January 2021

The Advanced Applications For Optical Coherence Tomography In Skin Imaging

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DEDICATION

To my beloved parents who support me with unconditional love.

To my grandfathers with whom I can no longer share my success and joy.
ACKNOWLEDGEMENTS

My highest appreciation to all the people who have helped and encouraged me along this PhD journey.

First, I would like to thank my advisor Dr. Kamran Avanaki, who has introduced the wonderful world of optical imaging. Thank you for providing me with an opportunity to learn research skills and sharpen my thinking. I would also like to express my deep gratitude to my co-advisor Dr. E Mark Haacke who helped, supported, and guided me throughout my last year of doctorate program. And it is an honor and a great pleasure for me to follow in your footsteps.

I also want to thank my committee members, Dr. Mai Lam, Dr. Zhifeng Kou, and Dr. Gholam-Abbas Nazri, for their time and efforts on this dissertation. In addition, I want to give special thanks to my colleagues, Dr. Rayyan Manwar, Dr. Karl Kratkiewicz, and Mohsin Zafar, who have been wonderful friends and great support during my PhD research.

Finally, I would like to thank my dearest parents who have been a strong and constant support during the challenges of graduate school and life. I am truly grateful for your unconditional love, trust, and encouragement.
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Chapter 1 INTRODUCTION

Optical coherence tomography (OCT), based on low coherent interferometry, is an FDA-approved non-invasive imaging modality. It uses a low coherent near-infrared laser around the wavelength of 1300 nm [1]. OCT has been widely used in ophthalmology to assess retina thickness and anatomical changes. In 1997, this technology was tested to image the cross-section of human skin in vivo, and it was able to visualize different skin layers and skin appendages [2]. Although, OCT has great potential in monitoring skin morphologic changes, skin cancer diagnosis, and tumor margin assessment [3], it is not well recognized and adopted by many dermatologists.

Therefore, in this research work, we aim to explore the potential clinical applications of OCT in dermatology, especially focused on melanoma detection. We began with a brief introduction of OCT and an overview of melanoma diagnosis in the clinic. We also discussed the application of different imaging modalities to assist melanoma detection.

OCT can offer real-time cross-sectional images of skin with high-resolution. It is also a contact-free and non-invasive imaging modality. Based on these unique features, OCT could aid and improve melanoma detection in four different aspects.

First, in chapter 2, we introduced OCT as an imaging aid to assist dermatological procedures, such as punch biopsy. Since no imaging aids are used during dermatological procedures, the accuracy of those procedures highly depends on the training and experience of dermatologists. Since OCT is a non-contact and real-time imaging modality, it can be used as a great visual aid to assist the procedures and improve their accuracy.

Second, in chapter 3, we aim to improve the sensitivity and specificity of OCT for melanoma detection. Due to the low contrast from intrinsic scattering characteristics of tissue microstructure, OCT can not provide accurate and robust diagnosis of melanoma. Therefore, to overcome this
limitation, we aim to use contrast agents, such as gold nanoparticles, to enhance the contrast of OCT images and improve the accuracy of melanoma detection.

Third, in chapter 4, to ensure the non-invasive administration of contrast agent and improve its penetration and diffusion in skin, we introduced a topical delivery method by combining chemical and physical penetration enhancers. And OCT was used to study and compare the delivery efficiency between different enhancers.

Fourth, in chapter 5, we aim to explore racial and age-related skin optical properties, which serves as a fundamental understanding of skin characteristics based on OCT images. Skin optical properties are a unique variable that depend on the degree of pigmentation, the location of the body, and the amount of water, collagen and blood in the skin. And such information is embedded in the OCT images. Our long term goal is to improve melanoma detection by analyzing tissue characteristics quantitatively based on OCT images and identifying unique radiomic signatures for future melanoma diagnosis. Hence, in this study, we extracted and quantified skin optical properties via post processing the OCT images.

1.1 Application of Optical Coherence Tomography (OCT) in Dermatology

1.1.1 Overview of OCT

Based on the principle of light interference, OCT uses infrared low coherence length (broadband) laser as the light source. As shown in Figure 1, the low coherence length light is split by a coupler evenly into two optical fibers: one of the fibers directs light to the tissue sample, and the other fiber directs light to a reference mirror [4]. The backscattered light from the tissue and the mirror return to the beam splitter, and then merge again. The interference patterns are generated when these two optical path lengths match [5]. Measurement of the interference pattern allows a
precise determination of the position within the tissue. And a cross-sectional image (B-scan) is produced by scanning the light beam across the surface of tissue.

**Figure 1. Principle of optical coherence tomography.**

As a new diagnostic method, its potential application in dermatology, especially in the field of skin lesion inspections, have been evaluated by many studies [3]. The advantages for using OCT in dermatology include moderate penetration depth, micro-scale resolution, use of near-infrared laser, and fast real-time non-invasive imaging, which makes OCT an ideal imaging modality to visualize the skin micro-morphology without any tissue removal.

OCT can image each layer of skin (including the epidermis, dermis, and dermoepidermal junction (DEJ)), the skin appendages (such as hair follicles and sebaceous glands), as well as blood vessels which are shown as low-scattering regions in the dermis of OCT images [6, 7]. As an emerging technology, it’s potential application for skin cancer diagnosis and treatment monitoring has been investigated by many studies [8-13]. The skin tumor cell aggregates are identifiable from the healthy tissue due to the different backscattering coefficient of light [13]. In addition, OCT has also been used for the tumor thickness measurement and margin assessment [14, 15].

There are commercial OCT systems available such as VivoSight (Michelson Diagnostics, Kent, UK), Skintell (Agfa Healthcare Mortsel, Belgium and München, Germany), Callisto
(Thorlabs, Lubeck, Germany), and NITID OCT (Dermalumics, Madrid, Spain) [16, 17]. Therefore, as a non-invasive imaging technique, OCT can provide valuable complementary information to the clinicians.

1.1.2 Overview of Skin Structure

As the largest organ of the body, skin has three layers, namely the epidermis, dermis, and subcutaneous tissue (hypodermis). The epidermal thickness ranges from 77 to 267 μm, and the dermal thickness ranges from 2115 to 5888 μm [18].

Epidermis is the superficial layer of skin, which has no blood or lymphatic vessels. It has five layers of stratified epithelium, which are stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, stratum basale from top to bottom [19]. The primary cell type in epidermis is keratinocyte that produces the protein keratin. Keratin is the structural protein for epidermis, hair, and nails. Another important cell type is melanocyte located in the stratum basale layer. It produces the pigment melanin, which is responsible for skin and hair color and helps protect the skin from UV radiation [20]. There are also nerve cells called Merkel cells for sensation and immune cells called Langerhans cells in epidermis.

Dermis is composed of two layers of connective tissue, which is the papillary layer and reticular layer from top to bottom [20]. It has no blood and lymphatic vessels, nerves, hair follicles, and sweat glands. The primary cell type in dermis is fibroblast that produces collagen and elastin fibers. The papillary layer intertwining with epidermis is the anchoring site for epidermis. The epidermal extensions called rete ridges extend downward into the papillary layer. The dermal extensions called dermal papillae extend upward into the epidermis.
Hypodermis is served as a layer of insulation and cushion between skin and fascia [20]. It is composed of loose connective tissue, adipose tissue, larger blood vessels and nerves. Two major cell types are fibroblast and adipocyte. Also the fat is stored within the adipocytes in hypodermis.

1.2 Overview of Melanoma Differential Diagnosis

1.2.1 Melanoma Development and Progression

Cutaneous malignant melanoma is the most dangerous and lethal form of skin cancer, which is still a diagnostic challenge in the dermatology clinic [21]. It is estimated that over 132,000 new cases of melanoma will be diagnosed globally each year [22]. Melanoma begins from pigment-producing cells, melanocytes, in the epidermis layer of skin, and progresses from the benign stage to metastasis stage through an initial radial growth followed by a vertical growth with deeper infiltration [23]. The transformation of melanocytes into metastatic melanoma is a very complex process involving exogenous, endogenous, tumor-intrinsic and immune-related factors [23-25]. And a stepwise acquisition of genetic abnormalities, i.e. increased number of point mutations, has been clearly seen during this progress [26]. Many studies have shown that B-Raf (BRAF) mutation is a typical feature for benign nevus formation [27, 28]; additional mutation of telomerase reverse-transcriptase (TERT) promoter is required for the in-situ melanoma [24, 28, 29]; the tertiary mutations of cyclin-dependent kinase-inhibitor 2A (CDKN2A) or chromatin-remodeling are required for invasive melanoma [30].

In clinic, based on the histopathological features, the melanoma progression is categorized into five different phases: congenital benign nevi (a mole) with normal structural melanocytes (Stage 0), dysplastic nevus with architectural disorder (Stage 1), early radial growth phase (Stage 2), advanced vertical growth phase with metastasis competence (Stage 3), and metastatic melanoma (Stage 4) [31]. As shown in Figure 2, in Stage 0 and 1, melanoma is still confined to
the epidermal region of skin, and not invasive. Stage 2 melanoma starts to grow through epidermis into the dermis, whereas in Stage 3 melanoma the neoplastic cells populate deeper into the dermis. As melanoma spreads into the dermis, it can invade the blood vessels and lymphatics. Finally, Stage 4 metastatic melanoma has metastasized to other distant organs, such as distant lymph nodes, brain, lungs, and liver [32].

![Figure 2. The progression of melanoma.](image)

For patients with early stage melanoma (Stage 0 and 1), they can be cured with surgical excision, and have an excellent long-term survival. At least 80% of patients are free of disease for 15 years [33]. For Stage 2 melanoma, the patients can be treated with wider and deeper excision, and a sentinel lymph node biopsy may be used to check if the cancer cells have spread to lymph
nodes [34]. However, the 15-year survival for Stage 2 melanoma patients dropped down to 50% [33]. For Stage 3 melanoma patients, they suffer higher risk of recurrence and metastases because the cancer cells have spread deeper into the dermis. Surgical excision and lymph node removal followed by additional adjuvant treatments, such as chemotherapy, radiation therapy, immunotherapies and targeted therapies, can be used for disease management [35]. And the long-term survival for Stage 3 melanoma patients is only about 35% [33]. For patients with Stage 4 melanoma, the cancer is considered unresectable. Biopsy and imaging are used to detect the range of metastasis. Both adjuvant treatments and clinical trials could be used for disease management [35]. Again, for Stage 4 melanoma patients, the 2-year survival is only about 20%, and the 15-year survival is only 10% [33]. Therefore, early diagnosis and treatment is crucial for disease management and saving lives.

Two types of classification methods have been used to determine the stages of melanoma [36, 37]. Cutaneous melanoma can be categorized into three stages using TNM (tumor-node-metastasis) designations [38]. T stage stands for the primary tumor stage. Based on the vertical depth of invasion, T stage can be subcategorized into Tis (in situ), T1, T2, T3, and T4. N stage involves the regional lymph node. Based on the number of tumor-involved regional lymph nodes, N stage can be subcategorized into N0, N1, N2, and N3. M stage involves the distant metastases. With a declining prognosis, M stage can be subcategorized into M0, M1a, M1b, M1c, and M1d. In addition to TNM classification, melanoma can also be categorized into stage I, stage II, stage III, and stage IV, based on the localization of melanoma and the metastatic status [39].

