissue oxygenation is 62.7% outside the ink-painted area and 43.0% inside the ink-painted skin area. A difference of 19.7 % is observed. This result presumably indicates that the additional absorption background has significant effect on the cutaneous oxygen saturation measurement using the OxyVu system but may not on our multispectral imaging system.

6.3.3. Statistical analyses

Figure 59. The influence of additional absorption background on the reliability of cutaneous tissue oxygenation measurements

Student's t-test was carried out to determine whether the additional absorption background had a significant effect on oxygenation measurements using our imaging
systems or the OxyVu system. Our null hypothesis is that the cutaneous tissue oxygenation measurement will not be affected by the change of skin color. This null hypothesis was tested on oxygenation maps generated by both imaging systems. For each system, the averaged cutaneous tissue oxygenations were calculated within and outside the ink-painted skin areas by randomly selecting 15 regions of interest (ROIs) in each area. P-value for each system was then calculated using a statistic analysis software MiniTab (MiniTab, Inc., State College, PA).

The test results, as plotted in Figure 59, show that the p value for the OxyVu measurements is less than 10^{-4}, indicating that it’s reasonable to reject the null hypothesis. Therefore, the additional absorption background does affect the cutaneous tissue oxygenation measurements using OxyVu imaging system. In comparison, the p value for our multispectral measurements is 0.128>0.05, indicating the additional absorption background is not very likely to affect cutaneous tissue oxygenation measurements.
Chapter 7: Discussion and conclusion

7.1. Major innovations of this research

In this dissertation, a multispectral imaging method for skin oxygen saturation measurement is reported. To develop the algorithm, spectral reflectance of skin with different conditions were simulated using a modified semi-infinite tissue model. From the simulation result, it was found that a second-derivative-ratio (SDR) calculated from reflectance at six specific wavelengths (544, 552, 568, 576, 592, 600nm) is dominantly determined by the oxygenation regardless of different blood concentration, scattering, and melanin concentration. Thus, skin oxygen saturation can be measured from skin reflectance at six wavelengths. This algorithm was verified by experiments using tissue phantom and in vivo human skin, demonstrating that skin StO$_2$ can be measured by our multispectral imaging method with less bias from different skin conditions.

There’re four major innovations of this study. First, multispectral imaging of skin oxygenation using these six specific wavelengths is firstly reported in this paper. Although wide-gap second derivative spectroscopy has been used to measure tissue oxygenation[21], it’s the first time that this method is implemented in imaging. Second, compared to other StO$_2$ measurement techniques, our method has the major advantage of being able to image StO$_2$ dynamics near-real-time because only six wavelengths are
required for one measurement. Currently our system has a recording speed of two seconds per measurement and a post processing speed of 15~45 seconds per measurement depending on the oxygen map resolution. Both speeds can be improved significantly by optimizing and customizing the engineering design of the imaging system. Third, our method is potentially able to quantitatively measure skin StO₂ with less measurement bias due to different skin types. Simulation result has shown that oxygenation measured by our method was not affected by blood concentration, tissue scattering and melanin concentration, which was validated by tissue phantom experiment. Then, a human subject test involving 10 human subjects with different skin darkness demonstrated that skin StO₂ measurement by our method was not affected by skin color. Another human subject test also showed that skin StO₂ measurement by our method was not affected by the additional background absorption. Fourth, the method in this paper may not be limited to the six wavelengths reported in this dissertation. The algorithm described in Chapter 3 is a general approach to find a parameter which is only related to the oxygenation regardless of different skin tissue conditions. Thus other wavelengths may be used in the future to test the feasibility of measuring oxygenation.

