Objective, quantitative assessment of burn scars with optical coherence tomography

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Abstract

Skin scarring arises from a wide range of traumatic injuries and can lead to significant physical discomfort, such as pain, itching and reduced mobility, and psychological problems for patients. There is a large body of work on treatment methods for preventing scarring, and improving the healing of scars. However, the outcomes of these methods vary greatly among patients. The understanding of scar responses to treatment is limited partially by the lack of robust scar assessment techniques. The aim of the research reported in this thesis is to develop an objective, quantitative scar assessment method to advance our understanding of scar characteristics and responses to treatment, using a high-resolution, non-invasive, three-dimensional optical imaging technique, called optical coherence tomography (OCT).

Scars, especially pathological scars, commonly show the abnormalities of an over proliferation of blood vessels and excess collagen growth. The research presented in this thesis first focuses on the development of OCT for clinical assessment of the scar vasculature. A method based on OCT speckle decorrelation was developed to image and automatically quantify the diameter and area density of the cutaneous microvasculature in scars. This method was applied to burn scar patients and prolific larger blood vessels were observed in the pathological hypertrophic scars as compared to normal skin. The feasibility of this method for longitudinal assessment was investigated by tracking the wound healing process in injured human skin. The injured skin showed an elevated level of vasculature at the beginning and a decrease to normal levels at the end of the healing process. The vasculature assessment method reported in this thesis provides not only an objective assessment of the scar vasculature, but also the basis for removing (masking) the effects of vasculature in the following assessment of scar collagen.

The second part of the work is on the development of OCT for clinical assessment of the collagen network in scars by using OCT to measure their optical properties using two separate approaches. The first approach quantifies the optical attenuation coefficient by fitting the corrected OCT A-scans to a single-scattering model. The second approach calculates the optical birefringence of scar tissue by measuring the rate of change of phase retardation with depth in polarisation-sensitive OCT scans. The vascular masking technique was incorporated into both approaches to remove the artefacts caused by the vasculature. The measured optical properties in both
instances were visualised as *en face* parametric images, showing lower attenuation coefficient and higher birefringence values for scars than for normal skin.

Finally, scar assessment with OCT was investigated for two different clinical applications. The first study applied OCT assessment of vasculature to the longitudinal monitoring of burn scars following fractional laser treatment. The scar tissue showed different responses to the treatment according to the scar type: decreased and increased degree of vasculature following treatment, respectively, in immature and mature scars. The second study investigated the feasibility of OCT for delineating scar boundaries by using another OCT-based method, optical palpation, to measure the stiffness at the surface of scar tissue. Case studies demonstrated the ability of OCT optical palpation to provide contrast between scar tissue and the surrounding normal skin.

The OCT scar assessment methods presented in this thesis provide a comprehensive measurement framework for both vasculature and collagen assessment in burn scars, and establish a basis for future clinical application of objective, quantitative OCT-based methods in scar assessment.
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Statement of Contribution

This thesis contains results of research performed by the candidate, Peijun Gong, whilst a member of the Optical+Biomedical Engineering Laboratory, within School of Electrical, Electronic and Computer Engineering at The University of Western Australia. The content is primarily derived from five full-length, peer-reviewed journal articles. PG is the first author of three of these articles and the sole author of any other content found in this thesis. The journal articles are presented in the form in which they were published with the formatting adapted for this document\(^1\). The contributions of each author to each article are listed below. [Note: Acronyms used for co-author names are PG (Peijun Gong), YML (Yih Miin Liew), RAM (Robert A. McLaughlin), FMW (Fiona M. Wood), DDS (David D. Sampson), PRTM (Peter R. T. Munro), LC (Lixin Chin), SE (Shaghayegh Es’haghian), KAH (Karl-Anton Harms), AM (Alexandra Murray), SR (Suzanne Rea), BFK (Brendan F. Kennedy) and KMK (Kelsey M. Kennedy)].

1. Yih Miin Liew, Robert A. McLaughlin, **Peijun Gong (15%)**, Fiona M. Wood and David D. Sampson, "**In vivo** assessment of human burn scars through automated quantification of vascularity using optical coherence tomography," *Journal of Biomedical Optics*, vol. 18, no. 6, pp. 061213, 2013.  
***(Section 3.3)***

*YML was the principal author of this article. YML designed the experiment setup, performed a series of experiments in the laboratory and at the Royal Perth Hospital, and developed the MATLAB codes for data post-processing. RAM organised collaboration to access patients, assisted with managing patients, assisted in conducting the experiments at the hospital, and mentored the research. PG assisted YML with the experiments and the data post-processing. FMW provided clinical input and access to patients. DDS initiated the project, provided the research facility, research equipment and research review and guidance. YML drafted the journal article, which was reviewed by all co-authors.*

2. **Peijun Gong (70%)**, Robert A. McLaughlin, Yih Miin Liew, Peter R. T. Munro, Fiona M. Wood and David D. Sampson, "Assessment of human burn

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\(^1\) This thesis was prepared according to The University of Western Australia’s guidelines on “Thesis as a series of papers” (http://www.postgraduate.uwa.edu.au/students/thesis/series, assessed on 15 April, 2015)
scars with optical coherence tomography by imaging the attenuation coefficient of tissue after vascular masking," *Journal of Biomedical Optics*, vol. 19, no. 2, pp. 021111, 2014.

(Section 4.3)

*PG was the principal author of this article. PG developed the method for calculating attenuation coefficient of the skin, performed the clinical data collection and data processing, and drafted the journal article. RAM organised the clinical collaboration, assisted with the clinical data collection and development of the attenuation coefficient calculation method. YML assisted with the clinical data collection. PRTM performed the optical simulation of tissue optical properties. FMW was responsible for the patient recruitment and management. DDS provided the research facility and equipment, and supervised this study and the drafting of the manuscript. The article was reviewed by all co-authors.*


(Section 5.3)

*PG was the principal author of this article. PG designed the experimental setup, collected and processed the clinical data, and drafted the journal article, which was reviewed by all co-authors. LC developed the MATLAB codes for data post-processing. SE assisted with the design of the imaging setup and clinical data collection. YML assisted with the clinical data collection. FMW was responsible for the patient recruitment and management. DDS initiated the project, provided the research facility, research equipment and research review and guidance. RAM organised and assisted with the clinical data collection, helped refine the data processing algorithm and supervised the research.*

(Section 6.2)
PG was the principal author of this article. PG designed the experiment setup, developed MATLAB codes for data post-processing, collected and processed the clinical data, and drafted this journal article, which was reviewed by all co-authors. SE assisted with the design of the experiment setup and clinical data collection. KAH, AM, SR and FMW, were responsible for the patient recruitment, treatment and management. BFK assisted with the clinical data collection. DDS provided the research facility and equipment, and reviewed and guided the research. RAM organised and assisted with the clinical data collection and supervised the research.


(Section 6.3)
SE was the principal author of this article. SE designed the experiment setup, developed the MATLAB codes for data post-processing, fabricated the imaging phantom, and collected and processed the data from phantom and skin lesions. SE also drafted the manuscript, which was reviewed by all co-authors. KMK assisted SE with the phantom fabrication and imaging. PG assisted SE with the design of the experiment setup and the data collection. DDS provided the overall research facility and equipment, and reviewed and guided the research. RAM contributed to providing the research facilities and supervised the research. BFK assisted SE with the design of the experimental setup, the data processing method and supervised the research.

The research in this thesis involves experiments on human subjects in vivo. YML prepared all written works required for human research ethics applications, under the supervision and guidance of RAM. The ethics applications were approved by the Human Research Ethics Committee of Royal Perth Hospital and The University of Western Australia (reference numbers: RA-10.020 and RA/4/1/4131) prior to the commencement of the study.

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List of Publication

Peer-reviewed journal articles:


7. Vipul Agarwal, Dominic Ho, Diwei Ho, Yuriy Galabura, Faizah M. D. Yasin, Peijun Gong, Weike Ye, Ruhani Singh, Alaa Munshi, Martin Saunders, Robert C.


**Conference papers:**

Key: * International | ^ Domestic | † Full paper | § Abstract | ‡ Refereed | ! Unrefereed or abstract refereed


12. *§! **Peijun Gong**, Yih Miin Liew, Lixin Chin, Shaghayegh Es’haghian, Peter R. T. Munro, Fiona M. Wood, David D. Sampson and Robert A. McLaughlin, "Improved attenuation coefficient and birefringence parametric optical coherence tomography imaging of burn scars using vasculature masking," presented at BiOS/Photonics

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<th>Description</th>
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<tbody>
<tr>
<td>2-D</td>
<td>Two-dimensional</td>
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<tr>
<td>3-D</td>
<td>Three-dimensional</td>
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<tr>
<td>ASR</td>
<td>Ablative skin resurfacing</td>
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<tr>
<td>BCC</td>
<td>Basal cell carcinoma</td>
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<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
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<tr>
<td>FOV</td>
<td>Field of view</td>
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<tr>
<td>FP</td>
<td>Fractional photothermolysis</td>
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<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
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<tr>
<td>LDF</td>
<td>Laser Doppler flowmetry</td>
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<tr>
<td>LDI</td>
<td>Laser Doppler perfusion imaging</td>
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<tr>
<td>LSPI</td>
<td>Laser speckle perfusion imaging</td>
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<tr>
<td>MAPD</td>
<td>Mean absolute percentage deviation</td>
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<tr>
<td>MIP</td>
<td>Maximum intensity projection</td>
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<tr>
<td>MTZ</td>
<td>Microscopic treatment zone</td>
</tr>
<tr>
<td>NDR</td>
<td>Non-ablative dermal remodelling</td>
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<tr>
<td>OCE</td>
<td>Optical coherence elastography</td>
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<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
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<tr>
<td>POSAS</td>
<td>Patient and observer scar assessment scale</td>
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<tr>
<td>PS-OCT</td>
<td>Polarisation-sensitive optical coherence tomography</td>
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<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>SD-OCT</td>
<td>Spectral-domain optical coherence tomography</td>
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<tr>
<td>SHG</td>
<td>Second harmonic generation</td>
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<tr>
<td>SLD</td>
<td>Superluminescent diode</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>SS-OCT</td>
<td>Swept-source optical coherence tomography</td>
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<tr>
<td>TBSA</td>
<td>Total body surface area</td>
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<td>TD-OCT</td>
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<td>TPEF</td>
<td>Two-photon excited fluorescence</td>
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<td>VSS</td>
<td>Vancouver Scar Scale</td>
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Chapter 1

Introduction

1.1 Research motivation

Burns give rise to approximately 11 million incidences of injuries requiring medical attention each year, accounting for an estimated 265 000 deaths [1]. The resulting scarring represents a long-term complication to these burns victims, as it can lead to significant physical discomfort, such as pain, itching and reduced mobility, and psychological issues [2-4]. Various treatment techniques are available or under investigation for preventing and treating scarring. However, the outcomes of these treatments vary greatly between patients. Improved assessment of the scarring would enable a better understanding of the scar responses to treatment and aid in the evaluation of treatment efficacy.

Current clinical approaches for assessing scars are most commonly performed using scar scales. For example, the widely used Vancouver Scar Scale [5] assesses a scar’s pliability, height, vascularity and pigmentation, scored through the observation and palpation by the examiner. The subjective nature of these scar scales can lead to large inter-observer and intra-observer variability [5]. In addition, these methods are not capable of providing insight into the cutaneous structure or function at a microscopic level, limiting our understanding of the scarring and how it changes in response to
treatment. There is a need for non-invasive, objective methods to assess the severity of scars, and the changes in scars in response to treatment.

One promising candidate for imaging and assessment of scarring is optical coherence tomography (OCT). OCT is a high-resolution (1-20 μm), non-invasive, three-dimensional imaging technique [6], which can probe up to ~1 mm deep into skin tissue. Whilst OCT provides an image of the tissue microstructure using the intrinsic optical contrast, extensions to OCT have shown the capability to image tissue vasculature [7] and optical properties, such as birefringence and optical attenuation [8, 9]. Both the qualitative and quantitative imaging abilities of OCT have been widely explored and used in various areas of medicine and biology, such as examining the eye [10-12], airway [13, 14], breast [15-17], lymph nodes [9, 18] and heart [19-21]. A large body of current work is seeking to extend OCT for applications in dermatology. OCT and its extensions have shown promise for the imaging of skin morphological structures and vasculature and for quantifying the optical properties of both normal skin and a range of skin conditions [22-24]. However, these techniques have yet to be applied to the assessment of human burn scars in vivo.

The aim of this thesis is to develop objective, quantitative approaches using OCT, which complement current methods for scar assessment. In so doing, it seeks to exploit the current feasibility to perform 3-D OCT imaging. One characteristic of pathological scarring is a red appearance, indicating an abnormally high degree of vascularity [25, 26]. The assessment of the vasculature in scars is first explored using a method based on OCT speckle decorrelation, combined with strategies to mitigate artefacts caused by motion during scanning. A second feature in pathological scarring is the over-production of collagen, leading to a raised scar surface in comparison to normal skin [27]. The spatial imaging resolution of typical OCT systems is not sufficient to be able to resolve the individual collagen fibres. Therefore, we develop parametric methods to assess the collagen by quantifying its optical properties at a scale of 10s-100s of microns, including the optical attenuation coefficient and birefringence, with the latter utilising polarisation-sensitive OCT (PS-OCT). This approach to automatically computing the optical properties of the skin is used to produce an image of the optical properties, referred to as a parametric image [28]. The vasculature induces artefacts in such parametric images. In order to mitigate this, we propose a method to segment and subsequently mask the blood vessels. To the best of our knowledge, this is the first example utilising a segmentation algorithm to generate improved parametric images. We demonstrate this approach with two types of parametric images; one based
on the attenuation coefficient, and the second measuring birefringence in the tissue. Finally, this thesis explores the use of OCT for clinical monitoring of skin with two examples of clinical measurements of two features of scars: applying OCT vasculature imaging to scars undergoing ablative fractional laser treatment; and using OCT-based optical palpation to delineate scar boundaries at a high resolution by quantifying the local mechanical properties of the skin.

1.2 Thesis outline

The content of each chapter is briefly summarised. Where journal papers are included in the chapters, they are reproduced as published, including with original language (US English) and reference list. Other references cited elsewhere in the chapters are listed in a separate list at the end of this thesis.

Chapter 2 - Background

Chapter 2 provides an introduction to the morphology of normal human skin, describing how it changes during the wound healing process after injury, and describing the characteristics of the resulting scarred tissue. This is followed by a brief summary of several commonly used scar treatment techniques, and scar assessment methods with a focus on microscopic imaging modalities. OCT is then introduced with a description of its working principle and its applications in dermatology.

Chapter 3 - Vasculature imaging for scar assessment

Chapter 3 provides a concise review of OCT-based vasculature imaging techniques and then develops a method using speckle decorrelation for clinical imaging of burn scar vasculature in vivo. Strategies for mitigating the artefacts caused by motion during scanning are incorporated into this method to enhance its clinical feasibility. Automatic techniques are then developed to quantify the morphological parameters of vasculature, including the vessel diameter and area density. Comparisons based on these parameters show differences between scars and normal skin. The feasibility of a high resolution vasculature assessment method for longitudinal applications is also demonstrated by monitoring the wound healing process of a burn injury on a human subject over time. This chapter is based on the journal paper in Reference [29].

Chapter 4 - Attenuation coefficient imaging for the assessment of collagen in scars

Chapter 4 begins with a brief introduction to parametric OCT imaging, focused on measurements of optical attenuation in tissue. A method for attenuation coefficient
imaging for scar assessment is developed based on a single-scattering model of tissue. The presence of blood vessels introduces artefacts to the attenuation coefficient measurement, so care is taken to mask the blood vessels from the OCT scans based on the vasculature imaging method presented in Chapter 3. The source of the difference between the attenuation coefficients of scars and normal skin is explored by performing optical simulation of the impact of variations in the collagen fibres. This chapter is based on the journal paper in Reference [30].

**Chapter 5 - Birefringence imaging for the assessment of collagen in scars**

Chapter 5 presents an introduction to PS-OCT, a variation on OCT to enable imaging of tissue birefringence, with a demonstration of its working principle. We develop a parametric imaging method using PS-OCT to assess tissue birefringence. This method calculates the birefringence by measuring the rate of change of the phase retardation with depth. We also explore the detrimental impact of the vasculature on these measurements, and demonstrate the use of vascular masking to mitigate these effects. The birefringence from different types of scars and normal skin are compared to indicate the degree of parallel orientation of the collagen fibres, with potential to provide a method of scar assessment. This chapter is based on the journal paper in Reference [31].

**Chapter 6 - Clinical monitoring of skin**

In this chapter, the utility of OCT for clinical monitoring of skin is investigated for two different applications. The first application extends the vasculature imaging method to assess changes in the scar vasculature over a time period of weeks to months following ablative fractional laser treatment. This longitudinal assessment develops scanning protocols to accurately track the same scar location at multiple time points over such extended periods. The results show different vascular responses to the treatment for immature and mature scars. The second application uses OCT-based optical palpation to provide a high-resolution *en face* map of the relative stiffness of the scar tissue. This method extends the use of OCT beyond assessing optical properties, and provides an indication of the mechanical properties of the tissue. The spatial distribution of the measured stress contrast is shown to correlate with scar boundaries. This chapter is based on the journal papers in References [32] and [33].

**Chapter 7 - Conclusion**
This chapter summarises the significance and limitations of the research presented in this thesis, and makes recommendations for future work. The thesis concludes with a summary of the key contributions and some final remarks.
Chapter 2

Background

2.1 Preface

This chapter provides background to the research work reported in this thesis. Firstly, a brief review of the structure and physiological properties of normal human skin is presented. This is followed by a review of the disruption of skin structure by injuries and the stimulated wound healing process which leads to scar formation. Methods for scar treatment and scar assessment, with a focus on microscopic imaging techniques, are then briefly reviewed. Lastly, a review of the imaging technique (optical coherence tomography) used in this thesis is given, along with a summary of the current applications of optical coherence tomography for various areas in dermatology.

2.2 Burn scars

2.2.1 Skin structure

Skin is the largest organ of the integumentary system that covers, protects and regulates the human body. The skin plays several important roles [34] in regulating and maintaining the internal conditions of the body. It firstly acts a barrier to protect the
body from external pathogens, mechanical impacts, radiation and chemicals. It regulates body temperature, fluid balance, and peripheral circulation, and is integral to the synthesis of vitamin D. It also provides many sensations through its network of nerve cells with receptors for touch, pressure, pain, heat and cold. These functions are essential to the normal functioning of the entire human body and are realised through a layered structure, including the epidermis, dermis and hypodermis for both hairless and hairy skin, as shown in Figure 2.1.

As shown in Figure 2.1, the outmost layer is the epidermis, which comprises squamous epithelium with five sublayers (from outside to inside, as shown in Figure 2.2): stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. The superficial stratum corneum, comprising anucleated corneocytes (dead cells that remain active) and surrounding keratin proteins, acts as a barrier to protect the underlying tissue from infection, dehydration, chemical and mechanical stress. The underlying stratum lucidum is also made of dead skin cells and is only present in hairless skin, such as on the palms or soles. In the stratum granulosum, the keratinocytes migrating from the stratum spinosum become granular cells, which contain keratohyalin granules to bind the intermediate keratin filaments. The stratum spinosum consists of polyhedral keratinocytes that synthesise fibrillar proteins, contributing to the strong connection of keratinocytes. In the deepest layer of stratum basale, the basal keratinocyte cells are connected to the melanocytes. These melanocytes are responsible for producing the melanin through melanogenesis, which

contributes to the brown colour tones of the skin and protects it from harmful radiation, such as the ultraviolet radiation in sunlight. The total thickness of the epidermis presents variation between the hairy (~100 \( \mu \text{m} \)) and hairless (~600 \( \mu \text{m} \)) regions [34-36].

![Structure of the epidermis](http://kreativestudios.com/Tooltip/05Integument/02epidermis.html. Accessed on 23rd March, 2015)

The underlying dermis is much thicker (1-4 mm) [37] than the epidermis and contains two sublayers: the papillary and reticular dermis. The papillary dermis is adjacent to the epidermis with a thickness less than 1/10 of the full dermal thickness [37]. The dermal papillae in this layer intertwine with the epidermis with their nipple-like structures. These structures increase the interface area between the epidermis and dermis, allowing the efficient transport of nutrients and oxygen from the dermis to the deep region of the epidermis, since the vasculature is only present in the dermis layer. The deeper reticular dermis represents a large portion of dermis. This layer has rich connective tissue to support the various skin appendages, such as the hair follicles, sebaceous glands and sweat glands, shown in Figure 2.1.

The connective tissue of the dermis (Figure 2.3) is rich in collagen, which accounts for 80% of the dry weight of the dermis [38]. The collagen molecule has a distinctive triple helix structure, in which there are three collagen polypeptide chains (i.e., \( \alpha \) chains) [39]. Each type of \( \alpha \) chain is encoded by distinct genes. Different combinations of the \( \alpha \) chains lead to different types of collagen molecules with Type I being the most common type in skin [37]. After being secreted into the extracellular matrix, the collagen molecules pack together to form fibrils. This fibril has a long thin structure, and is many 100s of \( \mu \text{m} \) in length for mature tissue. Bundles of these fibrils further assemble into collagen fibres that have a diameter of several \( \mu \text{m} \) [40]. These collagen fibres are organised to build a network to support the skin with structural
stiffness [38]. In normal human skin, this network usually shows a ‘basket-weave’ organisation with a degree of parallel orientation [41, 42], which can present unique optical properties, such as birefringence [39, 43]. The quantity and organisation of the collagen has been shown to vary for different skin locations [44]. Note that the dermis also contains an amount of elastin fibrous matrix that is 4% of the adult dermal proteins [37]. The elastin forms a branching network between the collagen to provide the elasticity of the skin [38].

The dermis also contains an extensive network of vasculature, comprised of two horizontal plexuses: the superficial subpapillary plexus and the deep vascular plexus. The superficial subpapillary plexus is situated at the junction between the papillary and reticular dermis. It supports the upper region of the dermis and the deeper region of the epidermis with nutrients and oxygen. The capillaries in this plexus typically have an outer diameter ranging from 8 μm to 10 μm [45, 46]. The deep reticular vascular plexus spreads close to the boundary of the reticular dermis and hypodermis to serve the deep region of dermis, including the deep hair follicles, sweat glands and sebaceous glands. This plexus is connected to the subpapillary plexus by communicating blood vessels: the ascending arterioles and the descending collecting venules, as shown in Figure 2.4. The arterioles and venules typically present outer diameters of, respectively, 10-26 μm and 10-35 μm [45, 46]. The whole vascular network in the dermis provides nutrition and oxygen for the skin, removes waste products and carbon dioxide from the skin, and also regulates body temperature by adjustment of the heat release through the skin.

The innermost layer is the hypodermis, which is connected to the dermis by collagen and elastin fibres. This layer is crucial for attaching the skin to the underlying bone and muscle. A major component of this layer is the adipose tissue to store excess

Figure 2.3 Scanning electron microscopy (a) and light microscopy (b) of the skin tissue. The red dashed lines delineate the approximate deep boundary of the papillary dermis. (Adapted from [37])
body energy and conserve body heat. There are also blood vessels, lymph vessels, nerves and hair follicles in this layer.


2.2.2 Skin injury and scar formation

The integrity of skin structure and function can be damaged by various injuries. Burns are the most common household injury, and can be caused by heat, electricity, chemicals, friction and radiation [47]. Burn injuries give rise to a serious global health problem. Statistics from the World Health Organization show that nearly 11 million people worldwide suffered serious burns that required medical attention in 2004 [1]. These burns lead to an estimated 265,000 deaths each year with the majority in low-income and middle-income countries [1].

Clinically, assessing the severity of a burn injury, which is largely based on the extent and the depth of the injury [48, 49], is a key step for optimising the treatment strategy. The extent of a burn injury is usually quantified by the percentage of the total body surface area (TBSA) that is affected by the injury. The depth assessment of the burns is further used to classify the severity of the injury into four different degrees: superficial, superficial partial-thickness, deep partial-thickness and full-thickness burns [49].

Superficial burns only involve the injury of the epidermis, such as that caused by sunburn. This type of burn takes only a few days to heal and causes minimal damage with possible discolouration but almost no scarring. The superficial partial-thickness burns extend the damage to the upper 1/3 of the dermis, where the papillary dermis is included. It usually takes weeks to heal via re-epithelialisation, typically giving rise to minimal scarring. Deep-partial thickness burn injuries occur when the damage extends into the deep dermis. This burn degree takes longer to heal and usually leads to scarring
and possible contracture (i.e., tightening of tissue). If the epidermis, entire dermis and the subcutaneous tissue are injured, a full-thickness burn is present. Note that a burn injury is called a subdermal burn when the fat, muscle and bone underlying the skin are also damaged. As with full-thickness burns, subdermal burns require surgical intervention, such as skin grafting, to cover the lost skin, as the body is not able to heal by itself. These two most severe burns present prolonged healing times and usually lead to significant scarring. The treatment of burns patients depends on the severity of the burns. Superficial burns can be treated with home care, including such tasks as cleaning, cooling, preventing infection and managing pain. Major burns require treatment by specialised clinicians.

The wound healing process is triggered immediately upon the burn injury. It comprises overlapping, sequential phases of inflammation, tissue formation (i.e., cell proliferation in Figure 2.5) and tissue remodelling (i.e., matrix remodelling in Figure 2.5), as shown in the upper panel in Figure 2.5(a) [50, 51]. Upon injury, the inflammation phase initiates the healing process and usually lasts for 3-4 days. Platelets gather at the injury site to achieve haemostasis through the formation of a fibrin clot acting as a scaffold for wound repair [52]. Foreign materials, bacteria and the damaged tissue, are cleaned by the infiltrating neutrophils and then extruded with the eschar or by the macrophages through phagocytosis [51]. The re-epithelialisation process also starts in this phase with epithelial cells migrating across the new tissue to form a barrier between the wound and the external environment [51]. In the tissue formation stage, fibroblasts propagate and proliferate at the wound site to generate new extracellular matrix materials, including collagen, acting as a new scaffold of reparative tissue [50]. New microvascular structures are also formed by assembling vascular endothelial cells through angiogenesis [51]. The tissue remodelling phase usually begins after week 2 and lasts weeks to months [51]. This healing phase removes the excess matrix materials, leading to the degradation of the extracellular matrix. The remaining collagen fibres are also cross-linked to form a network. This normal healing process, through the physiological responses to the injuries, leads to wound repair and scar formation.

However, the wound healing can become pathological due to the excessive skin responses to injury. This excessive healing process shows different tissue behaviours from normal healing, as summarised in Figure 2.5. In brief, the excessive healing usually shows stronger and more prolonged inflammation and tissue formation phases [50] than normal healing. It is characterised by prolific collagen deposits in the dermis.
due to the breakdown of the balance between matrix degradation and matrix biosynthesis, when compared against the normal healing and scarring process.

![Figure 2.5. Process of the normal wound healing (a) and excessive scarring (b). (Adapted from [50])](image)

When excessive healing occurs, pathological scarring, typically seen as hypertrophic scars and keloids, can develop [51]. Photographs of a representative hypertrophic scar and keloid are shown, respectively, in Figures 2.6(a) and (b). These two types of pathological scars usually present a red appearance with the tissue surface raised from the normal skin. Keloid scars are more problematic than hypertrophic scars, since they typically extend beyond the boundary of the wound site, whereas hypertrophic scars stay within the site of the original wound. Early differentiation of these two types of abnormal scarring is important, since they typically require different treatment strategies [53].

Pathological scars show different characteristics from the normal skin in their collagen network and blood vessels. The over-produced collagen in pathological scarring has been shown to have a higher density and larger fibres than in normal skin [40]. The organisation of the collagen presents a marked increase in parallel orientation [42]. Interestingly, the scar tissue can also show higher water content than the normal skin, depending on the stage of the scarring for the hypertrophic scars [54, 55]. These characteristics of the scar collagen might give rise to changes in the scar optical properties from those of the normal skin, such as the attenuation coefficient and birefringence [8, 9, 43], which can potentially be used as a measure of the pathological
condition. Meanwhile, the blood vessels in the pathological scars present abnormalities such as the visible red appearance, as shown in Figure 2.6, which has been widely used for scar assessment as described in the following section.

Figure 2.6. (a) Photograph of a 10-month-old hypertrophic scar on the right thigh and (b) 20-year-old keloid on the left shoulder. The hypertrophic scar was caused by thermite explosion on a 23-year-old male Caucasian patient. The keloid was caused by hot boiling water on a 21-year-old female Indian patient. Square outlines: 1 × 1 cm.

2.3 Scar treatment and assessment

2.3.1 Scar treatment

Because scars, especially pathological scars, often lead to significant discomfort, such as pain, itching, reduced mobility and cosmetic impairment [2-4], they usually require professional care and treatment. There are a variety of treatment options, and the selection of the treatments depends on the scar condition. Herein, we briefly describe some commonly used treatment methods. One particular treatment, referred to as fractional laser treatment, is investigated in greater detail in Chapter 6.