1.2.2 Current Clinical Diagnosis of Melanoma

It has been recognized that early detection of melanoma is essential in decreasing mortality from this malignant cancer [40, 41].
In clinical practice, visual inspection based on the “ABCDE” rules has been used as the first step to look for signs of melanoma. The “ABCDE” criteria stands for asymmetry, border irregularity, color variegation, diameter greater than 6 mm, and evolving in size and shape [42]. Although the effectiveness of this approach has been confirmed, the sensitivity and specificity range from 57% to 90% [43, 44].

Therefore, to further confirm a suspicious lesion to be melanoma, skin biopsy, as a standard of care, must be performed for histopathological examination [38]. Key features, such as thickness and spread of lesion, cytological atypia, pagetoid spread of melanocytes, cellular morphology, atypical mitoses, and lymphovascular and perineural invasion, have been used to determine the stage and extent of melanoma [45, 46]. However, skin biopsy is an invasive procedure, which can cause pain, bleeding, scaring and other complications. Moreover, the biopsy overuse increases expenses, the dermatology practitioners have been well trained to assure accurate examinations and avoid unnecessary biopsies.

1.2.3 Diagnostic and Prognostic Biomarkers for Melanoma

Although histological examination remains the standard procedure for melanoma diagnosis in clinic, researchers have invented other supplemental diagnostic methods to improve diagnostic accuracy, such as melanoma specific biomarkers. Biomarkers are considered as molecular signatures, which are made in response to different tumor stages or therapy. They can be expressed in tumor cells, or secreted in fluids, such as blood and urine [47]. And both tissue-based and serum-based biomarkers have been investigated for different stages of melanoma. Although biomarkers have great potential and value for melanoma detection, they have not been incorporated and recommended into clinical practice [48].
The tissue-based biomarkers can be detected by immunohistochemical staining after biopsy. They are expressed on cell nucleus or cytoplasm [48]. In 2005, after evaluating the current melanoma biomarkers, the investigators at The Markers and Tissue Resources for Melanoma Meeting in Gaithersburg compiled a list of 30 melanoma biomarkers of interest, including melanocyte inducing transcription factor (MITF), cyclin-dependent kinase inhibitor 2A (P16), cyclin-dependent kinase inhibitor 1A (P21), galectin 3 (GAL3), proliferation marker protein Ki-67 (Ki-67), mesothelin (MLSN), ADP-ribose glycohydrolase (ARH3), catenin beta-1 (CTNNB1), interleukin-6 receptor (IL6R), signal transducer and activator of transcription 1/3 (STAT1/STAT3), phosphatase and tensin homolog (PTEN), AKT serine/threonine kinase 1 (AKT1), pancreatic eIF-2alpha kinase (pERK), secreted phosphoprotein 1 (OPN), melanoma cell adhesion molecule (MCAM), nitric oxide synthases (iNOS), forkhead box P3 (FOXP3), CD3, CD4, vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1 (HIF-1), RELA proto-oncogene (RelA), transmembrane 4 superfamily (TM4SF), tyrosine-protein kinase kit (c-kit) [49, 50]. These biomarkers showed a differential expression in melanoma, and they can be detected using formalin-fixed paraffin-embedded tissues.

The serum-based biomarkers, or serological biomarkers, are tumor-derived small molecules constantly released into the circulatory system of cancer patients, including tumor cells, tumor DNAs and RNAs, proteins, metabolites, and vesicles [51]. According to the updated report from American Joint Committee on Cancer (AJCC), lactate dehydrogenase (LDH) is the only circulating protein biomarker with prognostic value for melanoma therapies [52]. The elevated serum LDH is associated with poor survival in stage IV melanoma and indicates negative response to immunotherapy [53-55]. Numerous other serological biomarkers have been studied their potential role to predict melanoma progression, including serum proteins (S100 [56], melanoma
inhibitory activity protein (MIA) [57], VEGF [58], and interleukin-8 (CXCL8) [59]), microRNAs (miRNA-15b, miRNA-17, miRNA-19a, miRNA-21, miRNA-33a, miRNA-126, miRNA-149, miRNA-150, miRNA-199a-5p, and miRNA-424) [60, 61], long non-coding RNAs (SPRY4 intronic transcript 1 (SPRY4-IT1) [62], BRAF-activated non-protein coding RNA (BANCR) [63], HOX transcript antisense RNA (HOTAIR) [64], urothelial cancer associated 1 (UCA1) [65] and metastasis associated lung adenocarcinoma transcript 1 (MALAT-1) [66]), and circulating melanoma cells [67-70]. Although many serological biomarkers have been proposed, limited studies have been performed to validate their clinical application.

But unlike tissue biopsies, which are invasive and have limited tumor accessibility, serological biomarkers can be collected from biological fluids, such as serum, blood, urine, saliva and cerebrospinal fluid with minimal invasion [71]. Then they can be analyzed through a repeatable liquid biopsy test. And various circulating components can be captured by liquid biopsy, providing more comprehensive information for a patient’s cancer growth and metabolism. Therefore, these biomarkers can be used routinely for tracking the patients response to treatment and monitoring cancer progression. The liquid biopsy tests, Guardant360 CDx and FoundationOne Liquid CDx, have been approved by FDA to identify specific genetic mutations in non-small cell lung cancer, ovarian cancer, breast cancer, and prostate cancer [72, 73].

1.3 Using Imaging Techniques to Augment Melanoma Diagnosis

The current applied method for melanoma diagnosis is visual inspection followed by histology or immunohistochemistry. To detect one melanoma, many benign lesions have to be excised which can be time consuming and expensive. At a specialized clinic, the number of excised lesions is about 4 for a dermatologist; but at a non-specialized clinic, this number can go up to 20-40 for a general practitioner [74, 75]. In addition, the ability of clinicians to identify melanoma by
visual inspection is variable, and the current diagnostic method is inadequate to screen large populations. Therefore, to save the high cost of unnecessary biopsies and achieve a faster patient management system, it’s essential to design non-invasive imaging techniques to facilitate melanoma detection.

1.3.1 Various Imaging Modalities Developed to Enhance Diagnosis

Multiple imaging modalities have been developed and adapted to use on melanoma detection, such as total body photography, dermoscopy, confocal microscopy, high frequency ultrasonography, magnetic resonance imaging (MRI), positron emission tomography (PET), spectroscopic imaging, and photoacoustic imaging.

Total body photography uses high-resolution digital camera to capture specific anatomical sites, such as face, abdomen, chest, back, arms, legs and feet [76]. This method is most suitable to track the evolution of pigmented lesions over time in high-risk patients, or to survey the patients with a large number of atypical melanocytic nevi [77-80]. However, the major limitation of this method is that it only captures the surface features of the lesions without any information about its depth.

Dermoscopy, or epiluminescence microscopy, a hand-held magnifier with 10 to 20 folds of magnification, has been widely used in clinical applications [81]. It uses LED for illumination and polarized filters to decrease light reflection[82]. This allows the visualization of the subsurface layer like papillary dermis (top layer of dermis), the vascular structures, and the distribution of pigments [83, 84]. Several meta-analyses have proven that melanoma diagnostic accuracy can be improved by 49% by using dermoscopy [85-87]. Also, many dermoscopy algorithms, such as Menzies method [88], ABCD (asymmetry, border irregularity, color, diameter) system [89], CASH method (color, architecture, symmetry, and homogeneity) [90], and 7-point checklist [91],
have been developed and tested to improve and simplify the diagnostic process [92]. Although dermoscopy has been a great aid for dermatologists, it requires extensive training to interpret the dermoscopic structures, which hinders its routine use for many clinicians. More importantly, due to its limited detection depth and resolution, dermoscopy couldn’t be used to observe the structures within dermis and any cellular patterns.

Confocal microscopy, including reflectance confocal microscopy (RCM) or confocal scanning laser microscopy (CSLM), has also been developed to visualize the *in vivo* dermal cellular structures without a biopsy [93, 94]. Confocal microscopy utilizes a laser light source, a condenser, an objective lens, and a detector to selectively collect the reflected light from different depths, which allows the user to see different layers of skin. Confocal microscopy features of melanoma have been proposed by many studies, including roundish-polygonal single cells, polygonal cells with a dark nucleus and bright cytoplasm, oval to round bright aggregates, broadened honeycomb pattern, mesh appearance at DEJ, etc [93-96]. VivaScope® (Caliber I.D, Rochester, NY) is a commercial confocal microscopy that offers high resolution real-time cellular-level images of skin [97]. However, the 200–300 µm imaging depth is the major limitation for confocal microscopy, since this only allows the visualization of epidermis and upper dermis [98]. Also, confocal microscopy is a high-cost equipment, and it needs extensive training to interpret the images.

High frequency ultrasonography with frequency of 3 to 100 MHz has been tested and used in dermatology [99]. Ultrasound images display the vertical cross section of the skin. The imaging depth and resolution depend on the frequency of ultrasound. As the frequency increases, the penetration depth decreases, the imaging resolution increases. Lower frequency (3 – 10 MHz) ultrasonography can image subcutaneous layer or lymph node, higher frequency (>20 MHz)
ultrasonography can image superficial layer at a depth between 3.8 mm to 1.1 mm [100]. The 20 MHz ultrasound scanner has been mainly used for melanoma detection with good resolution (80 µm) and imaging depth (3.8 mm) [101, 102]. And on ultrasound image, melanoma was seen as solid homogeneous hypoechoic lesion [103]. However, one common issue for ultrasound is overestimation of melanoma thickness due to the presence of hair follicles or glands, and lymphocytic infiltration [104, 105]. Although ultrasonography is a great aid for lymph node examination, it cannot distinguish benign nevi and melanomas [106].

Furthermore, some other non-invasive imaging techniques, such as MRI [107], PET [108], spectroscopic imaging [109], and photoacoustic imaging [110], have also been tested for melanoma detection. However, MRI, PET, and spectroscopic imaging are unable to detect melanoma at early-stage [111]. And photoacoustic imaging is still at the experimental stage and still needs considerable improvement for future clinical application [112].

### 1.3.2 OCT Imaging of Melanoma

OCT has been routinely used in the eye clinic, and it was introduced in dermatological applications since the 1990s [2, 113]. Although OCT is not able to visualize single-cell morphology, it can display a real-time 2D cross-section of skin at a depth of 1 to 2 mm with a resolution of 3 to 15 µm [114]. Various research has proved that OCT can clearly visualize the sub-anatomical structures of skin, such as epidermis, dermis, epidermal dermal junction, sweat glands, hair follicles, and blood vessels [115]. Moreover, OCT has been tested to detect non-melanoma skin cancers like basal cell carcinoma [116-119]. Therefore, compared to the other imaging modalities, OCT has a promising future for melanoma diagnosis.