7.2. Future work

There’re many unclear issues which will be investigated in the future work of this study. First, a systematic human subject test with larger sample size should be conducted to characterize the reliability and repeatability of our imaging method. Skin StO₂ will be
measured on subjects (different sex, age, race) with different skin types on different skin sites. From Figure 14, one can see that the reflectance spectra of all StO\textsubscript{2} levels almost overlapped in the highest melanin level. This indicates that it may be more difficult to use this algorithm to effectively reduce the measurement bias when the melanin is extremely high. Thus, a human test involving different skin types should be conducted to find out the level of skin darkness which does not affect our measurement. Second, the hardware design and the data processing algorithm will be optimized for better temporal resolution. Third, the imaging platform will be applied to a clinical case, such as wound debridement, to demonstrate the clinical utility.

7.3. Conclusion

In this dissertation, a multispectral imaging method capable of imaging dynamics of skin tissue oxygen saturation with minimal bias from different skin conditions is reported. A numerical model was first established to simulate skin tissue reflectance of different skin conditions including oxygen saturation, blood concentration, tissue scattering and melanin concentration. From the simulation result, a parameter SDR, which can be calculated from skin reflectance images of 544, 552, 568, 576, 592, 600nm, was found to be mainly determined by oxygenation regardless of different tissue conditions. Thus, skin oxygen saturation can be measured by taking multispectral images of six wavelengths. This method was first verified using a phantom which was a liquid mixture of different concentrations of blood, intralipid and ink. StO\textsubscript{2} measured by our
method was not affected by the different concentration of phantom materials. Then, multispectral imaging of skin oxygenation was demonstrated by a human subject test using a postocclusive reactive hyperemia (PORH) protocol. Totally 10 subjects with different skin types were recruited. The StO₂ dynamics imaged by our system was compatible with the oxygen measurements by other oximetry techniques. The relative variation of skin StO₂(%) measured by multispectral imaging is two times smaller than that of deep tissue StO₂(%) measured by ISS tissue oximeter and three times smaller than that of transcutaneous oxygen tension pO₂(mmHg) measured by TCM oxygen tension monitor. This result indicates that skin StO₂ measurement by our multispectral imaging system has minimal bias caused by different skin types. Finally, in order to test if our StO₂ imaging method is affected by additional background absorption, measurement was taken on the skin on which a layer of ink was painted. The averaged skin StO₂ measured by our system had 3% difference within and outside the skin painted area, while the StO₂ measured by a commercial imaging system showed 20% difference. This implies that the additional background absorption does not significantly affect the skin tissue oxygenation measurements by our system. The above result indicates that our multispectral imaging technique can be potentially used for quantitative imaging of dynamics of skin oxygen saturation with minimal bias from different skin conditions.


17. [http://omlc.ogi.edu/spectra](http://omlc.ogi.edu/spectra).


Appendix A: IRB approved clinical protocol

This is the clinical protocol approved by the OSU IRB (protocol No: 2010H0017). The whole protocol document is very long. Only the major experiment steps are shown here, which is item 17 Research Method and Activities in the IRB document. One should note that the ‘CWC hyperspectral imaging system’ is the multispectral/hyperspectral imaging system developed in this dissertation. Also, eventually we only recruit 10 subjects instead of 50. This is because we met other technical issues (mainly algorithm) and spent time solving those problems. Also, the part of study on subjects with chronic wounds is not reported in this dissertation since it belongs to the clinical research of other investigators.
This is an open-label, un-blinded, pilot study involving 50 healthy adult subjects and up to 100 subjects with chronic wounds. The test procedure is described below:

For the group of healthy adult volunteers: (using occlusion stimulation as the example)

1. Set up the Hypermed system, the CWC hyperspectral imaging system, the TcOM device, the Oxiplex tissue oximeter, and the Doppler blood flow meter. Calibrate each device.

2. Let the subject rest the left or right arm on the test station, with the forearm or hand exposed to the imaging system.

3. Place the sensor patches for the TcOM device near the area of interest on the forearm or hand and start the device. It takes 15 minutes to reach its stable readings.

4. Place the sensor patches for the Oxiplex tissue oximeter, and the Doppler blood flow meter around the area of interest on the forearm or hand. Adjust the fields of view for the hyperspectral imaging system and the Hypermed imager.