Surgical excision is one treatment option for keloids and hypertrophic scars, such as when the hypertrophic scarring results from an infected wound or delayed wound closure [56]. It is also the most practical and effective treatment for removing keloids, since they do not regress over time. This method removes and narrows the pathological scar tissue and may change the scar directions to reduce the skin tension [56]. However, excision alone can lead to a high recurrence rate of 45% to 100% [57]. The clinical approach to mitigate this problem is to combine excision with other follow-up treatments to affect the healing process in the newly formed wound, with the proven effect of reducing the recurrence rate to 8%-50% [58].

Pressure therapy, which entails wearing pressure garments [59], is a long-standing treatment method, although the mechanism of healing is not well understood. Previous studies have proposed a few working principles, such as: 1) hypoxia from the applied pressure results in the degeneration of fibroblasts and collagen; and 2) decreased
scar dehydration gives rise to mast cell stabilisation followed by decreased neovascularisation and matrix production [56]. Pressure garments usually apply a pressure of 5 to 50 mm of mercury [60], which may make body movement uncomfortable since it is recommended that they be worn for 8 to 24 hours a day for several months [56]. For children, this pressure may cause adverse effects, such as skeletal or dental deformity, since they are still in the process of growth and development [60, 61].

Silicone gel is a transparent gel containing long-chain silicone polymers (polysiloxanes), silicone dioxide and volatile components. It was first used for treating burn scars in the 1980s [57]. Since then, this scar treatment technique has been widely used for treating pathological scars, with widespread adoption because of its easy administration. The use of silicone materials in the form of a gel or a sheet may provide several benefits for the patients. For example, the silicone sheeting forms a layer on the skin that holds the moisture while allows penetration of oxygen. The resulting hydration can regulate fibroblast production and reduce collagen synthesis in pathological scarring [56]. This treatment can also reduce patient discomfort, such as itching [62]. Improvements in redness, itching, texture and thickness of pathological scars in 60% to 100% of cases treated with silicone [63] have been described in literature. Some studies correlate the good treatment efficacy of silicone with hydration, pressure, temperature, oxygen transmission and silicone absorption [62]. However, the application of silicone gel in warm climates can be problematic since it commonly leads to skin reactions and the interruption of the treatment [64].

Intralesional corticosteroid injection is either used alone or commonly combined with other scar treatment methods. It delivers a steroid solution to the scar tissue usually through needle injection. The most commonly used steroid solution is triamcinolone acetonide, which is injected into the upper dermis of scars at a dose ranging from 5 to 10 mg/mL [56]. It is believed that this treatment causes a decrease in fibroblast proliferation and collagen synthesis [56]. Previous studies have documented softening, flattening, and improvements in the symptoms of the scar tissue [65]. However, as well as the pain related with the injection, the application of this technique requires care to control the injection site precisely to avoid complications, such as fat atrophy [58].

Laser therapy provides a method for treating burn scars based on light-tissue interaction. The laser energy is absorbed by the chromophores in the scar tissue contents, such as haemoglobin, melanin, and water, depending on the wavelength used for treatment, and converted into heat [66]. The heat leads to thermal damage in the scar
tissue and causes a healing process which can result in improved scar tissue. There are two commonly used variations: ablative skin resurfacing (ASR) and the non-ablative dermal remodelling (NDR) [67]. Both of which are more widely used for treatment of other dermatological conditions, such as acne, psoriasis and photo-aged skin [68, 69]. As shown in Figure 2.7, ASR removes the entire epidermis and induces thermal damage to the underlying dermis, leading to a good treatment efficacy but with significant side effects, such as edema, oozing, crusting and burn discomfort [69]. NDR introduces thermal damage mainly to the deeper dermis layer, leaving the epidermis intact. This treatment usually gives rise to a lower degree of side effects, but also a worse treatment efficacy than ASR [69]. To achieve a good treatment efficacy with reduced side effects, fractional photothermolysis (FP) [69] has been applied to create microscopic thermal wounds as shown in Figure 2.7(c), instead of a large wound in scars. These microscopic thermal wounds are induced in microscopic treatment zones (MTZs, diameter of ~100 μm), where the scar tissue between these zones is spared. The local thermal damage then leads to a healing process to improve the scar tissue. Although studies have shown that this technique is promising for treating burn scars [68], more studies are needed to assess the treatment efficacy and elucidate the treatment mechanism.

Figure 2.7. Comparison of ASR (a), NDR (b) and FP (c). ASR removes the epidermis and induces thermal damage in the dermis. NDR causes thermal damage to the dermis with the epidermis intact. FP induces thermal damage to, and spares the tissue between, the MTZs. (Adapted from [69])

There are a number of other treatment options. For example, radiation therapy applies ionising radiation to treat burn scars and can be used in combination with surgical excision [57]. It is believed to destroy fibroblasts to establish a balance between the fibrogenesis and fibrolysis, in contrast to the excessive fibrogenesis in pathological scarring [56]. Cryotherapy freezes the scar tissue with a cold substance, such as liquid nitrogen, to destroy the pathological scar tissue. It is suggested this treatment causes freezing-induced ischaemic damage to the microcirculation to improve the scar tissue [65]. These scar treatment methods typically show a large variation in treatment outcomes among patients with pathological scarring [57]. In clinical practice, they are
usually combined to construct a multiple treatment approach based on the scar conditions.

2.3.2 Scar assessment

This section presents a brief review of scar assessment methods, including both those that are in clinical use and some that are still under investigation. These methods can be broadly categorised as subjective or objective. The subjective methods are primarily clinical scar scales based on manual assessment by clinicians and/or patients. The objective techniques refer to imaging techniques to quantify scar features, as observed using a physical probe, such as light or sound. Many objective methods are still under investigation, but they have shown promise for future clinical application.

(a) Clinical scar scales

Current scar assessment is most commonly performed using clinical scar scales, in which scar properties are assessed through manual observation and palpation [70]. Among clinical scar scales, the most widely used is the Vancouver Scar Scale (VSS) [5]. The VSS is composed of four parameters: pigmentation, vascularity, pliability and height; assessed by comparing the scar with normal skin. These four parameters are scored manually by an examiner using a defined lookup table, such as the example shown in Table 2.1. An overall score is then calculated by summing the four scores to give a comprehensive assessment of the scar condition, with higher scores corresponding to poorer condition.

<table>
<thead>
<tr>
<th>Vascularity</th>
<th>Pigmentation</th>
<th>Height</th>
<th>Pliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Flat</td>
<td>Normal</td>
</tr>
<tr>
<td>Pink</td>
<td>Hypopigmentation</td>
<td>&lt;2mm</td>
<td>Supple</td>
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<tr>
<td>Red</td>
<td>Mixed</td>
<td>2-5mm</td>
<td>Yielding</td>
</tr>
<tr>
<td>Purple</td>
<td>Hyperpigmentation</td>
<td>&gt;5mm</td>
<td>Firm</td>
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<td></td>
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<td>Ropes</td>
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<td>Contracture</td>
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Table 2.1. Vancouver Scar Scale. (Adapted from [71])

However, the VSS (and similar scar scales [5]) is based on the clinical observer’s evaluation and neglect the opinion of the patient. Thus, the patient and observer scar assessment scale (POSAS) was proposed to incorporate the perception of the patient by adding a separate patient scale to the observer scale [5, 71]. In this scheme, the observer scale has five parameters, including vascularisation, pigmentation, thickness, relief and pliability of the scar. The patient scale comprises the parameters of
pain, itching, colour, stiffness, thickness and irregularity. POSAS provides patients with the opportunity to reveal the impact of the scarring through their own evaluation. Although these two and other alternative clinical scar scales are widely used, they are inherently subjective and prone to high inter-observer and intra-observer variability [5, 72], which limits their reliability and utility.

(b) Microscopic imaging

Several imaging modalities have been investigated for objective scar assessment to complement the subjective scar scales. Among these techniques, light microscopy and electron microscopy are two early, invasive imaging methods explored for investigating and assessing scars [27, 41]. They use visible light photons and electrons, respectively, to illuminate the sample, excised and processed through fixation, staining and dehydration, to generate a magnified image. The magnified images allow inspection of the microstructures within the scar tissue, such as the structure and the organisation of the collagen network [41]. Figures 2.8(a) and (b) show representative images from an early work using light microscopy, where the collagen presents a “basket-weave”-like organisation in normal skin and, by contrast, a dominant parallel orientation in mature scar tissue [41]. Compared with light microscopy, electron microscopy is capable of much higher resolution imaging, which can differentiate and quantify the diameter of individual collagen filaments, as shown in Figure 2.8(c) (predominant diameter of 100 nm). Linares et al. showed that the scar tissue tends to have smaller collagen filaments (diameter ~60 nm) than corresponding normal skin tissue (diameter ~100 nm), as measured from the electron microscopy images [41].

Figure 2.8. Normal skin (a) (∼575 magnification) and mature scar (b) (∼625 magnification) imaged with light microscopy and skin collagen filaments (c) (∼105,000 magnification) imaged with electron microscopy. (Adapted from [41])

Utilising histological techniques, particular scar features can be highlighted from the microscopic images by tuning the staining methods. For example, the scar
vasculature can be better differentiated from other tissue with the aid of anti-CD31 immunostaining [73, 74]. This method has been used to quantify the vascularity in mature hypertrophic scars treated with fractional laser and an average increase in vascularity of 82.6% post-treatment was observed [73]. Two representative images of the scar tissue before and after the laser treatment are shown in Figures 2.9(a) and (b), demonstrating the increased vasculature after treatment. Combined with image processing techniques, the blood vessels can be segmented from the 2-D cross-sectional image, and a 3-D blood vessel image may be generated by extending the segmentation to multiple 2-D images. Figure 2.10 shows one such imaging example, where the hypertrophic scar in Figure 2.10(b) presents more capillaries than the keloid scar in Figure 2.10(a) [74]. Further quantification of the vasculature in such images can be used as an objective assessment of the scar’s vascularity.

Light microscopy and electron microscopy are performed \textit{ex vivo} on biopsied tissue and typically require laborious histological processing of the scar tissue. This process may cause not only patient discomfort, but also the deformation of the scar tissue during the processing of the sample, leading to possibly inaccurate information. In addition, this invasive method may interfere with the healing process.
There are alternative, non-invasive imaging methods for scar imaging to mitigate these adverse effects, such as confocal microscopy and multiphoton microscopy with commercially available systems for non-invasive skin imaging (e.g., *Vivascope* confocal microscope by Lucid Inc., and *DermaInspect* multiphoton microscope by JenLab GmbH). Confocal microscopy builds an optically sectioned image (in depth) by raster scanning a focused light beam and placing a pinhole in the optically conjugate plane in front of the detector to eliminate the light that is out of focus. This confocal setup provides several advantages over conventional microscopy, including improved imaging resolution, the ability to control the depth of field by optical sectioning and the capability to acquire 3-D images by acquiring optical sections at multiple depths. These attributes facilitate the visualisation of microstructures in scar tissue, as shown in Figure 2.11 [40, 42]. The confocal microscopy images in Figures 2.11(a)-(d) show the representative organisation of the collagen network, respectively, in normal skin, normotrophic scar, hypertrophic scar and keloid. Verhaegen *et al.* applied image processing techniques to such images to assess the diameter, density and degree of parallel orientation of the collagen [40, 42]. They found that scar tissue has denser, larger collagen fibres with a significantly higher degree of parallel orientation than those in normal skin. The mechanical properties of scar tissue can also be investigated with confocal microscopy by measuring the change in the orientation of the collagen after mechanical stimulation, such as stretch. One such example is shown in Figures 2.11(e) and (f), where an increase of the parallel orientation of the collagen is observed by comparing the image of the scar tissue before (e) and after (f) the stretch. This increase was quantified and shown to be significant [40], providing a possible solution for assessing the scars’ mechanical properties.

Although the images in Figure 2.11 were acquired *ex vivo*, the optical sectioning ability of confocal microscopy allows non-invasive assessment of *in vivo* scar tissue. Lange-Asschenfeldt *et al.* used confocal microscopy to monitor the wound healing process and demonstrated its ability to visualise the features of wound healing *in vivo* [75]. Koehler *et al.* applied confocal microscopy to scar tissue *in vivo* and observed higher reflectivity of the scar tissue than the normal skin, indicating the high collagen content in the scar tissue [76]. The reflectivity of normal skin has been shown to decrease after fractional photothermolysis, assessed through monitoring with *in vivo* confocal microscopy [77], which can be potentially used for assessing the responses of the scar tissue to laser treatment.
The ability of confocal microscopy to perform high-resolution, non-invasive imaging has also shown promise for assessing blood flow in skin. Figure 2.12(b) shows a confocal microscopy image acquired *in vivo* with a commercial confocal microscopy system in (a). The papillae appear as the dark regions in the bright circular basal layer, and the lumina of the capillary loops are visible as black holes containing blood cells, which are brighter spots. Altintas *et al.* quantified the blood flow in patients following free-tissue transfer by counting the blood cells over time using images such as that shown in Figure 2.12(b) [78].

Multiphoton microscopy is based on the emission of a photon after the simultaneous absorption of two or more excitation photons with longer wavelength (i.e., lower energy). It is similar to confocal microscopy, since it collects localised emission
light within a thin raster-scanned plane to perform optical sectioning [79]. The pinhole in confocal microscopy is not necessary in this technique (although sometimes used as an adjunct), since the effect requires high excitation power scaling with the square (or higher) of the intensity, thus, only the region very close to the focus has sufficient excitation power to generate a signal.

Figure 2.13. SHG and TPEF images of a keloid in the upper (a)-(c) and deep dermis (d)-(f): (a) and (d) are the SHG images of the collagen; (b) and (e) are the TPEF images of the elastin; (c) and (f) are the overlaid SHG and TPEF images. Scale bar is 20 μm. (Adapted from [80])

Although there are several variants of multiphoton microscopy based on different nonlinear optical effects, two commonly used ones for imaging scar tissue are two-photon excited fluorescence (TPEF) and second-harmonic generation (SHG). TPEF involves the absorption of two photons to emit a photon with energy higher than the excitation photons, but less than their summation. In biological tissue, elastin is an effective source for generating the TPEF signal. SHG emits a photon with energy exactly equal to the sum of the energies of the two excitation photons (so, at exactly half the wavelength), for which collagen produces a strong signal. These properties make multiphoton microscopy a powerful tool for imaging and assessing the scar collagen and elastin network. Chen et al. investigated keloids with SHG and TPEF, as shown in Figure 2.13 [80]. Briefly, they noticed that the collagen content increased in both the upper (a) and deep dermis (d), whilst the elastin content only appeared in the deep dermis (e) with a larger quantity than the normal skin. Similar to confocal microscopy, multiphoton microscopy has the potential for non-invasive imaging. The collagen in keloids has been imaged non-invasively by collecting the SHG signal between 397 nm and 408 nm, with excitation by an 800 nm tunable titanium sapphire pulsed laser [81].
The generated images show that keloids possess larger collagen bundles, with a distinctive arrangement in complex swirls, when compared against normal skin.

The aforementioned optical imaging techniques typically provide high imaging resolutions, but have shallow imaging depths. To overcome this limitation for assessments requiring greater imaging depth, such as the assessment of the scar thickness, ultrasound imaging provides an alternative. Ultrasound imaging sends an ultrasound pulse to the sample and detects the strength and arrival time of the reflected echoes to create an image of the sample structure. Its larger imaging depth compared to optical imaging allows the visualisation of deep scar tissue, although its imaging resolution is generally poorer than that of optical imaging. Figure 2.14 shows a representative ultrasound image of a scar in a paediatric patient used to assess the scar thickness [82]. The maximum thickness of the scars measured by Wang et al. was 4-6 mm [82], corresponding to a depth range not typically achievable by optical imaging.

![Figure 2.14. Ultrasound imaging of a 6-month-old scar in a paediatric patient: (a) Photograph of the scar; (b) Cross-sectional ultrasound image of the scar. (Adapted from [82])](image)

There are also non-invasive imaging techniques specifically for imaging and assessing skin vascularity with the potential for scar assessment. Among these techniques, video capillaroscopy is a non-invasive, high-resolution technique for imaging the superficial blood vessels. It provides a 2-D magnified projection of the capillaries within the superficial layer of the skin. Its high imaging resolution allows the

![Figure 2.15. Video capillaroscope (a) and a representative image (b) (~200 magnification) acquired from the normal skin at the nail fold. (Adapted from [83])](image)
visualisation of individual capillaries, such as the vasculature imaging of normal skin shown in Figure 2.15 [83]. This imaging technique has been applied to human scars to show the increased neo-angiogenesis in post-burn scars, compared to normal skin [84]. However, due to the limited imaging depth (~200 μm) [85], its clinical applications in dermatology are mainly confined to superficial capillaries in relatively transparent skin.

Laser Doppler flowmetry (LDF) is a method to measure flow velocity using the Doppler frequency shift of a laser beam, with a larger frequency shift indicating a large flow velocity. Previous studies have shown the promise of this technique for assessing burns [86]. In addition, it has been applied to the subsequent scarring to investigate the microcirculation properties in human subjects at a single point [87, 88], although the value of such local measurements can be limited due to the tissue heterogeneity. This point measurement has been extended by laser Doppler perfusion imaging (LDI) to generate a 2-D en face perfusion map by collecting measurements from multiple points. Clinically, LDI is capable of scanning a large tissue area to avoid the bias caused by the tissue heterogeneity, such as the images (7.5 × 7.5 cm) shown in Figures 2.16(a) and (b) taken at the skin/scar boundary [89]. The scar shows greater perfusion [in the right half of the image in Figure 2.16(b)] than the adjacent normal skin tissue.

Figure 2.16. Blood perfusion imaging of hypertrophic scarring surrounded by normal skin: (a) Moor LDI total reflected light intensity map; (b) Moor LDI perfusion map; (c) LSPI CCD camera photograph; (d) LSPI perfusion image. (Adapted from [89])

Similarly, laser speckle perfusion imaging (LSPI) analyses the decorrelation caused by the blood between successive images of the speckle pattern to generate a 2-D perfusion map. In Figure 2.16(d), a representative LSPI image of scar tissue also shows higher perfusion of the scar (the lower right corner) than the adjacent normal skin tissue.
Compared with video capillaroscopy, LDI and LSPI can scan a large field of view with a larger penetration depth, but lack the spatial resolution to resolve individual capillary vessels.

All of the above vasculature imaging techniques have commercial systems, such as the capillaroscopy systems by Optilia Instruments AB, and LDI and LSPI devices by Moor Instruments. One common limitation of these techniques is that they are incapable of providing the depth information of the captured blood vessel, which may present bias for longitudinal comparison of the vasculature at the same site. In the next section, we introduce an imaging technique, called optical coherence tomography to visualise the depth-resolved vasculature in addition to the tissue microstructure.

2.4 Optical coherence tomography

2.4.1 Working principle
Optical coherence tomography (OCT) is a 3-D, non-invasive imaging modality capable of micro-scale spatial resolution (1-20 μm) up to 1-3 mm into tissue [6]. A near-infrared light beam is directed into the sample, and the detected backscattered light is used to form a depth-resolved signal (A-scan), analogous to the detection of the sound echoes in ultrasonography. Translating the probing beam to different lateral locations of the sample generates a cross-sectional 2-D image (B-scan), and a sequence of B-scans may be acquired to construct a 3-D data set (C-scan). For skin imaging, OCT offers a good trade-off between imaging resolution and depth with a relatively large FOV to account for skin heterogeneity, as compared to other alternative imaging techniques in Figure 2.17.

![Figure 2.17](http://obel.ee.uwa.edu.au/research/fundamentals/introduction-oct/) (Accessed on 23rd March, 2015)
Since its emergence in 1991 [90], several variants of OCT have been developed [6]. The first-generation OCT systems used a technique referred to as time-domain OCT (TD-OCT). A schematic of a typical TD-OCT system is shown in Figure 2.18. TD-OCT utilises a near-infrared broadband light source as the imaging light to probe the tissue. The imaging light is input into the Michelson interferometer using a beam splitter. The light is then split into the reference arm and the sample arm. The reference arm has a mirror to reflect the incoming light, which is then coupled back into the interferometer. In the sample arm, as the OCT light penetrates into tissue, elastic scattering occurs due to the variation in the tissue refractive index, and the backscattered light along depth is coupled by the objective into the interferometer. This backscattered light signal from the sample then interferes with the reference beam for detection.

![Figure 2.18. Schematic of TD-OCT. (Adapted from: http://obel.ee.uwa.edu.au/research/fundamentals/introduct ion-oct/. Accessed on 23rd March, 2015)](image)

Constructive interference only occurs when the optical path length of the sample light and reference light are matched (i.e., to within less than the coherence length of the light source). This means that only the sample light for a particular tissue depth can be detected at a single time point. The mirror in the reference arm, which is mounted onto a translation stage, is then translated over a certain range to change the optical path length of the reference light in order to match the sample light from various depths in the tissue. During translation of the reference arm mirror, the interference signal is detected by a photoreceiver and recorded electronically, leading to a depth profile of the backscattered light from various depths of the tissue, referred to as an A-scan. Since the backscattered light is proportional to the tissue reflectivity associated with each depth, the A-scan is a map of the depth-resolved tissue reflectivity at a particular lateral tissue location.
A limitation of TD-OCT is that it requires the translation of the mirror in the reference arm to achieve depth profiling, imposing a limit to the imaging speed of the system. To overcome this problem, alternative techniques have been developed, including spectral-domain OCT (SD-OCT). As seen in the schematic of a typical SD-OCT system in Figure 2.19, this has a similar setup to that of a TD-OCT system. However, a key difference is that the mirror in the reference arm is fixed, instead of being translated as in the TD-OCT configuration. A second distinction is with the detection unit, in which the spectral components of interferometric light are separated by a spectrometer and detected with a single exposure of the detector, such as a photodiode array or charge-coupled device. The depth profile (A-scan) in this configuration is constructed through an inverse Fourier transform of the interferometric spectrum.

![Figure 2.19. Schematic of SD-OCT. (Adapted from: http://obel.ee.uwa.edu.au/research/fundamentals/introduction-oct/. Accessed on 23rd March, 2015)](image)

Alternatively, in a technique known as swept-source OCT (SS-OCT), a swept-wavelength source can be used to sweep through a broadband spectral range with a light beam with a narrow instantaneous spectral line-width. This removes the need for a spectrometer, using instead a photodetector to measure the signal associated with each spectral component as the light source sweeps. This system also constructs an A-scan through an inverse Fourier transform. Since both SD-OCT and SS-OCT do not require the translation of the mirror in the reference arm, they typically offer faster scanning speeds up to MHz A-scan rate [91-93], which is critical for many clinical applications.

To describe the OCT signal formation mathematically [6], the imaging light from the light source in Figure 2.20 can be expressed by its complex electric field:

$$E_i = s(k, \omega)e^{(ikr - \omega t)},$$  

(2.1)
where \( s(k, \omega) \) is the electric field amplitude at wavenumber \( k \) and angular frequency \( \omega \). After passing through the beam splitter and being reflected by a reference mirror with an electric-field reflectivity of \( R_R \) (power reflectivity: \( R_r = |r_s|^2 \)), the electric field from the reference back into the interferometer is

\[
E_r = \frac{E_i}{\sqrt{2}} r_s e^{2\pi i z_R},
\]

(2.2)

where \( z_R \) is the distance from the beam splitter to the reference mirror and a splitting ratio of 50/50 is assumed for the beam splitter.

The sample usually presents variable reflectivity of the incoming electric field along the light path, depending on the distribution of the sample refractive index. This depth-dependent reflectivity function is usually continuous in biological tissues. Mathematically, this field reflectivity function can be modelled as a series of \( N \) discrete, real delta functions at each tissue depth:

\[
r_s(z_s) = \sum_{n=1}^{N} r_{s_n} \delta(z_s - z_{s_n}),
\]

(2.3)

where \( z_{s_n} \) represents a discretised depth in the sample with a field reflectivity of \( r_{s_n} \). This field reflectivity is related to the power reflectivity by \( R_{s_n} = |r_{s_n}|^2 \). The electric field coupled back into the interferometer from the sample arm can be calculated as:

\[
E_s = \frac{E_i}{\sqrt{2}} \left[ r_s(z_s) \otimes e^{2\pi i z_s} \right] = \frac{E_i}{\sqrt{2}} \sum_{n=1}^{N} r_{s_n} e^{2\pi i z_{s_n}}.
\]

(2.4)
The detector then generates a photocurrent, \( I_{\rho}(k, \omega) \), which is proportional to the square of the sum of the electric field of the light from the reference and sample arms as:

\[
I_{\rho}(k, \omega) = \frac{\rho}{2} \left| \langle E_R + E_S \rangle \right|^2 = \frac{\rho}{2} \left| \langle E_R \rangle + |E_s|e^{i\omega t} + E_s^* \right|^2.
\]

where \( \rho \) is the responsivity of the detector, and the angular brackets denote the integration of the detector during its response time. Since the electric field oscillates much faster than the response of the detector, temporal averaging removes the temporal angular frequency. Therefore, we can derive the following equation by substituting \( E_R \) and \( E_s \) with Equations (2.1) to (2.4),

\[
I_{\rho}(k) = \frac{\rho}{4} S(k)(R_{s_R} + R_{s_S} + \ldots + R_{s_{S_n}}) + \frac{\rho}{4} S(k) \sum_{m=1}^{\infty} \sqrt{R_{s_R} R_{s_S}} \cos[2k(z_{s_R} - z_{s_S})] + \frac{\rho}{2} S(k) \sum_{m=1}^{\infty} \sqrt{R_{s_R} R_{s_S}} \cos[2k(z_{s_R} - z_{s_S})] \]

where \( S(k) = \left| \langle |s(k, \omega)|^{\gamma} \right|^\gamma \) describes the power spectral distribution of the light source.

The photocurrent signal then goes through the inverse Fourier transform to generate an A-scan as:

\[
i(z) = \frac{\rho}{4} \gamma(z)(R_{s_R} + R_{s_S} + R_{s_{s_R}} + \ldots + R_{s_{s_{S_n}}}) + \frac{\rho}{8} \gamma(z) \otimes \sum_{m=1}^{\infty} \sqrt{R_{s_R} R_{s_S}} \delta(z \pm 2(z_{s_R} - z_{s_S}))
+ \frac{\rho}{4} \gamma(z) \otimes \sum_{m=1}^{\infty} \sqrt{R_{s_R} R_{s_S}} \delta(z \pm 2(z_{s_R} - z_{s_S}))
= \frac{\rho}{4} \gamma(z)(R_{s_R} + R_{s_S} + R_{s_{s_R}} + \ldots + R_{s_{s_{S_n}}}) + \frac{\rho}{8} \sum_{m=1}^{\infty} \sqrt{R_{s_R} R_{s_S}} [\gamma[2(z_{s_R} - z_{s_S})] + \gamma[-2(z_{s_R} - z_{s_S})]]
+ \frac{\rho}{4} \sum_{m=1}^{\infty} \sqrt{R_{s_R} R_{s_S}} [\gamma[2(z_{s_R} - z_{s_S})] + \gamma[-2(z_{s_R} - z_{s_S})]],
\]

where \( \otimes \) represents convolution; and \( \gamma(z) \) is the inverse Fourier transform of \( S(k) \).

The first component contains only the DC terms and does not profile the tissue reflectivity \( R_{s_{s_R}} \) at each tissue depth. The second component includes the autocorrelation terms, which is proportional to \( \sqrt{R_{s_R} R_{s_S}} \). Note that the tissue power reflectivity is usually relatively low, with a typical value on the order of \( \sim 10^{-4} \) to \( 10^{-5} \) [6]. Therefore, this component is typically neglected, since it is negligible in comparison to the third component, which comprises the cross-correlation terms. The cross-correlation, which builds up the main OCT A-scan signal, is proportional to the square root of the tissue power reflectivity (i.e., \( \sqrt{R_{s_R}} \)). Therefore, the OCT A-scan
essentially maps the depth-dependent tissue reflectivity, which is the basis for the work presented in Chapter 4.

There are also extensions of OCT to complement the conventional imaging of the tissue reflectivity, such as polarisation-sensitive OCT for imaging tissue birefringence [94], techniques for imaging vasculature [7], and techniques for imaging tissue optical properties [9]. A review of these extensions will be provided in the following chapters.

2.4.2 OCT for dermatology

OCT has been explored for a range of applications in dermatology. A review of its applications to normal skin, skin injuries, wound healing and scarring, psoriasis and skin cancer is presented in this section.