OCT operating at the wavelength between 830 nm to 1700 nm have been used for melanoma diagnosis. Many studies found that melanoma displays unique features in OCT images [120]. The
atypical melanocytes in the upper layer of epidermis and dermis have a more chaotic architectural disarray [121-124]. More atypical cell clusters are found at the epidermal dermal junction that distort the normal and well-defined rete ridges [122, 125, 126]. For those superficial melanoma, a prominent feature is the upward invasion of highly reflective roundish pagetoid cells into the epidermis from below, which is called the pagetoid growth [118, 123]. These compact infiltrations appear as large vertical icicle-shaped structures on OCT images [122, 127]. In addition, the vasculature of melanoma appears to be more tortuous, irregular and invasive [128, 129]. Moreover, Moraes et al. also showed that a loss of bright collagen which appeared as a lack of hypo-reflective bands on OCT images has a correlation with invasive melanoma [130]. And multiple studies have reported a significant correlation between histopathological findings and OCT features [120].

Although there are only limited studies exploring melanoma diagnosis with OCT, they have shown promising results that OCT can be used as a quick and relatively inexpensive method for cutaneous melanoma detection and longitudinal tracking of lesion progression.

1.3.3 Potential Use of Molecular Imaging for Melanoma Diagnosis

Although these aforementioned non-invasive imaging techniques have been developed to visualize cutaneous melanoma, they only can provide high-resolution anatomical images, and these anatomical images alone are not enough for guiding melanoma diagnosis. As for OCT, current studies showed that it is still difficult to differentiate melanoma from benign nevi with OCT alone [120]. This is because the contrast of OCT images coming from the intrinsic scattering of different tissue compartments is insufficient to tell them apart [121]. Therefore, another imaging technique, molecular imaging, has also drawn lots of attention and been tested for melanoma detection.
The unique advantage for molecular imaging is its ability to visualize the expression of specific biomolecules and quantify the biological processes at cellular levels [131]. This information can have a huge impact on melanoma management and individualized treatment. Another advantage for using molecular imaging on melanoma diagnosis is that it is a non-invasive procedure, and it can potentially replace the invasive biopsy and histopathology procedures [132]. Also, the anatomical changes displayed by other imaging techniques could be discernible only at late stage of cancer, but molecular imaging can identify and detect a diseased tumor at its early stage [133].

Molecular imaging combines traditional imaging modalities with molecular imaging agents to visualize and track the precise localization of a lesion [132, 134]. A variety of imaging agents have been developed and used to visualize the activity and structure. Such imaging agents can be oxygen, blood, and chemical molecules involved in metabolism; or a radioactive atom and isotope for nuclear medicine; or a contrast agent such as nanoparticles, fluorophores, and dyes for optical imaging [131]. Typically, a synthetic molecular agent consists of the targeting and signaling components [132]. An ideal molecular probe should be specific for its target of interest and have suitable pharmacokinetics for visualization [135, 136].

For melanoma diagnosis, the commonly used molecular imaging techniques are lymphoscintigraphy and positron emission tomography (PET) [137]. However, these two imaging techniques use radioactive tracers, and they are mainly used for metastatic melanoma [138].
Chapter 2 SWEPT-SOURCE OPTICAL COHERENCE TOMOGRAPHY SUPERVISED BIOPSY

2.1 Introduction

Skin biopsy still serves as the decisive diagnostic tool in dermatology [139]. It is an essential procedure, in which skin samples are removed and examined under the microscope to provide diagnostic information [46, 140]. To improve the diagnostic accuracy and to assist the dermatologist, various imaging techniques have been added to the examination of skin [141]. To date, several new techniques have been used to examine the skin, including reflectance confocal microscopy (RCM), multiphoton microscopy (MPM), high-frequency ultrasonography (HFUS) and optical coherence tomography (OCT).

RCM is the only imaging modality which has category I current procedural terminology (CPT) reimbursement codes [142], and it has been tested and implemented to guide diagnosis [143, 144]. RCM can guide margin assessment prior to excision for lentigo maligna [145] and confirm laser ablation of superficial and nodular basal cell carcinoma [146]. However, the main drawbacks for RCM include high cost, large size of the device, long imaging time (20 to 30 minutes) and limited penetration depth of 200 to 500 microns, which includes the epidermis and superficial papillary dermis [147]. Moreover, although confocal microscopy is better established, it is still not widely used due to the operator difficulties.

Multiphoton microscopy (MPM) is another imaging modality used to obtain cellular morphology and extracellular matrix structure information [115, 148]. MPM can separate collagen and elastin in extracellular matrix through differences in autofluorescence [149]. However, low intracellular autofluorescence makes it difficult for MPM to differentiate between cancerous and healthy cells [115].
High-frequency ultrasonography (HFUS) is also a non-invasive, real-time imaging modality that has been assessed in dermatology [150]. Studies have been carried out to assess skin tumors using HFUS. But the image resolution of HFUS is relatively low [151]. While it can be used to delineate the margins of a skin tumor, HFUS is not precise enough to distinguish the specific diagnosis of skin lesions [152, 153].

Limitations such as coarse resolution and low penetration depth remain the major issues for these methods. As the technologies continue to improve, there may come a time when they replace skin biopsy. At present, skin biopsy continues to be the standard procedure for diagnosis, but non-invasive imaging modalities hold the potential to aid in skin biopsy. In this regard, optical coherence tomography (OCT), provides safe 2D and 3D images with micrometer-scale resolution and high penetration depth, and can be used as an imaging guide during biopsy.

OCT is a non-invasive optical imaging technique based on interferometry, which measures backscattering of near infrared light [3]. OCT enables visualization of skin layer architecture including stratum corneum, epidermis and dermis [154]. In addition, skin appendages, such as hair follicles and sebaceous glands, as well as blood vessels are shown as low-scattering regions in the dermis of OCT images [114]. OCT was introduced to study human skin in 1997. Its use increasingly grew in skin tumor imaging, especially in the application of skin cancer diagnosis and tumor margin assessment [3, 114, 155]. Skin tumor cell aggregates are identifiable from the healthy tissue due to the different backscattering coefficient of light, and also in some cases the tumor borders are detectable [114, 139]. Alawi et.al. has suggested that OCT imaging can be used to define the tumor margin prior to excision [15].

We investigated the feasibility of using OCT imaging for real-time visualization of needle insertion and punch biopsy techniques in both a tissue phantom and biological tissue.
2.2 Materials and Methods

2.2.1 OCT system configuration

The OCT system used in this study is a swept-source OCT (SS-OCT) from Michelson Diagnostic TM. The central wavelength of the laser is 1305 nm with a sweep range of 150 nm. The penetration depth of the system was measured as 1.5 mm in healthy human skin. The images obtained with this OCT system have a size of 5 mm × 2 mm with lateral and axial resolutions of 7.5 and 10 microns, respectively. The schematic diagram of the SS-OCT system is shown in Figure 3.

![Schematic diagram of the SS-OCT system used in this study.](image)

Figure 3. Schematic diagram of the SS-OCT system used in this study.
2.2.2 Imaging needle insertion in mouse skin

A 30-gauge insulin needle was inserted into the back skin of a euthanized mouse. The back of the mouse skin was shaved prior to the insertion. To track the movement of the needle, the needle was held by hand and moved in and out of the skin. The OCT imaging plane was held perpendicular as well as parallel to the needle. Figure 4 illustrates the relative position between the imaging plane and needle.

Figure 4. Demonstration of the position of the imaging plane over the inserted needle. (A) the imaging plane parallel to the needle; (B) the imaging plane perpendicular to the needle.

2.2.3 Needle insertion guidance using OCT

To demonstrate the utility of OCT imaging to guide the tip of a needle in reaching a target, we utilized a gelatin phantom with the same scattering coefficient as skin. The detailed skin scattering coefficient calculation and phantom fabrication methods are described in supplementary material. To create a target, 10% gelatin phantom was made with a tiny dot of TiO₂ powder inside
it. India Ink, an absorber, was added to the phantom, so that the TiO$_2$ was not visible to the naked eye. OCT imaging was used to guide the insertion of a 30-gauge needle to reach the target. Figure 5 shows a schematic illustration of the procedure.

![Figure 5. Needle biopsy procedure is supervised using the OCT. The black line indicates the TiO$_2$ “lesion”.

### 2.2.4 Punch biopsy OCT piercing tip

A probe/piercing tip was designed and fabricated to perform the imaging and punch biopsy at the same time (Figure 6A). The piercing tip is an aluminum device that was designed to fit tightly over the OCT handheld probe tip, so the laser will not be blocked by the metal (Figure 6C and D). The size and schematic design was shown on Figure 6B. To perform the punch biopsy, the sharpened piercing tip is rotated while the OCT probe remains stationary. The punch biopsy was performed on 10% gelatin phantom. A small amount of TiO$_2$ powder was added to the gelatin phantom to resemble a lesion. India ink was also added in the phantom, so that the lesion can only
be seen using OCT. After locating the lesion by OCT imaging, punch biopsy was performed immediately by the OCT probe to remove the lesion area.

**Figure 6.** Punch biopsy probe /piercing tip. (A) picture of piercing tip; (B) schematic illustration of piercing tip design, unit in mm; (C) front view of the probe attached to the OCT probe; (D) side view of the probe attached to the OCT probe.
2.3 Results

2.3.1 Needle in mouse skin

To test the needle insertion in real skin tissue, we performed the needle insertion in mouse skin. A needle was inserted inside mouse skin and then imaged using OCT (Figure 7A). The mouse skin structure, both dermis and epidermis, can be clearly seen on the OCT image. When the imaging plane was perpendicular to the needle, the cross section of the needle was a bright half-circle (Figure 7B). As the needle went inside the skin, the cross section of the needle appeared as a less bright curved line (Figure 7C). The curvature decreased as the needle goes from outside to inside the skin. When the imaging plane was parallel to the needle, the side view of the needle was seen on OCT image. The needle inside mouse skin was shown as one bright line with a slight bend at the air-tissue interface (Figure 7D).
2.3.2 OCT supervised needle insertion

Figure 8A is the gelatin phantom with TiO$_2$ powder to serve as a target. When a 30-gauge insulin needle was inserted in the phantom (Figure 8B), a bright line was visualized on the OCT image. The bright line on the image, as seen in Figure 8C, is the top outer surface of the needle. Next, we tested the idea of supervised needle insertion using OCT. During the procedure, the tip of the needle was used as a guide to lead the whole procedure. Figure 8C demonstrates tip of the needle reaching the target and Supplemental Digital Content 1, which demonstrates the procedure and corresponding OCT imaging on video.
2.3.3 OCT supervised punch biopsy

To test the idea of OCT supervised punch biopsy, we performed the procedure on a gelatin phantom using the fabricated piercing tip (Figure 9A and B). The TiO₂ powder was a bright line on the OCT image (Figure 9D). The OCT images were taken to locate the position and shape of the suspected area (Figure 9D). The punch biopsy was performed immediately during the imaging procedure.
to sample the “lesion” (Figure 9C). After sampling the “lesion”, we cut it open to check, and the TiO$_2$ powder was found inside (Figure 9E).

Figure 9. Supervised punch biopsy using OCT. (A) Gelatin phantom used for punch biopsy; (B) procedure of punch biopsy on gelatin phantom; (C) the TiO$_2$ “lesion” removed after punch biopsy; (D) B-scan OCT image for the punch site during the procedure; (E) TiO$_2$ “lesion” inside the phantom.