5. Place a pressure cuff of a blood pressure meter around the upper arm of the subject.

6. Start image acquisition for the hyperpspectral imaging system and the hypermed system.

7. Take the baseline measurements of tissue oxygenation, hemoglobin concentration and blood flow without occlusion.
8. Inflate the pressure cuff to occlude the brachial artery (at the systolic pressure) or brachial vein (at a pressure between systolic and diastolic) and hold the pressure for 60 seconds to 300 seconds.

9. Release the pressure and keep the subject’s arm still for 5 to 7 minutes.

10. Stop image acquisition, and save the raw data.

11. Stop TcOM device, Oxiplex tissue oximeter, and the Doppler blood flow meter, and save all the data.

12. Exam subject’s arm for any possible discomfort and report any adverse events.

For a group of patients with chronic wounds: (using occlusion stimulation as the example)

If the wound location is on hands or forearms, the test procedure is the same as the above procedure for healthy subjects. If the wound location is different from hands or forearms, then following procedures will be used:

1. Set up the Hypermed system, the CWC Hyperspectral imaging system, the TcOM device, the Oxiplex tissue oximeter, and the Doppler blood flow meter. Calibrate each device.

2. Let the subject rest the part of the body with wound on the test station, with the wound facing up to the imaging systems.

3. Place the sensor patches for the TcOM device near the area of interest of the wound and start the device. It takes 15 minutes to reach its stable readings.
4. Place the sensor patches for the Oxiplex tissue oximeter, and the Doppler blood flow meter around the wound. Adjust the fields of view for the hyperspectral imaging system and the Hypermed imager.

5. Take the measurements of tissue oxygenation, hemoglobin concentration and blood flow.

6. Stop TcOM device, Oxiplex tissue oximeter, and the Doppler blood flow meter, and save all the data.

7. Start the hyperpspectral imaging system, take the images of oxygen, hemoglobin and perfusion, stop the system, and save the data.

8. Start the hypermed system, take the images of oxygen, hemoglobin and perfusion, stop the system, and save the data.

9. Examine subject’s arm for any possible discomforts and report any adverse events.
Appendix B: Selecting an oximetry technique for oxygen measurement comparison

Many oximetry techniques have been introduced in Chapter 2. Among these techniques, two were selected for qualitative comparison with our measurement by multispectral imaging: near-infrared frequency modulated tissue oximeter and TCM transcutaneous oxygen tension monitor. Also, one was selected for quantitative comparison which is hyperspectral imaging, while the other was found completely not suitable for comparison: fluorescence spectrometer based oxygen sensor.

Near-infrared frequency modulated tissue oximeter has been introduced in Chapter 2. It’s selected for qualitative comparison with our multispectral imaging because it also measures tissue StO₂. It’s not for quantitative comparison because it measures deep tissue StO₂ while our system measure superficial skin StO₂.

The rationale of choosing other three devices is introduced in the followings.

B.1. Principle of TCM

TCM measures tissue oxygen partial pressure using microelectrodes base on electrochemical principle. This oxygen meter usually consists a membrane-type electrode and a galvanic cell[60]. A Galvanic cell is an electrochemical cell that derives electrical current from spontaneous redox reaction taking place within the cell. Figure 60 shows an
illustration of the configuration of oxygen microelectrodes. The Galvanic cell has two electrodes: a cathode (a noble metal such as silver Ag) and an anode (base metal such as lead Pb). The cathode is placed close to the tissue while the anode is placed far away from the tissue. A highly oxygen-permeable membrane (such as Teflon) is placed between the cathode and the tissue. An electrolyte solution, which is usually an alkaline solution such as Potassium hydroxide (KOH), is filled between two electrodes. The voltage between the two electrodes will be measured.

Figure 60. Illustration of the configuration of oxygen microelectrodes

When oxygen passes through the membrane, it will be reduced with the cathode. A current between two electrodes will be generated and measured. Since this current is proportional to the concentration of the dissolved oxygen, oxygen tension pO$_2$ can be measured.
The technique of this type of oxygen meter has been very mature and there’re a lot of products already in the market. One well-known product is the TCM transcutaneous oxygen tension meter by Radiometer[61]. This technique has been used in many biomedical and clinical applications including infant heal monitoring[62] and wound care [63].