(a) Normal skin

OCT has been applied to investigate normal skin properties since its early development [22, 95]. OCT has been used to explore the layered structure of skin tissue, particularly the epidermis and a portion of the dermis. Figures 2.21(a)-(c) show, respectively, a representative C-scan, en face image [from CS1 position indicated in Figure 2.21(a)] and B-scan [from CS2 position indicated in Figure 2.21(a)] of hairy forearm skin. The epidermis is seen as a thin grey layer on the top of the dermis with strong backscattering, as shown in Figure 2.21(c) [96]. The thickness of the epidermis, which is important for medical and biological research, can be measured from the OCT data. In a study by Gambichler et al., the thickness of the epidermis in hairy skin was measured with OCT to range from 60 to 80 μm for different body sites [97].

![Figure 2.21. OCT imaging of normal skin on the forearm: (a) C-scan; (b) En face image from CS1 position in (a); (c) B-scan from CS2 position in (a); (d)-(f): B-scans from approximately the same skin location acquired, respectively, without index matching, with glycerol, and ultrasound gel for index matching. (Adapted from [96])]
**In vivo** imaging of the skin tissue is subject to several sources of artefacts, such as the intensity distortions shown as vertical streaks (indicated by the arrows) in Figure 2.21(c) in the dermis [96]. These artefacts can distort the skin structures, such as the blood vessels (B) and hair follicles (H), shown in Figure 2.21(d). There are ways to reduce these artefacts to optimise the imaging quality, such as the application of an index matching liquid, in (e) glycerol and (f) ultrasound gel.

The vasculature network of the normal skin has also been imaged with OCT through further processing of the OCT intensity or phase signal. One representative en face projection image of the vasculature in normal skin is shown in Figure 2.22, where the vasculature is detected using a speckle decorrelation method [29, 98]. Vasculature images of normal skin can act as a baseline to indicate the deviation of the vasculature network under pathological conditions, such as hypertrophic scarring. OCT is also capable of providing the flow velocity for the vasculature in the skin tissue, using a technique referred to as Doppler OCT [12]. For example, Zhao et al. investigated Doppler OCT for human skin imaging and mapped the velocity profile across vessels in the finger, with a typical vein showing a flow velocity of ~3 mm/s [99].

![Image](image.jpg)

Figure 2.22. OCT vasculature image of normal skin in the upper arm of a 42-year-old male Caucasian: (a) Photograph of the skin; (b) En face projection of blood vessels (from surface to a depth of 300 μm into the skin) in the centre area of the 10 × 10 mm blue outline in (a).

The optical properties, such as the optical attenuation coefficient and birefringence, of the skin tissue, have also been explored using OCT. Schmitt et al. first quantified the attenuation coefficient of human skin tissue in vivo, based on a single-scattering model for OCT [100]. The calculated attenuation coefficient showed a large variation by body location, ranging from 1 to 5 mm⁻¹ at 1300 nm centre wavelength. The birefringence of human skin tissue at various body locations has also been quantified by Pierce et al. at a centre wavelength of 1310 nm [44]. The results demonstrated a similarly large variation for different anatomical sites, with a mean phase retardation rate of 0.340, 0.250 and 0.592 deg/μm, respectively, for the skin at the
dorsal hand, temple and lower back regions, corresponding to the range of birefringence \(0.5 \times 10^{-3}\) to \(1.1 \times 10^{-3}\). These large variations in the optical properties of normal skin indicate that it is useful to quantify the corresponding normal skin as a baseline when using these properties to assess pathological skin conditions.

(b) Skin injury, wound healing and scarring

OCT is capable of imaging changes in skin structures associated with injury and monitoring of the wound healing process. For example, a loss of the layered structure has been observed in the structural B-scans of deep burns [101]. The injury-induced changes in the structure and organisation of the collagen demonstrated a reduction of the birefringence through polarisation-sensitive OCT (PS-OCT) scans 4 and 6 days post-injury, as compared to that of the normal skin [101]. Using this property, Srinivas et al. assessed the burn depth with PS-OCT on animal burns models [102].

The subsequent changes of skin tissue during the healing process have also been imaged with OCT. For example, Cobb et al. investigated the feasibility of OCT for monitoring the cutaneous wound healing process in a mouse model with a high-resolution OCT system with and axial resolution of 2.8 \(\mu\)m (in air) and lateral resolution of 5.6 \(\mu\)m [103]. Their OCT images show the transition from inflammation to the formation of granulation tissue, the migration of the epidermis across the wound and the formation of a blister, changes in the collagen content and the formation of the dermal-epidermal junction, respectively, 3, 7 and 11 days post wound induction, validated with histology, as shown in Figure 2.23. The changes in vasculature during the healing process have been presented by Jung et al. with OCT microangiography [104]. In their experiment, an injury was induced by a 0.5 mm biopsy punch through the skin in the pinna of a mouse. The generated vasculature images in Figure 2.24 show the removal of the vessels after the biopsy punch and the regrowth of the vessels during the wound healing process. These studies on animal models show the potential of OCT to image dynamic changes in skin vasculature.

Scars that form as a result of the wound-healing process have received relatively little investigation with OCT imaging. Among the few studies published, Liew et al. presented image processing techniques to mitigate the motion artefact in \textit{in vivo} OCT scans and applied these techniques to scarred skin, showing different surface texture and subsurface structures from normal skin [105]. Pierce et al. presented a phase retardation B-scan, acquired by PS-OCT, across a mature scar, showing much higher birefringence of the scar tissue than the adjacent normal skin [106]. Their finding indicates that
optical properties of scar tissue have potential to provide insight into scar formation and healing.

(c) Psoriasis

Psoriasis is a chronic, immune-mediated skin disease with the common form, psoriasis vulgaris, characterised by red, scaly, raised plaques [107]. It affects approximately 25 million people in North America and Europe [107]. The visible redness of psoriatic skin lesions are attributed to the marked dilation of the blood vessels. In order to assess this abnormality, Qin et al. used OCT microangiography to image the vasculature in psoriatic skin with representative images from the normal skin and psoriatic skin shown in the upper and lower row in Figure 2.25 for several depths [108]. The images demonstrated the presence of elongated capillary loops, such as those highlighted by the
arrows in Figures 2.25(f) and (g). The psoriatic skin also had a denser vasculature network than the normal skin, which led to the significantly higher value (0.53 ± 0.06) for the quantified blood vessel area density (i.e., the ratio of the blood vessel area to the total tissue area) than that (0.37 ± 0.04) in normal skin [108].

Figure 2.24. OCT vasculature imaging of wound healing in skin in the pinna of a mouse: (A) Serial vasculature imaging (1.2 × 1.2 mm) before (Control) and during (30 min to Day-60) the wound healing; (B) Mean and standard deviation of the fractal dimension value at the periphery of a small and large circle around the wound in the ‘30 min’ vasculature image. (Adapted from [104])

In addition to the characteristics in vasculature, psoriatic skin also shows deviation of the tissue structure, visible in the structural OCT images. Welzel et al. applied OCT imaging to psoriatic skin before and after treatment with various regimens [24]. A representative OCT image of psoriatic skin with the presence of severe scaling is shown in Figure 2.26. The superficial OCT signal for psoriatic skin showed the
presence of several parallel layers with a thickening of the epidermis (267 ± 94 μm), compared to the normal skin (176 ± 50 μm). After therapy, the thickness of the dermis in psoriasis diminished (217 ± 61 μm), but was still higher than the normal skin level (181 ± 58 μm). The attenuation coefficient in the psoriatic skin dermis was quantified to show lower values of 2.9 ± 0.9 mm⁻¹ compared to the normal skin (3.6 ± 1.5 mm⁻¹), possibly due to the increase of water content and decrease of collagen density associated with the inflammatory reactions in psoriasis. This difference in attenuation coefficient was narrowed after the therapy, which may indicate the improvement of the skin tissue.

![Figure 2.26. OCT imaging of psoriatic skin with severe scaling (4 × 1.8 mm). The appearance of several layers in the superficial tissue is indicated by the arrow. (Adapted from [24])](image)

(d) Skin cancer

Skin cancers arise from the development of abnormal cells in the skin, with three commons types being basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma. BCC is typically observed as a raised, smooth, pearly bump within skin that is subject to sun exposure, such as the face. It grows slowly and is rarely fatal, but it can damage the surrounding tissue and the bones, if not detected and treated early [109]. SCC presents as a red, scaling, thickened patch, typically seen in sun-exposed, skin as well. It is more invasive than BCC with a higher chance of damaging surrounding tissue and undergoing metastasis, but it is treatable if detected early [109]. BCC and SCC are classified as non-melanoma skin cancers. Malignant melanoma originates from the melanocytes and is an aggressive type of skin cancer, which can lead to significant mortality.

Non-invasive visualisation of the skin structures with OCT provides a means to examine skin cancers. A loss of the normal skin structure and disarrangement of the epidermis and dermis in BCC have been demonstrated by Gambichler et al. using OCT imaging [110]. Notably, the honeycomb-like, signal-poor areas in OCT images were found to correspond to the tumour lobules of nodular BCC, as shown in Figure 2.27.
The changes of the structures in skin cancers can be further characterised by PS-OCT scans. Mogensen et al. applied PS-OCT to BCC, with representative images shown in Figure 2.28, in which the upper, non-birefringent, homogeneous band in the normal epidermis and papillary dermis [white bar in (d)] disappeared in the PS-OCT image of BCC in (b) [111]. This change in the birefringence properties can be potentially used for delineating the cancer boundaries.

Figure 2.27. Histology (a) and OCT image (b) of a nodular BCC. The multiple tumour lobules in (a) (indicated by *) is corresponding to the honeycomb-like signal-poor structures in (b). ED: epidermis; UD: upper dermis. (Adapted from [110])

Figure 2.28. PS-OCT imaging of a BCC: (a) Structural OCT image of the BCC with the corresponding phase retardation image in (b). (c) Structural OCT image of the normal skin with the phase retardation image in (d). (Adapted from [111])

As with other cancers [112], skin cancers are associated with abnormalities in the vasculature network due to rapid angiogenesis. OCT has been used to image the abnormal characteristics of vasculature in BCCs with an enhanced OCT depth of focus by Blatter et al. [23]. Figure 2.29 shows the OCT B-scan in (b) and en face vasculature image in (c) of a BCC in the photograph in (a), compared to the normal skin in (d)-(f). The vasculature in normal skin commonly appeared as small capillaries within a flat
vasculature bed in the upper layers [Figure 2.29(e)] and larger vessels in deep regions [Figure 2.29(f)]. In comparison with the normal skin vasculature, they summarised vasculature characteristics of the BCCs, including a denser network of unorganised vessels with chaotic branching, larger vessels close to the skin surface, and less pronounced and visible capillary structures, as shown in Figure 2.29(c). Combining these vasculature characteristics and the birefringence of the collagen network, provided by OCT, may provide a useful assessment of skin cancers for future applications.

OCT has been applied to the imaging of other skin lesions and conditions, such as bullous diseases [95], contact dermatitis [24], skin photoaging [113], keratosis [22, 114], hemangioma [22, 115, 116], and porokeratosis [22]. These skin conditions reveal characteristic features in the OCT images, which can extend our understanding or even aid the assessment of these conditions. These studies have shown the promise of OCT for dermatological applications. However, there is still a lack of validation of OCT in clinical applications, requiring a significant amount of future work to establish the value of OCT in the clinic.

2.5 Chapter summary
This chapter presented a review of the background to the research in this thesis, firstly on the physiological properties of human skin, skin injuries, wound healing, scar formation, methods for scar treatment and scar assessment. This was followed by an introduction to OCT imaging and its applications in dermatology. Based on OCT, quantitative methods for scar assessment will be developed in subsequent chapters. Specifically, we will present a method to assess scar vasculature in Chapter 3, and methods to assess the skin’s optical attenuation coefficient and birefringence in Chapters 4 and 5 respectively. These methods will be applied to burn scar patients to demonstrate the feasibility of OCT for quantitative scar assessment, as a step towards the validation of OCT for clinical applications.
Vasculature imaging for scar assessment

3.1 Preface

Human skin is perfused with a network of blood vessels, referred to as vasculature, to support the skin functions [51]. Pathological conditions can alter the characteristics of this vasculature network. One example of the altered characteristics is the prolific vasculature present in pathological burn scars. To assess the abnormality of vasculature in burn scars, clinical approaches typically involve manual observation of the scar’s colour to give a score for the vascularity (i.e., the quantity and characteristics of the blood vessels) [5]. However, this approach is highly subjective [5] and lacks information regarding the structure and organisation of the blood vessels in the scar tissue. Optical coherence tomography (OCT) [6] has the potential to enable objective assessment of the microvasculature in scar tissue.

This chapter investigates the feasibility of OCT imaging for the assessment of scar vasculature using image processing algorithms based on speckle decorrelation. The chapter begins with a brief review of current OCT vasculature imaging techniques. The following section, published as a paper in the Journal of Biomedical Optics, develops a method for imaging and quantifying the vasculature in human burn scars and demonstrates its feasibility through clinical measurements. Following this section, key
aspects of the speckle decorrelation method are then discussed, including a more
detailed description of our vasculature imaging algorithm. The feasibility of OCT
speckle decorrelation for longitudinal assessment of the vasculature is then investigated
through the monitoring of the wound healing process. The chapter concludes with
results from studies showing both one-off assessment of scar vasculature and
longitudinal assessment of a scar, demonstrating changes in vasculature during the
wound healing process.

3.2 Vasculature imaging using OCT

There is a large body of work describing the use of OCT to image vasculature, with
potential applications for various tissues, such as the retina [7, 12], brain [117], tumours
[118], and skin [23, 98, 108]. These papers note that conventional OCT on its own is
not sufficient to detect blood vessels as they often have a similar appearance to the
surrounding tissue. To isolate blood vessels from the surrounding tissue, extensions of
OCT have been developed to increase the contrast of vessels, including two main
groups of techniques based on temporal changes in either the OCT phase or intensity
[7].

3.2.1 Phase-based vasculature imaging

Phase-based OCT vasculature imaging techniques, including Doppler OCT and Doppler
variance OCT, take advantage of the Doppler effect [6, 7]. Figure 3.1 shows a schematic
of the orientation of the OCT beam and flow of blood cells within a vessel [6]. For
incoming and backscattered wave vectors of the OCT light of $k_i$ and $k_s$, the detected
light undergoes a Doppler frequency shift of
\[ f = \frac{1}{2\pi} (k_x - k_y) \cdot \mathbf{v} = \frac{2vn\cos\theta}{\lambda_0}, \] (3.1)

where \( \mathbf{V} \) is the velocity vector of the flowing cells; \( \theta \) is the angle between the flow and the imaging beam; \( n \) is the tissue refractive index; and \( \lambda_0 \) is the wavelength of the light. With Doppler OCT, this Doppler frequency shift is captured by the phase difference, \( \Delta \phi(z) \), between sequential complex OCT measurements, with the following relationship [119]:

\[ f(z) = \frac{\Delta \phi(z)}{2\pi T} = \frac{\phi_{j+1}(z) - \phi_{j}(z)}{2\pi T}, \] (3.2)

where \( T \) is the time interval between successive collocated \((j\) and \((j+1)\) A-scans. Based on Equations (3.1) and (3.2), the velocity component along the depth direction can be calculated from the measured phase difference as:

\[ v\cos\theta = \frac{\Delta \phi(z)\lambda_0}{4\pi n T}. \] (3.3)

Additionally, averaging can be incorporated into the calculation of \( \Delta \phi(z) \) to enhance the ratio of the flow signal to the background noise [120]. The Doppler phase difference is calculated at each location in a B-scan, proving an image of blood flow. In such an image, blood vessels show a large phase difference, whilst the static tissue shows phase difference values close to zero.

As Equation (3.3) shows, the Doppler OCT method is only sensitive to movement along the beam propagation direction since only this directional component contributes to the Doppler frequency shift and phase difference. The total velocity \( (v) \) can only be calculated when the direction of the flow relative to the beam propagation direction (i.e., \( \theta \) ) is known or by using more complex imaging setups, such as dual-beam bidirectional OCT imaging [12]. The velocity cannot be determined when the imaging direction is perpendicular to the direction of movement. Besides this angle-dependent property, phase wrapping imposes another challenge for accurate flow speed quantification, requiring phase unwrapping techniques to be overcome.

Alternatively, Doppler variance OCT [120, 121] uses the variance of the Doppler frequency spectrum to provide contrast for blood flow imaging, given as:

\[ \sigma^2 = \frac{(f - \bar{f})^2 P(f)df}{\int P(f)df} = \bar{f}^2 - \bar{f}^2, \] (3.4)

where \( P(f) \) is the Doppler power spectrum; and \( \bar{f} \) is the mean Doppler frequency shift. In contrast to Doppler OCT [122], Doppler variance is not capable of determining the flow directly without the use of a pre-determined variance-speed calibration curve.
Note that accuracy and sensitivity of phase-based OCT vasculature imaging is affected by the phase-stability of the OCT system, limiting its implementation to certain systems.

### 3.2.2 Intensity-based vasculature imaging

Intensity-based vasculature imaging is similar to laser speckle perfusion imaging [123] analysing the dynamics and the structure of the speckle pattern to measure the motion within a sample. In OCT, the speckle arises from the partially coherent backscattered light [124] and is temporally invariant for a stationary object. However, this speckle pattern varies temporally for moving particles, such as flowing blood cells. A number of measures have been proposed to measure the change in speckle over time. For example, the variance of the OCT speckle can be used [125]:

\[
SV_{jk} = \frac{1}{N} \sum_{i=1}^{N} \left[ I_{jk}(x,z) - \frac{1}{N} \sum_{i=1}^{N} I_{jk}(x,z) \right]^2 = \frac{1}{N} \sum_{i=1}^{N} \left( I_{jk} - I_{\text{mean}} \right)^2, \tag{3.5}
\]

where \( i \) is the index of the OCT B-scan; \( j \) and \( k \) are, respectively, the index of the of transverse (\( x \)) and depth (\( z \)) position in the B-scan; \( I \) is the OCT signal intensity; and \( N \) is the number of B-scans for calculating the variance. A large variance is caused by changes in the speckle pattern between acquisition of consecutive OCT measurements, indicating movement (e.g., blood flow).

An alternative measure of speckle change, referred to as correlation mapping [98], is calculated by the following formula [29]:

\[
corrMap(x, z) = \frac{\sum_{p=1}^{N} \sum_{q=1}^{M} \left[ I_a(x, p, z + q) - \overline{I_a}(x, z) \right] \left[ I_b(x, p, z + q) - \overline{I_b}(x, z) \right]}{\sqrt{\sum_{p=1}^{N} \sum_{q=1}^{M} \left[ I_a(x, p, z + q) - \overline{I_a}(x, z) \right]^2} \sqrt{\sum_{p=1}^{N} \sum_{q=1}^{M} \left[ I_b(x, p, z + q) - \overline{I_b}(x, z) \right]^2}}, \tag{3.6}
\]

where the correlation is calculated for two frames (\( I_a \) and \( I_b \)) in a window with a size of \( M \times N \), respectively, in the fast scanning and depth directions; and \( \overline{I_a}(x, z) \) and \( \overline{I_b}(x, z) \) are, respectively, the mean of the OCT intensity within the window in the two frames. The high speckle variance induced by moving blood cells corresponds to low correlation (i.e., high decorrelation), indicating the presence of blood vessels [29].

Measures such as these, quantifying change in speckle intensity, do not suffer from the Doppler methods’ angle-dependence since the flow component perpendicular to the beam propagation direction will also lead to detectable changes in the speckle pattern. Moreover, these intensity-based vasculature imaging techniques do not require high phase stability in the OCT scanner, and, therefore, are readily implemented with a wider variety of OCT systems. A faster flow speed generally leads to a higher variance
due to the faster change of scatters, and methods have been proposed to use the rate of speckle change to estimate variations in blood flow speed [126]. However, the flow speed is not available without additional calibration [127], and positive and negative flows are indistinguishable [126]. The intensity-based techniques provide an alternative for vasculature imaging when mapping the morphology of the vasculature is the primary objective rather than the determination of the flow speed.

Note that this group of vasculature imaging methods usually presents significant shadowing artefact, where the tissue below a vessel also undergoes temporal change in speckle even when this tissue is stationary. Projecting the 3-D vasculature image along the depth direction to form a 2-D en face image, such as the maximum intensity projection (MIP) of the decorrelation, is a typical way to minimise the impact of this artefact. In the following section, a vasculature imaging technique is developed using speckle decorrelation for clinical assessment of vascularity of burn scars patients. The section is adapted from a paper published in the Journal of Biomedical Optics (v18, no.6, art. no. 061213, 2013) and contains a separate bibliography to list all the citations in the paper.

3.3 In vivo assessment of human burn scars through automated quantification of vascularity using optical coherence tomography

[Yih Miin Liew, Robert A. McLaughlin, Peijun Gong, Fiona M. Wood and David D. Sampson; Journal of Biomedical Optics, vol. 18(6), 061213, 2013]

Abstract: In scars arising from burns, objective assessment of vascularity is important in the early identification of pathological scarring, and in the assessment of progression and treatment response. We demonstrate the first clinical assessment and automated quantification of vascularity in cutaneous burn scars of human patients in vivo that uses optical coherence tomography (OCT). Scar microvasculature was delineated in three-dimensional OCT images using speckle decorrelation. The diameter and area density of blood vessels were automatically quantified. A substantial increase was observed in the measured density of vasculature in hypertrophic scar
A proliferation of larger vessels (diameter ≥ 100 μm) was revealed in hypertrophic scarring, which was absent from normal scars and normal skin over the investigated physical depth range of 600 μm. This study establishes the feasibility of this methodology as a means of clinical monitoring of scar progression.

3.3.1 Introduction

Burn injury is an important global health issue, with nearly 11 million incidences worldwide of fire-related burn injuries reported annually [1]. Burn damage to the skin may also arise from a range of other causes, including contact with hot liquids or surfaces, electricity, radiation, chemicals and moving surfaces (friction) [2].

A broad spectrum of scars may result in response to a burn injury. A normotrophic scar is the best clinical endpoint as the scarred tissue assumes similar characteristics (thickness, color, pigmentation and pliability) to those of the surrounding, unscarred normal skin [3]. For some individuals, pathological scar conditions, such as hypertrophic scarring, can develop. A hypertrophic scar is characterized by a high degree of angiogenesis and an over-proliferation of collagen synthesis that is restricted to the original wound margin. It presents clinically as a red, raised, and rigid lesion, which may produce scar contractures (i.e., tightening of tissue) when located over joints [4-6]. Hypertrophic scarring is associated with delayed healing of burns [7] and occurs in more than 60% of patients [3, 6, 8]. It is a common outcome of a deep dermal burn and occurs within weeks of the injury. This type of scarring often increases in size for 3-6 months [9] and subsequent regression and maturation may take more than two years.

Examination of scar progression is important in the early diagnosis and treatment of pathological scarring, and in the assessment of response to treatment. A potent clinical indicator is the redness of the scar, which is indicative of the degree of angiogenesis [10, 11]. Angiogenesis occurs initially during the proliferation phase of the wound healing process and may continue long after wound closure. A red scar has a higher risk of becoming hypertrophic than a pale scar [10]. Regression of vascularity is expected towards scar maturity with notable reduction of redness, becoming comparable to the surrounding normal skin [12, 13]. Visual assessment of color is a standard clinical approach to rate scar vascularity, and is used in protocols such as the Vancouver Scar Scale [14, 15]. However, the unaided visual assessment of scar redness can be highly subjective, with limited sensitivity and significant inter-observer variability. Previous
attempts to objectively quantify scar vascularity have been performed within histological studies [16-19], although the invasive nature of histological analysis precludes its use in longitudinal assessments.

Several non-invasive techniques to measure scar vascularity have been investigated. These include the use of a reflectance meter to measure the erythema index of scars [11] and photography or videography to record scar color [10]. Video capillaroscopy has been used to evaluate the differences in the vasculature of hypertrophic scars and healthy skin [20]. It acquires image data with high resolution but is limited to extremely superficial vessels (within ~200 μm) [21]. Laser Doppler flowmetry (LDF) has been used to non-invasively measure blood flow at a single point in a hypertrophic scar [22, 23]. Laser Doppler perfusion imaging (LDI) [24] and laser speckle perfusion imaging (LSPI) [25] extend the point measurement of LDF into 2-D en face perfusion imaging. Whilst these techniques provide insight into the overall level of perfusion in a scar, they are unable to provide a depth-resolved perfusion map. With the exception of video capillaroscopy, these non-invasive techniques lack the micrometer-scale resolution required to distinguish individual capillary vessels and, therefore, are unable to resolve the microvasculature in tissue.

Optical coherence tomography (OCT) [26] is a non-invasive, micrometer-resolution 3-D tomographic imaging technique for in vivo tissue imaging. The capability of OCT for depth-resolved, microvasculature mapping has been demonstrated in previous in vivo studies of normal and psoriatic human skin [27-30]. It has also been applied to in vivo vasculature mapping in the human eye [31-33] and oral tissues [34]. Vasculature contrast in OCT is realized through the endogenous scattering properties of moving blood cells without the requirement of exogenous contrast agents. Several vasculature extraction techniques have been developed for 3-D OCT imaging, including methods based on the Doppler shift (such as phase-resolved Doppler OCT [35], optical microangiography [30], and Doppler standard deviation [36]) and based on speckle temporal dynamics (such as correlation mapping [27], speckle variance [37] and spatial speckle frequencies [38]). The choice of method is dependent upon the phase stability of the OCT system, and whether quantitative flow information is required.

The aim of the study reported here was to investigate the utility of OCT for clinical assessment of human cutaneous burn scars as a complement to visual assessment. We describe novel techniques to automatically quantify vessel diameter and density, and illustrate the methodology through a series of case studies, comparing a variety of scar tissues against contralateral or adjacent normal tissues. The results
constitute the first *in vivo*, non-invasive automated quantification of scar vascularity using OCT. We show that the use of these techniques enables 3-D OCT imaging to be used as a means of objective assessment of scar vascularity.

### 3.3.2 Material and methods

#### (a) Imaging system

Imaging was performed with a fiber-based swept-source polarization-sensitive OCT system (PSOCT-1300, Thorlabs, USA), comprising a broadband swept-source laser and a Michelson interferometer with balanced detection. The laser source has a central wavelength of 1325 nm and the average optical power of the probing beam incident on the skin is 3.2 mW. The measured full width at half maximum (FWHM) axial resolution is 17 μm in free space and the lateral resolution is 16 μm. The working distance and numerical aperture of the objective lens are 25.1 mm and 0.056, respectively. The system was operated at an axial scan rate of 5.4 kHz.

The sample arm was terminated in an imaging head mounted on an articulating arm [Figure 3.2(a)]. An adjustable sample spacer was affixed to the imaging head to enable a constant distance between the objective lens and the skin to be maintained. This spacer had an 18 mm diameter hole and a grooved base in order to attach a glass cover slip (22 × 40 × 0.13 mm), which was used as a window for imaging the scar through a layer of ultrasound gel [Figure 3.2(b)]. The use of ultrasound gel for refractive index matching has been shown to reduce a range of morphological and intensity artifacts common in OCT imaging of skin [39].

![Figure 3.2. (a) Imaging head mounted on an articulating arm, and (b) Imaging head-tissue interfacing setup with the lower right inset showing the *en face* view of the fiducial marker and scanning region.](image)

#### (b) Clinical image acquisition
The clinical protocol was approved by the Human Research Ethics Committee of Royal Perth Hospital and The University of Western Australia. In total, eight patients (four male and four female, Caucasian, mean age: 32 years) were enrolled with prior informed consent. Patients were undergoing follow-up examination of burn scars.

Prior to imaging, any hair on the area to be imaged was trimmed using an electric shaver to avoid shadowing artifacts in the OCT scans. To enable removal of motion artifacts, a small metal fiducial marker was adhered securely to the scar using double-sided adhesive tape, as described in Ref. [40]. The marker is a 1-cm square brass shim (thickness: 170 μm) with a 3-mm diameter hole in its center through which the scar was scanned [Figure 3.2(b)]. For each patient, scarred skin and normal unscarred skin (at a site adjacent or contralateral to the scar) were scanned. Each 3-D OCT scan was 4 × 1.5 × 3 mm (x × y × z) in size and consisted of 1088 × 1088 × 512 pixels, where x and y represent the lateral dimensions, and z the axial depth dimension. This resulted in a distance of 1.4 μm between two adjacent B-scans. All scans were performed in a quiet, temperature-controlled examination room and patients were rested for at least 20 mins prior to scanning.

To reduce the intensity of detected specular back reflections, the axis of the scanning beam was tilted at ~1° to the surface normal of the marker and glass cover slip. The OCT beam focus was set at ~350 μm below the scar surface (in physical distance) for optimal subsurface imaging. The B-scan acquisition time was ~200 ms, and the acquisition time for a 3-D volume was ~3 min 40 s.