2.4 Discussion

In this study, we evaluated the feasibility of using OCT for supervised needle insertion and punch biopsy. We demonstrated that the movement of needle insertion can be monitored using OCT in real time, using mouse skin and a skin phantom. There is no time delay during the
procedure, allowing the movement of the needle to be clearly captured by the OCT images. The needle was a single bright line in the mouse skin (Figure 7), but appears bent at the skin surface. This is due to the difference between the refractive index of air and skin. Refractive index is 1 in air. The refractive index of human skin has different values for different layers of skin, ranging from 1.37 to 1.5. In our OCT system we assume that the refractive index is constant and equals 1.3. We have devised a method to compensate for the refractive index error, and the corrected OCT image is shown in Figure 10.

![Figure 10](image1.png)

**Figure 10.** The uncorrected and correct OCT image for refractive index error. (A) Uncorrected OCT image; (B) Corrected OCT image.

Due to the easy accessibility of the skin, intralesional injection is a convenient and effective method of delivering medication. Intralesional therapy can be used for various dermatologic diseases, including inflammatory, infectious and neoplastic [156-158]. OCT guidance will allow visualization for more accurate drug delivery. In neoplastic disease, OCT can not only aid in the treatment, but can also be utilized as a non-invasive means of assessing the response to treatment [8]. Real time visualization of needle placement also holds potential in the field of cosmetic filler injection.
Here, we introduce OCT for image-guided skin biopsy. With OCT image guidance, biopsy can be performed with specific targets within a lesion, or the boundaries of the lesion can be assessed during the procedure. In large tumors, such as lentigo maligna, it is not always possible to biopsy the lesion in its entirety. Imaging with ultraviolet light and confocal microscopy has proven utility in biopsy and margin delineation [159, 160]. We designed a piercing tip so imaging and biopsy can be performed at the same time. This new technique will allow the clinician to biopsy various parts of the tumor quickly and conveniently. Imaging at the time of biopsy will also allow the clinician to ensure that each representative morphology within the lesion is sampled. At present, the biopsy tool is 6.8mm in diameter. In the future, the development of tools of various sizes will expand the application of the technique. Further clinical studies to elucidate the applications of OCT during skin procedures are indicated.

2.5 Conclusion

Optical coherence tomography holds potential not only as a diagnostic tool in dermatology, but also to assist in procedures. OCT can provide guidance for skin injections as well as real time imaging to assist in the performance of punch biopsy.
Chapter 3 CONTRAST-ENHANCED OPTICAL COHERENCE TOMOGRAPHY FOR MELANOMA DETECTION: AN IN VITRO STUDY

3.1 Introduction

Optical coherence tomography (OCT), with a high spatial resolution (<10 microns), intermediate penetration depth (1 to 2 mm), and volumetric imaging capability has become a popular diagnostic-assistant modality in tumor diagnosis [7]. Tissue contrast in OCT images is generated by the intrinsic scattering characteristics of tissue that are proportional to the density, size and shape of the tissue microstructures. Because malignant cells show pleomorphism, with different refractive indices and absorption characteristics than normal cells, based on light-tissue interaction theories, OCT images should discriminate malignant tissues from normal tissues and benign neoplasms. However, the sensitivity and specificity of OCT for tumor detection and differentiation from benign tissue is lower than anticipated. Several groups including ours have attempted to increase the specificity by OCT image enhancement, texture analysis, even implementing sophisticated configurations of OCT, including polarization-sensitive, phase-sensitive, and dynamic OCT; these have also failed to adequately discriminate between tumor and benign lesions [120, 161]. Therefore, novel contrast agents are needed to selectively enhance the OCT radiomic features of the tumor and improve the diagnostic sensitivity and specificity of OCT. In this study, we introduce the SMall nanoparticle Aggregation-enhanced Radiomics of Tumors OCT (SMART-OCT), and evaluate its potential use for melanoma differential diagnosis in vitro.

As shown in Figure 11, the SMART-OCT approach utilizes an OCT-specific contrast agent, which is made by conjugating ultra-small gold nanoparticles (USGNPs) to single-chain antibody (AB). The AB specifically binds to the melanoma biomarker, which is overexpressed in melanoma, but is absent in benign skin lesions. Therefore, after extravasation and diffusion into the tumor tissue, the AB-USGNPs bind to the tumor cells, while the unbound fraction clears more rapidly
and effectively to minimize background signals. Although individual unbounded AB-USGMPs scatter the light isotropically, they only weakly reflect the scattered light back to the detector, which is significantly attenuated. In contrast, AB-USGNPs bind to and aggregate on melanoma cell membranes, forming much larger reflective surfaces of irregular shape and significantly reflect back the scattered light, which considerably enhances the OCT signal.

Figure 11. Principle of tumor detection using SMART-OCT imaging

Several cell membrane biomarkers used in histopathology to differentiate malignant melanoma from benign nevi suite our purpose, including: Galectin-3 (Gal3), Prame, p16, Nestin, SOX2, COX2, Collagen XVII and MMP2. We choose Gal3 due to its greater expression in primary melanoma compared to benign pigmented skin lesions [162]. Gal3 is a cellular surface protein, expressed in benign cutaneous melanocytic lesions as well as melanoma lesions. This expression is significantly greater in melanomas than in benign pigmented skin lesions (i.e., compound nevus,
intradermal nevus, junctional nevus and solar lentigo) [163]. On the molecular level, Gal3 regulates autotaxin expression by modulating the expression of the transcription factor NFAT1 [164]. In addition, Gal3 directly interacts with transcription factor AP-1 and facilitates the binding of this complex to the matrix metalloproteinase-1(MMP-1) promoter that drives MMP-1 transcription [165].

Among different types of nanoparticles, gold nanoparticles (GNPs) have been extensively studied, largely because they are FDA approved. More importantly, GNPs are photostable, tunable in the NIR region, nontoxic, and chemically inert for all biological processes and remain unoxidized [166]. Moreover, biomolecules, such as antibodies can easily be conjugated to the surface of the GNP as diagnostic agents for multiple applications. GNPs can exhibit a cytotoxic profile, when the size is below 2 nm [167], therefore we choose to use 10 nm GNPs. The ultra-small nanogold particles have a high labeling efficiency, fast diffusion rate, deep tumor penetration, and efficient accumulation and extravasation [168].

3.2 Materials and Methods

3.2.1 OCT system configuration

A multi-beam, hand-held swept-source OCT system (Vivosight, Michelson Diagnostic Inc., Kent, United Kingdom), was used in this study. The lateral and axial resolutions are 7.5 and 10 μm, respectively. The scan area of the OCT system is 6 mm width by 6 mm length by 1.5 mm depth (in tissue). A tunable broadband laser source with the central wavelength of 1305 and a bandwidth of 30 nm, successively sweeps through the optical spectrum and leads the light to four separate interferometers and forms four consecutive confocal gates.
3.2.2 Molecular probe preparation

Gal3-USGNP conjugates were synthesized by Nano Hybrids Inc. (Austin, TX) by covalently attaching 10 nm Gold Nanospheres via the 3.4 kDa PEG tether to Galectin-3 (B2C10) mouse IgG1 kappa light chain antibody fragment (31 kDa; sc-32790; Santa Cruz Biotechnology Inc.) using free lysine groups.

3.2.3 Cell culture preparation

B16-F10 melanoma cells (CRL-6475™) and PCS-200-011 primary normal human epidermal keratinocytes were obtained from ATCC. B16 melanoma cells were cultured in DMEM + 10% FBS + 1% NEAA + 1% Pen & Strep. PCS-200-011 keratinocytes were cultured in Keratinocyte Growth medium (ATCC PCS-200-040) containing Extract P 0.4%, rhTGF-alpha 0.5 ng/mL, L-glutamine 6 mM, hydrocortisone 100 ng/mL, insulin 5 μg/mL, epinephrine 1.0 μM, apo-transferrin 5 μg/m. These cells were used between passages 3-5. Both cell lines were seeded at the density of 0.16 M cells/cm2 in triplicates and were left for 48 hrs to adhere and grow. Then, Gal3-USGNPs were added to the cells in increasing concentrations relative to the number of cells in culture: $10^3$/cell, $10^4$/cell, $10^5$/cell, $2	imes10^5$/cell, $5	imes10^5$/cell, and $10^6$/cell. Cells were incubated with Gal3-USGNPs for 2 hrs. Cells not exposed to Gal3-USGNPs were used as a negative control. After 2 hrs, the cells were washed two times with PBS to remove the non-attached Gal3-USGNPs (to mimic the physiologic washout over 2 hours of non-bound fraction from the tumor tissue) and the cells were detached from the plate using Hanks solution.

3.2.4 Data analysis

A triaxial holder maintained the OCT probe fixed and perpendicular to the sample surface. 120 cross-section OCT images were collected from a 6×6×2 mm volume of medium containing the cells. The cells that are at the same depth (±50 μm) were considered for processing. We made
sure that the OCT objective focal point is almost at the same depth for all acquired images. Data processing was done using Matlab (MathWorks, Terrence, CA). The OCT signal amplitude was normalized and expressed as a fraction of maximum detectable signal (that equals 1) and expressed as mean ± standard error.

3.3 Results and Discussion

The goal of this in vitro study was to demonstrate the ability of the proposed method for significant differentiation between melanoma cells and nonmelanoma cells. The results in Figure 12A shows that using OCT and the contrast agent, Gal3-USGNPs conjugates, B16 melanoma cells can be well differentiated from normal skin keratinocytes in vitro. Binding parameters of Gal3-USGNPs to B16-F10 melanoma cells expressing Gal3 was determined by plotting the normalized OCT signal intensity values over the concentration of Gal3-USGNPs/cell and fitting data in GraphPad Prizm 4 (GraphPad, La Jolla, CA) using Hill equation for one-site saturable binding: $Y = B_{\text{max}} \times X^h/(K_d^h + X^h)$, where $B_{\text{max}}$ is the maximum specific binding, $K_d$ is the concentration of ligand needed to achieve half maximum binding at equilibrium and $h$ is the Hill slope.

We found that Gal3-USGNPs binding to B16-F10 melanoma cells was saturable (Figure 12B), with an apparent $B_{\text{max}}$ of 0.98 ± 0.09 and $K_d$ of 18 093 ± 7926. In contrast, nonspecific background OCT signals detectable from normal skin keratinocytes did not increase after incubation even with high concentrations of Gal3-USGNPs ($B_{\text{max}} = 0.09 ± 0.12; K_d = 0.001 ± 0.004$).

Importantly, the SMART-OCT was able to reliably discriminate B16-F10 melanoma cells from normal keratinocytes even at the lowest concentrations of Gal3-USGNPs used in this study ($10^3$/cell) (Figure 12C) and that the half-maximum saturation of Gal3-USGNPs to B16-F10 melanoma cells was already achieved after exposure to less than $2 \times 10^4$ particles per cell (although
the actual number of particles bound to each melanoma cell remains to be determined). These results are consistent with high levels of Gal3 expression in B16-F10 melanoma cells that has been published previously by other investigators [169] and retested by us; Western blot analysis of the expression level of Gal3 in PCS-200-011 and B16F10 melanoma cells are shown in Figure 12E. The absence of Gal3-USGNPs signals from normal skin keratinocytes is also consistent with previous reports about the lack of Gal3 expression in normal skin [170, 171].