B.2. Fluorescence/ phosphorescence based oxygen sensor

Fluorescence and phosphorescence based oxygen sensor is based on the principle of photophysical intermolecular deactivation process. In such a process, the fluorescence / phosphorescence of a fluorophore (such as ruthenium fluorophor) is quenched by the oxygen molecular due to the energy transfer from the excited state of the fluorophore to certain molecular state of oxygen. Since the degree of quenching is in proportion to the oxygen concentration, the oxygen concentration can be quantified by measuring the fluorescence / phosphorescence intensity.

This principle is arithmetically described by the Stern–Volmer relationship.

\[
\frac{I_0}{I} = 1 + k \cdot \tau_0 \cdot [O]
\]

Where \( I_0 \) is the fluorescence / phosphorescence intensity when no oxygen is presented, \( I \) is the fluorescence / phosphorescence intensity with oxygen, \( k \) is the quencher rate coefficient, \( \tau_0 \) is the fluorescence lifetime of the fluorophor molecule without oxygen and \([O]\) is the oxygen concentration.
Let $K = k \cdot \tau_0$. Then, $K$ and $I_0$ are two constants which can be obtained by calibration of the oxygen sensor at two points: $[O]=0\%$ (by adding excess sodium hydrosulfite) and $21\%$ (balanced with air). Then oxygen concentration at other levels can be measured by measuring the fluorescence or phosphorescence intensity $I$.

There’re some fluorescence based oxygen sensors in the market. An oxygen sensor by Ocean Optics Inc.[64] measures oxygen in the air or liquid environment. OxyLite [65] is a fluorescence based tissue oxygen meter developed by Oxford Optronix to measure skin tissue oxygen tension. Phosphorescence based oxygen sensing has also been used in many clinical applications [66-69] such as the measurement of dynamic changes of oxygen tension in the brain and the cerebral metabolic rate of $O_2$ during functional activation.

Fluorescence based oxygen sensor is not suitable to measure oxygen concentration in solution containing blood of hemoglobin. The issue for fluorescence based oxygen sensor will be briefly explained here since this is a problem we encountered during our study. Figure 61 shows the oxygenated and deoxygenated hemoglobin absorption spectra (top) and excitation and fluorescence spectra of oxygen sensor (bottom). At the excitation (470nm) and fluorescence emission (600nm) wavelengths, the absorptions of oxygenated hemoglobin and deoxygenated hemoglobin are different. This means when the StO$_2$ changes, the absorption of hemoglobin will change. Since the sensor is emerged in the liquid phantom, the fluorescence detected by the sensor is not only affected by the quenching effect of oxygen to the fluorescent
material but also affected by the absorption of the phantom media. Thus, oxygen saturation of our blood phantom cannot be measured accurately using this type of fluorescence oxygen sensor.

![Graph of hemoglobin absorption spectra and fluorescence spectra of oxygen sensor](image)

Figure 61. Comparison of hemoglobin absorption spectra (top) with fluorescence spectra of oxygen sensor (bottom)

B.3. A hyperspectral imaging method suitable for quantitative comparison with our multispectral imaging method

The selected technique for side by side comparison with our multispectral imaging method is a hyperspectral imaging method with whole spectra fitting algorithm. This hyperspectral imaging algorithm [31] was selected because it measure skin StO₂
quantitatively after considering different tissue conditions including melanin concentration and scattering.

The principle of this algorithm is introduced here.

Define I and TR to be the image intensity of skin and a 99% reflected calibration diffuser.

Reflectance R and apparent absorption A will be

\[
R = \frac{I}{TR}
\]

Equation 31: \[A = -\log_{10}(R)\]

Then an important assumption is made here. The contribution from melanin absorption and scattering are considered as a linear team within the apparent absorption.