(c) Data post-processing

(i) Segmentation of vascular network

The vascular network was segmented from the 3-D OCT scan using speckle decorrelation, based on the algorithm presented by Enfield et al. [27]. A linear intensity volume was produced by adding the squared magnitude of the data from the two detection channels of the OCT system. The segmentation algorithm then involves computing a correlation map between each pair of adjacent B-scans. Correlation coefficients, \( corrMap(x,z) \), were computed using the standard normalized cross-correlation equation [Equation (3.7)]:

\[
corrMap(x,z) = \frac{\sum_{p=1}^{N} \sum_{q=1}^{M} [I_a(x+p,z+q)-\bar{I}_a(x,z)][I_s(x+p,z+q)-\bar{I}_s(x,z)]}{\sqrt{\sum_{p=1}^{N} \sum_{q=1}^{M} [I_a(x+p,z+q)-\bar{I}_a(x,z)]^2} \sqrt{\sum_{p=1}^{N} \sum_{q=1}^{M} [I_s(x+p,z+q)-\bar{I}_s(x,z)]^2}}, \tag{3.7}
\]
where the window size is defined by a grid of \( M \times N \), and \( I_A \) and \( I_B \) are a pair of neighboring subimages in adjacent B-scans in an OCT linear intensity volume, which are defined by the window. \( \overline{I_A} \) and \( \overline{I_B} \) are the mean values of the subimages \( I_A \) and \( I_B \), respectively. Correlation values lie in the range \([-1, 1]\). A window of \( 3 \times 7 \) pixels \((x \times z)\) was empirically chosen for our system, and the computation was performed for each pair of adjacent B-scans in the 3-D OCT scan to yield a 3-D correlation map. Small absolute values of correlation reflect rapid speckle decorrelation, indicative of blood flow. Large positive correlation values indicate stationary tissue. In areas with low backscatter, there is a poor signal-to-noise ratio (SNR) and both stationary tissue and moving blood will exhibit rapidly decorrelating speckle. To reduce this confounding effect, the 3-D correlation map values were weighted by the OCT signal intensity. Specifically, correlation map values were inverted \((0 \leftrightarrow \text{correlated}, 1 \leftrightarrow \text{decorrelated})\) and then weighted with the median filtered, log-intensity back-scatter values. This reduced the estimated decorrelation in areas with low SNR.

To reduce decorrelation due to tissue bulk motion, each pair of adjacent B-scans was aligned using a cross-correlation intensity-based registration algorithm prior to calculation of the correlation map. After calculation, the shape of the vessels was corrected using a fiducial-based registration algorithm. Both registration algorithms have been described in Ref. [40].

Vessel visualization was performed using a 2-D en face maximum intensity projection (MIP) image calculated from the 3-D correlation map. First, the surface of the tissue was automatically extracted using a Canny edge detector [41]. The MIP was generated over the \( xy \) plane, including data to a physical depth of \(~600\ \mu m\) below the skin surface. Depth measurements were corrected assuming an average group refractive index of skin of 1.43 [42] at a wavelength of 1325 nm. In addition, a 2-D depth-encoded en face vessel image was produced by thresholding the correlation values and color-coding vessel pixels by their depth. Automatic quantification of vessel diameter and area density was performed on the MIP, as detailed in Section 3.3.2(c)(ii). The flow of data post-processing is summarized in Figure 3.3.

(ii) **Automatic quantification of blood vessel diameter and area density**

To measure blood vessel diameter, the MIP of the 3-D correlation map was thresholded and skeletonized [43]. The branch points (i.e., bifurcation points) of the skeleton were subsequently identified and eliminated. This step decomposed the vasculature skeleton into distinct vessel segments, with the skeleton points marking the center line of each
section of vessel. At each skeleton point, the orientation of the vessel was calculated by finding the line of best fit to skeleton points within a small circular neighborhood. The vessel diameter at each point was estimated by tracing a line perpendicular to the vessel orientation, identifying the edges of the vessel by the rapid increase in speckle correlation. An example illustrating the process is shown in Figure 3.4.

![Diagram of data post-processing steps.](image)

**Figure 3.3. Flow of data post-processing.**

In OCT, blood flow will cause speckle decorrelation at all subsequent axial depths in an A-scan. For this reason, superficial vessels may occlude deeper vessels and vessels which cross at different depths will appear to intersect. To reduce the impact on the quantification, we computed two MIPs: the first extending from the tissue surface to an empirically chosen physical depth of 300 μm; and the second extending from 300 to
Vessel pixels identified in the shallower MIP were subtracted from those in the deeper MIP. Vessel diameters across both MIPs were calculated and combined. As the distribution of vessel diameters is not symmetric, we report the median diameter instead of the mean.

To measure the area density of vessels, each MIP was thresholded to identify locations within the vasculature, with the threshold empirically set to 60% of the mean correlation values present within the tissue region of the MIP (i.e., excluding the fiducial marker). The percentage of total surface area of vasculature per unit area of tissue was then computed as an indicator of vessel density.

All computation was implemented in MATLAB (vR2009b, Mathworks, Natick, Massachusetts) on an Intel Quad Core i7@3.07GHz computer.

### 3.3.3 Results

In this section, we present four case studies to demonstrate qualitatively and quantitatively the capacity of OCT to measure the differences in vascularity between scar tissue and normal skin.

Case Study 1 is shown in Figure 3.5: a 5-month-old hypertrophic scar (left column) on the left anterior thigh of a 28-year-old female patient, and the corresponding contralateral healthy skin (right column). This scar was formed after a friction burn, and vascularity was categorized as ‘red’ on the Vancouver Scar Scale (VSS). Note that on the VSS, the degree of vascularity is categorized into one of four classes, in ascending degree: normal, pink, red and purple. The patient underwent three courses of laser treatment (localized thermal vascular damage) between three and four months after injury to flatten and improve the appearance of the scar.

In each photo, the imaging area is located in the center of a 1 × 1 cm square drawn in ink on the skin surface. *En face* MIPs of the correlation maps are shown in Figures 3.5(c)-(f). The latter two have been thresholded and color-coded by depth. Despite laser treatment, an abundant supply of large blood vessels is present in the scar. The histogram in Figure 3.5(g) illustrates the frequency of blood vessel diameters in both acquisitions. These distributions appear right-skewed, especially for the scar tissue, leading us to report the median, as foreshadowed in Section 3.3.2(c)(ii), rather than the mean for each distribution. The median diameter of the blood vessels in this hypertrophic scar was measured to be 44 μm, whereas that of the contralateral normal skin was 23 μm. The area density of vasculature was estimated to be 47% in the scar.
tissue, corresponding to a ~70% increase compared with the vasculature density in the contralateral normal skin (28%).

Figure 3.5. Case Study 1. Photographs of: (a) a 5-month-old hypertrophic scar due to a friction burn on the left anterior thigh; and (b) the contralateral normal skin, showing the 1 × 1 cm square shim outline. The en face MIPs of the 3-D correlation maps of the scar and normal skin over a depth of ~600 μm from the tissue surface are shown in (c) and (d), respectively. The vasculature from (c) and (d) is color-coded by physical depth (μm), respectively, in (e) and (f). Histograms of blood vessel diameter measurements and the quantified vascular parameters are shown in (g). Scale bar indicates a distance of 0.5 mm.

Figure 3.6 shows Case Study 2, a 12-month-old hypertrophic scar resulting from a scald with hot oil on the left lateral forearm of a 21-year-old male patient. The patient was diagnosed with a deep-partial thickness burn and the scar was graded ‘red’ on the VSS. The patient was treated after injury with ReCell® Spray-On Skin™ and received a split-thickness skin graft. ReCell® Spray-On Skin™ is an autologous epithelial cell suspension cultured from the patient’s own skin [44]. A split-thickness skin graft includes the epidermis and part of the dermis obtained from the patient’s own skin [45]. During treatment, the skin graft was meshed to cover the entire wound and the cell suspension was applied over this to assist wound healing. The MIPs in Figures 3.6(c)-
(d) show that the hypertrophic scar is more densely infiltrated with large blood vessels compared to the contralateral normal skin. This correlates with the histogram of Figure 3.6(g). The color-coded depth image shows that the vessels present in the scar are located at greater depths (>200 μm from the surface) than those in the normal skin [Figures 3.6(e) and (f)]. The median scar vessel diameter is 46 μm, almost twice the 24 μm diameter in normal skin. This corresponds to a similar increase in the vessel density: 45% for scar tissue versus 24% for normal tissue.

Figure 3.6. Case Study 2. Photographs of: (a) a 12-month-old hypertrophic scar resulting from a scald caused by hot oil on the left lateral forearm; and (b) the contralateral normal skin. En face MIPs of the vasculature over a physical depth of ~600 μm are shown in (c) and (d). The physical depths (μm) of the vessels in (c) and (d) are color-coded, respectively, in (e) and (f). Histograms of blood vessel diameter measurements and other quantification results are shown in (g). Scale bar indicates a distance of 0.5 mm.

Figure 3.7 shows Case Study 3, a 24-month-old, flame burn hypertrophic scar on the right medial thigh of a 20-year-old female patient. The wound was classified as a deep partial-thickness burn. The patient had received treatment comprising ReCell® Spray-On Skin™ and a split-thickness skin graft 20 days after injury. She was subsequently treated with three courses of intrallesional injections of corticosteroid to
flatten the scar. Corticosteroids are believed to reduce scarring by several mechanisms [46, 47], which include mediating vasoconstriction to reducing the supply of nutrients to the scar tissue and activating the endogenous collagenase to break down the scar matrix. The scar color was graded as ‘red’ on the VSS. Several large vessels are apparent at a superficial depth (~100-150 μm), as seen in Figure 3.7(d). Consistent with the other cases, the median vessel diameter in the scar (37 μm) is notably greater than that of the corresponding normal skin (23 μm). Vessel density is also correspondingly greater: 35% in scar tissue versus 19% in normal tissue.

Figure 3.7. Case Study 3. (a) Photograph of a 24-month-old hypertrophic scar due to a flame burn on the right medial thigh, and the adjacent scanned normal skin. (b) and (c) are the en face MIPs of the vasculature in scar tissue and normal skin, respectively. The physical depths (μm) of the vessels in (b) and (c) are color-coded in (d) and (e), respectively. Histograms of blood vessel diameter measurements and other quantification results are shown in (f). Scale bar indicates a distance of 0.5 mm.

Case Study 4, shown in Figure 3.8, is a 12-month-old normotrophic scar on the left lateral forearm of a 58-year-old female patient, originating from a flame burn. The scar had been treated with pressure garments and massage, but without surgery. The
scar appears hypopigmented but the scar color was rated ‘normal’ on the VSS. Vessels in this scar were found to have a similar diameter to those in normal tissue, although were far more prolific [Figure 3.8(b), (c)], with a density of 32% in the scar tissue versus 18% in the contralateral normal skin (photograph not shown).

Figure 3.8. Case Study 4. (a) Photograph of a 12-month-old normotrophic scar due to a flame burn on the left lateral forearm. The extent of the scar is outlined in dotted green. The photograph of the contralateral normal skin is not shown. (b) and (c) are the en face MIPs of the vasculature in scar tissue and contralateral normal skin, respectively. The vasculature in (b) and (c) is color-coded by physical depth (μm) in (d) and (e), respectively. Histograms of blood vessel diameter measurements and other quantification results are shown in (f). Scale bar indicates a distance of 0.5 mm.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Area density, % mean (±SE)</th>
<th>Median vessel diameter, μm mean (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scar</td>
<td>38 (± 3.2)</td>
<td>34 (± 3.2)</td>
</tr>
<tr>
<td>Normal skin</td>
<td>22 (± 1.4)</td>
<td>23 (± 0.7)</td>
</tr>
</tbody>
</table>

Table 3.1. Quantification results for all patients (n = 8).

Table 3.1 summarizes the results of the quantification of vascular parameters for all eight scar tissue and eight normal skin data sets. Overall, scar tissue showed a 73%
increase in vascular density over normal skin (38% vessel area density versus 22%). Combining the median diameter values from all scars, the average of these values is 34 μm, which is ~48% larger than the measured vessel diameter in normal skin (23 μm).

Figure 3.9 depicts a scatter plot of median vessel diameter versus area density for all data sets. Scars were categorized by a medical clinician as normotrophic or hypertrophic according to appearance and clinical history. One scar data set was excluded from this scatter plot as it was too early (three weeks old) in the scar formation process for the scar to be accurately categorized. Figure 3.9 suggests a correlation between the clinical assessments and the quantitative vascularity parameters. Our overall finding that the hypertrophic scars exhibited more and larger vessels correlated well with a redder appearance which forms the basis of clinical assessment.

![Figure 3.9](image)

**Figure 3.9.** Vessel diameter and area density for normal and scarred skin measured over a physical depth of ~600 μm from the tissue surface.

### 3.3.4 Discussion

In this pilot study, we have investigated the feasibility of non-invasively quantifying vascularity in scars to complement subjective clinical assessment as a step towards more evidence-based scar management. We demonstrated that OCT is indeed capable of providing depth-resolved microvasculature maps, which are a prerequisite for the understanding of angiogenesis in scarring. We demonstrated the feasibility of using automated techniques to extract quantitative measures of the vascularity from these maps. Such quantification offers great potential to minimize intra- and inter-observer variability in the longitudinal assessment of scar response to treatment.
We extracted maps of the vasculature from tissues using a speckle decorrelation technique [27]. In this technique, flow regions are extracted based on speckle variations (due to the movement of highly scattering blood cells) which manifest as fluctuations in the intensity of the backscattered OCT signals. As opposed to techniques based on the Doppler shift, flow contrast based on speckle decorrelation does not rely on the phase of the backscattered signal and, therefore, is applicable to systems vulnerable to phase instability, such as swept-source OCT. Although speckle decorrelation does not provide information on the flow velocity and direction, it is Doppler angle independent (i.e., sensitive to both axially and transversely oriented vessels), simple to implement and computationally efficient [27, 48]. Blood vessels were extracted only up to a physical depth of ~600 μm from tissue surface to avoid the confounding effect of noise at depths with poor SNR.

In this study, we observed that scar tissue in general, and hypertrophic scar tissue in particular, has a rich vascularization. Vascular density in scar tissues was found, on average, to be ~73% greater than in normal skin. Whilst normal skin and normotrophic scars are predominantly perfused with fine vasculature (diameter ~20-30 μm), clinically graded hypertrophic scars included larger vessels (diameter ≥ 100 μm) over the physical depth range investigated. The findings of this study are consistent with previous histological studies showing that hypertrophic scars are permeated with a higher density of blood vessels, some of which are enlarged, as compared to normal skin and normal scarring [16, 18]. Our results also correlate well with earlier studies using laser Doppler flowmetry, which found increased vascularity in hypertrophic scarring [10, 11].

Thickening of the epidermis, which is believed to be due to acanthosis (i.e., a thickening of the stratum spinosum) in some hypertrophic scarring [49], was observed in some data sets (e.g., Case Study 2) and can be implicated in a corresponding increase in the physical depth of the vasculature [see, e.g., Figure 3.6(e)]. Examination of the log-intensity OCT B-scans for this case study showed an epidermal thickness of ~260 μm in the scar tissue, as opposed to ~100 μm in the normal skin.

Various management techniques are currently in clinical use for prophylaxis and treatment of hypertrophic scarring. These include surgical excision, intralesional corticosteroid injections, pressure garment therapy [50], silicone gel sheeting, pulsed-dye laser treatments [51], radiotherapy [52, 53] and cryotherapy [54, 55]. Many of these techniques are primarily proven through extensive clinical use, with the effectiveness of only a few of them supported by a prospective study [9]. This is, in part, due to the
difficulty in objectively quantifying changes of scarring before and after treatments [9, 56]. Our preliminary study suggests the potential use of OCT to monitor and compare different treatment modalities in such a longitudinal prospective study.

A particular potential use of OCT vasculature imaging is in guidance for pulsed-dye laser treatment [51]. This treatment improves the appearance of scars by reducing erythema using localized injury of microvessels. Laser treatment of hypertrophic scarring has previously been postulated to alter the scar remodeling process by causing tissue ischemia and hypoxia, the release of cytokines, and a reduction in mast cell degranulation [51]. Previous studies [57] have demonstrated the promising use of OCT to guide laser treatment of human port wine stain, a congenital disease characterized by capillary dilation and malformation in the upper dermis. We note that the ability of OCT to quantify vasculature makes it a good candidate imaging modality to guide such treatments in scar tissue.

Scars often exhibit regional variation in severity. Within this study, scans were acquired in the severely affected areas of each scar. In clinical practice, multiple acquisitions across the scar would be required during the assessment because of the limited field of view of the OCT system. However, recent improvements in OCT image acquisition rates [58] could potentially allow larger scans to be acquired in real-time.

3.3.5 Conclusion
We have demonstrated the first *in vivo* clinical assessment of microvasculature in cutaneous burn scars of human patients using OCT. Using novel quantification techniques, vessel diameter and density were automatically extracted from OCT speckle decorrelation data. Through a series of case studies, our results showed a proliferation of larger vessels in hypertrophic scars when compared against normal contralateral or adjacent skin. These results correlate well with the established pathology of hypertrophic scars [16, 18]. The average median diameter of the vessels in scars was quantified as 34 μm, compared with 23 μm in normal skin. Scar tissue was also found to be more richly supplied with blood vessels than the normal skin; with an average area density of 38% measured for scars versus 22% for normal skin. The results of this work establish a basis for pathological scarring to be assessed in larger OCT clinical studies.

3.3.6 Acknowledgements
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### 3.3.7 References


3.4 Additional technical considerations in vasculature imaging

3.4.1 OCT sampling density

In the paper presented in Section 3.3, the OCT speckle was analysed by calculating the decorrelation of densely sampled adjacent B-scans since the OCT system was not capable of acquiring repetitive B-scans from the same location. By dense sampling, we refer to lateral spacing between adjacent B-scans that is significantly smaller than the lateral resolution. As the sampling space between each pair of adjacent B-scans increases, the speckle decorrelates, even in static tissue, as there is less overlap in the beam path [128, 129]. This decorrelation reduces the contrast between the blood vessels and static tissue. Thus, an important scanning parameter is the sampling density. We used a spacing between B-scans of 1.4 μm, which may be compared with the measured lateral imaging resolution of 16 μm. This sampling density was empirically chosen based on the observation that a less dense sampling would lead to a significant increase of the background decorrelation. The drawback of this dense sampling is a corresponding increase in memory requirements for the OCT C-scan. In practice, this limits the field of view which may be imaged. This limitation can be mitigated by
acquiring multiple B-scans at each location for the computation of speckle decorrelation, allowing a larger spacing between each group of co-located B-scans. This approach is explored in Chapter 6.

### 3.4.2 Detailed data processing flow

A detailed flow chart of the speckle decorrelation algorithm used for vasculature imaging in the previous section is shown in Figure 3.10. A PS-OCT system was used in the previous section so as to enable the analysis of tissue birefringence from the same scans in the following Chapter 5. The first step of the data processing is to build the OCT signal from the recorded raw data. The raw spectral data from the two detection channels of the PS-OCT system is linearly sampled in wavenumber space, followed by computation of the inverse Fourier transform. The OCT signal is then calculated as the sum of the square of the amplitudes of the two complex OCT depth profiles. The subsequent data processing chain to extract blood vessels is shown in Figure 3.10.

![Flow chart of vasculature imaging using speckle decorrelation. MIP: maximum intensity projection.](image)

In vivo clinical OCT scans are often subject to corruption by motion artefacts, which induce artificially high decorrelation. Our scans are easily corrupted by such artefacts due to the slow scanning speed (B-scan rate: 5 Hz) of our PS-OCT system. To mitigate these artefacts, each pair of adjacent B-scans are registered with an intensity-based registration algorithm, described in [105]. This algorithm maximises the correlation coefficient between the adjacent B-scans, defined as:

$$
CC = \frac{\sum_{x_y, x'_y} [A(x_y) - \bar{A}][B^T(x_y) - \bar{B}]}{\sqrt{\sum_{x_y, x'_y} [A(x_y) - \bar{A}]^2 \sum_{x_y, x'_y} [B^T(x_y) - \bar{B}]^2}},
$$

(3.8)
where $A$ and $B$ are the fixed reference B-scan and B-scan to be aligned with $A$ respectively; $x_A$ is the pixel position in B-scan $A$; $A(x_A)$ and $B^T(x_A)$ are the pixel intensity values of these B-scans at position $x_A$; $\bar{A}$ is the mean pixel intensity value in B-scan $A$ and $\bar{B}$ is the mean of $B^T |_{\Omega_{AB}}$, where $B^T$ is B-scan $B$ transformed to the coordinate space of B-scan $A$, and $\Omega_{AB}$ is the overlap domain of B-scans $A$ and $B^T$ for a given transformation estimate $T$ [105]. The output of this registration is the optimal transformation parameters to align the pair of B-scans.

The registered pairs of adjacent B-scans are used as inputs for calculating the cross-correlation by Equation (3.7). A window in the cross-sectional plane is defined for calculating the cross-correlation. This value is subsequently subtracted from one, to give a measure in which large values indicate blood flow (decorrelated), and small values indicate static tissue (correlated). The size of this window was empirically chosen so that the contrast of the vasculature is enhanced without being significantly blurred. The window size that we typically use is $\sim 11 \mu m \times 41 \mu m$ (lateral $\times$ depth), while the corresponding OCT imaging resolutions are, respectively, $16 \mu m$ and $17 \mu m$. Note that the sampling resolution was $3.7 \mu m \times 5.9 \mu m$. The window size along the fast scanning axis direction is smaller than the imaging resolution to avoid blurring of the blood vessels in the $en face$ plane. In the depth direction, although the window size is much larger than the imaging resolution, the vasculature image is not degraded by this strong averaging since there is unavoidable depth decorrelation shadowing and only the projection of the decorrelation in the $en face$ plane is displayed.

Absolute positioning of each image is then corrected using a feature-based registration method [105]. This method uses the shape of the round hole in the fiducial marker to align all images in a volume to remove the possible distortion caused by motion. First, the fiducial marker surface, which appears as a line of high backscatter on either side of the OCT B-scan, and the inner edges of the fiducial marker, are segmented from each B-scan using Hough transform [130], as shown in Figure 3.11(a). The rotation for registering each pair of adjacent B-scans is calculated as:

$$\theta_{ab} = \tan^{-1}\left(\frac{m_A - m_B}{1 + m_A m_B}\right),$$

(3.9)

where $m_A$ and $m_B$ are the slopes of the fiducial marker surfaces in the fixed reference B-scan $A$ and B-scan $B$ to be aligned with $A$, respectively, as shown in Figure 3.11(b).
The translation of the B-scan $B$ relative to $A$ along the lateral ($t_x$) and depth ($t_z$) directions are then determined from:

$$
\begin{pmatrix}
  t_x \\
  t_z
\end{pmatrix} = \begin{pmatrix}
  x_A \\
  z_A
\end{pmatrix} - \begin{pmatrix}
  \cos \theta_{AB} & \sin \theta_{AB} \\
  -\sin \theta_{AB} & \cos \theta_{AB}
\end{pmatrix} \begin{pmatrix}
  x_B \\
  z_B
\end{pmatrix}.
$$

(3.10)

($x_A, z_A$) and ($x_B, z_B$) are, respectively, the midpoints of the two inner edges in the two B-scans. Knowing the transformation rotation and translation parameters between adjacent B-scans, all B-scans are then registered relative to the first B-scan to align the whole 3-D OCT C-scan and decorrelation volume. As this registration is limited to image transformation in the cross-sectional ($x$-$z$) plane, it is unable to mitigate possible motion in the en face ($x$-$y$) plane.

To remove the artificially high decorrelation in regions with poor signal-to-noise ratio, the decorrelation data is weighted by the corresponding logarithmic OCT signal-to-noise ratio. Prior to the weighting, the logarithmic OCT data is first smoothed and thresholded with an empirically determined value to reduce background noise. The skin surface is then delineated by applying a Canny edge detector [131] to the thresholded, logarithmic OCT B-scans. The maximum of the weighted decorrelation within an empirically chosen depth (600 μm) below the skin surface is then projected into the en face plane as a MIP of the vasculature for visualisation and quantification, as shown in Figure 3.5(c).

![Figure 3.11 Feature-based registration: (a) Segmentation of the fiducial marker surface ('Detected line') and inner edges of the hole ('End point'); (b) Alignment of B-scan B ('Image B') to A ('Image A'). ($x_A, z_A$) and ($x_B, z_B$) are the coordinates of the central point of the inner edges of the fiducial marker. (Adapted from [105])](image)

### 3.5 Vasculature imaging of wound healing

Wound healing to repair wounded tissues after injuries, such as those caused by burns [51], involves changes in the vascular network and leads to the scar formation. Whilst the level of vascularity in the resulting normotrophic scars tends to approach that of normal, undamaged skin, pathological hypertrophic scars, caused by excessive healing, are usually accompanied by excessive blood vessel proliferation [29], giving the scar a characteristic red appearance. Imaging and quantifying the vasculature longitudinally
during healing may provide a timely indicator of healing progression and the resulting scar severity, therefore, enabling early intervention. The work in Section 3.3 showed the feasibility of OCT vasculature imaging for one-off measurements of the scar vascularity. In this section, the ability of OCT vasculature imaging to be used for longitudinal assessment is demonstrated. We apply the vasculature imaging technique presented in Section 3.3 to a case of wound healing in human skin *in vivo* and report the quantified vessel diameter and area density during the healing process.

![Figure 3.12](image)

The injured skin on the outer right forearm of a 29-year-old female was imaged with the same OCT scanner as that used in Section 3.3, on Days 5, 10, 16 and 22 [Figures 3.12(a)-(d)] following a moderate injury by contact with a hot surface. Care was taken to target the same skin region for OCT imaging using physical landmarks apparent on the surface of the skin, such as variations in pigmentation. The contralateral normal skin was also imaged to provide baseline measurements, with one example shown in Figures 3.12(e), (j) and (o). Each 3-D OCT scan (4 × 1.5 × 3 mm) was acquired from the centre of the blue 10 × 10 mm outlines of the fiducial markers on the skin in Figures 3.12(a)-(e) and processed to image the vasculature using the speckle decorrelation method described in Sections 3.3 and 3.4. A 2-D (4 × 1.5 mm) vasculature
MIP was generated by projecting the maximum speckle decorrelation values (weighted by the OCT intensity, as described in Section 3.4) from the surface to a depth of 600 μm below the skin surface. The vasculature quantification algorithm in Section 3.3 was then applied to the MIPs to automatically quantify the vessel diameter and area density.

Figures 3.12(f)-(j) and (k)-(o), respectively, show the vasculature MIP images and the corresponding colour-coded depth of the vasculature in the injured and normal skin. The vessel pattern delineated by the circles in Figures 3.12(f)-(i) is present in the vasculature MIPs of the injured skin at different time points. The appearance of common patterns of vessels validates that the same injured skin tissue region was successfully located in each longitudinal OCT scan. On Day 5, the injured skin presents dilated blood vessels [Figure 3.12(f)] with a higher vessel area density compared to normal skin [Figure 3.12(j)]. The surface scab of the wound started to detach on Day 10, creating an air gap between the scab and underlying skin, which gave rise to shadowing artefacts in the structural OCT images (not shown) and the artificial loss of vasculature in Figure 3.12(g). 16 days post-injury, the injured skin shows reduced vessel diameters [Figure 3.12(h)] compared to that on Day 5 [Figure 3.12(f)]. On Day 22, the healed skin exhibited a similar appearance [Figure 3.12(d)] to the normal skin [Figure 3.12(e)] with a significantly reduced vessel diameter and density [Figure 3.12(i)] in comparison to those on Day 5. The quantified vessel diameter and area density are summarised in Figure 3.13, showing the increased vasculature 5 days after the injury and its decrease to the level of normal skin on Day 22. Note that the quantified parameters of the injured skin on Day 10 shown in Figure 3.13 contain artefacts caused by shadowing, leading to an unreliable estimation.

Figure 3.13. Quantified vasculature area density and vessel diameter during wound healing. OCT FOV of the normal skin was shifted by several mm to investigate the heterogeneity in vasculature.
In this case study, we used the speckle decorrelation method for \textit{in vivo}, longitudinal monitoring of the changes in vascular morphology during the wound healing process. The quantified vessel parameters showed an elevated vascularity 5 days post-injury and decreased to the normal level by Day 22. These results indicate the regular vascular changes expected in the classic model of normal wound healing \cite{132}. When abnormal healing occurs due to the excessive synthesis of collagen, pathological scarring, such as hypertrophic scars, is formed. The hypertrophic scars have shown, by contrast, the presence of a prolific vasculature network even 1-2 years after the burn injuries, as seen in Section 3.3. Applying the presented imaging method to the abnormal healing process associated with hypertrophic scarring presents the opportunity to show the possible presence and maintenance of an elevated level of vascularity over a much longer duration. Thus, this imaging method can be potentially extended to investigate the vascular characteristics in abnormal healing longitudinally and aid in the early diagnosis and treatment of pathological scarring. This is explored through longitudinal imaging of hypertrophic scars in Chapter 6.