To study OCT signal enhancement at different incubation times, Gal3-USGNPs were added to the cells with a concentration of $10^6$/cell. The results are shown in Figure 12D. It appeared that with only 30 minutes incubation time, 87% of the maximum OCT signal is achieved, which still yields 100% OCT signal enhancement compared to when there is no contrast-enhanced OCT signal; this conclusion will slightly be changed in in vivo experiments when the conjugates are administered either topically or systemically.
Figure 12. Contrast enhanced OCT imaging of normal keratinocytes and B16-F10 melanoma cells with Gal3-USGNPs, \textit{in vitro}. (A) Contrast-enhanced B-scan OCT images of increasing concentrations of Gal3-USGNPs; (B) Binding saturation analysis of Gal3-USGNPs; (C) same data in a loglinear format; (D) OCT signal improvement at different incubation times; (E) Western blot analysis of the expression level of Gal3; (F) transmission electron microscopy of USGNPs.
An IR800 (LI-COR 929-70 020) dye conjugated with Gal3-USGNPs (Gal3-USGNPs-IR800) was used to test the expression of Gal-3 on melanoma cell surface. The conjugates were applied on 4% formaldehyde fixed melanoma cells in cell culture disk. Bright-field image (×40) of 4% formaldehyde fixed melanoma cells in cell culture disk mixed with and without dye-AB-USNPs are shown in Figure 13A, B. The fluorescent images of the cells were taken using Fluorescence Microscope BZ-X800 (Keyence, Inc.) with a 750 to 800 nm filter, that is, ET775. The surface of the melanoma cells that were exposed to Gal3-USGNPs-IR800 reflected light, indicating the attachment and aggregation of Gal3-USGNPs on the surface of the melanoma cells, while the ones which did not expose to the conjugates, showed no reflection (see Figure 13C, D). The bright spots in Figure 13C may be due to the aggregation of some Gal3-USGNPs that are not attached to the melanoma cells. These initial results indicated that contrast-enhanced OCT has a great potential in melanoma detection and differentiation of it from nonmelanoma cells.
Figure 13. Evaluation of nanoparticle aggregation on the melanoma cell surface. Bright-field image (×40) of 4% formaldehyde fixed melanoma cells in cell culture disk mixed, (A) with dye-AB-USNPs, (B) without; (C) fluorescence microscopy images of the sample in (A); (D) fluorescence microscopy images of sample in (B); (E) absorbance and emission spectrums of IR800 dye as well as the accepting spectrum of the optical filter (ET775).

To study the accuracy ((true positive + true negative)/(true positive + false negative + false positive + true negative)) of OCT in differentiating melanoma and nonmelanoma cells, we imaged
both PCS-200-011 and B16F10 cells, with and without contrast enhancement. The accuracy without enhancement was 87.5% while increased 10% when contrast enhancement was utilized with 30 minutes incubation time; increasing the incubation time or/and concentration of the conjugates may improve the accuracy which is needed for \textit{in vivo} experiments.

3.4 Conclusion

We demonstrated the ability of the SMART-OCT imaging for differentiating melanoma and nonmelanoma cells with a high accuracy. And we believe the combination of SMART-OCT imaging with some OCT radiomic feature extraction methodology [172], could increase the specificity and sensitivity of OCT for tumor diagnosis.
Chapter 4 MONITORING THE TOPICAL DELIVERY OF ULTRASMALL GOLD NANOPARTICLES USING OPTICAL COHERENCE TOMOGRAPHY

4.1 Introduction

In dermatology, the early detection of pre-malignant and malignant lesions is crucial for disease detection and treatment [140]. Currently, the excisional biopsy is the gold standard for skin cancer diagnosis. However, since excisional biopsy is invasive, susceptible to sampling errors, and can cause patient discomfort, the development of imaging-based non-invasive diagnostic techniques with high sensitivity and specificity is gaining attention [173, 174].

Among all the imaging modalities, optical coherence tomography (OCT) has shown great promise for skin cancer detection [3, 114]. OCT is a non-invasive and FDA approved optical imaging technique that enables micrometer-scale imaging [139]. It is particularly promising for dermatological applications because it provides real-time 2-D and 3-D cross sectional images of skin subsurface structures [175, 176]. OCT is based on the principle of Michelson interferometry and uses low coherent near infrared light at the wavelength of about 1300 nm [177]. However, the primary limitation for using OCT in early cancer detection is the low contrast between normal and neoplastic tissue.

To overcome this limitation, the contrast agents such as nanoparticles have been developed and explored [178]. Various nanoparticles have been designed and invented to possess many beneficial characteristics, such as transport vehicles for drug delivery [179, 180], contrast agent for imaging [181, 182], and photosensitizer for photodynamic therapy [183, 184]. Specifically, Gold nanoparticles (Au NPs) have been used as contrast agents for a variety of applications [185, 186]. Au NPs have lots of advantages as in vivo OCT contrast agents, such as biocompatibility, and surface functionalization with additional biomolecules [187-189]. Au NPs are effective as OCT contrast agents because of their flexible optical properties which are tailored by modifying
the size and shape of nanoparticles [190-192]. To ensure sufficient OCT signal from Au NPs and to improve sensitivity, it is crucial to deliver the Au NPs efficiently, evenly, and specifically to the targeted area [193]. For dermatological applications, therefore, a topical administration is preferable.

In our study, a commercialized OCT was used to monitor the diffusion of sub-resolution ultrasmall 10 nm gold nanoparticles by topically applying the particles on pig ear skin which mimics human skin. We hypothesized that DMSO and ultrasonic force would improve the penetration and distribution of Au NPs and thus enhance topical delivery. The intensity of OCT images over 40 minutes at different depths of skin was analyzed and compared.

4.2 Dimethyl sulphoxides (DMSO)

Dimethyl sulphoxides (DMSO) is one of the most prevalent penetration enhancers. It is a powerful aprotic solvent which hydrogen bonds with itself rather than water. DMSO helps facilitate diffusion through the stratum corneum, activates the formation of deposits in the dermis, and facilitates transport into the local blood vessels. The stratum corneum acts as the major barrier to any active substance delivered from the surface designated for deeper layers of the skin. The four factors that influence penetration of any given material through any membrane are as follows: (a) membrane's diffusion coefficient [194], (b) the agent's concentration in the vehicle [195], (c) the partition coefficient between the membrane and the vehicle [196], and (d) the membrane thickness [197]. Penetration enhancers are intended to influence all or some of these factors without causing permanent structural or chemical modification of the membrane. Since modifying the thickness of the membrane is not practical, most penetration agents, including DMSO, are used to reversibly modify the factors (a) to (c). DMSO increases diffusion through the stratum corneum by disruption of the barrier function in the stratum corneum layer, aprotic interactions with
intercellular lipids, and reversible distortion of lipid head groups which generate a more permeable packing arrangement. DMSO also contributes to partitioning by forming solvent microenvironments within the tissue. Lastly, DMSO has a solubilizing effect on less soluble agents, improving penetration by delivering a higher concentration to the membrane barrier [198, 199].

4.3 Sonophoresis

Sonophoresis is a method that disrupts the stratum corneum lipid bilayer by facilitating the delivery of molecules with low molecular weight through the skin. It increases the fluidity of lipids and thus intracellular drug permeation. Sonophoresis is typically used in the form of low frequency pressure waves < ~100 kHz. Ultrasound creates microbubbles which collapse at the surface of the stratum corneum and produces many shock waves or acoustic microjets rendering the skin permeable [200, 201]. Other mechanisms have been reported, including thermal effects and radiation pressures that further add to the sonophoretic permeation enhancement [202].

4.4 Materials and Methods

4.4.1 OCT system setup

A commercialized high resolution swept-source OCT (SS-OCT) scanner with handheld probe from Michelson Diagnostic™ was used (Figure 14A). The imaging system is FDA approved for dermatological study, has a central wavelength of 1305 nm, and a bandwidth of 30 nm. The A-line rate is 10 kHz, and the frame rate is 6 fps. The B-scan images obtained with this OCT system have a size of 6 × 2 mm with lateral and axial resolutions of 7.5 and 10 μm, respectively. The penetration depth of the system was measured as 1.5 mm in healthy human skin.

4.4.2 Nanoparticles delivery on ex vivo pig skin

Pig ear skin was used due to its similarity to the human skin with particular emphasis on the epidermal structure, thickness, and the epidermal/dermal junction. The pig ear was purchased from
a local slaughterhouse, the skin was removed, and the hair was carefully shaved prior to the experiments. 2 μL of 10 nm Au NPs (NanoHybrids. Inc.) was topically administered on top of the pig skin. DMSO was used as a chemical penetration enhancer for transdermal drug delivery, and the pig skin was treated with it (#472301, Sigma Aldrich Inc.) prior to Au NP application. 200 μL DMSO was applied and rubbed on pig skin for 3 - 5 minutes before adding the nanoparticles on top of that. Next, the sonophoretic method was used. After the Au NPs topically dropped on the skin, 1 MHz sonicator (Sonifier 250, Branson) applied ultrasonic force by placing the sonicator tip about 2 mm and 45 degree away from the Au NPs drop surface (Figure 14A, iii and iv).
Figure 14. Optical coherence tomography (OCT) imaging setup and OCT images of pig ear skin. (A) The OCT machine from Michelson Diagnostic\textsuperscript{TM} (i) and imaging setup for Au NPs topical delivery with (iii, iv) and without (ii) sonophoresis; (B) the OCT images of skin with a drop of Au NPs on top; (C) the OCT images of skin after applying Au NPs at 0 min (i, iii, v) and 40 min (ii, iv, vi) for no DMSO treatment and sonophoresis (i, ii), using DMSO treatment only (iii, iv), and using DMSO treatment and sonophoresis (v, vi). Au NPs, gold nanoparticles; HSL, high speed scanning laser.
4.4.3 Image acquisition and processing

The OCT probe was stably fixed using a clamp to acquire images from the same area of pig skin, and diffusion of the nanoparticles was monitored using OCT. The first set of OCT images were taken when the nanoparticles disappeared on the skin surface and were completely diffused inside the skin. The second set of OCT images were taken 40 minutes later. The intensity of these two sets of OCT images was analyzed over 13 different depths and analyzed using Matlab.

The OCT images were analyzed over 13 different depths and at two different time points. In terms of depth, the images started from the top surface of the skin to 0.565 mm (130 pixels) downwards with 0.044 mm (10 pixels) steps. The two time points were the disappearance of the Au NPs on the skin surface, and 40 minutes after that.

To compensate for the effect of laser energy drop over time, the OCT signal was calibrated at 40 minutes to the time point at which we collected the first set of images. Six areas of pig skin without DMSO treatment and Au NPs administration were used for compensation. The mean OCT signal intensity over 13 different depths was calculated at those two different time points. The calibration factor for each depth was calculated as the intensity at 40 minutes divided by the intensity at the initial time point. This calibration factor was used for all the data at 40 minutes to correct the laser energy drop. For each depth, a region of interest (ROI) was chosen with the size 400 by 10 pixels. The mean intensity of the ROI was calculated and normalized as OCT signal intensity. The standard deviation was calculated and normalized as the homogeneity of the ROI.