Equation 113: \[A = a + b\cdot\lambda + C_1\cdot\varepsilon_1(\lambda) + C_2\cdot\varepsilon_2(\lambda)\]

In which a and b are two constants, \(C_1\) and \(C_2\) are the concentration of oxygenated and deoxygenated hemoglobin, and \(\varepsilon_1(\lambda)\) and \(\varepsilon_2(\lambda)\) are the molar extinction coefficient of oxygenated and deoxygenated hemoglobin. The term \((a + b\cdot\lambda)\) is the contribution from melanin and scattering.

There’re four isosbestic points \((\lambda = 530, 545, 570, 584\text{nm})\) at which the molar extinction coefficient of oxygenated and deoxygenated hemoglobin are the same. At these isosbestic points, Equation 113 can be simplified as

Equation 114: \[A = a + b\cdot\lambda + C_1\cdot\varepsilon(\lambda) + C_2\cdot\varepsilon(\lambda) = a + b\cdot\lambda + C\cdot\varepsilon(\lambda)\]

In which \(C = C_1 + C_2\) is the concentration of the total hemoglobin.

Then we will have a group of linear equations:

Equation 115
\begin{align*}
A(530) & = a + 530b + C \cdot \varepsilon(530) \\
A(545) & = a + 545b + C \cdot \varepsilon(545) \\
A(570) & = a + 570b + C \cdot \varepsilon(570) \\
A(584) & = a + 584b + C \cdot \varepsilon(584)
\end{align*}

Absorption A can be measured. Then there’re three unknowns a, b and C, which can be solved from Equation 115 using linear least square fit method.

Then, define a corrected absorption spectrum as

Equation 116: \( A_{\text{correct}} = A - (a + b \cdot \lambda) \)

Figure 62. Spectral reflectance of the blood phantom and the 99% reflected diffuser.
Figure 63. Apparent absorption spectrum at different oxygenation levels

Figure 64. Absorption spectrum before and after being corrected.

Figure 62 shows the spectral reflectance of the blood phantom (indicated as ‘TR’) and the 99% reflected diffuser (indicated as ‘I’). Absorption spectra of different
oxygenation levels were calculated from TR and I, as shown in Figure 63. Four isosbestic points are clearly seen. The dash line shows the linear component contributed by the scattering and melanin absorption. This linear component is subtracted from the original absorption to get the correct absorption spectrum, as shown in Figure 64.

Remember the correct absorption spectrum is

Equation 117: \[ A_{\text{correct}}(\lambda) = C_1 \cdot \varepsilon_1(\lambda) + C_2 \cdot \varepsilon_2(\lambda) \]

Then \( A_{\text{correct}}(\lambda) \) will be fitted with the linear combination of \( \varepsilon_1(\lambda) \) and \( \varepsilon_2(\lambda) \) by some linear least square fitting algorithm such as Levenberg-Marquardt Algorithm. From the fitting \( C_1 \) and \( C_2 \) can be solve. Finally skin tissue oxygen saturation is calculated as

Equation 118: \[ \text{StO}_2 = \frac{C_1}{C_1 + C_2} \]

After linear fitting the corrected absorption spectrum using the extinction coefficient of oxygenated and deoxygenated hemoglobin, the concentration of hemoglobin can be solved.
Figure 65. Compare fitted absorption spectrum of phantom with hemoglobin extinction coefficient spectrum

Figure 65 shows the comparison of the fitted absorption spectrum of phantom with hemoglobin extinction coefficient spectrum. Several interesting things can be observed here. First, at those four isosbestic points, three spectra crossed as expected. Second, the phantom absorption spectrum is expected to be between the two extinction coefficient spectra. This is true in the wavelength range from 530 to 585 nm, which is the range defined within the four isosbestic points. However, the phantom absorption
spectrum is below both hemoglobin spectra beyond 585nm as shown in (a) and (b), and above both hemoglobin spectra prior to the 530nm as shown in (c). This indicates it may not be strictly correct that scattering and melanin absorption contribute linearly to the total absorption. This statement of linear contribution may be a good approximation in some wavelength range or for some skin tissue type. Thus, one may need to select a certain wavelength range to fit the spectra, rather than the whole spectra. As described by Seo [31], the spectra from 520 to 585 nm should be fitted. But other ranges were also proposed such as from 500 to 600nm [35, 36]. Thus, this kind of hyperpsectral imaging algorithm is also under investigation.