### 3.6 Chapter summary

Scar vascularity has been widely assessed through manual subjective scar assessment using scar scales \cite{5}, but there is still a lack of objective assessment methods in clinical use. OCT imaging has demonstrated the potential to provide objective quantification of skin vasculature \cite{98}, with particular value in assessing burn scars. In this chapter, we presented a method for clinical assessment of burn scar vascularity using OCT speckle decorrelation, as part of an imaging protocol optimised to reduce imaging artefacts through the use of refractive index matching media (ultrasound gel) and algorithms to correct for motion artefacts. We demonstrated the feasibility of this approach through imaging of hypertrophic scars, normotrophic scans and contralateral normal skin (Section 3.3). The quantified vessel diameter and area density showed a proliferation of larger vessels in hypertrophic scars, compared to normal skin. We then demonstrated the feasibility of using this approach for longitudinal assessment by assessment of a burn injury over a period of 22 days (Section 3.5). This final study showed elevated vascularity 5 days post-injury and its decease to normal levels 22 days post-injury. The vasculature imaging technique presented in this chapter shows great promise for clinical applications, and these will be further explored in subsequent chapters.
Chapter 4

Attenuation coefficient imaging for the assessment of collagen in scars

4.1 Preface

The collagen network in the dermis is a significant component of human skin, occupying approximately 18% to 30% of the volume of the dermis [133]. In burn scars, this collagen network is remodelled by the wound healing process following burn injuries [51]. In the case of pathological scarring, such as hypertrophic scars, the tissue undergoes an over-production of collagen [51], leading to raised, stiff scar tissue. Clinical scar assessment scales, such as the Vancouver Scar Scale, incorporate assessment parameters related to the presence of abnormal collagen, including manually assessed height and pliability of the scars [5]. However, this type of assessment is limited by high inter-observer and intra-observer variability due to its subjective nature [5].

The collagen comprises long, cylindrical fibres with a “basket-weave”-like organisation in normal skin [41, 42]. The structure and organisation of these collagen fibres give rise to characteristic optical properties of the skin tissue. In pathological scars, the resulting prolific collagen fibres have been shown to have a denser, more
parallel organisation in a more hydrated tissue environment than in normal skin [40-42, 54, 55]. Changes in the structure and organisation of collagen can lead to characteristic changes in the optical properties of scar tissue. Whist the optical properties of normal skin have been widely investigated [134], the optical properties of scar tissue are poorly understood. In this chapter, we use parametric optical coherence tomography (OCT) imaging to investigate the feasibility of utilising the optical properties, specifically the attenuation coefficient, of scar tissue for objective, quantitative scar assessment.

This chapter begins with a review of parametric OCT with a focus on the optical attenuation coefficient at near-infrared wavelengths. The following section comprises a journal paper, published in the Journal of Biomedical Optics. In the paper, a specific method, based on the single-scattering model, for imaging the skin attenuation coefficient is demonstrated and applied to burn scar patients. For accurate measurement of the attenuation coefficient, a novel vascular masking technique is presented to remove artefacts caused by blood vessels. This chapter concludes with a description of the characteristic attenuation coefficients of burns scars.

4.2 Attenuation coefficient imaging using parametric OCT

Parametric OCT is a method of extracting optical properties of tissue from OCT scans, typically presenting the calculated values as a parametric image, where the intensity of each pixel indicates the parameter values at that location in the tissue [9, 28]. Parametric OCT fits a model to the measured A-scan signal, modelling aspects such as the intensity attenuation or phase retardation, to calculate a physical parameter such as the attenuation coefficient [9], backscattering coefficient [100], or birefringence [8], associated with this A-scan. Repeating this with each A-scan allows the computation of the parametric image, typically an en face image (if 3D OCT data has been recorded). Parametric OCT complements the structural information in conventional OCT, providing additional insight into tissue structure through the calculated optical properties.

Parametric OCT was first demonstrated by Schmitt et al. with the measurement of several optical properties, including the backscattering coefficient and attenuation coefficient [100]. They developed a single-scattering model for OCT, which was used to calculate the optical properties from A-scans for several tissues, including the human skin, liver, adipose and cartilage [100]. In later work based on the single scattering model, van Leeuwen et al. proposed an approach to determine the attenuation coefficient by characterising and removing the effect of the axial point spread function.
point associated with the focusing optics in OCT [135]. Using this method, they extended attenuation coefficient calculations to OCT B-scans to characterise atherosclerotic plaque constituents [136]. Xu et al. further extended the parametric calculation to whole C-scans for characterisation of atherosclerosis plaques. An example is shown in Figure 4.1, where the calcification shows low backscattering and low attenuation (red); the fibrous plaque has high backscattering and low attenuation (green); and the lipid plaque has high backscattering and high attenuation (blue) [21]. This additional contrast provided by parametrisation of the optical properties allowed differentiation of additional tissue types in Figures 4.1(c) and (f). More recently, Scolaro et al. explored parametric imaging of human axillary lymph nodes and demonstrated differences between healthy and reactive nodes based on calculation of the attenuation coefficient [9]. Note that the aforementioned work fitted the model to the measured OCT signal within a depth range to calculate the underlying property, resulting in a reduction in axial resolution. To address this limitation, Vermeer et al. proposed a depth-resolved approach to calculate the attenuation coefficient at each corresponding pixel, providing improved retinal tissue characterisation as compared to the conventional OCT imaging [137].

These and other studies have investigated the application of parametric OCT to various tissues, including the attenuation coefficient of lymph nodes [9, 28], the relative attenuation coefficient of musculoskeletal structures [138], the scattering coefficient and
phase retardation of ovarian tissue [139], the attenuation coefficient and backscattering coefficient of atherosclerotic plaques [21, 136, 140, 141], the relative attenuation coefficient of oral mucosa [142], the attenuation coefficient of retina [137], the attenuation coefficient, backscattering coefficient and birefringence of normal human skin [44, 100, 143, 144], and the attenuation coefficient of skin with dermatitis and psoriasis [24]. These studies quantified optical properties, but more broadly, parametric imaging has been performed to image other tissue properties, including mechanical properties using elastography [145]. In this chapter, we focus on the attenuation coefficient-based parametric OCT which will be employed in Section 4.3.

The attenuation coefficient is the rate at which the measured backscattered light decreases with depth in the tissue. This attenuation at a given wavelength is indicative of the structure and organisation of the scatterers in the medium. This parameter can be calculated by fitting the OCT A-scan intensity signal to a specific OCT model [9, 100]. Two main scattering models have been used to calculate the attenuation coefficient from the OCT signal: the single-scattering [100] and the multiple-scattering [146] models. The single-scattering model assumes that the detected OCT light has been subjected to a single scattering event, while the multiple-scattering model takes into account the contribution of the light that has been scattered multiple times before being detected by the OCT scanner. The single-scattering model is relatively simple, allowing rapid calculation of an estimate of the attenuation coefficient. However, care should be taken to perform the fitting only for a depth range less than a few mean free paths to stay within the single-scattering regime [100]. In contrast, the multiple-scattering model is more complex, imposing a heavier computational requirement for the model fitting. One advantage of this model is that it allows the extraction of not only the attenuation coefficient, but also the scattering anisotropy factor [146], which is a measure of the scattering directionality. The majority of the aforementioned publications estimating attenuation coefficients have utilised the single-scattering model.

Prior to the model fitting for calculating the attenuation coefficient, preprocessing for removing OCT system-dependent factors is essential. The measured OCT A-scan, which is proportional to the tissue reflectivity according to Equation (2.7) in Chapter 2, is further modified by two system-dependent depth factors. Firstly, OCT systems usually use a weakly focused light beam with low numerical aperture in a confocal setup. This confocal setup induces a depth-dependent irradiance modulation to the A-scan, which indicates that there is a variation of the coupling efficiency of the light backscattered from different depths within the tissue. Secondly, the OCT depth
scan response also impacts the generated A-scan. In time-domain OCT, this depth scan response is caused by the coupling loss due to scanning the reference arm. In Fourier domain OCT, this is due to the sensitivity fall-off associated with the finite spectral resolution of the detector in spectral-domain OCT and the instantaneous lineshape of the swept-source laser in swept-source OCT [6].

The confocal function and the depth scan response function are both depth-dependent and can be represented, respectively, by $F(z)$ and $S(z)$. Based on Equation (2.7), we can incorporate these two functions to obtain the following relationship:

$$i(z) \propto F(z)S(z)R(z),$$  \hspace{1cm} (4.1)

where $i(z)$ is the linear OCT intensity (i.e., the detected photocurrent for time-domain OCT or the amplitude of the complex A-scan from inverse Fourier transform for spectral-domain and swept-source OCT); and the discrete power reflectivity function of the tissue is replaced by the continuous version $R(z)$. For calculation of the tissue attenuation coefficient, the effects of $F(z)$ and $S(z)$ are removed by correction. There are two common approaches to remove these two system-dependent factors: by measuring (or calculating) these two functions [135, 136] or by mapping these two functions in a phantom with well known optical properties [9]. In this thesis, we adopted the approach of using a phantom for calculating the scar attenuation coefficient, described in detail in the following section.

The calculation of the attenuation coefficient from the OCT signal assumes homogeneity of the tissue along the depth direction within the fitting window. However, when applied to the assessment of burn scars in vivo, this assumption is problematic due to the presence of prolific vasculature [29], which introduces heterogeneity and creates artefacts in the attenuation coefficient estimates. In order to overcome this problem, the attenuation coefficient imaging is combined with a vascular making technique for the clinical assessment of burn scars in the next section. The work in the next section has been published in the Journal of Biomedical Optics (v19, no.2, art. no. 021111, 2014). All citations in the paper are listed in the chapter bibliography instead of the thesis bibliography.

4.3 Assesment of human burn scars with optical coherence tomography by imaging the attenuation coefficient of tissue after vascular masking
Abstract: The formation of burn-scar tissue in human skin profoundly alters, among other things, the structure of the dermis. We present a method to characterize dermal scar tissue by the measurement of the near-infrared attenuation coefficient using optical coherence tomography (OCT). To generate accurate en face parametric images of attenuation, we found it critical to first identify (using speckle decorrelation) and mask the tissue vasculature from the three-dimensional OCT data. The resulting attenuation coefficients in the vasculature-masked regions of the dermis of human burn-scar patients are lower in hypertrophic (3.8 ± 0.4 mm$^{-1}$) and normotrophic (4.2 ± 0.9 mm$^{-1}$) scars than in contralateral or adjacent normal skin (6.3 ± 0.5 mm$^{-1}$). Our results suggest that the attenuation coefficient of vasculature-masked tissue could be used as an objective means to assess human burn scars.

4.3.1 Introduction

Wound healing after burn injury involves the sequential phases of inflammation, tissue formation, and tissue remodeling [1]. Scar formation during this process is a natural facet of healing, and hypertrophic scars are one pathology arising from an excessive response to injury during healing [2]. Such scars are characterized by prolific neovascularization; and excessive content [3-5] and abnormal arrangement of collagen [6]. In normal skin, collagen is arranged in a “basket-weave”-like structure, whereas collagen in hypertrophic scars is more unidirectionally aligned [6, 7]. Previous studies have shown that, compared with normal skin, hypertrophic scars contain collagen fibrils with reduced diameter [6, 8] and have higher water content [9, 10].

Various techniques are available or under investigation for the treatment of pathological scars including surgery, pressure therapy, corticosteroid injections, silicone gel sheeting, radiotherapy, and laser therapy [11]. Evaluating the efficacy of these clinical treatments typically requires the longitudinal assessment of a scar’s severity. Current scar assessment methods are primarily based on the direct observation and palpation of the features of scars. For example, the Vancouver Scar Scale uses observer assessment of pigmentation, vascularity, pliability, and height to characterize the scar
Assessment with such methods is inevitably somewhat subjective, leading to high inter-observer variability. There is reported research on alternative, less subjective techniques. Electron microscopy has been used to study the characteristic morphological profiles of pathological scars but requires histological slices of excised scar tissue [6, 13]. Multiphoton [14, 15] and confocal microscopies [16] have been applied to scar tissue or wounds, *ex vivo* and *in vivo*, including for high-resolution imaging of collagen and elastic fiber components. Ultrasound imaging has been investigated as a means of assessing the thickness of burn scars in pediatric patients noninvasively [17]. Other techniques, such as laser Doppler perfusion imaging and laser speckle perfusion imaging, have used the degree of vascularization as a surrogate indicator of scar status [18].

Changes in tissue morphology in pathological scarring can result in changes in the optical properties of the tissue in the near-infrared wavelength range. This has driven a growing body of work exploring the use of optical coherence tomography (OCT) in scar assessment [19-26]. OCT is a noninvasive imaging modality capable of acquiring three-dimensional (3-D) scans of tissue at resolutions from 1 to 20 μm from superficial tissues at depths of up to 1 to 2 mm [19]. The morphological features of normal and diseased skin have been studied *in vivo* with OCT imaging [20, 21]. Wound healing has been evaluated noninvasively by high-resolution OCT [22], and polarization-sensitive (PS) OCT has been used to measure the birefringence of both normal and burn-damaged human skin [23, 24]. OCT has also been used to noninvasively assess vascularity as a measure of normal skin and scar status [25, 26].

For objective scar assessment, absolute quantities that characterize the tissue are required to monitor the progression of healing. One such quantity that may be extracted from OCT scans is the attenuation rate (parameterized by the attenuation coefficient), which is the rate at which the OCT signal decreases with depth in the tissue. This rate can be extracted by fitting the measured OCT signal versus depth (an A-scan) to a specific model [27-29]. Previous studies have shown that the attenuation coefficient measured with OCT can be used to distinguish between different types of tissue [30-33]. Previous research has also explored the attenuation coefficient of normal human skin tissue [27, 34, 35]. A very effective means of visualizing attenuation coefficients is through the presentation of *en face* maps, i.e., parametric images of the attenuation coefficient [30, 31]. We hypothesize that differences in tissue microstructure between scarred and normal skin give rise to differences in the attenuation coefficient of near-infrared light. In this article, we extend the measurement of OCT attenuation
coefficients and their presentation in parametric images to the case of pathological scarred tissue.

A difficulty arises when applying the attenuation coefficient method to in vivo OCT scans of skin. The vasculature of scarred and normal skin generates strong forward scattering at near-infrared wavelengths due, in part, to the high anisotropy and high scattering cross-section of red blood cells [36], causing artificially high attenuation of the OCT signal that is not representative of the surrounding nonvascular component of the tissue. Here, we present an algorithm that automatically removes areas of vasculature from the calculations, allowing more accurate quantification of the attenuation coefficient of scarred and normal skin tissue. We present results from six clinically assessed scars that demonstrate a systematically lower attenuation coefficient of scarred tissue as compared with normal skin tissue and propose a mechanism explaining this observation.

4.3.2 Materials and methods

(a) OCT imaging

A polarization-sensitive swept-source OCT system (PSOCT-1300, Thorlabs, Newton, New Jersey) with a central wavelength of 1325 nm was utilized to perform the scanning on patients. The measured axial and transverse resolutions (full-width-at-half-maximum) of the system are, respectively, 17 μm (in air) and 16 μm. Light illuminated the skin through an objective lens with a working distance of 25 mm to deliver a scanning beam with a numerical aperture of 0.03 and a measured optical power of 3.2 mW to the skin.

The tissue-imaging setup has been described in detail previously [26]. Briefly, a 10 × 10 mm outline was drawn on the skin to guide the attachment of a metal fiducial marker, which had a central hole (3-mm diameter) for imaging. The marker was used as a guide to remove motion artifacts, as previously described [37]. An adjustable spacer between the OCT scanner and the skin was used to maintain a suitable distance from the objective lens of the OCT scanner to the skin surface. A layer of ultrasound gel was applied to reduce the refractive index mismatch at the skin surface, thereby reducing imaging artifacts and enhancing the imaging depth [38]. The scanner was positioned on the skin and handheld during data acquisition.

Scans were acquired on six patients (age: 19 to 58 years, three females, three males) undergoing follow-up examination 12 or 24 months after initial treatment for burns. The scanning protocol was approved by the Human Research Ethics Committee of Royal Perth Hospital and The University of Western Australia. Written consent was
obtained from all patients prior to scanning. For each patient, locations on the scar and on the contralateral or adjacent normal skin region were selected for 3-D OCT imaging. Each OCT data volume covered a region of $4 \times 1.5 \times 3 \text{ mm} (1088 \times 1088 \times 512 \text{ pixels})$ in the $x$, $y$ (lateral), and $z$ (axial into the skin) directions, respectively, acquired within the center of the outlined region, including a portion of the fiducial marker to enable motion correction. With these scanning parameters, over-sampling was used in order to record the speckle decorrelation for vasculature segmentation. The system was operated at an A-scan rate of $\sim 4.9 \text{ kHz}$, leading to a B-scan rate of $4.5$ frames per second and 3-D data volume acquisition time of $\sim 4 \text{ min}$.

(b) Vasculature masking

The flow chart for vasculature masking is shown in the left column of Figure 4.2. We denote by $i^2$ the sum of the squared OCT signals from the two detection channels of the PS-OCT system. This is equivalent to the square of a conventional OCT signal and is sometimes also referred to as the OCT signal power. In the remainder of this article, the term OCT data refers to $i^2$. Blood vessels were automatically segmented from this OCT data using speckle decorrelation [39]. In brief, the correlation between OCT data within a small moving window was calculated between pairs of adjacent B-scans, $A$ and $B$, using the standard normalized cross-correlation equation:

$$
\text{corrMap}(x,z) = \frac{\sum \sum \sum \hat{x}(x+p,z+q) - \bar{i}^2_A(x,z) \hat{y}(x+p,z+q) - \bar{i}^2_B(x,z)}{\sqrt{\sum \sum \sum \hat{x}(x+p,z+q) - \bar{i}^2_A(x,z) \hat{x}(x+p,z+q) - \bar{i}^2_B(x,z)}}
$$

(4.2)

where the window size is $M \times N$, and $\bar{i}^2_A$ and $\bar{i}^2_B$ are OCT data from a pair of neighboring subimages in adjacent B-scans in an OCT data volume that are defined by the window. $\bar{i}^2_A$ and $\bar{i}^2_B$ are, respectively, the mean values of $i^2_A$ and $i^2_B$.

Regions of low cross-correlation in the resulting correlation volume image were identified as blood vessels. Motion artifacts caused by patient movement during scanning were then removed from this correlation volume by the use of a registration algorithm utilizing the metal fiducial marker. Details of the motion correction are described elsewhere [37]. By identifying the voxel of the lowest correlation (i.e., the highest decorrelation) along each A-scan ($z$-direction) at depths in the skin with adequately high OCT signal-to-noise ratio, a two-dimensional (2-D) en face maximum intensity projection (MIP) image of the vasculature was produced, showing the ($x$, $y$) locations of blood vessels. Further details of the algorithm were given previously [26]. This MIP image was then thresholded to form a binary mask of blood vessels,
indicating \((x, y)\) locations to be removed from subsequent calculation of the attenuation coefficient.

(c) Calculation of the attenuation coefficient

Assuming that single scattering is the dominant scattering process, the rate of attenuation of the OCT signal with depth in a homogeneous sample follows a negative exponential function [27]. In addition, this signal is modulated by the confocal function of the focusing optics, and the sensitivity fall-off of our swept source OCT system. The skin OCT data, \(i^2\), as a function of depth \(z\) can be expressed as follows:

\[
i^2(z) \propto F(z)S(z)\rho e^{-2\mu z}
\]

where \(F(z)\) and \(S(z)\) are, respectively, the confocal and sensitivity fall-off functions, and \(\rho e^{-2\mu z}\) represents the single-scattering component, where \(\rho\) is the initial value of the reflectance and \(\mu\) is the attenuation coefficient [31].

To extract the attenuation coefficient, we first corrected our \textit{in vivo} scans for the confocal function and sensitivity fall-off of the system using a calibration scan of a low-scattering suspension of polystyrene microspheres (Polybead®, Polysciences, Inc., Warrington, Pennsylvania), as described by Scolaro \textit{et al.} [31]. The calibration OCT data can be written as
\[ i_0^2(z) \propto F(z)S(z)\rho_0e^{-\mu_zz} \] (4.4)

where \( \rho_0 \) is the initial value of the reflectance, and \( \mu_{i0} \) is the attenuation coefficient of the calibration suspension. \( \mu_{i0} \) was calculated to be 0.1 mm\(^{-1} \) at our OCT wavelength using Mie theory [40] and assuming negligible absorption. Each voxel value of the skin scans was corrected by division by the corresponding voxel value in the calibration scan volume. After taking the logarithm of the corrected data, the resulting logarithmic OCT data at each \((x, y)\) location as a function of depth \((z)\) is given by

\[
\ln\left[\frac{i^2(z)}{i_0^2(z)}\right] = -2(\mu_i - \mu_{i0})z + a,
\] (4.5)

where \( a \) is a constant for each A-scan dependent on the ratio \( \rho/\rho_0 \).

The aforementioned fiducial marker-based registration algorithm was applied to the corrected OCT data to reduce the motion artifacts and to maintain the spatial consistency between the vasculature mask and the corrected OCT data. A Canny edge detector [41] was used to identify the surface of the skin. For each dataset, the average depth to the dermis from the tissue surface within the scan field-of-view was empirically estimated. Speckle was reduced using a moving window averaging function with a uniform kernel of 40 × 40 \( \mu \)m within the \( x \)-\( y \) plane over regions not masked out. The attenuation coefficient \( \mu_i \) was calculated from a linear least-squares fit to the averaged logarithmic OCT data over a depth of 200 \( \mu \)m from the average depth into the dermis [taking the refractive index of skin to be 1.43 (Ref. [42]) to correct for the difference between physical and optical path length]. The goodness-of-fit of the least-squares regression was calculated to assess the fitting quality.

After performing the calculation on each A-scan at vasculature-masked locations covered by the central 1 × 1 mm \((x \times y)\) region, a 2-D image in the \( x \)-\( y \) plane was generated with the attenuation coefficient represented graphically as the pixel intensity for each location. In addition, a one-dimensional normalized histogram was computed, comprising all attenuation values in the image (not including locations masked by the vasculature). Each histogram contained typically 100,000 to 170,000 values, corresponding to the number of vasculature-free A-scans in each dataset. The histogram shows the distribution of attenuation coefficients for scar tissue and the contralateral or adjacent normal skin tissue. All data processing was performed using MATLAB® (vR2012a, The MathWorks, Inc., Natick, Massachusetts).

### 4.3.3 Results
(a) Vasculature-induced artifacts

Figure 4.3 shows the calculated attenuation coefficient and related data for normal skin on the lower right leg of a 19-year-old Caucasian male patient. The OCT scanning area (4 × 1.5 mm) was in the center of the region marked with a square outline (10 × 10 mm) on the skin, as shown in Figure 4.3(a). Figure 4.3(b) shows the 2-D en face vasculature MIP of the scanning region. The blue square (1 × 1 mm) delineates the region over which the attenuation coefficient map in Figure 4.3(c) was extracted using the corrected, registered, and averaged structural OCT data. The attenuation coefficient determined for all (vascular and nonvascular) tissue regions is shown. The corresponding goodness-of-fit (0 → poor fitting, 1 → good fitting) is presented in Figure 4.3(d). By comparing Figures 4.3(b) and (c), it is apparent that the attenuation coefficient values in the circled vascular regions are much lower than in the surrounding tissues, even giving rise to nonphysical negative values. Figure 4.3(e) compares the normalized attenuation coefficient distribution between the vascular (i.e., blood vessels) and nonvascular (i.e., all tissue excepting blood vessels) regions and highlights this problem. The attenuation coefficient distribution in the vascular region is artificially broadened and extended to low values.

Figures 4.3(f) and (g) show two representative fitting examples, respectively, for nonvascular and vascular tissues, marked by the two small purple squares in Figure 4.3(c). The region of OCT signal used for the fitting (200 μm in length) is shown in blue, while the remainder of the A-scan is rendered partially transparent. The attenuation coefficient is extracted from the line of best fit (red) for each A-scan. Figure 4.3(f) demonstrates the applicability of the single-scattering model to the nonvascular dermal tissue with a high goodness-of-fit value of 0.91. In Figure 4.3(g), the presence of two blood vessels at different depths gives rise to the sharp drops in the observed signal. The subsequent increase in the measured OCT signal below the first blood vessel gives rise to an apparent negative attenuation coefficient value with a poor goodness-of-fit value of 0.06. It is also evident from Figure 4.3(d) that the low attenuation in the outlined vascular regions is accompanied by poor goodness-of-fit to the single-scattering, log-linear model. Since scarred skin has been shown to have larger blood vessel diameters and higher vasculature density than normal skin [26], this vascular artifact is expected to be still more prominent in scar tissue. Consequently, we incorporated a vasculature-masking step in the determination of the attenuation coefficient in order to minimize this corrupting effect.
Figure 4.3. Attenuation coefficient of normal skin showing the corrupting effect of the vasculature: (a) Photograph and (b) vasculature MIP of normal skin. (c, d) En face attenuation coefficient map and corresponding goodness-of-fit of the vascular and nonvascular tissues in the region outlined in blue in (b) (each image 1 × 1 mm). Examples of vascular regions are circled by the dotted lines in (b), (c), and (d). (e) Comparison of normalized attenuation coefficient distribution between vascular and nonvascular tissue regions. (f, g) Fitting examples, respectively, from nonvascular and vascular regions marked by the small purple squares and indicated by arrows in (c).

(b) Case study of a hypertrophic scar

Figure 4.4 shows a 12-month-old hypertrophic scar originating from a hot water burn on the medial left inner forearm of a 30-year-old Caucasian female patient. Figures 4.4(a) and (b) are photographs of the contralateral normal skin and scar tissue, respectively. OCT scans were performed in the central 4 × 1.5 mm of the outlined regions. Figures 4.4(c) and (d) show the vasculature MIPs for the normal skin and scar, respectively, with the central blue squares (1 × 1 mm) identifying the regions for attenuation coefficient calculation. As noted by Liew et al. [26], the vasculature is observed to be much more prolific in the scar tissue. Figures 4.4(e) and (g) depict the masks generated (black: vascular region; white: nonvascular region) after thresholding the vasculature MIPs. Figures 4.4(f) and (h) present the 2-D en face attenuation coefficient maps,
respectively, for normal skin and scar. The attenuation coefficient is visibly lower in the hypertrophic scar than in the contralateral normal skin. From the distribution of attenuation coefficient values for each scan plotted in Figure 4.4(i), we observe that the attenuation coefficients in scar tissue have a distribution of similar width to normal skin tissue but are on average much lower, with the mean attenuation coefficients of scar and normal skin being 3.8 and 6.4 mm\(^{-1}\), respectively.

**Figure 4.4.** Case study of the attenuation coefficient of a hypertrophic scar: (a, b) Photographs of the contralateral normal skin and scar. (c, d) Vasculature MIPs of the normal skin and scar in (a) and (b), respectively. (e, g) Vasculature masks for normal skin and scar (1 × 1 mm), respectively. (f, h) *En face* attenuation coefficient (mm\(^{-1}\)) maps of, respectively, normal skin and scar (1 × 1 mm). (i) Histogram of attenuation coefficients for normal skin and scar.

(c) **Case study of a normotrophic scar**

The results of a case study on a 12-month-old normotrophic scar are shown in Figure 4.5. The scar, outlined in red in Figure 4.5(a), was caused by a wood fire burn on the
left lateral forearm of a 58-year-old Caucasian female. Figures 4.5(b) and (c) show MIPs of the vasculature of the contralateral normal skin (photograph not shown) and the scar, respectively. After masking of the vasculature using Figures 4.5(d) and (f), the calculated attenuation coefficient maps are shown in Figures 4.5(e) and (g). Figure 4.5(h) plots the normalized attenuation coefficient distribution of the scar and normal skin, which have mean values of 5.4 and 6.7 mm\(^{-1}\), respectively. Note the increased degree of overlap of the distributions between this (nonpathological) normotrophic scar (red) and the contralateral normal skin (green), in comparison to the (pathological) hypertrophic scar in Figure 4.4(i).

Figure 4.5. Case study of the attenuation coefficient of a normotrophic scar. (a) Photograph of the scar (which is outlined in dotted red). (b, c) Vasculature MIPs of the normal skin and scar, respectively. (d, f) Vasculature masks for normal skin and scar (1 × 1 mm), respectively. (e, g) En face attenuation coefficient (mm\(^{-1}\)) maps of, respectively, normal skin and scar (1 × 1 mm). (h) Histogram of attenuation coefficients for normal skin and scar.

(d) Results from all subjects
The mean (± standard error) attenuation coefficient of the vasculature-masked dermal tissue in hypertrophic scars (n = 3), normotrophic scars (n = 3), and normal skin (n = 6), calculated from data on all subjects, is summarized in Figure 4.6. The hypertrophic scars give rise to the lowest attenuation coefficient (3.8 ± 0.4 mm\(^{-1}\)); the normotrophic scars values are greater (4.2 ± 0.9 mm\(^{-1}\)); and the contralateral or adjacent normal skin has the largest values (6.3 ± 0.5 mm\(^{-1}\)). Student’s t-test shows that the difference between the attenuation coefficients of the scar and normal skin is statistically significant (p < 0.001).