4.5 Results

The OCT monitored the Au NPs topical diffusion in real time. The results in Figure 14B clearly show the Au NP drop on the pig skin surface. The first set of OCT images ($t = 0$ minute) were acquired when the Au NPs were completely diffused inside the skin surface. The OCT probe
was fixed during the entire imaging period to image the same location after 40 minutes ($t = 40$ minutes). The skin structure can be clearly seen up to 0.565 mm (130 pixels) below the top surface. Therefore, we analyzed the OCT signal intensity of pig skin at 13 different depths with the steps of 0.044 mm (10 pixels). Each depth is considered as one ROI with a width of 400 pixels. The intensity of each ROI was expressed as the mean intensity value of all the pixels. The mean intensity values were plotted over 13 different depths for two different time points $t = 0$ minute (blue line) and $t = 40$ minutes (red line) (Figure 15A, B).

The OCT signal decreased over depth for two time points (Figure 15A, B). In Figure 15A, i, without adding any Au NPs, the OCT signal dropped over time for the same location, especially at depths 0.05 mm to 0.25 mm. Thus, the signal at 40 minutes for each depth was calibrated to reach the OCT signal at initial time point ($t = 0$ minute) (Figure 15A, ii) and the calibration factors were calculated and later applied to the corresponding depth.

First, the spontaneous diffusion of Au NPs on the skin surface was tested without any enhancers. 2 μL Au NP was added on the skin surface. After the calibration, the OCT signal at 40 minutes was still lower than the signal at $t = 0$ minute (Figure 15B, i). On the OCT images, it is important to note that the intensity dropped at 40 minutes (Figure 14C, ii) compared to the OCT image at 0 minute (Figure 14C, i).

Next, two non-invasive approaches, namely DMSO and sonophoresis, were tested. DMSO was applied and rubbed on the skin surface before adding the Au NPs. After the calibration, the OCT signal at 40 minutes did not decrease significantly (Figure 15B, ii). The OCT signal at depth 0.044 mm and 0.088 mm dropped at 40 minutes compared to the signal at $t = 0$ minute. For the following depth below 0.088 mm, however, the OCT signal at 40 minutes is higher than the signal at $t = 0$ minute.
Then, the two methods were combined. DMSO was rubbed on the skin surface first, the Au NPs were dropped on top of skin, and then sonophoresis was administered until the Au NPs were completely diffused inside the skin. Following calibration, the OCT signal at depth 0.044 mm and at 40 minutes is slightly lower than the signal at t = 0 minute (Figure 15B, iii). For the depths below 0.044 mm, the OCT signal at 40 minutes is much higher than the signal at t = 0 minute (Figure 15B, iii). On the OCT images, the intensity of the skin increased at 40 minutes (Figure 14C, vi) compared to the OCT image at 0 minute (Figure 14C, v). The use of sonophoresis on the DMSO treated skin greatly enhanced the results (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>NP alone</th>
<th>NP + DMSO</th>
<th>NP + DMSO + US</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1144</td>
<td>0.5612</td>
<td>1.0628</td>
</tr>
</tbody>
</table>

Table 1. The nanoparticle diffusion rate over time. NP alone diffused at 0.1144, while adding DMSO increased the relative rate to 0.5612 and adding both DMSO and sonophoresis (US) increased the diffusion rate to 1.0628. NP: nanoparticles, DMSO: dimethyl sulfoxide, US: ultrasound.

Additionally, the homogeneity of skin with and without enhancing methods was compared. In Figure 15C, i, the homogeneity of skin when using Au NPs alone decreased over time. However, the homogeneity of skin was almost unchanged over time (Figure 15C, ii, iii) when the DMSO and sonophoresis were applied as enhancers.
Figure 15. The optical coherence tomography (OCT) signal change over depth at different time points. (A) The OCT signal at two different time points before (i) and after (ii) calibration; (B) The OCT signal change over depth for no DMSO treatment and sonophoresis (i), using DMSO treatment only (ii), and using DMSO treatment and sonophoresis (iii); (C) The homogeneity of pig skin at two different time points for no DMSO treatment and sonophoresis (i), using DMSO treatment only (ii), and using DMSO treatment and sonophoresis (iii).
4.6 Discussion

Despite a growing number of current studies involving nanomedicine in dermatology and the cosmetic industry, the penetration and distribution of nanoparticles inside the skin requires further investigation. Additionally, efficient delivery of Au NPs is crucial to obtain a sufficient signal in a variety of applications. Many imaging-based techniques including light microscopy, confocal microscopy, and electron transmission microscopy have been used to evaluate the penetration and distribution of nanoparticles. However, these techniques are limited in penetration depth and field of view.

Optical coherence tomography can be used to visualize nanoparticle diffusion real time inside the skin. The OCT signal for the same location was observed to drop over approximately 40 minutes because of reduced OCT laser energy and dehydration of the skin. Since skin is excised and exposed in the air during surgery, and the imaging period lasts 40 minutes, this dehydration can result in an OCT signal drop. Six different areas from the skin without DMSO and Au NPs were used in order to compensate for this effect. The calibration factors were calculated for each depth and were applied to the corresponding depth in our analyses.

It was found that the addition of DMSO and sonophoresis increased the diffusion rate and penetration of Au NPs in skin. Over time, the OCT signal showed an increase after using DMSO and sonophoresis, whereas the Au NPs alone showed an OCT signal decrease. These findings indicate that the combination of DMSO and sonophoresis may be an effective method for nanoparticle delivery. Additionally, the topical administration can minimize the concerns of toxicity, drug overdose, and frequent drug administration. Advances in nanotechnology such as these have consistently expanded the field of nanomedicine in various applications.
4.7 Conclusion

The present study demonstrates an effective approach to improve the topical delivery of gold nanoparticles and enhance OCT image contrast. The combination of DMSO and sonophoresis was demonstrated to be an effective method to improve the penetration and diffusion rate of gold nanoparticles in skin. These findings may provide a new paradigm for enhancing \textit{in vivo} OCT images and, ultimately, improve the capability of OCT in the early diagnosis of cancer.
5.1 Introduction

Skin consists of two layers: epidermis and dermis. The stratified epidermis is primarily made of keratinocytes. Additionally, the melanin producing cell melanocytes are also in this layer. And melanin determines the color of skin and hair. Dermis, the underlying layer of epidermis, also has two layers: the upper layer is papillary dermis, and the deeper one is reticular dermis [203]. Dermis is made of collagen fibers and elastic fibers. In the papillary dermis, the collagen fibers are thinner, more loosely arranged and wavy. Whereas, in the reticular dermis the collagen fibers are thicker, denser, and more horizontal [204]. A younger skin is full of collagen and elastin fibers that work together like a mesh to support its firmness and hold its shape. As one ages, these collagen and elastin fibers begin to deteriorate, causing the appearance of fine lines, wrinkles and sagging on the surface of the skin [20]. Some factors impact the process of aging are smoking, sun UV light, diet, sleep, dehydration, gravity, pollution, and climate [46].

Various imaging techniques have been introduced to aid skin disease diagnosis, such as optical coherence tomography (OCT), multispectral imaging, photoacoustic imaging, polarized light imaging, confocal scanning laser microscopy, and ultrasound imaging [205-210]. Among all these imaging modalities, OCT holds great potential and is gaining use and acceptance [116, 211]. It has the added benefit of providing higher resolution images due to using low coherent near infrared light around the wavelength of 1300 nm. OCT is a non-invasive and high resolution medical imaging technique that allows visualization of different skin layers including epidermis and upper dermis, as well as the superficial vasculature of the skin [212-214].
OCT can display the anatomical structure of skin, and visual examination of OCT images could assist clinicians to diagnose many skin diseases. Moreover, OCT images also contain information that can be extracted using some post-image processing algorithms to aid in diagnosis [161, 215, 216]. Tissue attenuation coefficient is one of the extractable information. The attenuation coefficient is an optical property that correlates with how easily light penetrates a tissue after taking into account the light scattering and absorption [203, 217, 218]. A large attenuation coefficient indicates a quick exponential decrease of incident light with depth which happens in highly scattering or absorbing tissue types, and small attenuation happens when the tissue sample has low absorption and weakly scattering properties [219, 220]. Therefore, the attenuation coefficient could give us a better understanding of the skin composition and condition.

The optical attenuation coefficient has been used as a quantitative measurement of tissue optical properties. It reflects the optical properties of different tissues or the tissues with different abnormalities. The depth-resolved attenuation coefficient of OCT signal is one extension of OCT. Vermeer et al. developed a model-based method to spatially resolve the attenuation coefficient for each pixel from an ocular OCT image. It was shown that this method can effectively remove common OCT image shadowing artifacts [221]. Amaral et al. developed a new model to estimate the depth-resolved attenuation coefficient using OCT for thin and membrane-like structure, i.e. tympanum. The obtained OCT image is based on the tissue optical properties instead of intensity, which has additional benefit for tissue differentiation [222]. Liu et al. developed an optimized depth-resolved estimation method to estimate the attenuation coefficient from OCT signals. They showed that this optimized method can calculate attenuation coefficient at any depth, even though the light is completely attenuated. They applied this method in OCT angiography to detect cerebral damage [223]. Since the attenuation coefficient reflects tissue properties at different depths, it has
been used for OCT image segmentation, such as retinal pigment epithelium, choroidal stroma, and lamina cribrosa [224, 225]. In addition, the attenuation coefficient measured from OCT signal has also been used for tissue discrimination. Freek et al. measured the intrinsic attenuation coefficient of atherosclerotic tissue. They proposed that such quantitative analysis of OCT image can be used to discrimination between plaque constituents and different plaque types [226]. Xu et al. used the attenuation coefficient as a quantitative OCT feature to enhance the differentiation between fibrous, lipid and calcific atherosclerotic plaques [227]. Xu et al. showed that the viscous properties during blood coagulation is related to the changes of attenuation coefficient. And OCT can be used to monitor the blood coagulation status by measuring the viscosity-related attenuation coefficient [228]. Ali et al. developed an enhancement algorithm for OCT skin images by using skin layer detection and attenuation coefficient compensation. In this study, the attenuation coefficients of different skin layers are estimated based on the exponential decay of light optical power. The results show that the attenuation compensation algorithm can increase the SNR and image contrast [229].