Figure 66. RGB image of the tissue-simulating phantoms at different oxygenation plateaus
Figure 66 shows the RGB image of the tissue-simulating phantoms at different oxygenation levels. The oxygenation level is from higher on the left to lower on the right, as indicated by the brighter red color on the right to the darker red on the right. The corresponding spectral reflectances are plotted in Figure 67. When the oxygenation is high (such as 93%) the spectral reflectance is more characterized by the absorption of oxygenated hemoglobin, and when the oxygenation is low (such as 4%) the spectral reflectance is more characterized by the absorption of deoxygenated hemoglobin.
The Labview GUI plays a very important role in our multispectral/hyperpectral imaging system. The main function is to synchronize the camera and the liquid crystal tunable filter (LCTF) which is mounted in front of the camera to filter light of specific wavelengths. The following modules have been developed to make a GUI with comprehensive functions including living imaging and image acquisition. Figure 68 shows the front panel of the GUI.
Figure 68. Front panel of the GUI. (a) overview; (b) ‘Basic Setting’; (c) ‘Advanced Setting’; (d) ‘Acquisition’.
C.1. Module 1: Live imaging module

Figure 69 shows the back panel of the live imaging module. This includes the following two main feathers.

First, the basic settings of the whole imaging system. The port number of the camera and LCTF, and the image format will be set. They’re put outside the live imaging ‘for loop’ thus they’re fixed system settings. The only system setting which can be adjusted interactively is the gain of the camera, which is inside the ‘for loop’. The corresponding front panel is shown in Figure 68(c).

Second, the live imaging settings: exposure time and the wavelength of the image. The LCF wavelength selection function is realized by a sub module as shown in Figure 70. The front panel of the exposure time and the wavelength control is shown in the top part of Figure 68(b).
Figure 69. Labview GUI module: live imaging
Figure 70. The sub module of controlling LCF for wavelength selection

C.2. Wavelength selection module

For multispectral imaging, users just type in the wavelengths desired for image acquisition. But for hyperspectral imaging, there will be many wavelengths so it’s better to have a module for wavelength selection. Figure 71 shows the diagram of this module. One may input the beginning wavelength $\lambda_1$, the ending wavelength $\lambda_2$ and the wavelength interval $\Delta \lambda$. Then the wavelengths will be set. For example, if $\lambda_1=500\text{nm}$, $\lambda_2=600\text{nm}$ and $\Delta \lambda = 2\text{nm}$, then the hyperspectral images will be taken at 500, 502, 504, … 598, 600nm with a spectral resolution of 2nm.
Figure 71. The wavelength selection module for hyperspectral imaging
C.3. Auto-exposure module

The function of the Autoexposure module is to automatically set the exposure time (i.e. shutter speed) of the camera so that the intensity of a monochromatic image is at an optimal level. Figure 72 shows the flowchart of the autoexposure procedure.

![Flowchart of Autoexposure procedure](image)

In the beginning, we can input a random shutter speed for each of the wavelength they choose. So now we have a monochromatic image with wavelength $\lambda$ and shutter
speed $t$ (1). Also we will define the optimal intensity we want every image to have: $I_o$. Then the intensity of an area of interest (ROI) of the image will be measured(2). A judgment will be made to check whether the intensity $I$ falls within the linear range of the camera (3) which is between 10 to 250 for an AVT camera. If the image is overexposure ($I>250$), the shutter speed will be decrease until the ROI intensity drops below 250 (4c). If the image is too dark, the shutter speed will be increase until the ROI intensity rises above 10(4b). Step 4b and 4c may undergo several iterations. Then a new shutter speed $t'$ will be calculated based on the linear behavior (4a):

$$t' = \frac{t}{I} \cdot I_o$$

Then the ROI intensity will be checked(5). If the difference between the current intensity and the target intensity is less than 2(6), the current shutter speed will be stored (7). If the difference is bigger than 2, another new shutter speed will be calculated using Equation 79. Steps (4a), (5) and (6) will be iterated until measured intensity and the target intensity is less than 2. Usually less than 3 iterations are needed since the operating point is already within the linear range of the camera.