![Figure 4.6. Mean (± standard error) attenuation coefficient of hypertrophic scars (n = 3), normotrophic scars (n = 3), and normal skin (n = 6) of all subjects.](image)

4.3.4 Discussion

In this study, we specifically focused on characterization of the dermis, since it undergoes very significant changes in content and structure during the wound healing process [2] and occupies most of the OCT imaging depth range. As the epidermis is only about 100-μm thick and is largely restored with new epidermal tissue during the wound healing process, we chose to exclude it. Our results show on average 36% lower dermal attenuation in scarred skin compared with normal skin. This strong effect can potentially be used as the basis for an objective method of scar characterization. As pathological scarring commonly leads to excessive production of both collagen fibers and blood vessels, the present method complements the scar assessment method solely based on vasculature that we have recently reported [26]. The integration of both methods could potentially be used to track the efficacy of various treatment strategies targeting both collagen fibers and blood vessels.
To explain the contrast that we observe in the vasculature-masked tissue, we have employed a model proposed by Jacques which has been shown to accurately describe the attenuation of light in the dermis [43]. This model was verified against measurements of the reduced scattering coefficient, \((1-g)\mu_s\), where \(\mu_s\) is the scattering coefficient and \(g\) is the anisotropy factor, of *ex vivo* samples of dermis [44]. Jacques’ model treats scattering in the dermis as being equivalent to single scattering by a collection of infinite cylinders, each of which represents a collagen fiber. Jacques also considered the contribution of Rayleigh scattering by subwavelength scatterers, but it has been shown that Rayleigh scattering is insignificant compared with scattering by the collagen fibers at the wavelength of interest [44]. The key parameters in the model are, thus, the refractive index of the collagen fibers and the background material, the distribution of fiber diameters, and the volume fraction of collagen fibers. The refractive index of the collagen fibers was calculated by assuming that the fibers are composed of a combination of water and collagen. Mie theory, describing the scattering of light by infinite cylinders, was used to calculate \(\mu_s\) and \(g\).

In relating Jacques’ model to the mathematical form of the OCT signal in Equation (4.3), we note that \(\mu_s\) is proportional to \(\mu_s a(g)\), where \(a(g)\) represents the directional nature of scattering [45]. We, however, found that \(g\) varies insignificantly compared with \(\mu_s\) when contrasting normal skin and scarred tissue, enabling \(a(g)\) to be neglected when assessing the relative magnitudes of \(\mu_s\) for the normal and scar cases. We did not have access to data on the distribution of fiber diameters for the particular cases presented in this article. We, thus, approximate the two ensembles of fibers, i.e., scar and normal, by their mean diameter and spacing from the literature [7, 46]. In particular, the mean spacing of collagen fibers has been found to be 10.37 \(\mu m\) and 8.53 \(\mu m\) for normal and scar tissues, respectively, while the mean diameter was found to be 5.39 and 5.77 \(\mu m\), respectively. Thus, if all other parameters remained equal, the higher density of fibers would result in \(\mu_s\) for scar tissue being larger than that for normal tissue. It has, however, been noted that the percentage of water content is greater in hypertrophic scar tissue than in normal tissue (approximately 64% in normal skin) [9, 10]. The water content of hypertrophic scar tissue has been found to vary with scar age and, in one study, varied monotonically between 85.1% for a 0.5-year-old scar to 63.8% for a 2-year-old scar [9]. Another study noted that the water content of hypertrophic scar was 82%, although the age of the scar was not noted [10]. We cannot suppose that this water content is identical to that likely to be found in the collagen fibers. We note,
however, that Jacques [43] assumed a water content of 65% in his model, which is in agreement with the measured figure for normal skin tissue.

Our approach was, thus, to calculate the ratio $\mu_{\text{scar}}/\mu_{\text{normal}}$, where $\mu_{\text{scar}}$ and $\mu_{\text{normal}}$ are the scattering coefficients for scar and normal skin tissues, respectively, for different percentages of water content in scar collagen fibers, while the water content percentage of collagen fibers in normal skin was fixed at 64% consistent with the literature. We used refractive indices for water and collagen of 1.3241 and 1.4367 at a wavelength of 1325 nm, respectively [47]. The scattering coefficients were calculated using Mie theory for scattering by cylinders, at normal incidence, using the mathematical formulation presented by Bohren and Huffman [40]. Figure 4.7 shows the simulated ratio of scattering coefficient between scar and normal skin, $\mu_{\text{scar}}/\mu_{\text{normal}}$, as a function of water content in the scar collagen fibers. The green triangle and red circle highlight simulated scattering coefficient ratios between scar and normal skin of particular interest: respectively, for the water content percentage of 64% (2-year-old hypertrophic scars) and 85% (0.5-year-old hypertrophic scars) [9]. The purple square corresponds to the ratio derived from our calculated attenuation coefficients of the scars and normal skin, and the resulting inferred water content (~76%) lies between that of the 0.5- and 2-year-old hypertrophic scars. This level is consistent with the 1.2-year-old mean age of the scars in this study but not with the lower reported water content of 1-year-old hypertrophic scars (66.5%) [9]. Although requiring further study, our model and previous reports suggest that the contrast in attenuation coefficient may come from higher water content in scars. Thus, results of the type plotted in Figure 4.7 could be used to study the water content in scar tissue noninvasively from measurement of the OCT attenuation coefficient ratio.

The masking of blood vessels removed a significant artifact from the calculation of the attenuation coefficient caused by the strong scattering by blood vessels. To the best of our knowledge, this is the first reported incorporation of vasculature masking into the calculation of attenuation coefficients from OCT data. Blood comprises plasma and other constituents, among which erythrocytes dominate the optical properties. Erythrocytes have a biconcave, disk-like shape with a size considerably larger than our OCT wavelength, which leads to strong forward scattering. This, together with the high-scattering cross-section and strong absorption of light [36], generates a strong drop in the OCT signal, causing blood to exhibit a much higher attenuation coefficient than the nonvascular tissue in the skin. A previous study on fresh porcine blood, using 1300 nm
OCT light, reported an attenuation coefficient of 12.15 mm$^{-1}$ [48], which is almost twice the mean value of the vasculature-masked skin tissue measured in this study. This is particularly important in the evaluation of hypertrophic scars, which are known to have enhanced vasculature [26]. Analysis of the attenuation in these masked regions of blood flow may potentially provide additional information on metabolism such as the noninvasive monitoring of blood glucose concentration [49].

![Figure 4.7. Simulated $\mu_s^{\text{scar}}/\mu_s^{\text{normal}}$ ratios versus water content in scar collagen fibers. The triangle and circle represent water content values reported in the literature [9], and the square represents the value calculated from the data in this study.](image)

Skin comprises a collection of microstructures with varying scattering and absorption properties. Quantification of scar and normal skin attenuation coefficients is complicated by tissue heterogeneity. Even after masking out the vasculature, heterogeneity in depth can invalidate the single-scattering exponential decay model and results in modified attenuation coefficient values, even negative values when the fitting window initiates in a low-signal region and extends into a high-signal region. We empirically found a fitting range of 200 $\mu$m in the dermis to be an appropriate compromise between including sufficient data in the estimate of the attenuation coefficient and minimizing the effects of tissue heterogeneity. The average attenuation coefficient of normal skin we determined using this fitting length is 6.3 ± 0.5 mm$^{-1}$, which lies between the results reported by Schmitt et al. [27] (4.6 to 4.7 mm$^{-1}$) and Kholodnykh et al. [34] (10 to 13 mm$^{-1}$) for forearm at 1300-nm wavelength. As discussed by Kholodnykh et al. [34], the variation in attenuation coefficient could be caused by factors such as differences in experimental protocol, the use of a clearing agent, and variations in the skin tissue among individuals. One different feature of our
experimental protocol from that of others is that we removed the contribution of blood vessels to target the nonvascular tissue.

In vivo OCT skin scanning can be affected by the motion between the scanner head and the skin. Such motion introduces image artifacts that distort the structural OCT images [37]. We used a fiducial marker fixed to the skin to guide a feature-based registration algorithm to remove such artifacts. However, motion artifacts also artificially increase speckle decorrelation, resulting in errors in blood vessel segmentation. An example of such an artifact appears as a pattern of horizontal lines in the vasculature MIP of Figure 4.3(b). Alternative solutions have been proposed, such as rigidly affixing the scan head to the patient’s skin [50, 51], although this can present additional difficulties when scanning acute burn wounds. Another feasible method is to reduce the 3-D data acquisition time, although this may reduce the sensitivity of vessel detection in areas of slow blood flow due to reduced speckle decorrelation between rapidly acquired B-scans.

4.3.5 Conclusions

In this article, we have presented a method for in vivo assessment of near-infrared attenuation coefficients of the dermis using OCT. We corrected for artifact caused by the presence of blood vessels using an automated method to segment and mask vasculature. We demonstrated the method of assessment on several in vivo human burn scars. Our results indicate that scars of mean age 1.2 years have on average 36% lower attenuation coefficients than contralateral or adjacent normal skin, and our modeling suggests that the lower attenuation coefficients arise from the previously reported increased water content in scars. This method could potentially be integrated with an automated vascularity quantification method in an objective scar assessment protocol to characterize both the vasculature and the connective tissue in scars. Future work will involve the investigation of this objective assessment protocol for the longitudinal study of human burn scars.

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4.3.7 References


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4.4 Chapter summary

In response to a burns injury, scars are formed by tissue regrowth and remodelling processes. This includes synthesis of both collagen fibres and blood vessels to close the wound. The newly synthesised collagen results in changes in structure and organisation of the tissue [40, 42], in comparison to normal skin. These changes are difficult to assess with OCT alone, since the relative OCT signal intensity does not provide a quantitative measurement. Measurement of optical properties, such as the attenuation coefficient, provides a quantitative approach to assess the impact of these changes to the tissue’s structure. The estimation of these types of optical parameters from the OCT signal is referred to as parametric OCT.

In this chapter, the parametric imaging of the tissue attenuation coefficient was investigated for the non-invasive, objective assessment of the burn scars. While the prolific blood vessels provided an indication of the scar severity in Chapter 3, they also introduced artefacts to the measurement of the attenuation coefficient, as shown in Section 4.3. In order to generate accurate attenuation coefficient maps, the vasculature was identified and removed prior to the calculation of the attenuation coefficients. The clinical results showed scars of mean age 1.2 years have on average 36% lower attenuation coefficient than normal skin, which is statistically significant. Our modelling suggested the lower attenuation coefficients are indicative of increased water content in scar tissue [54, 55]. Our results suggest that this type of attenuation coefficient imaging, when combined with characterisation of the vasculature as presented in Chapter 3, provides new insight into scar status, with the potential to assess changes during the healing process.
Chapter 5

Birefringence imaging for the assessment of collagen in scars

5.1 Preface

Birefringence is an optical property of materials, where the refractive index differs as a function of the polarisation state and propagation direction of light [43]. This results in changes in the polarisation state of light passing through the material. Birefringence is exhibited by many biological tissues, such as the muscle, nerve, bone, cartilage, tendon and skin [43]. It reflects the degree of anisotropy and structural organisation of the tissue.

This structure is altered by many pathologies, resulting in a change in tissue birefringence. In human skin, collagen forms fibrils comprising a distinctive triple helix of collagen molecules, and bundles of these fibrils form fibres. The high aspect ratio of these fibrous structures gives rise to the birefringence of the skin tissue, including the intrinsic birefringence, due to the ordered configuration of chemical groups of the collagen molecules, and form birefringence, caused by the ordered organisation of these molecules [39]. Burn injuries tend to decrease this birefringence since the structure and arrangement of the collagen network can be significantly changed by the tissue coagulation [101]. The collagen in the resulting burn scars after the wound healing process shows changes in the quantity [51] and arrangement of the collagen network [41, 42], which may lead to their characteristic birefringence properties.
This chapter investigates the feasibility of birefringence imaging with polarisation-sensitive optical coherence tomography (PS-OCT) to characterise scar tissue. It begins with a review of PS-OCT techniques, including a description of its working principle, and a brief summary of its applications for dermatology. The following section is adapted from a paper that has been published in the Journal of Biomedical Optics (vol. 19, no. 12, art. no. 126014, 2014). The paper demonstrates an algorithm for calculating skin birefringence from the PS-OCT signal, adapted from that presented by Chin et al. [8], and applies it to human burn scars. The vascular masking technique introduced in the previous chapter is incorporated into the birefringence calculation, providing a novel method to remove vasculature-induced artefacts in the measurement of birefringence. A brief summary concludes this chapter, identifying key findings from the paper.

5.2 PS-OCT

5.2.1 Working principle of PS-OCT

Birefringent tissues alter the polarisation state of propagating light. The rate at which the polarisation state changes with distance travelled through the tissue is related to the degree of birefringence. PS-OCT combines the depth sectioning ability of OCT with the ability to resolve the polarisation state of light at each depth [94, 147].

There are several different implementations of PS-OCT. Early systems used a bulk-optic design with a representative schematic shown in Figure 5.1 [6]. Key changes from a regular bulk-optic OCT system are the addition of a polarising beam splitter in

Figure 5.1. Schematic of a time-domain PS-OCT system. L: lens; Pol: polariser; BS: beam splitter; QWP: quarter wave plate; PBS: polarising beam splitter. (Adapted from [6])
the detector, to separately resolve the orthogonally polarised components of the interferometric signal, the linear polariser at the source, and the quarter-wave plates in the reference and sample arm, which act to generate specific polarisation states for the reference and incident sample light. Bulk-optic PS-OCT systems have generally been superseded by fibre-based systems, but are described here to illustrate the basic principles of PS-OCT.

The working principle of the PS-OCT system in Figure 5.1 has been described in the literature [6, 94]. Briefly, the collimated imaging light from the source first passes through a polariser to generate a linear horizontal polarisation state. This incoming light is split evenly into the reference arm and sample arm by a non-polarising beam splitter. The reference light passes through a zero order quarter wave plate twice, oriented at an angle of 22.5° to the horizontal direction, and returns to the beam splitter. This results in a linear polarisation state with an angle of 45° to the horizontal direction to interfere with the backscattered light from the sample.

In the sample arm, the light passes through a quarter wave plate with an angle of 45° relative to the horizontal direction to generate a circularly polarised light for probing the tissue. This circular polarisation state is then modified depending on the tissue birefringence properties. The returning backscattered light passes through the quarter wave plate again and is then directed to the beam splitter. The combined light from the reference arm and sample is then split by a polarising beam splitter into horizontal and vertical components. These two components are detected separately and processed to generate the PS-OCT signal.

To demonstrate the detailed configuration of the polarisation state of light in a PS-OCT system, Jones formalisation can be used as one means to describe the light, although other formalisms are possible [94]. Here, we follow the treatment by de Boer to describe the generation of the PS-OCT signal [94]. In Figure 5.1, the horizontal, linearly-polarised imaging light after the polariser is described by the Jones vector as:

\[
E(z) = E(z) \begin{bmatrix} 1 \\ 0 \end{bmatrix}
\]

\[ E(z) = \int \tilde{e}(k)e^{-ikz} dk, \]  \hspace{1cm} (5.2)

where \( \tilde{e}(k) \) represents the field amplitude and \( k \) is the wavenumber.
The non-polarising beam splitter then splits the light evenly into the reference arm \( E_{\text{r}}(z) \) and sample arm \( E_{\text{s}}(z) \) with the Jones vector of:

\[
E_{\text{r}}(z) = E_{\text{s}}(z) = \frac{E(z)}{\sqrt{2}} \begin{bmatrix} 1 \\ 0 \end{bmatrix}.
\]  (5.3)

The Jones matrix of the quarter wave plate oriented at an angle of \( \theta \) relative to the horizontal direction is given by:

\[
J_{\rho}(\eta, \theta) = \begin{pmatrix} \cos(\theta) & -\sin(\theta) \\ \sin(\theta) & \cos(\theta) \end{pmatrix} \begin{pmatrix} e^{i\eta} & 0 \\ 0 & e^{-i\eta} \end{pmatrix} \begin{pmatrix} \cos(-\theta) & -\sin(-\theta) \\ \sin(-\theta) & \cos(-\theta) \end{pmatrix},
\]  (5.4)

where \( \eta = \pi/4 \) for the quarter wave plate; the second matrix gives the Jones matrix of a quarter wave plate with its fast and slow axes aligned, respectively, along the horizontal and vertical directions; and the first and third matrices represent the rotation of the Jones matrix due to angle \( \theta \). Thus, the light in the reference arm after passing the quarter wave plate twice, oriented at an angle of 22.5° to the horizontal direction, has a Jones vector of:

\[
E_{\text{r}}(z_r) = J_{\rho}(\pi/8, \pi/8) J_{\rho}(\pi/8, -\pi/8) E_{\text{r}}(z) = \frac{E(2z_r)}{2} \begin{bmatrix} 1 \\ 1 \end{bmatrix}
\]  (5.5)

(i.e., linearly polarised at angle of 45°), where \( z_r \) is the length of the reference arm.

In the sample arm, the sample for imaging can be modelled as a homogeneous linear phase retarder with a constant orientation of the optic axis, characterised by its Jones matrix:

\[
J_{\alpha} = e^{-ik\alpha} J_{\rho}(k\Delta n/2, \alpha),
\]  (5.6)

where \( \bar{n} \) and \( \Delta n \) are, respectively, the average, \((n_s + n_f)/2\), and difference, \( n_s - n_f \), of the refractive index along the fast \((n_f)\) and slow \((n_s)\) axis; and \( \alpha \) is the angle of the fast axis relative to the horizontal direction. The light returned from the sample arm to the non-polarising beam splitter is

\[
E_{\text{s}}(z_s + z) = J_{\rho}(\pi/4, \pi/4) J_{\rho}(\pi/4, -\pi/4) E_{\text{s}}(z)
\]

\[
\propto \sqrt{R(z)} \int e(k) e^{-i2k(z_s + z)} \begin{bmatrix} e^{i2\alpha} \sin(kz\Delta n) \\ \cos(kz\Delta n) \end{bmatrix} dk,
\]  (5.7)

where \( R(z) \) is the reflectivity of the tissue as a function of depth \( z \) in the tissue, and \( z_s \) is the optical path length along the sample arm to the sample surface.

The interfering light is split by the polarising beam splitter, and the horizontal and vertical components are respectively detected as:
\[ i_\mu(z, \Delta z) = E_{\mu,0} E_{\mu,0}^* + E_{\mu,1} E_{\mu,1}^* \propto \sqrt{R(z)} \sin(kz\Delta n) \cos(2k\Delta z + 2\alpha) S(k) dk, \] (5.8)

\[ i_v(z, \Delta z) = E_{v,0} E_{v,0}^* + E_{v,1} E_{v,1}^* \propto \sqrt{R(z)} \cos(kz\Delta n) \cos(2k\Delta z) S(k) dk, \] (5.9)

where \( \Delta z = z_r - z_s - zm \), is the difference of the optical path length between the sample and reference arms; and \( S(k) \) is the power spectral density of the light source and related to \( \tilde{e}(k) \) through the Wiener-Khintchine theorem [148] as:

\[ \langle \tilde{e}^*(k)\tilde{e}(k') \rangle = S(k) \delta(k - k'). \] (5.10)

For a Gaussian power spectral density \( [S(k) \propto e^{-k^2/(\kappa^2)}] \), the integration in Equations (5.8) and (5.9) can be performed analytically, leading to:

\[ i_\mu(z, \Delta z) \propto \sqrt{R(z)} \sin(k_0 z\Delta n) \cos(2k_0 \Delta z + 2\alpha) e^{-1/(\kappa^2)} \] (5.11)

\[ i_v(z, \Delta z) \propto \sqrt{R(z)} \cos(k_0 z\Delta n) \cos(2k_0 \Delta z) e^{-1/(\kappa^2)}, \] (5.12)

where \( \Delta l = \frac{1}{\kappa} = \frac{\lambda_0 \sqrt{\ln 2}}{\pi \Delta \lambda} \) and \( \Delta \lambda \) is the full width at half maximum of the wavelength of the Gaussian source. The \( \cos(2k_0 \Delta z) \) component is then eliminated by the signal demodulation, leading to the following equations:

\[ I_\mu(z) = \left| i_\mu(z) \right|^2 \propto R(z) \sin^2(k_0 z\Delta n), \] (5.13)

\[ I_v(z) = \left| i_v(z) \right|^2 \propto R(z) \cos^2(k_0 z\Delta n). \] (5.14)

The total intensity is the sum of the vertical and horizontal components as:

\[ I(z) = I_\mu(z) + I_v(z) \propto R(z). \] (5.15)

With the two components in Equations (5.13) and (5.14), the corresponding phase retardation is calculated as: [94]

\[ \varphi(z) = \arctan \left[ \frac{I_v(z)}{I_\mu(z)} \right] = k_0 z\Delta n, \] (5.16)

which is wrapped in the range \([-\pi/2, \pi/2]\). When represented graphically, this phase wrapping appears as a characteristic banding pattern with depth, \( z \). The phase retardation signal with depth produces a PS-OCT A-scan, and multiple A-scans are used to construct a 2-D or 3-D tomogram with tissue birefringence characterised by the appearance of the banding pattern with depth. The birefringence, \( \Delta n \), of the sample can
be calculated from the measured depth-dependent phase retardation \( \phi(z) \) using Equation (5.16).

Retrieval of the phase retardation in fibre-based PS-OCT systems is more complex, since the optical fibres introduce an unknown amount of birefringence into the sample and reference signals. There are several ways to recover the phase retardation. Many fibre-based PS-OCT systems introduce multiple polarisation states in the sample and/or reference arm, to determine both the optic axis and the phase retardation [149-151]. Polarisation-maintaining single-mode fibre also provides one solution for the reconstruction of the phase retardation signal [152, 153]. Alternatively, in Section 5.3, we utilise a simple method for measuring sample phase retardation and birefringence using a fibre-based PS-OCT system possessing only a single incident polarisation state, as described in the next section. This was implemented in a commercial OCT system, schematically shown in Figure 5.2 [8]. Regardless of implementation, all PS-OCT systems acquire phase-retardation measurements simultaneously with measurements of standard OCT backscatter, allowing for complementary detection of tissue properties.

![Figure 5.2. Schematic of the employed PS-OCT system (PSOCT-1300, Thorlabs, New Jersey). Abbreviations: SS, swept source; PC: polarisation controller; CIR: circulator; FC: fibre coupler; C: collimator; VA: variable attenuator; M: mirror; AL: alignment laser; PBS: polarising beam splitter; D: detector; S: vertical polarisation state; P: horizontal polarisation state; MZI: Mach-Zehnder interferometer; TRIG: trigger; DAQ: data acquisition board; AO: analog output card; SD: XY scanner driver. (Adapted from: https://www.thorlabs.de/thorcat/18100/PSOCT-1300-Manual.pdf. Accessed on 6th April, 2015)](image)

### 5.2.2 PS-OCT for dermatology

The ability of PS-OCT to detect the phase retardation and quantify the birefringence [Equation (5.16)] has shown promise for dermatological applications. Early PS-OCT work has investigated the birefringence of the normal human skin \textit{in vivo} and indicated a large variation of skin birefringence across various body locations [44]. PS-OCT has also been used to image changes in the skin birefringence caused by burns, both in
animal models [154], and human skin ex vivo [155] and in vivo [101]. The ability of PS-OCT to detect the changes associated with burn injuries has been assessed for the determination of burn depth in animal models [102, 156]. The wound healing process following injuries has also been imaged with PS-OCT to measure the collagen regeneration [157]. Although not widely explored, the birefringence in the resulting scars after the healing process indicates an increase in the birefringence measured from the PS-OCT signal [106]. In addition to burn scars, PS-OCT has also been shown as a potentially useful tool for assessing the collagen in photo-aged skin [113] and skin cancers [111].

Similar to the attenuation coefficient imaging of human skin in the previous chapter, we found that the quantification of tissue birefringence in skin in vivo is confounded by the presence of blood vessels. When quantifying the tissue birefringence, the averaging of the phase retardation signals [44, 101] prior to data fitting may reduce this artefact for some small vessels. However, we found that this did not sufficiently remove vasculature-induced artefacts, particular in scar tissue where there is prolific vasculature. Thus, in the next section, we extend the vascular masking technique to the estimation of birefringence in burns scars with PS-OCT.

5.3 Imaging of skin birefringence for human scar assessment using polarization-sensitive optical coherence tomography aided by vascular masking


Abstract: We demonstrate the in vivo assessment of human scars by parametric imaging of birefringence using polarization-sensitive optical coherence tomography (PS-OCT). Such in vivo assessment is subject to artifacts in the detected birefringence caused by scattering from blood vessels. To reduce these artifacts, we preprocessed the PS-OCT data using a vascular masking technique. The birefringence of the remaining tissue regions was then automatically quantified. Results from the scars and contralateral or adjacent normal skin of 13 patients show a correspondence of birefringence with scar type:
the ratio of birefringence of hypertrophic scars to corresponding normal skin is 2.2 ± 0.2 (mean ± standard deviation), while the ratio of birefringence of normotrophic scars to normal skin is 1.1 ± 0.4. This method represents a new clinically applicable means for objective, quantitative human scar assessment.

5.3.1 Introduction

Skin scarring arises from a wide range of traumatic injuries and can lead to significant physical discomfort, such as pain, itching and reduced mobility [1, 2], and psychological problems [3] for patients. The severity of these issues, and consequently the degree of treatment required [4], is related to the type and severity of the scar. Normotrophic scars are the least severe, and heal to an appearance very similar to normal skin. Hypertrophic and keloid scars result from a pathological healing response to injury and are likely to present issues for the patient. Evaluation of scar pathology and response to treatment requires longitudinal assessment of scars during their formation and progression. Current clinical assessment techniques, such as the Vancouver Scar Scale, typically involve a medical clinician performing a manual visual-tactile assessment of the scar, and include factors such as scar pigmentation, vascularity, height, and pliability [5]. Such assessments are inherently subjective and prone to a high degree of inter-observer variability [5, 6], which limits their utility.

Excess collagen is a major constituent of scar tissue. The quantity and arrangement of the collagen impacts scar appearance, size, and pliability [7]. Normal skin contains abundant collagen in the dermis, organized in a “basket-weave”-like structure of partially parallel collagen fibers [8, 9]. Normotrophic scars have a slightly increased parallel orientation of the collagen fibers compared to normal skin [9]. In hypertrophic scarring, there is an overproduction of collagen, causing the scars to be raised above the normal skin. The collagen fibers are generally thicker, denser [10], and arranged with a marked increase in parallel orientation [9]. In keloid scarring, the excessive synthesis of collagen extends beyond the site of the initial wound. As in hypertrophic scarring, the collagen fibers in keloids exhibit a much higher degree of parallel organization than in normal skin [9].

Collagen in skin forms fibrils comprising a distinctive triple helix of collagen molecules, and bundles of these fibrils form fibers. The quantity and arrangement of the collagen fibrils affects several optical properties of scar tissue, including its scattering coefficient [11] and birefringence. Because of their high aspect ratio, collagen fibrils are
birefringent, and the high degree of organization into networks of collagen fibers gives rise to measurable birefringence [12]. The degree of birefringence is indicative of the organization of the collagen fibers, potentially providing an indicator of scar structure.

Polarization-sensitive optical coherence tomography (PS-OCT) [13, 14] is a promising technique for \textit{in vivo} imaging of scar birefringence. PS-OCT is an extension of OCT in which the polarization state, as well as the reflectance of light backscattered from the tissue, is determined. Thus, PS-OCT is sensitive to the phase retardation induced between two orthogonally polarized components of light propagating in the tissue. Birefringent tissue causes a change in the phase retardation with depth, which can be quantified from PS-OCT scans. The birefringence of normal human skin determined by PS-OCT has been shown to vary with body location [15]. Previous work on assessing the birefringence of burn-injured skin, using PS-OCT measurements acquired 4 and 6 days post-injury, has shown that skin acutely exhibits lower birefringence because of denaturation of the collagen upon injury [16]. This contrast mechanism has also been explored for the determination of the burn depth in animal models of burn injuries [17]. As the tissue heals after such injuries, scars are formed in which the collagen is restored or over-produced [18] and reorganized, leading to potentially restored or increased birefringence compared to normal skin. We propose that quantification of scar birefringence using PS-OCT has the potential to provide novel insight into collagen organization, and hence scar structure.

There are several confounding factors in performing such \textit{in vivo} measurements; predominantly, the presence of blood vessels. On the one hand, the vascular network is indicative of scar severity and its characterization provides a means of scar assessment [19]. On the other hand, the high optical scattering from the moving blood cells causes artifacts in PS-OCT estimates of scar birefringence. This is particularly pronounced in pathological scarring, where there is generally a proliferation of blood vessels, giving the scar a red appearance. These blood vessels are interspersed in the collagen matrix and strong scattering from the flowing blood cells corrupts the polarization measurements of the static tissue beneath the vessels, thereby confounding the quantification of birefringence. While averaging may reduce the impact of small blood vessels, many pathological scars are permeated by larger blood vessels [19], requiring more sophisticated techniques to mitigate their impact on the measured birefringence.