We propose that the attenuation coefficient will be influenced by differences in skin type (due to different amounts of melanin in the tissue [230]) and age (due to the integrity and density of collagen and elastin fibers [231]). By analyzing the attenuation coefficient, we develop a baseline of this property in different skin types and at different ages which may assist in correct utility of analyzing this property in the diagnosis of various skin diseases, including skin cancer. In our study, we quantified the skin attenuation coefficient for different skin type and different ages using OCT and studied the change of attenuation coefficient between epidermis and dermis, and between head and hip as well.
5.2 Materials and Methods

5.2.1 Participants

Individuals with healthy skin on the forehead between the ages of 1 and 100 were eligible to participate in the study (see Table 2 for the distribution of participants based on age and skin type). Participants are asked about their response to sun exposure regarding tanning and burning to determine their Fitzpatrick skin type. Fitzpatrick skin photo type is a numerical classification system based on a person's skin color, degree of sun burning and tanning, which is determined by the amount of melanin pigment in their skin [232]. The participants were categorized in 3 different age groups, from <30, 31 to 60, and >60. The participants were also divided into 3 different skin type groups based on their Fitzpatrick skin type: I & II, III & IV, and V & VI. OCT images were collected from their forehead and hip. The attenuation coefficient from both epidermis and dermis were calculated and averaged separately.

Inclusion criteria were as follows: (i) age 18 years or older; (ii) able to provide written informed consent prior to any trial related procedure. The exclusion criteria were as follows: (i) failure to give written informed consent; (ii) skin not intact (e.g., open sores, ulcers, bleeding); (iii) lesion containing foreign matter (e.g., tattoo ink, splinter, marker).

All the participants were recruited at the Oakwood Hospital. All the imaging procedures and experimental protocols were approved and carried out according to the guidelines of the Wayne State's Institutional Review Board (IRB #: 061818MP4E). Written informed consent was obtained from all subjects before enrollment in the study. The patients neither refused to sign the written informed consent nor were excluded from the study.
<table>
<thead>
<tr>
<th>FITZPATRICK TYPE</th>
<th>AGE</th>
<th>TOTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I and II</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>III and IV</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>V and VI</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>

**Table 2. Distributions of participants.**

### 5.2.2 OCT system specifications

We use a swept-source OCT system (SS-OCT) (VivoSight, Michelson Diagnostic™ Inc., United Kingdom) for this study. The swept source laser is with the central wavelength of 1305±15nm. The lateral and axial resolutions are 7.5 μm and 5 μm, respectively. The scan area of the OCT system is 6 mm (width) × 6 mm (length) × 2 mm (depth).

### 5.2.3 Attenuation coefficient calculation from OCT images

Loss of light in tissue can be caused by absorption, scattering, or a combination of both. When propagating through a medium, the irradiance of the light beam follows the equation: $I(z) \propto e^{-\mu_a z}$, where $\mu_a$ is the attenuation coefficient, and $z$ is the depth. Fitting an exponential curve to the averaged OCT A-line (or sometimes smoothed averaged A-line) in a region of interest (ROI) is a commonly used method to calculate the attenuation coefficients from OCT images [233]. For the fitting, a built-in function “fit” in Matlab was used, in which the Levenberg–Marquardt least-square method was used. This function adjusts the attenuation coefficient in the equation above in order to obtain a curve that best fits the (smoothed) averaged A-lines.

Three sets of OCT images from each participant were chosen for analysis. In each set, five different ROIs were selected from the epidermis and five from the dermis. The values from five ROIs (in all three sets) were averaged to get the final attenuation coefficient of epidermis or dermis of each participant.
5.2.4 Statistical analysis

Descriptive statistics such as mean and standard deviations were provided for continuous endpoint. Comparison between groups of patients by age and skin type were made by two-way ANOVA. Repeated measurements from different locations (forehead vs. hip) and skin layers (epidermis vs. dermis) were averaged for each patient for the ANOVA analysis. If the global tests were significant, pairwise comparison tests were performed with Tukey HSD adjustment for multiple testing. Comparison between forehead and hip or epidermis and dermis were performed with paired T test. All statistical analysis was carried out at a 5% level significance. The statistical analysis was carried out using SPSS software (version 17.0; SPSS, Chicago, IL, USA).

5.3 Results

As shown in Figure 16, skin structures, such as epidermis, dermis, stratum corneum (SC), and dermoeidermal junction (DEJ), are clearly discernible on OCT images. The averaged A-line from the selected ROI is calculated and plotted as a function of pixels in axial direction. The first order linear fit is applied to the smoothened average A-line.
Figure 16. Attenuation coefficient calculation from OCT images. (A) OCT image of a lateral hip skin of 37-year-old male, with skin type II, demonstrating the skin layers including epidermis and dermis; Fitting 1st order polynomial equation on the smoothened averaged OCT A-line in (B) epidermis, and (C) dermis. SC: Stratum corneum (SC); DEJ: Dermoepidermal junction

The OCT images from three different age groups, dark vs. light skin, and head vs. hip were shown in Figure 17. It is difficult to tell the skin structural differences among three different age groups. Comparing the structural differences between light and dark skin, the DEJ for dark skin appears less obvious. However, comparing the OCT images of head and hip, we noticed that the vasculatures were more apparent on the hip OCT images than on the head ones.
Figure 17. OCT images of head and hip for different age groups and skin tone. (A) < 30 age group, (B) 31 to 60 age group, (C) > 60 age group.

Paired sample t-test revealed there is a significant difference when comparing the attenuation coefficient between epidermis and dermis, both in head and hip (Figure 18A, B). But there is no
significant variation in attenuation coefficient within the epidermises at head or hip (Figure 18C, D). The error bars indicate 95% confidence interval.

![Figure 18](image)

**Figure 18.** Attenuation coefficients for (A) epidermis and dermis at head; (B) epidermis and dermis at hip; (C) epidermis at head and hip; (D) dermis at head and hip. The attenuation coefficient of epidermis is higher than that of dermis. P<0.01 for paired t-test. The attenuation coefficient of epidermis or dermis at head and hip shows no statistically significant difference. P=0.976 for paired t-test.

Two-way ANOVA was performed for four groups, namely head epidermis, head dermis, hip epidermis, and hip dermis, to compare the difference of attenuation coefficient among three different skin types. The results were shown in Table 3. The ANOVA test revealed there is no significant variation for attenuation coefficient across age groups and skin types in dermis (Figure
19B. D). There is a significant difference of attenuation coefficient in epidermis between three different age groups (Figure 19A, C). For epidermis only, the skin attenuation coefficient increases with advancing age (Figure 19A, C). And there is a significant variation at head epidermis across age groups and skin types (Figure 19A). And there is a significant variation of attenuation coefficient at hip epidermis across age groups, but not skin types (Figure 19C).

Furthermore, the Post Hoc test revealed that there is no difference in attenuation coefficient between skin type I & II, and III & IV (Figure 19A, red and blue line), the attenuation coefficient in skin type V & VI is significantly different from the other two skin types as shown in Figure 19A (green line). And the skin type V & VI have a lower attenuation coefficient than the other skin types.
<table>
<thead>
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<td></td>
<td>4.452</td>
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<td>Skin type</td>
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<td>.695</td>
<td>0.501</td>
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<tr>
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<td></td>
<td>.794</td>
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<td>0.455</td>
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<tr>
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<td>Epidermis</td>
<td>Skin type</td>
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<td>1.613</td>
<td>0.205</td>
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<td></td>
<td>3.452</td>
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<tr>
<td>Total</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td>1.506</td>
<td>0.227</td>
</tr>
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</table>

Table 3. Two-way ANOVA for three different age groups and skin types. $P < 0.05$
Figure 19. The mean attenuation coefficient of forehead and hip skin of different age groups and skin types. (A) head epidermis, (B) head dermis, (C) hip epidermis, (D) hip dermis. The error bars indicate 95% confidence interval. $P < 0.05$

5.4 Discussion

The light transport through skin is determined both by the scattering effects of fibrous proteins such as collagen, and by the absorbing effects of chromophores such as melanin and hemoglobin [219, 234, 235]. The attenuation coefficient is an optical property of biological tissue that indicates how quickly the OCT signal intensity drops over depth.
We compared the attenuation coefficient of the skin from the forehead and lateral hip since these areas represent photo-exposed and photo-protected skin, respectively [236]. We observed a significant difference in the attenuation coefficient comparing epidermis and dermis for all groups (Figure 18A, B). Regardless of the age differences, we noticed that there was no significant difference of attenuation coefficient between the forehead and hip (Figure 18C, D).

First, this is because the structure of the epidermis and dermis are quite different, with the epidermis composed of a structured layering of keratinocytes and the dermis composed of larger collagen and elastin fibers. The arrangement, size, and shape of these structures results in different interactions with light and results in different attenuation coefficients.

Furthermore, the absorption of the light in epidermis is mainly caused by the melanin in melanosomes in epidermis. The amount of melanin is about 10 times higher in darkly pigmented skin (skin type V and VI) than light pigmented skin (skin type I and II) [237, 238]. Unlike epidermis, dermis is perfused with blood. Hence, the absorption of the light in dermis is mainly caused by the hemoglobin in blood [239, 240]. The absorption coefficient of melanin is much higher than that of hemoglobin [241-243]. This explains that the epidermis has much higher attenuation coefficient than the dermis as we shown in Figure 18. Campbell et al. also showed that epidermis has higher attenuation coefficient than the dermis using 3D Monte Carlo modelling [244].

As light penetrates a turbid medium like skin, multiple scattering events take place. The OCT system uses a super luminescent diode as a light source with 1305 nm central wavelength. As light penetrates deeper, it gradually loses spatial coherence because of the scattering of different cellular structures such as cellular organelles or collagen fiber [245, 246]. The size of cellular organelles is less than the wavelength of light, which causes the Rayleigh scattering of light [247, 248].
Whereas the size of collagen fiber is greater than the wavelength of light, causing the Mie scattering of the light [249]. The scattering behavior of skin is accounted for by the combination of Rayleigh and Mie scattering [250]. However, the scattering behavior is dominated by Mie scattering from collagen fibers above 650 nm [251].

In the epidermis, increasing age was associated with a significant increase in the attenuation coefficient. As age increases, the skin becomes more dehydrated, and the amount of collagen and elastin will decrease in dermis [252]. Moreover, UV exposure begins to damage the structural integrity of skin [253]. Skin cell renewal process starts to slow down. The skin appears thinner due to the dehydration and loss of lipids barrier. Studies have shown that dehydration causes scattering particles like collagen and organelles to become more densely packed and reduces scattering in soft tissue [254-256]. Therefore, the OCT signal intensity dropped quickly in the aged group, and the aged group has a higher attenuation coefficient.

In evaluating the dermis, we found no significant difference in the attenuation coefficient across age groups and skin types. There are changes seen in the dermis with both aging and chronic sun exposure. It is possible that these changes do not result in a detectable change in attenuation coefficient in the dermis. Alternatively, our sample size may have been too small to detect a change.

The color of skin is influenced by several factors: such as melanin, carotene, and hemoglobin. The amount of melanin varied in different skin types. Melanin is produced by specialized skin cells called melanocytes in the lower layers of the epidermis and is contained inside a melanosome. The melanin is transferred into the keratinocytes via a cellular vesicle called a melanosome. As shown in Figure 19 A and C, dark skin has lower attenuation coefficient. This is more likely due to the melanosome being evenly distributed, whereas in light skin the melanosome forms clumps [257]. The clumps of melanosomes will increase the random scattering events of light. But in dark
skin, without the melanosome clumps, the light can propagate in forward direction. Moreover, since the dark skin has more compact structure, the intensity changes on OCT images appear not dramatic compared to the light skin [258]. Therefore, the dark skin has lower attenuation coefficient.