Figure 73 shows the back panel of the Autoexposure module. Figure 73(a) is the case for a starting intensity/shutter speed already falls within the linear range of the camera. It shows the iteration using Equation 79 to get to the designated ROI intensity. The iteration is control using a case structure with a decision criteria of whether the difference of measured and target intensity is less than 2. It’s also worth noted that a background dark intensity has been subtracted during the calculation. The background
dark intensity (or dark current) is the ROI intensity when the camera shutter speed is set to 0. So Equation 79 becomes:

\[
    \text{Equation 120: } t' = \frac{t}{I - I_{dark}} \cdot (I_o - I_{dark})
\]

Figure 73(b) is the case when the starting image is overexposure. The only difference between (b) and (a) is that an iteration functionality of decreasing the shutter speed is added before the iteration functionality of setting new shutter speed using Equation 80. This new iteration functionality is terminated using a decision criteria of whether the ROI intensity is less than 250. Similarly, Figure 73(c) is the case when the starting image is too dark. An iteration functionality of increasing the shutter speed is added and controlled by a decision criterion of whether the ROI intensity is more than 10.

Figure 74 shows the ‘check intensity’ submodule which is used to determine whether the ROI intensity is above, within or below the linear range of camera. Figure 75 shows the ‘ROI mean’ submodule which acquire the ROI intensity.

Finally, the whole Autoexposure procedure is realized automatically by hitting the button ‘Autoexposure’, as shown on the front panel (Figure 68(b)).
(a) Autoexposure: when the starting image intensity falls in the linear range of camera

Figure 73. Back panel of ‘Autoexposure’ Moduel
(b) Autoexposure: when the starting image intensity is above the linear range of camera.
(c) Autoexposure: when the starting image intensity is below the linear range of camera
C.4. Image acquisition module

The acquisition and recording of multispectral and hyperspectral images are realized by the ‘image acquisition’ module. When this module is run, multispectral
images will be taken repetitively at designated wavelengths and corresponding exposure times.

Multispectral images will be taken at six designated wavelengths (544, 552, 568, 576, 592, 600nm). The corresponding exposure times have been determined and stored using the autoexposure module. Then the multispectral images can be taken using two

Figure 76. Flow chart of image acquisition by repeat time

Multispectral images will be taken at six designated wavelength (544, 552, 568, 576, 592, 600nm). The corresponding exposure times have been determined and stored using the autoexposure module. Then the multispectral images can be taken using two
different methods: (a) by repeat time, and (b) by total time. Six monochromatic images taken from 544nm to 600nm are considered as one measurement. If the images are taken by repeat time N, totally N measurement will be made; if the images are taken by total time, measurements will be continued until the total amount of time reaches the designated time.

Figure 76 shows the flow chart of the image acquisition module when the images are taken by repeat time. The first wavelength and corresponding shutter speed will be retrieved from the stored data (1, 2). Then the liquid crystal tunable filter (LCF) is set to this wavelength and the camera is configured to the shutter speed (3). The program will wait for 200 milliseconds in order to make sure all the configurations are done (4). Then monochromatic image will be taken (5). After that, another monochromatic at the next wavelength and corresponding shutter speed is taken. After all six images are taken, one measurement is finished (6). Then the next measurement will begin and the above steps (2 to 5) will be repeated. Measurements are continued until N measurements are made (7).

Before taking the image, a folder and name of image will be typed in by users, which is shown in the front panel in Figure 68(c). It also shows the option to take image by repeat time or total time.