In this study, we investigate the assessment of human scars using the magnitude of birefringence determined by three-dimensional (3-D) PS-OCT. To enable accurate quantification of the birefringence, we first identify the blood vessels using a speckle
decorrelation method [19], and mask them from the PS-OCT scans. We then automatically quantify the rate of change of phase retardation with depth in the remaining vascular-free data, and use this to generate an *en face* parametric image of birefringence. We demonstrate our assessment method on 13 clinically assessed scars compared against the contralateral or adjacent normal skin of the same patients *in vivo*.

### 5.3.2 Materials and methods

(a) Polarization-sensitive optical coherence tomography scanning

PS-OCT scanning on scar patients was performed *in vivo* using a portable, fiber-based, swept-source PS-OCT system (PSOCT-1300, Thorlabs, Newton, New Jersey) with a central wavelength of 1325 nm and a spectral bandwidth of 100 nm. The system illuminates the sample using a single incident polarization state, and detects the phase retardation, induced by the sample, between two orthogonal, linearly polarized components of the backscattered light. The system has a measured axial and transverse resolution (full-width at half-maximum irradiance) of, respectively, 17 μm (in air) and 16 μm. The scan lens in the sample arm has a working distance of 25 mm that enables a custom imaging interface to be placed between the imaging probe and the skin to account for the effects of motion and refractive index mismatch [20, 21].

The imaging interface comprises a probe spacer and a ring plate, which rigidly affixes the imaging probe to the skin, and provides an improved probe-to-skin coupling over our group’s earlier design [20, 21]. The imaging probe with its probe spacer was mounted onto an articulating arm, as shown in Figure 5.3(a). The ring plate in Figure 5.3(a) was first firmly attached to the skin using double-sided adhesive tape. The ring plate was fitted into the groove in the spacer, as shown in Figures 5.3(a) and (b), and attached to the spacer with locking screws. Figure 5.3(c) shows a sketch of the imaging probe-skin interface corresponding to the region in Figure 5.3(b) delineated by a dashed rectangle.

![Figure 5.3. PS-OCT imaging interface for coupling the imaging probe and the skin. (a) and (b) Photographs of the imaging interface before and after coupling the imaging probe and the skin. (c) A magnified sketch of the probe spacer and ring plate, corresponding to the region in (b) delineated by the dashed blue rectangle.](image-url)
blue rectangle. A thin metal plate (10 × 10 mm with a central round hole of 3-mm diameter) was aligned with the center of the ring plate and also attached to the skin, acting as a fiducial marker to correct for residual bulk motion, as previously described [20]. Ultrasound gel was used as the index-matching medium to reduce imaging artifacts and to improve coupling of light into and out of the tissue [21].

The clinical scanning protocol was approved by the Human Research Ethics Committee of Royal Perth Hospital (Perth, Western Australia) and The University of Western Australia. 13 scar patients (aged 18 to 80 years; 7 hypertrophic burn scars, 1 hypertrophic surgical scar, and 5 normotrophic burn scars) undergoing follow-up examinations were recruited for this study, with written consent obtained from all patients. For each patient, a region from the scar and a corresponding region of either contralateral or adjacent normal skin were selected for PS-OCT scanning; the adjacent region was chosen if the contralateral region was also scarred. The selected regions were first trimmed of hair with an electric shaver to minimize shadowing artifacts. Each PS-OCT scan was then acquired, with a field of view (FOV) of 4 × 1.5 mm (1088 × 1088 pixels) in the lateral (x, y) directions, and a depth (z) scan in air of 3 mm (512 pixels). Dense sampling was utilized along the slow scanning (y) axis to minimize the decorrelation between adjacent B-scans of the static tissue. Prior to each scan, the polarization state of the light incident on the sample was manually adjusted by adjusting the polarization controllers in the system, following a standard procedure defined by the manufacturer [22], to maximize the dynamic range of the PS-OCT phase-retardation signal. This is achieved when the polarization is circular or linear at 45 deg to the tissue optic axis. We have assumed that the optic axis within the small FOV is approximately constant. Note that the optic axis is parallel to the collagen fibers [12]. In the case of pathological scarring, it has previously been noted that the collagen fibers tend to be largely parallel. For normal skin, the collagen fibers have been shown to generally be orientated along the Langer’s lines (topological lines of tension or cleavage) within the skin [23].

(b) Vascular masking
Our PS-OCT system illuminates the sample using a single incident polarization state, and records the two orthogonal, linearly polarized components of the complex interferometric signal [14, 24, 25]. The measured signal from every voxel in the data volume can be represented as a real-valued Stokes vector, \( S = [I, Q, U, V]^\top \). The structural OCT signal, contained in the total irradiance component, \( I \), was used for the
purposes of vascular masking. The vasculature in the imaging volume was first identified by applying a speckle decorrelation method [26] to the total irradiance, as previously described [19]. In brief, this method calculates the normalized cross-correlation of the total irradiance between each pair of adjacent B-scans to generate a correlation volume. The low correlation (i.e., high decorrelation) regions were identified as blood vessels, since the blood flow causes rapid changes in the OCT speckle and, thus, high decorrelation between B-scans in those regions. A two-dimensional en face maximum intensity projection (MIP) of vasculature was then generated by projecting the highest decorrelation at each lateral location. The projection was computed from the skin surface (automatically identified using a Canny edge detector) to a depth of 600 μm. A binary vascular mask was formed by thresholding the vasculature MIP image and was used to remove lateral locations containing blood vessels from the calculation of birefringence. We have previously used a similar approach to improve the calculation of the optical attenuation coefficient in burn scars [11].

(c) Birefringence imaging
The birefringence in the remaining (vascular-free) tissue regions was calculated using an automated algorithm, extending our earlier work [27, 28], and is illustrated in Figure 5.4(a). PS-OCT detects a fully polarized signal; hence, the polarization state of the complex, interferometric OCT signal is fully described by the normalized, reduced Stokes vector (hereafter, “Stokes vector”), \( \hat{S} = [\hat{Q}, \hat{U}, \hat{V}]^T \), where \( \hat{Q} \), \( \hat{U} \), and \( \hat{V} \) are obtained by dividing \( Q \), \( U \), and \( V \), respectively, by \( I = (Q^2 + U^2 + V^2)^{1/2} \). To reduce noise, the Stokes vectors were spatially averaged in the \( x-z \) plane through convolution with a Gaussian kernel of width equal to twice the resolution of the PS-OCT system. Changes in polarization resulting from the PS-OCT system itself were considered to be lossless (i.e., no polarization-dependent attenuation) and constant during imaging, allowing us to estimate the birefringence of the skin from the rate of change of the phase retardation of the Stokes vectors with depth into the skin [27, 29]. The original phase retardation with depth, \( \phi_i(z) \), in radians, between the smoothed reference Stokes vectors at the skin surface, \( \hat{S}_{ref} \), and at a depth \( z \), into the skin, \( \hat{S}(z) \), is given by

\[
\phi_i(z) = \cos^{-1}\frac{\langle \hat{S}_{ref} \cdot \hat{S}(z) \rangle}{\| \hat{S}_{ref} \| \| \hat{S}(z) \|},
\]

(5.17)

where \( \cdot \) is the 3-D vector dot product, \( \| \| \) is the Euclidean norm, and the subscript \( i \) denotes the discretization of the PS-OCT signal in the \( z \) dimension.
Figure 5.4. (a) Flow diagram of the birefringence imaging algorithm described in the text. (b) Fitting example 1: the original phase retardation, $\phi(z)$, is shown in blue, along with the wrapped linear fit to the unwrapped phase retardation, $\phi_{\text{w}}(z)$, shown in red, with a MAPD of 27%. (c) Fitting example 2: the original phase retardation, $\phi(z)$ (blue), and the best fit using the two-component, piecewise-linear model (red) with the first component ($L_1$) showing a high $R^2$ value of 0.94 and the second component ($L_2$) showing a low $R^2$ value of 0.37.

The Hilbert transform, $H$, was used to demodulate $\phi(z)$ to minimize errors resulting from the effects of possible misalignment of the incident polarization, any tissue diattenuation, and possible changes in the orientation of the optic axis with depth into the tissue. This resulted in the wrapped phase,

$$\phi_{\text{w}}(z) = \tan^{-1}(H[\cos(\phi(z))] / \cos(\phi(z))).$$  \hspace{1cm} (5.18)

The wrapped phase was unwrapped by considering the difference between successive values, $\phi_{\text{diff}}(z) = \phi_{\text{w}}(z) - \phi_{\text{w}}(z_{i-1})$. Phase unwrapping was performed by first identifying the values of $z_i$ for which $\left|\phi_{\text{diff}}(z_i)\right|$ exceeds a threshold, then interpolating $\phi_{\text{diff}}(z_i)$ at these locations. The unwrapped phase retardation is then $\phi_{\text{u}}(z_i) = \sum_{j=i}^{z_i} \phi_{\text{diff}}(z_j)$.

Weighted least-squares linear regression was used to fit a line, $\phi_{\text{r}}(z_i)$, with constant
slope, $\delta \phi_i$, to the unwrapped phase retardation over a depth range of 350 $\mu$m (270 $\mu$m for one scan due to the limited depth range of its banding pattern) in the dermis (where, for each dataset, a single depth below the tissue surface was chosen as denoting the start of the dermis based on the start of the increasing phase retardation, typically in the range 40-270 $\mu$m). The weights were the effective OCT signal-to-noise ratio after spatial averaging associated with each phase measurement. To evaluate the goodness of fit of the calculated linear fit to the recorded data, $\phi_i(z_i)$ was first wrapped, such that $\phi_w(z_i) = \cos^{-1} \left[ \cos \left[ \phi_i(z_i) \right] \right]$, and the mean absolute percentage deviation (MAPD) of the wrapped fit, $\phi_w(z_i)$, to the original phase retardation, $\phi(z_i)$, was calculated as a measure of the fitting error. One such fitting example is shown in Figure 5.4(b), where the wrapped fit, $\phi_w(z_i)$ (red) has a MAPD value of 27% from the original phase retardation (blue).

The calculated line was considered to be a poor fit, and thus rejected, when the MAPD exceeded an empirically chosen threshold (80%), or when the calculated slope of the linear fit was negative. This typically occurred in regions of low birefringence, or in regions showing just the ascending part of a band of phase retardation, possibly caused by axial tissue heterogeneity, or limited imaging depth. In such circumstances, the removal of amplitude modulations using the Hilbert transform failed. We found the amplitude modulations to be minimal here, and recalculated the birefringence without the Hilbert transform. Instead, the original phase retardation, $\phi_i(z_i)$, was modeled using a continuous, two-component, piecewise-linear model. The phase retardation was modeled as linearly increasing over an automatically computed range, and then linearly decreasing over an adjoining range when phase wrapping occurs. The model is specified by: $L_1 \delta \phi_i$, the slope of the first component; $c_{1i}$, the offset of the first component; $z_{z_1}$, the $z$-index where the first component ends and the second component begins; and $L_2 \delta \phi_i$, the slope of the second component. The offset of the second component was constrained to the value of the first component at $z_{z_1}$ so that the combined model was continuous. The slope of each linear component, and the point at which the model transitioned from the first to the second linear component were automatically calculated so as to minimize the weighted least-squares difference of the model to the phase retardation, $\phi_i(z_i)$. The fitting example shown in Figure 5.4(c), shows the optimized fit (red) to the original phase retardation (blue) with an $R^2$ value of 0.94 for the first component ($L1$).
derivative of the first linear function, $\delta \phi_1$, was used as the estimate of the rate of phase retardation for the skin. If this value was also negative, then this location was discarded as being unable to provide a reasonable estimate of birefringence.

The birefringence, $\Delta n$, was then calculated from the rate of change of the fitted phase retardation, $\delta \phi$, which was equal to either $\delta \phi_\pi$ (using the Hilbert transform) or $\delta \phi_1$ (using the two-component, piecewise-linear model) based on the above criteria, as

$$\Delta n = RI \times \delta \phi \times \lambda_0 / (4\pi)$$

(5.19)

where $RI$ is the refractive index of tissue, which, in this study, is assumed to be 1.43 [30] and $\lambda_0$ is the central wavelength of the PS-OCT light source. In Section 5.3.3, we graphically represent the birefringence as an en face parametric image, where the pixel value at each lateral location in the image is defined by the measured birefringence. The masked vascular areas are represented as black and the areas where the rate of phase retardation could not reliably be computed as white. The normalized distribution of the birefringence was also quantified and compared between the scar and the contralateral or adjacent normal skin tissue.

5.3.3 Results

In this section, we present representative examples of birefringence imaging from two hypertrophic scars and one normotrophic scar. The measured scar birefringence from all patients is then aggregated by scar type, and the results highlight a correspondence between scar type and measured birefringence.

(a) Birefringence imaging across a scar-normal skin boundary

The birefringence in the vicinity of the boundary of a 3-year-old hypertrophic scar on the outer right upper arm of a 32-year-old male Caucasian patient is shown in Figure 5.5. Figure 5.5(a) is a photograph of the hypertrophic scar, which appears raised and slightly redder than the surrounding normal skin. The scanning region is at the center of the area delineated in blue, which marks the outline of the metal fiducial marker. The scar boundary lies within the scanning region and is indicated by the dashed line (black). A representative cross-sectional (B-scan) phase retardation image across the scar boundary [in the position and orientation given by the arrow in Figure 5.5(a)] is shown in Figure 5.5(b). The contrast in birefringence between the scar and the normal skin region is qualitatively visualized in Figure 5.5(b), in which the banding pattern is clearer and denser in the case of the scar tissue (right).
Figure 5.5. Birefringence and vascular imaging across a hypertrophic scar boundary. (a) Photograph of the scar with the portion of the scar boundary included in the PS-OCT scan indicated by the dashed line. (b) A cross-sectional phase retardation image across the scar boundary from the position and orientation shown by the arrow in (a). (c) En face birefringence map without masking the blood vessels, which are shown by the vasculature MIP in (d). The two long arrows in (c) label the position of (b). Several representative vasculature-induced low-birefringence artifacts in the birefringence map are highlighted by the short black arrows in (c) and correlated with the presence of vasculature (white arrows) in (d). The dashed lines in (c) and (d) identify the same scar boundary as shown by the short dashed line in (a). Scale bars: 0.5 mm.

An en face birefringence image calculated without vascular masking is shown in Figure 5.5(c). The scar boundary is marked by the purple dashed line. Long arrows mark the location of the phase-retardation B-scan [Figure 5.5(b)]. The normal skin at this scanning location mainly shows low birefringence (dark blue) with some local variation. In contrast, the scar region is characterized by much higher birefringence (cyan, yellow, and red) possibly due to the overgrowth of more organized collagen in the visibly raised hypertrophic scar tissue. The scar region, to the right of the white dashed line marking the scar boundary in Figure 5.5(d), also reveals a more prolific network of blood vessels than in the normal skin, in agreement with the results found in a previous study [19]. Short arrows in Figure 5.5(d) mark several representative blood vessels, which appear as low-birefringence artifacts (short black arrows) in the birefringence map [Figure 5.5(c)]. Although phase retardation was smoothed through averaging the Stokes vectors with a Gaussian kernel prior to the birefringence calculation, the confounding effect of blood vessels is still apparent in these regions. The vascular masking method, which identifies and removes these artifacts from the birefringence image, was thus applied in all subsequent analyses to enable more accurate quantification of scar birefringence.

(b) Birefringence imaging of a hypertrophic scar

Figure 5.6 shows the birefringence images from a 1-year-old hypertrophic scar on a 59-year-old female patient, which was caused by a fire burn injury. A region of the scar on
the outer left forearm, located at the center of the left-hand side 10 × 10 mm blue outline in Figure 5.6(a), and the adjacent normal skin (located more proximally, on the right of the photo) were selected for PS-OCT scanning. Compared to the normal skin vasculature MIP in Figure 5.6(b), the scar region shows a prolific collection of large blood vessels with a complex pattern, visualized in Figure 5.6(c). This feature in the underlying scar tissue is not easily discernible through scar assessment by clinical observation, as the scanned scar and normal skin regions are similar in color. Birefringence images of both normal skin [Figure 5.6(d)] and scar [Figure 5.6(e)] are shown, with areas of vasculature masked in black. It is evident that the scar region is characterized by higher birefringence (red) than the normal skin (cyan). The normalized distributions of birefringence in the vasculature-masked regions are shown in Figure 5.6(f) (histogram bin size: $2.5 \times 10^{-5}$) where the median (lower quartile, upper quartile)

Figure 5.6. Birefringence and vascular imaging of a hypertrophic scar and adjacent normal skin. (a) Photograph. (b) and (c) Vasculature MIPs of the normal skin and scar, respectively. (d) and (e) En face birefringence maps of the normal skin and scar, respectively. (f) Histogram of birefringence for the normal skin (green) and scar (red). Scale bars: 0.5 mm.
value in the scar region is $2.6 \ (2.2, 2.9) \times 10^{-3}$, compared to $1.1 \ (1.0, 1.5) \times 10^{-3}$ in the normal skin. The scar birefringence distribution (red) demonstrates a clear contrast from that of normal skin (green) with its median value being more than twice that of normal skin.

(c) Birefringence imaging of a normotrophic scar

![Birefringence Imaging](image)

Figure 5.7. Birefringence and vascular imaging of a normotrophic scar and contralateral normal skin. (a) and (b) Photographs of the contralateral normal skin and scar, respectively. (c) and (d) Vasculature MIPs of the normal skin and scar, respectively. (e) and (f) En face birefringence maps of the normal skin and scar, respectively. (g) Histogram of birefringence for the normal skin (green) and scar (red). Scale bars: 0.5 mm.

The birefringence images from a 1-year-old normotrophic scar on the left side of the back of a 28-year-old male Caucasian patient are shown in Figure 5.7. Figures 5.7(a) and (b) are photographs of, respectively, the contralateral normal skin and scar, with the scanning FOV centered within each blue square outline. The scar in Figure 5.7(b) was caused by scalding (boiling water and hot coals) and shows a similar appearance to the normal skin in Figure 5.7(a), although there is slight hypopigmentation. The vasculature MIPs shown in Figures 5.7(c) and (d) indicate a similar pattern of blood vessels. The
birefringence maps in Figures 5.7(e) and (f) of, respectively, the normal skin and scar tissue, are comparable, although the normal skin has some local areas of high birefringence (yellow and red). Quantification of the birefringence in Figure 5.7(g) also shows a similar distribution (histogram bin size: $2.5 \times 10^{-5}$) of values for both scar (red) and normal skin (green). The median (lower quartile, upper quartile) scar birefringence is $0.9 \ (0.8, 1.1) \times 10^{-3}$, which is comparable to the value of $1.2 \ (1.0, 1.8) \times 10^{-3}$ in the normal skin, suggesting the amount of collagen and its organization in the scar approach that of the normal skin tissue. These results are consistent with the very similar appearance of the scar and normal skin apparent in Figure 5.7.

(d) Birefringence of scars by type from all patients

![Birefringence ratio of scars](image)

Figure 5.8. Ratio of thresholded birefringence for normotrophic and hypertrophic scars to the median birefringence of normal skin from 13 scars. Error bars indicate the upper and lower quartiles. The written values represent the mean(± standard deviation) of the median birefringence ratio for the normotrophic and hypertrophic scars, respectively. The surgical scar is highlighted by the green circle.

The results of birefringence measurements on all 13 patients included in this study are shown in Figure 5.8 as the ratio of the thresholded birefringence of the scar to the median birefringence of contralateral or adjacent normal skin (\( \Delta n_{\text{scar}}/\Delta n_{\text{skin}} \)). The birefringence of each scanned scar and normal skin region is thresholded at $0.2 \times 10^{-3}$, which is the estimated minimum reliable birefringence detected by our algorithm. The birefringence of the scar is then normalized to the median birefringence of the corresponding normal skin for each patient. The median, lower, and upper quartiles of the normalized scar birefringence are shown by, respectively, the markers (triangles and circles) and bars. The mean(±standard deviation) of the median birefringence ratio for each scar type (normotrophic and hypertrophic) is also shown in Figure 5.8. The
hypertrophic scars (n = 8) show the highest birefringence, ~2.2 times that of contralateral or adjacent normal skin tissue (n = 8); whereas, the normotrophic scars (n = 5) have a much lower birefringence, ~1.1 times that of normal skin (n = 5). The median birefringence ratio of hypertrophic scars to normal skin was found to be statistically different from the median birefringence ratio of normotrophic scars to normal skin (Student’s t test, p < 0.001).

5.3.4 Discussion

The results presented in this study demonstrate the feasibility of using PS-OCT, incorporating the automatic masking of the blood vessels, to quantify scar birefringence in vivo and confirm the correlation of birefringence with scar type. Having calculated the median birefringence ratio within each hypertrophic scar relative to normal skin, we find that the mean of this value across all patients is much higher than that of normotrophic scars to normal skin, and the difference was shown to be statistically significant. The increased birefringence observed in hypertrophic scars is most likely due to an increase in the density and parallel organization of collagen over normal skin, as has previously been reported [9, 10]. Normotrophic scars do not suffer from the overproduction of collagen, but do exhibit collagen fibers with a slightly more ordered organization than normal skin [9]. Thus, their birefringence is expected to be slightly higher than that of normal skin, but lower than that of hypertrophic scars, which agrees with the quantified birefringence ratios summarized in Figure 5.8. We note that the extracellular matrix in the dermis also contains elastin, which is an additional source of birefringence [31]. However, as elastin is much less abundant than collagen in the human dermis [32], we expect its influence to be negligible in this study.

In regions rich with collagen, quantification of birefringence may be complicated by the presence of tissue heterogeneity within the depth-fitting window, which can give rise to artifacts. Both normal skin and scars have complex structures with several sources of heterogeneity. We have found the vasculature to be a significant source of artifacts in the quantification of collagen birefringence, particularly in burn scars where neovascularization is prolific. This artifact is largely accounted for by vascular masking. However, some residual, nonvascular artifacts remain, as seen by the artificially low birefringence of a thin vertical streak in the middle lower section of Figure 5.7(f). This artifact is caused by shadowing by a short hair still present after trimming. Further postprocessing may be able to remove such artifacts.
Another prominent source of artifacts for *in vivo* PS-OCT scanning is motion, including small involuntary motions due to respiration and heartbeat. Besides image distortion, motion causes artificially high speckle decorrelation even in the static tissue regions [33], and corrupts the blood vessel identification. We found that mechanically coupling the scanning probe and the skin using our imaging interface significantly reduces this effect [34]. We observed that smaller geometrical transformations, determined by the use of the fiducial marker, were required to align the 3-D PS-OCT volume compared to a previous version of the imaging interface [19].

A fiber-based PS-OCT system enables ready access of the OCT imaging probe to different parts of the body. However, there are a number of sources of potential systematic error. If the optic axis of the tissue is not perpendicular to the light beam, then the tissue birefringence is underestimated. Large misalignment of the incident polarization state of the light beam also impacts the measured birefringence. Additionally, the fiber-based system is potentially affected by the polarization changes induced by the optical fibers, which may confound the measurement of the change of polarization due to sample birefringence. Our algorithm assumes the polarization changes due to the optical fibers in the system are lossless and constant during imaging, and uses the relative angles between the reference Stokes vector at the skin surface and the Stokes vectors with depth into the skin as an estimate of phase retardation in order to eliminate the confounding effect of polarization changes caused by the optical fibers. To satisfy this assumption, we gently attached the optical fiber in the sample arm to the articulating arm to avoid bending or movement of the fiber during scanning.

With our scanning protocol and birefringence imaging algorithm, the calculated median birefringence of normal skin ranges from $0.4 \times 10^{-3}$ to $1.3 \times 10^{-3}$ for various body locations, which is in agreement with previously published values (equivalent to $0.5 \times 10^{-3} - 1.1 \times 10^{-3}$) measured at $1.3 \mu m$ wavelength [15]. Such large variations in birefringence are consistent with the known variation in the quantity and organization of collagen at different body locations [15]. Therefore, it is important to compare the birefringence of the scar with its contralateral or adjacent normal skin as the baseline in order to minimize the influence of this variation.

### 5.3.5 Conclusions

In this paper, we investigated *en face* birefringence imaging of human scars, which we expect to be indicative of scar collagen structure. We presented a parametric imaging method, aided by the automatic masking of the confounding blood vessels, for accurate
quantification of the scar birefringence. The resulting birefringence from 13 patients, normalized to contralateral or nearby normal skin, showed the highest values for hypertrophic scars and values slightly higher than normal skin for normotrophic scars. The characteristic birefringence of scar tissue highlighted in this study can potentially be used as the basis for an objective scale for scar assessment. When combined with vascular assessment [19], the longitudinal monitoring of scars via PS-OCT may provide a viable new clinically applicable means to assess treatments of burns and other wounds.

5.3.6 Acknowledgments

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5.3.7 References


5.4 Chapter summary

The collagen network in the dermis of scar tissue shows abnormalities in both quantity and organisation, in comparison to normal skin. Although conventional OCT is a useful tool to image the scar tissue structures [105, 158], the individual collagen fibres (i.e., the assemble of collagen fibrils) are below the discernible spatial resolution of standard OCT systems [6]. PS-OCT is capable of characterising the organisation of the collagen through measurement of changes in the polarisation state of the backscattered light. These measurements enable estimation of the birefringence of the tissue. This ability makes PS-OCT a potential tool for clinical assessment of the organisation of collagen in burns scars.

This chapter has investigated the feasibility of using PS-OCT, incorporating vascular masking, to quantify the scar birefringence in vivo. An algorithm was developed to calculate the birefringence in scar and normal skin tissue. The measured birefringence was visualised through en face birefringence maps, showing high values in hypertrophic scars (~2.2 times that of normal skin), while values in normotrophic scars approached those in normal skin (~1.1 times that of the normal skin). This birefringence imaging method represents a promising new technique for objective, quantitative clinical scar assessment. The computation of scar birefringence provides complementary information to estimates of the optical attenuation coefficient (Chapter 5) and assessment of the vasculature (Chapter 4), for a more objective, quantitative assessment of burn scar tissue.
Chapter 6

Clinical monitoring of skin

6.1 Preface

In this chapter, we investigate the utility of OCT for clinical monitoring of skin with two examples of clinical measurements: longitudinal monitoring of vasculature in ablative fractional laser treatment and imaging scar tissue using mechanical contrast. They represent two attributes (i.e., vascularity and pliability) commonly utilised in clinical scar scales and are both demonstrated through the proof-of-principle studies in this chapter. With further development, these measures have the potential to be integrated into a comprehensive OCT-based method for clinical scar monitoring.

Section 6.2 is adapted from a paper published in the Journal of Biophotonics. In this section, we perform longitudinal scar assessment for monitoring the laser fractionation treatment of burn scars by extending the vasculature assessment method presented in Chapter 3. This study develops the scanning protocol to track the vasculature at the same scar site, with millimetre accuracy, over a treatment period of several months. The longitudinal vasculature images are compared and quantified (by the area density) to highlight the acute and long-term vascular changes associated with the treatment. Section 6.3 demonstrates the application of OCT-based optical palpation (i.e., tactile imaging) for mapping the mechanical contrast of the scar tissue, adapted
from a paper published in the Journal of Biomedical Optics. It develops a handheld OCT imaging probe to allow the optical palpation of scars and imaging of the tissue stress in the 2-D en face plane, aided by a translucent compliant silicone layer. The contrast in stress between the scar and adjacent normal skin is highlighted in the generated stress images. This chapter concludes with a summary of the key results from these clinical skin monitoring applications.

6.2 Optical coherence tomography for longitudinal monitoring of vasculature in scars treated with laser fractionation


Abstract: This study presents the first in vivo longitudinal assessment of scar vasculature in ablative fractional laser treatment using optical coherence tomography (OCT). A method based on OCT speckle decorrelation was developed to visualize and quantify the scar vasculature over the treatment period. Through reliable co-location of the imaging field of view across multiple imaging sessions, and compensation for motion artifact, the study was able to track the same scar tissue over a period of several months, and quantify changes in the vasculature area density. The results show incidences of occlusion of individual vessels 3 days after the first treatment. The subsequent responses ~20 weeks after the initial treatment show differences between immature and mature scars. Image analysis showed a distinct decrease (25 ± 13%, mean ± standard deviation) and increase (19 ± 5%) of vasculature area density for the immature and mature scars, respectively. This study establishes the feasibility of OCT imaging for quantitative longitudinal monitoring of vasculature in scar treatment.