In addition, there are differences of attenuation coefficient between light skin and dark skin only at head epidermis not hip epidermis (Figure 19 A and C). This might indicate sun exposure has some effect on skin, compared to sun protected regions.

5.5 Conclusion

Tissue attenuation coefficient is a quantitative measure that has been used in evaluating the health status of tissue in the literature. It is affected by the amount of pigmentation in different races, as well as the changes in collagen fibers in the aged. The attenuation coefficient increased with aging. Furthermore, it was observed that darker skins have a lower attenuation coefficient, especially in the epidermis.
Chapter 6 CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusions

These studies presented here sought to explore some advanced applications for OCT in dermatology. We introduced the application of OCT in four different directions: first, we explored the feasibility of OCT imaging for assisting real-time visualization in skin biopsy; second, we investigated the concept of contrast-enhanced OCT for melanoma detection in an *in vitro* study; third, we tested the capability of monitoring the topical delivery of nanoparticles using OCT; and finally, we looked into extracting quantitative information such as skin optical properties from OCT images.

In chapter 2, we showed that a needle insertion in mouse skin can be monitored using OCT in real-time. The structure of skin and the movement of needle can be clearly seen on the OCT images without any time delay during the procedures. Next, we proved that using the OCT imaging is a reliable technique to delineate the margin of lesion in a test phantom. And it is possible to perform the punch biopsy using OCT hand-held probe attached to a piercing tip.

In chapter 3, we tested the performance of contrast-enhanced OCT in differentiating melanoma cells from non-melanoma cells in an *in vitro* study. In this initial study, we showed that the contrast-agent, Gal3-USGNPs, can differentiate B16 melanoma cells and normal skin keratinocytes *in vitro*.

In chapter 4, we proved that OCT can be used to monitor the topical delivery of nanoparticles on pig skin over time. And the diffusion and penetration of nanoparticles in skin can be improved by applying chemical and physical enhancers such as DMSO and sonophoresis.

In chapter 5, we proved that quantitative information such as skin attenuation coefficient can be extracted from OCT images. We measured and compared the skin attenuation coefficient in the
of forehead and lateral hip, the skin of three different age groups, and the skin of three different Fitzpatrick types. The results showed that epidermis has much higher attenuation coefficient than dermis. And the skin type V & VI have a lower attenuation coefficient than the other skin types.

6.2 Limitations and Future directions

As a fast, safe, and inexpensive imaging, OCT has not been widely adopted by dermatologists. The main challenges are, first, the OCT radiomic features for many skin diseases are still unclear; second, there are no consensus guidelines for diagnosis of skin diseases using OCT; third, it requires extensive training for the clinicians to interpret the information.

Although OCT could not be used as a robust imaging technique for melanoma diagnosis for now, the work described in chapter 3 proposed a potential future direction by using contrast agents to enhance the specificity and sensitivity of OCT imaging. More research is needed to understand the optimal dosage of contrast agents and their incubation time for in vivo applications in the future. Moreover, melanoma biomarkers are a limitation for melanoma diagnosis. Currently, there are no biomarkers that have 100% specificity and sensitivity for melanoma. Future directions may include biomarkers discovery and validation using large cohorts across multi-centers, and analyze combinations of multiple putative markers to identify specific prognostic footprints. In addition, to fully understand the topical delivery of contrast agent, the uptake kinetics of contrast agent through skin should be investigated in future.

Imaging depth is another limitation for OCT. This is due to the limited penetration of light in a turbid media like skin tissue. The penetration depth for the OCT system from VivoSight is 1 to 2 mm, which is sufficient to image the whole epidermis (0.077 to 0.267 mm) and the shallow layer of dermis. However, the thickness of the dermis ranges from 2.1 to 5.9 mm. And as we mentioned in chapter 1, the Stage 3 melanoma will grow vertically into the deeper layers of dermis.
Hence, it is crucial that the imaging system has a higher penetration depth to cover the whole thickness of the dermis. Therefore, to improve the imaging depth, a dual-modality system combining OCT and high frequency ultrasound could be one of the future research directions.
APPENDIX: PRINCIPLES OF OPTICAL COHERENCE TOMOGRAPHY

1. Swept-Source OCT

As illustrated in Figure 1, the light from low coherence source is split evenly into sample arm and reference arm. The backscattered light from the sample mixes and interferes with the returning light from the reference arm in the fiber coupler. Then the interference signal is detected by a photodetector and processed to generate depth-scans called A-scans.

A swept-source OCT (SS-OCT) is used in this research work. The SS-OCT uses a spectral interferometric method that relates the spectral interference signal with the depth profile by Fourier transform [259, 260]. Without moving the reference mirror, the spectral interference signal, \( I_D(k) \), is generated as a function of wavenumber \( k \) to reconstruct the sample reflectivity profile (A-scan).

The recorded A-line intensity collected by the detector is proportional to the modulus-squared time average of the sum of the reference electric field, \( e_r \), and backscattered electric field \( e_s(z_m) \) of each reflector \( m \) at depth \( z \) in the sample [176, 261].

\[
I_D(k) = \eta \left[ (e_r + e_s(z_m))(e_r + e_s(z_m))^* \right] = \eta [e_re_r^* + e_re_s^* + e_s^*e_s + e_s^*] \tag{1.1}
\]

where, \( \eta \) is the detector responsivity.

The amplitudes of electric fields for reference and sample arms were written as:

\[
e_r(k) = \frac{S(k)}{\sqrt{2}} r_r e^{j2kz_r}, \text{ and } e_s(k, z_m) = \frac{S(k)}{\sqrt{2}} \sum_{m=1}^{M} r_m e^{j2kz_m}
\]

where, \( S(k) \) is the spectral density of the light source, \( r_r \) is the electric field reflectivity of the reference mirror, \( r_m \) is the electric field reflectivity of reflector \( m \) in sample, and \( M \) is the total number of reflectors along depth \( z \) in sample.

Equation 1.1 can be simplified into
\[ I_p(k) = \eta \frac{S(k)}{4} \left[ (R_r + \sum_{m=1}^{M} R_m) \right] \quad \text{“Continuous term (DC)”} \]

\[ + 2 \sum_{m=1}^{M} \sqrt{R_r R_m} \cos(2k(z_r - z_m)) \quad \text{“Cross-correlation term”} \]

\[ + \sum_{m \neq p=1}^{M} \sqrt{R_m R_p} \cos \left(2k(z_m - z_p)\right) \] \quad \text{“Auto-correlation term”}

where, \( p \) is a reflector in sample that is different from reflector \( m \), \( z_p \) is the position of reflector \( p \) in sample, \( R_r \) is the power reflectivity of the reference mirror, and \( R_m \) and \( R_p \) are the power reflectivity of reflector \( m \) and \( p \) in sample.

The continuous term is an optical pathlength-independent offset to detector current. The cross-correlation term is the desired interferometric signal of OCT images, which is proportional to the square root of power reflectivity of the reference mirror and each reflector. The auto-correlation term is the interference signal between different reflectors that brings noise and artifacts.

2. Axial and Lateral Resolution in OCT System

An A-scan represents the reflectivity profile of the sample over depth at a fixed lateral position. And the axial resolution of OCT imaging system, also known as round trip coherence length, \( l_c \), is defined as,

\[ l_c = \frac{2 \ln{(2)} \lambda_0^2}{\pi \Delta \lambda} \]

where, \( \lambda_0 \) is the central wavelength of the incident light source, \( \Delta \lambda \) is the wavelength bandwidth.

Multiple A-scans are acquired by scanning the light exiting the sample fiber in lateral direction. The cross-sectional B-scans are formed by assembling the A-scans into a two-dimensional image. And the lateral resolution, \( \delta_x \), is defined as,
\[ \delta_x = 0.37 \frac{\lambda_0}{NA} \]

where, \( \lambda_0 \) is the central wavelength of the incident light source, \( NA \) is the numerical aperture of the objective lens inside the optical probe.

3. Error Rate in OCT

Holmes et. al. measured the FWHM spot diameter of the OCT system in air. The measured FWHM was 9.6 µm in air, while the calculated theoretical value is 8.4 µm, which gives us a 12.5% error rate [262]. The error rate in OCT imaging needs to be considered and compensated in future clinical applications to ensure high accuracy during the dermatological procedures and measurements.
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ABSTRACT

THE ADVANCED APPLICATIONS FOR OPTICAL COHERENCE TOMOGRAPHY IN SKIN IMAGING

by

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December 2021

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Optical coherence tomography (OCT), based on the principle of interferometry, is a fast and non-invasive imaging modality, which has been approved by FDA for dermatologic applications. OCT has high spatial resolution up to micrometer scale compared to traditional ultrasound imaging. In addition, OCT can provide real-time cross-sectional images with 1 to 2 mm penetration depth, which makes it an ideal imaging technique to assess the skin micro-morphology and pathology without any tissue removal. Many studies have investigated the possibilities of using OCT to evaluate dermatologic conditions, such as skin cancer, dermatitis, psoriasis, and skin damages. Hence, OCT has tremendous potential to provide skin histological and pathological information and assist differential diagnosis of various skin diseases. In this study, we used a swept-source OCT with 1305 nm central wavelength to explore its advanced applications in dermatology.

This dissertation consists of four major research projects. First, we explored the feasibility of OCT imaging for assisting real-time visualization in skin biopsy. We showed that OCT could be used to guide and track a needle insertion in mouse skin in real-time. The structure of skin and the movement of needle can be clearly seen on the OCT images without any time delay during the...
procedures. Next, we tested the concept of performing the punch biopsy using OCT hand-held probe attached to a piercing tip in a phantom. We proved that using the OCT is a reliable technique to delineate the margin of lesion in phantom. And it is possible to perform the punch biopsy with the OCT probe. Second, we tested the performance of contrast-enhanced OCT in melanoma detection in an in vitro study. Melanoma is the most lethal type of skin cancer. Early detection could significantly improve the long-term survival rate of patients. In this initial study, a contrast agent (Gal3-USGNPs) is developed by conjugating melanoma biomarker (Gal3) to ultra-small gold nanoparticles (USGNPs). We showed that the contrast agent can differentiate B16 melanoma cells from normal skin keratinocytes in vitro. To avoid systemic administration of USGNPs, the third project continues to explore the enhanced topical delivery of USGNPs. In this study, we used OCT to monitor the topical delivery of nanoparticles on pig skin over time. And the diffusion and penetration of USGNPs in skin can be improved by applying chemical and physical enhancers such as DMSO and sonophoresis. Finally, in addition to image the cross-sectional structure of skin, we also aim to extract quantitative information from OCT images. The skin optical properties such as attenuation coefficient can be measured from OCT images. We measured and compared the skin attenuation coefficient in the skin of forehead and lateral hip, the skin of three different age groups, and the skin of three different Fitzpatrick types. The statistical analysis showed that epidermis has much higher attenuation coefficient than dermis. And the skin type V & VI have a relatively lower attenuation coefficient than the other skin types.

These studies could aid the detection of skin cancer using imaging techniques and provide some new insights into the future applications of OCT in dermatology.
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