Figure 77 shows the back panel of the image acquisition module. The module will first save the wavelengths and corresponding shutter speeds in a configuration text file, which is realized by the functions in the bottom left corner. Then the i\textsuperscript{th} wavelength and the corresponding shutter speed are retrieved by the ‘select wave’ submodule. The image
is taken and saved to the designated folder by the ‘take image’ submodule as shown in
Figure 78. Figure 77(a) and (b) are the modules for image taken by repeat time and total
time. The major difference is that a timer is added in (b) to track the time used for the
image acquisition.
Figure 77. Back panel of image acquisition module (a) by repeat time
(b) by total time
Figure 78. ‘Take image’ submodule
Appendix D: Other tissue simulating phantoms

Other kinds of tissue-simulating phantoms have been explored during this study. Here listed the fabrication methods of these phantoms.

(1) A phantom using hemoglobin solution extracted from fresh animal blood is introduced here. The procedure is called Red blood cell (RBC) lysis. First, the fresh whole blood is diluted from 15ml blood to 50ml in PBS solution and the centrifuge at 600 x g (600rcf) for 10 minutes to let the RBC precipitates. The supernatant is discarded and the precipitated RBCs were collected. Then the RBCs were re-dispersed in 50ml PBS, which is called the ‘wash’ step. The centrifuge, re-disperse and wash steps were repeated once. After that, the RBCs were re-dispersed in distilled water for lysis. The sample tubes will be kept in ice for 15 minute. After that, RBC will be lyzed and hemoglobin will be released into the water. Then the RBC solution will be centrifuged at 12,000 x g (12,000rcf) for 10 minutes to let the cell membrane precipitate. The supernatant which is just the hemoglobin solution was collected and then filter through a 0.2μm size filter paper using a vacuum pump. Finally, the filtered hemoglobin solution will be collected and store in 4ºC. The advantage of extracting hemoglobin solution is that it can be used for a longer time (usually 2 weeks compared to 3~5 days for whole blood).
(2) An agar-agar gel phantom which can resist heating up to 60°C can be fabricated using the following recipe. Agar-agar gel (Barry Farm, Wapakoneta, OH) has a gelling temperature of 32°C and a melting temperature of 65°C. To prepare the phantom, 0.09g agar-agar gel powder was fully dissolved in 10mL water (70°C) in a 10mL beaker. Other absorption and scattering materials can be added when the temperature of agar-agar gel dropped to 37°C. These absorption and scattering materials should be fully dispersed using a glass stir bar. Then the gel solution was kept in a refrigerator until complete gelling.

(3) A simple phantom simulating blood vessels in tissue can be fabricated in the following way. Intralipid - 10% (Kabivitrum, CA) was filled into a transparent acrylic box of 6.4 cm x 5 cm x 2 cm. A transparent plastic tube (radius 2.6 mm) was embedded in the Intralipid solution. The center of the tube was placed 5.6 mm away from the inner wall of the box. The tube has one end open and the other end connected to a 3 mL syringe through a 3-way Luer stopcock. The syringe was filled with blood or dyes and was installed on a NE-500 injection pump (New Era Pump System Inc, Wantagh, NY) for automatic control of flow direction and flow rate.

(4) A gel wax phantom can be fabricated using the following recipe. The phantom system has the following ingredients: (1) transparent gel wax (Gel-Wax, Yaley Enterprises, Redding, CA) as the host material, (2) titanium dioxide (TiO2) powder (Ti-Pure, Dupont, DE) for scattering control, (3) liquid candle dyes (Yaley Enterprises) for
absorption control, and (4) solid wax (Premium wax, Yaley Enterprises) for mechanical property control. Gel wax phantoms because has the following advantages: (1) the fabrication process does not involve any chemical reactions, therefore the resultant phantom properties can be readily controlled with reproducibility; (2) gel wax phantoms have a relatively long shelf time and can be recast for multiple times without significantly changing their properties; (3) gel wax is non-hazardous, inexpensive, and has relatively small shrinkage for dimensional accuracy.