6.2.1 Introduction
Burns by fire, radiation, electricity, chemicals and friction give rise globally to approximately 11 million injuries requiring medical attention each year [1]. Hypertrophic scarring arises from pathological wound healing of the tissue following burns, leading to a red, raised lesion within the original burn wound margin. It represents a major long-term complication of burn injuries, often severely disfiguring the patient, restricting normal movement, and resulting in psychological trauma. Ablative fractional laser treatment [2] is an emerging treatment for hypertrophic scarring [3]. It inflicts an array of small, focused regions of laser-induced ablation on the scarred tissue, with the goal of scar micro-reconstruction. However, the reported clinical efficacy varies greatly between patients [3-6]. The understanding of the response to treatment is limited by a lack of longitudinal studies in humans. Studies based on clinical scar scales are problematic because of their subjective nature, leading to large inter-observer and intra-observer variability [7]. For example, the Vancouver Scar Scale (VSS), scored by an independent examiner, is based on scar pliability, height, vascularity and pigmentation, as determined by observation and palpation [8]. The assessed scores are subjective, affected by the experience and skill of the examiners [9]. A review by Tyack et al. concluded that the VSS possessed only “indeterminate evidence of construct validity, reliability and responsiveness” [7]. Histological examination via skin biopsy has been used for assessing tissue responses to fractional laser treatment in normal skin [10], but the invasive nature of this method limits its use in clinical scar assessment. There is a need for a non-invasive, objective method to assess changes in scars over time and in response to such treatments.

One characteristic of hypertrophic scarring is an over-proliferation of the vasculature. Several optical techniques have been proposed to assess the vasculature, with the potential to provide an objective measure of scar progression. Video capillaroscopy [11] is capable of projecting individual capillaries into a two-dimensional (2-D) image at high resolution. However, due to the limited imaging depth, its clinical applications in dermatology are confined to superficial capillaries in relatively transparent skin, such as the nail fold. Laser Doppler perfusion imaging (LDPI) [12, 13] uses the Doppler effect with scanning laser light scattered from flowing blood cells to generate a perfusion image in the 2-D en face plane. Clinically, LDPI is capable of scanning a relatively large field of view (FOV), but typically offers a low spatial resolution image of vessels without depth information. Laser speckle contrast imaging (LSCI) [12-15] visualizes tissue perfusion by analyzing the decorrelation of the speckle intensity pattern caused by the blood. In general, the relative nature of laser
Doppler measurements (and of related techniques such as LSCI) limits their ability to compare flow at different sites, or at the same site under varying conditions [16]. Confocal scanning laser microscopy has been investigated to monitor microvasculature at high resolution (0.4-1.9 \( \mu \text{m} \)) [17], although it is typically limited to very superficial depths in the range of 100-350 \( \mu \text{m} \) [17, 18] with a relatively small FOV. Multiphoton microscopy has also been shown to be a useful tool to image the vasculature at high resolution [19] with the potential to be applied to human subjects.

Optical coherence tomography (OCT) [20] is a high-resolution (1-20 \( \mu \text{m} \)) three-dimensional (3-D) imaging technique, probing up to \(~\text{1 mm}\) into human skin. Its extensions have shown promise for imaging microvasculature using either the Doppler effect [21, 22] or temporal changes in the speckle, utilizing measures such as speckle variance and speckle decorrelation [22, 23]. In this paper, we explore the use of a speckle decorrelation technique. OCT speckle is formed by the summation of multiple backscattered optical wavefields from the sample [24] and, in static tissue, does not vary with time. Regions within the sample containing moving scatterers, such as the flowing blood in vessels, modify the speckle, with the rate of change indicative of the blood flow velocity [25]. This movement-induced variation in speckle is captured by OCT and can be analyzed by a speckle decorrelation algorithm to highlight blood vessels. Previous work has demonstrated that OCT speckle decorrelation techniques have the potential to provide insight into human scar microvasculature [26, 27], although these studies were restricted to one-off measures of the scar and did not demonstrate its potential for longitudinal assessment in humans.

In this study, we investigate the feasibility of OCT speckle decorrelation for longitudinal monitoring of burn scars. In particular, we assess changes in the superficial microvasculature in response to ablative fractional laser treatment on seven human burn scar patients over a period of several months. In addition to the image analysis technique required to perform quantification of the scar microvasculature, we have developed non-invasive imaging protocols that enable us to accurately track the same location with estimated millimeter-accuracy over a time period of weeks to months. In this paper, we demonstrate changes in individual capillaries across multiple imaging sessions. Our results show both short-term changes, including occlusion of individual vessels, and long-term changes, such as angiogenesis, in response to ablative fractional laser treatment.

### 6.2.2 Methods
(a) Burn scar patients

The clinical study protocol was approved by the Human Research Ethics Committee of Royal Perth Hospital and The University of Western Australia. Seven burn scar patients (age: 23-55 years; mean age: 38 years; 6 males and 1 female; age of scar: 6 months-29 years) were recruited for this study and written consent was obtained from all patients. The scars in these patients were caused by full-thickness burns and are classified as mature scars (n = 3, scar age >2 years) that have stabilized and immature scars (n = 4, scar age ≤2 years) that are still undergoing healing. All scars had a VSS total score greater than 5.

(b) Ablative fractional laser treatment

A 10.6 μm-wavelength fractional CO₂ laser (Ultra-Pulse®, Lumenis Inc., San Jose, CA, USA) equipped with the DeepFX™ microscanner was used for treating the burn scars. All treatments were performed with a pulse energy of 50 mJ/pulse, a beam density of 5% and a microscopic treatment zone spot size of 0.12 mm, and were performed by a laser-accredited consultant burns surgeon. With the chosen laser parameters, the penetration depth is estimated to be larger than 1 mm based on a previous study [28]. For each patient, a 5 cm × 10 cm confluent scar area was selected for treatment. Patients received general anesthesia, followed by single-pass laser treatment for the selected scar region. Each patient received three courses of laser treatment with a time separation of ~6 weeks, as shown in the diagram in Figure 6.1.

![Figure 6.1. Timing of laser treatments (1, 2 and 3) and OCT scanning (A, B and C) of burn scars.](image)

(c) OCT scanning

OCT scanning was performed using a spectral-domain OCT scanner (TELESTO II, Thorlabs Inc., Newton, NJ, USA) with a center wavelength of 1300 nm and a manufacturer-specified axial and lateral resolution of, respectively, 5.5 μm (in air) and 13 μm. The axial resolution is achieved by combining two superluminescent diodes to form a broad bandwidth light source, as shown in the system schematic in Figure 6.2(a). The reference arm is encapsulated within the imaging probe. A small metal fiducial marker was attached to the skin to allow the assessment and correction of motion
artifact during scanning. The fiducial marker comprises a 10 mm × 10 mm thin metal square with a round hole (5 mm diameter) through which imaging was performed, as described by Liew et al. [29]. Ultrasound gel was applied to the skin to reduce the refractive index mismatch between the imaging probe and the skin, and improve coupling of the light beam into and out of the skin [30]. The probe includes a customized spacer that holds the skin at a fixed distance from the focusing optics. The spacer was attached to the skin firmly with double-sided tape (not shown) to reduce motion artifact during imaging, as shown in Figure 6.2. The OCT imaging probe was attached to a five-degrees-of-freedom articulating arm [Figure 6.2(b)] to further reduce motion artifact during OCT acquisition.

Figure 6.2. OCT system and imaging interface. (a) Schematic of the OCT system. Thin red and green lines, respectively, represent the optical and electrical signal path. (b) Photograph of the imaging interface with a sketch of the region delineated by the blue rectangle shown in (c). Abbreviations: BS, beam splitter; CCD, charge-coupled device; L, lens; M, mirror; SLD, superluminescent diode; SM, scanning mirror; VA: variable aperture.

Scanning was performed over a (x × y) 6 × 3 mm FOV so as to include the fiducial marker at the edges of the scan, allowing for correction of motion artifact in post-processing. Pairs of co-located B-scans (1024 × 1024 pixels in x and z) were acquired, sampling at a pixel size of 5.9 × 3.5 μm (z scan depth of 3.6 mm in air), and assessed for speckle decorrelation. Each C-scan consisted of 400 B-scan pairs, acquired at a spacing of 7.5 μm, and at an A-scan line rate of 48 kHz, leading to a data
acquisition time of ~20 s for each C-scan. The motion during this acquisition time led to an accumulated translation of the B-scan of up to 100 μm in the cross-sectional plane. This translation distorts skin features such as blood vessels and is corrected in the data processing, as described below.

OCT scans were acquired for each patient at three time points: (A) before the first treatment (pre-treatment); (B) 3 days after the first treatment (acute changes); and (C) ~7 weeks after the third treatment (i.e., ~20 weeks after the first treatment) showing long-term changes, as illustrated in Figure 6.1. To record and position the scan FOV at the same location at different time points, a thin, transparent sheet of plastic was held on the skin and physical landmarks (moles, freckles, ridges or crevices in the skin and changes in pigmentation) were transcribed onto the plastic with an indelible ink marker, along with the position of the fiducial marker that framed the scan FOV. This plastic sheet was then used at time points B and C to obtain collocated scans. Photographs of the scar were also taken and used as a complementary means to finely adjust the location of the OCT FOV. A contralateral or adjacent normal skin region was also scanned, acting as a control for changes in the vasculature in unscarred, untreated normal skin. It also provided a means to validate our ability to track the same skin region, by correlating common vessel patterns across multiple time points, as described in the following section. All scans were performed in a quiet, temperature-controlled examination room and patients were rested for at least 20 mins prior to scanning.

(d) Vasculature imaging and quantification
The scar vasculature was automatically extracted from the OCT data using the speckle decorrelation method described in [26, 31]. The speckle decorrelation is calculated as:

\[
D(x,y,z) = 1 - \frac{\sum_{\text{vert}} \sum_{\text{horiz}} \left( I(x+m,y,z+n,t) - \overline{I(x,y,z,t)} \right) \left( I(x+m,y,z+n,t') - \overline{I(x,y,z,t')} \right)}{\sqrt{\sum_{\text{vert}} \sum_{\text{horiz}} \left( I(x+m,y,z+n,t) - \overline{I(x,y,z,t)} \right)^2} \sqrt{\sum_{\text{vert}} \sum_{\text{horiz}} \left( I(x+m,y,z+n,t') - \overline{I(x,y,z,t')} \right)^2}},
\]

where \( I \) is the amplitude of the complex OCT data; \( M \times N \) defines the window size in the cross-sectional (x-z) plane for calculation; \( t \) and \( t' \) differentiate the two B-scans acquired sequentially from the same lateral y location; and \( \overline{I} \) represents the mean value of \( I \) in the \( M \times N \) window, respectively, in the two B-scans. In this formulation, static skin will yield small positive values, whereas areas of decorrelated speckle (blood flow) will yield values ranging from 0 to 2. Note that to reduce the impact of tissue bulk motion on speckle decorrelation, each pair of co-located B-scans was aligned using a
cross-correlation intensity-based registration algorithm prior to calculation of speckle decorrelation.

The resulting speckle decorrelation images were weighted with their smoothed, thresholded OCT logarithmic intensity values to mitigate decorrelation noise, which arises in areas with low OCT signal-to-noise ratio. The relative position of each image was then corrected to remove the distortion of blood vessels due to motion artifacts, using a feature-based registration algorithm. The registration identified the fiducial marker from each B-scan using the Hough transform and aligned the images based on the shape of the round hole in the fiducial marker, as described in [29]. The skin surface was identified in the underlying OCT logarithmic intensity data using a Canny edge detector, and an \textit{en face} maximum intensity projection (MIP) image of vasculature was calculated by finding the highest decorrelation value at each \((x, y)\) location, to a depth 300 \(\mu\)m (assuming a refractive index of 1.43 for the skin [32]) below the skin surface. The value of 300 \(\mu\)m was empirically chosen based on considerations that will be described in the Discussion section.

Longitudinal vasculature MIP images from each scar region were manually co-registered using image-viewing software. The registration involved the recognition of several distinctive vessel patterns and bifurcations as reference structures, and the manual alignment (2-D rotation and translation) of the vasculature MIPs at time points B and C with that at A using these structures. The registration was validated by the presence of a high degree of overlap of the vessel patterns in the MIPs. When the reference structures could not be recognized at time point C, no registration was performed, and scan co-location was based purely on identification of characteristic marks and shapes on the skin surface. This registration indicated that when suitable reference structures could be identified, the accuracy for our imaging protocol to track the same scar location is of order 1-2 mm. It allowed assessment of acute effects (time points B vs A from Figure 6.1) and long-term effects (time points C vs A) on the vasculature of laser treatment. The microscopic treatment zones (MTZs) at time point B (i.e., acute effect 3 days after the first treatment) were readily delineated as a regularly spaced array of areas of low backscatter in the OCT logarithmic intensity data. An \textit{en face} image in which the zones are clearly evident is presented in Figure 6.3(a). The low backscatter observed within each MTZ indicates the scale of changes in the optical scattering properties of tissue caused by laser treatment. The MTZs were then superimposed upon the vasculature MIP at time points A and B [Figure 6.3(b)] to help interpret changes due to localized ablation. To monitor the vasculature quantitatively,
the vasculature MIPs were segmented by thresholding the speckle decorrelation to identify pixels that lay within the vessels. The threshold value was selected to best represent blood vessels while suppressing the background noise for each scan. The vasculature area density was calculated as the ratio of the area of the vasculature to the total tissue area. Calculating this value at each time point allowed quantification of the overall changes in vasculature.

![Figure 6.3. Microscopic treatment zones at time point B. An example of a low backscatter region is indicated by the blue arrow in the en face OCT image (a) at the scar surface, and marked in the corresponding vasculature MIP (b) by cyan ovals. Scale bars: 0.5 mm.](image)

6.2.3 Results

In this section, we present detailed results from three representative cases demonstrating the feasibility of undertaking longitudinal OCT imaging of vasculature. Quantification results comparing pre-treatment (time point A) and post-treatment (time point C) images are then presented, suggesting differences in response between mature and immature scars.

(a) Vasculature imaging of a mature scar

The vasculature of a 12-year-old mature scar on the outer left upper arm of a 38-year-old male Caucasian patient is shown in Figure 6.4. This scar was caused by a flash (i.e., strong thermal radiation) and explosion, resulting in scarring with a total body surface area of 60%. The 10 mm × 10 mm blue squares on the scar at time point A [Figure 6.4(a)], B [Figure 6.4(d)] and C [Figure 6.4(g)] outline the fiducial marker locations, with OCT imaging performed across a 5 mm diameter circle centered within the blue square. The corresponding vasculature MIPs are shown, respectively, in Figures 6.4(b), (e) and (h). A common vascular structure is visible in all three data sets, highlighted by the green circles.

Prior to treatment, the scar tissue shows a higher degree of vasculature [Figure 6.4(b)] than the contralateral normal skin [Figure 6.4(k)]. This is in agreement with earlier findings comparing normal skin and hypertrophic scars [26]. Three days after the first laser treatment [Figure 6.4(e)], we observe a slight reduction in vasculature area
density from 28% (pre-treatment, time point A) to 23% (time point B). In this figure, locations of MTZs are indicated by cyan and purple circles, respectively, corresponding to two spatially adjacent scan paths of the laser treatment microscanner. These regions are apparent as regions of low backscatter in the OCT intensity image. Figures 6.4(c) and (f) show the results from time points A and B after being aligned with a manual rigid registration. A comparison of the registered data sets reveals the loss of several vessels after treatment, particularly those vessels that pass through MTZs. At time point C, however, the vascular density has increased (area density: 35%), as shown in Figure 6.4(h). One possible cause of this increase is the process of angiogenesis, part of the wound-healing pathway, and in this case, occurring in response to the laser treatment.

Figure 6.4. Vasculature imaging of a mature scar (a)-(i) and adjacent normal skin (j)-(k). (a), (d) and (g) Photographs and (b), (e) and (h) corresponding vasculature MIPs of the center regions in the blue square outlines (10 mm × 10 mm) on the scar, respectively, at time points A, B and C. The green dashed circles show a prominent example of the same vessel pattern in each image. (c), (f) and (i) Registered, overlapped regions in (b), (e) and (h). (j) Photograph and (k) vasculature MIP of normal skin. (l) Vasculature area density of scar at time points A, B and C, and normal skin. The small cyan and purple solid circles in (e) and (f) show the MTZs of the first laser treatment, corresponding to two adjacent scan paths of the laser microscanner. The corresponding locations, after registration, are shown as a guide in (c) and (i) (dashed circles). Scale bars: 0.5 mm. All vasculature MIPs are taken from the surface to a depth of 300 μm into the skin.

(b) Vasculature imaging of two immature scars
Figure 6.5 shows the OCT vasculature images of a 10-month-old immature hypertrophic scar on a 23-year-old male Caucasian patient, caused by a thermite explosion. In contrast to the previous example, this immature scar on the right thigh is still undergoing significant scar maturation. The photographs show scans at all three time points to be centered on a red Y-shaped structure on the skin surface. Pre-treatment, the scar exhibited prolific vasculature, as shown in Figure 6.5(b), with a high area density of 40%, compared to 18% for the adjacent normal skin shown in Figure 6.5(k). At time point B, although a small number of vessels were occluded by the laser treatment, the vasculature MIP in Figure 6.5(e) showed comparable but increased area density of 47%.

The final OCT scan, at time point C, shows the vasculature to have a notably reduced area density of 34% in Figure 6.5(h). As this is an immature scar, the organization of the vasculature in Figure 6.5(h) presents significant changes after the 20-week maturation period, compared to Figures 6.5(b) and (e), making it difficult to identify corresponding vessel patterns. Based on the prominent Y-shaped pattern in the...
vasculature, we registered the orientation of the vasculature MIP as shown in Figure 6.5(i). Longitudinal OCT vasculature imaging highlights the dilation and constriction of a representative microvessel between the arrows in Figures 6.5(f) and (i), respectively, at time points B and C, compared to Figure 6.5(c) at time point A. We hypothesize that the long-term decrease in the vasculature area density, in contrast to the previous example, is due to the natural scar maturation process occurring in this immature scar. This process may also be affected by the laser treatment.

The vasculature of a second immature (10-month-old) hypertrophic scar of a 23-year-old male Caucasian patient is shown in Figure 6.6. The patient suffered a thermite explosion with a total body surface area of 55%. The treated scar region on the left lower quadrant of the abdomen in Figure 6.6(a) was imaged. The common vessel patterns highlighted within the green ovals in Figures 6.6(b) and (e) indicate that the OCT scanning was performed at the same region at time points A and B. The vessels in the MTZs (cyan ovals) have largely disappeared three days after the treatment, as shown in Figure 6.6(e), leading to the reduced vasculature area density from 40% [Figure

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Figure 6.6. Vasculature imaging of an immature scar (a)-(h) and adjacent normal skin (j)-(k). (a), (d) and (g) Photographs and (b), (e) and (h) corresponding vasculature MIPs of the center regions in the blue square outlines (10 mm × 10 mm) on the scar, respectively, at time points A, B and C. (c) and (f) Registered, overlapped regions in (b) and (e). (i) Vasculature area density of scar at time points A, B and C, and normal skin. (j) Photograph and (k) vasculature MIP of the adjacent normal skin. The small cyan solid ovals in (e) and (f) show the MTZs of the first laser treatment. The corresponding locations, after registration, are shown as a guide in (c) (dashed ovals). The green and white dashed ovals show two prominent examples of the same vessel patterns. Scale bars: 0.5 mm. All vasculature MIPs are taken from the surface to a depth of 300 μm into the skin.
6.6(b)] to 28% [Figure 6.6(e)]. We also note the reduction in diameter of the highlighted vessel (white ovals) in Figure 6.6(f) after the laser treatment.

As with the previous example of an immature scar, the final OCT follow-up at time point C shows a significant reduction in vasculature [Figure 6.6(h)]. The photo at this time point [Figure 6.6(g)] is also visually less red. The large changes in vasculature due to scar maturation and laser treatment make it challenging to identify the corresponding vasculature patterns as the morphology of the blood vessels has significantly altered. At the final time point C, the vasculature shows a further decreased area density (22%), which is similar to that of normal skin (22%), as shown in Figure 6.6(k).

(c) Vasculature area density of all patients

![Vasculature area density of all patients](image)

Figure 6.7. Change in vasculature area density after fractional laser treatment for each case reported here.

The post-treatment vasculature area density of all patients is summarized in Figure 6.7 with the mature and immature scars and normal skin, respectively, represented by the blue, red and green circles. The green baseline indicates the case of post-treatment area density being equal to that pre-treatment. Normal skin tissue presents little change in the vasculature area density as shown by the close distribution of green circles to the baseline. The mature scars (all located above the baseline) show an increase in the post-treatment area density of 5 ± 2% (mean ± standard deviation) compared to their pre-treatment values. This absolute increase corresponds to a relative (post-treatment area density divided by pre-treatment area density) change of 19 ± 5%. The immature scars are located below the baseline, with the area density having decreased by 9 ± 5% (relative decrease: 25 ± 13%). Possible reasons for this trend are discussed in the next section.
6.2.4 Discussion

This pilot study demonstrates the feasibility of OCT for in vivo, longitudinal monitoring of the vasculature in human burn scars through a series of case studies of patients undergoing laser treatment. Although the one-off imaging of vasculature in previous work has shown the potential of OCT for scar assessment [26, 27], longitudinal monitoring of vasculature is still challenging due to the limited imaging FOV (6 × 3 mm in our study). In this study, we have presented a method using physical landmarks on the skin as reference points to locate the same scar region over the treatment period of several months. This method was validated by the identification of common vessel patterns, observed in the vasculature MIPs before and three days after the first laser treatment and, in several cases, ~20 weeks after the first treatment. Visual assessment of the accuracy of registration of these vessel patterns indicated order 1-2 mm accuracy of our imaging protocol in tracking the same scar region. We observe that such registration is dependent upon finding suitable vessel patterns as landmarks, which was found to be occasionally not possible, particularly in normal skin which has a largely homogenous distribution of vessels [26]. To the best of our knowledge, this is the first demonstration in human subjects of longitudinal tracking of scar vasculature through the use of OCT speckle decorrelation over such a long time period.

Upon burn injury to skin, wound healing is triggered to repair the damaged tissue. Wound healing has three sequential, overlapping phases: inflammation; proliferation; and maturation; which give rise to scar formation. The vascularity increases through angiogenesis and decreases via vascular regression [33, 34], respectively, during the proliferative and maturation phases. The immature scars in this study were in the maturation phase, whilst the mature scars (>2 years old) had completed this process such that the level of vascularity had stabilized. Although at different stages, both classes of scar showed the acute occlusion of vessels three days after the first treatment. The occlusion is caused by ablation and may be caused by subsequent heating though the strong absorption of the CO₂ laser radiation at 10.6 μm by water [35] in the blood, leading to damage of blood and/or vessel walls, whether by vaporization due to ablation, or denaturation of proteins due to heating [36].

The long-term effect of ablative fractional laser treatment on the vasculature of mature and immature scars is distinct. The mature scars present with an increase in vascularity after three courses of treatment, in agreement with a previous histology-based study [37] and supported by the observation in our study that these scars typically appeared more red at time point C. This increase can be attributed to angiogenesis
during the wound healing process triggered by the laser-induced damage of the tissue. In contrast, the immature scars in this study present with a significant decrease in vasculature area density after treatment. The decrease in vasculature area density as the scar matures is further modified by the additional wound healing induced by the laser fractionation. The net effect measured in this study is a decrease in vasculature area density, correlated with a reduction in the red appearance of the scar. In addition, we found that the vascular patterns present in immature scars changed significantly between the first and last image acquisitions, potentially due to scar maturation and laser fractionation.

The 3-D imaging afforded by OCT allows for longitudinal quantification and comparison of vasculature over a constant depth range. For the results presented here, we chose evaluation of vessels to a depth of 300 μm below the tissue surface. This depth was empirically chosen to avoid possible bias in assessment of the changes of vasculature area density. Deep scar tissue has large vessels and they commonly overlap in the x-y plane of the vasculature MIP with the prolific superficial small vessels (area density ~50% up to 300 μm deep in some cases). This overlap can give rise to errors in estimating the changes in the vasculature area density and make the changes in small vessels difficult to discern when they lie directly above deeper, larger vessels. The comparatively shallow depth of 300 μm was set to provide a reliable assessment of the vascular changes by reducing the extent of overlap. In addition, the OCT signal in the MTZs three days after the first treatment attenuates more quickly than that in the surrounding regions. A depth of 300 μm ensures high signal-to-noise ratio in the MTZs.

We note a number of limitations of the vasculature assessment method adopted here, including the quantification of 2-D vasculature MIPs (not the 3-D structure) and the limited imaging depth used for assessment. The OCT speckle decorrelation technique commonly presents shadowing artifacts (i.e., high decorrelation in static tissue beneath a vessel). The use of a 2-D en face projection of the blood vessels mitigates the impact of these artifacts on the quantification. However, such an approach neglects the depth extent of the vessels, which may lead to errors in quantification of the vascular changes. It is possible that developing a method to segment and quantify the vasculature in 3-D will enhance the reliability of longitudinal scar assessment. By mitigating the effect of overlapping vessels described earlier, such a method may allow robust assessment of vasculature over a larger depth range. We note that there may be a mismatch between the OCT imaging depth and the treatment depth of the CO₂ laser, depending on the laser treatment parameters and tissue optical properties. In such a
situation, OCT imaging may only provide an incomplete estimate of the vascular changes. There is potential to increase OCT imaging depth using techniques such as Bessel beam illumination [38] and multi-beam OCT [39].

In addition to the prolific vasculature, hypertrophic scars show over-production of collagen due to excessive healing [40]. This collagen is also significantly impacted by the damage induced by the laser treatment and the subsequent healing response, as indicated by studies on normal (unscarred) skin [41, 42]. A previous study identified that the collagen is denatured when heated above 60 °C to 70 °C [36]. This denaturation can give rise to significant changes in the tissue optical properties, such as optical attenuation and birefringence, due to the changes in tissue structure and organization. Previous work has shown that the denaturation of collagen in porcine tendon leads to a decrease in birefringence [43]. A change in the attenuation coefficient has also been identified through OCT scanning in a mouse model after laser thermal therapy [44]. In the present work, we observed similar changes in the attenuation coefficient within the treated scar regions in the acute scans. In future work, OCT parametric imaging of the attenuation coefficient [45] and birefringence [46] of scar tissue could be included within our longitudinal imaging protocol to provide a more complete quantitative assessment of the tissue changes induced by ablative fractional laser treatment, and complement the assessment of the vasculature presented here.

6.2.5 Conclusion
In this article, we have demonstrated the feasibility of using OCT for longitudinal monitoring of change in the vasculature of burn scars in response to ablative fractional laser treatment. Based on a speckle decorrelation method, we developed a stable imaging setup and protocol to mitigate motion artifact and an imaging protocol to track a single scar location over the treatment period. The longitudinal vasculature MIPs were examined to reveal changes in individual vessels and automatically quantified using the vasculature area density to evaluate the overall changes to the vasculature. A series of clinical case studies on burn scar patients showed the acute occlusion of blood vessels three days after treatment, and long-term decreases and increases of the area density in immature and mature scars, respectively. The OCT imaging method presented here provides a feasible option for clinical longitudinal monitoring of vasculature to assess scar treatment.

6.2.6 Acknowledgements
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6.2.7 References


6.3 **Optical palpation in vivo: imaging human skin lesions using mechanical contrast**

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**Abstract:** We demonstrate the first application of the recently proposed method of optical palpation to in vivo imaging of human skin. Optical palpation is a tactile imaging technique that probes the spatial variation of a sample’s mechanical properties by producing an en face map of stress measured at the sample surface. This map is determined from the thickness of a translucent, compliant stress sensor placed between a loading element and the sample and is
measured using optical coherence tomography. We assess the performance of optical palpation, using a handheld imaging probe on skin-mimicking phantoms, and demonstrate its use on human skin lesions. Our results demonstrate the capacity of optical palpation to delineate the boundaries of lesions and to map the mechanical contrast between lesions and the surrounding normal skin.

6.3.1 Introduction

The pathologies of skin often modify its mechanical properties. For example, scleroderma [1, 2], skin cancer [3, 4] and burn scars [5, 6] all give rise to large variations in the pliability of skin. For this reason, clinicians commonly manually palpate skin lesions to obtain a subjective assessment of the pathology. In the case of burn scars, pliability, as assessed by palpation and observation, is one of four parameters considered in the Vancouver Scar Scale, which is an assessment scale commonly used by clinicians [7]. Limitations of manual palpation include its subjectivity, low resolution and low sensitivity. To address these limitations, a number of objective methods have been proposed to measure the mechanical properties of skin [8, 9].

Typically in such methods, the skin is subjected to a mechanical perturbation and its surface deformation, or the force required to produce a specific deformation, is measured. Examples of mechanical perturbation for in vivo studies include: suction [10], compression [11] (or indentation) [12-14], torsion [15-17], extension [5, 18, 19] and acoustic wave propagation [20]. These loading mechanisms have been incorporated into a number of devices to characterize the mechanical properties of skin in vivo. Examples of devices that apply static or quasi-static loads orthogonal to the skin’s surface include: the cutometer, which is an optical system that measures the skin surface displacement versus time under suction [21]; the tonometer, which measures the extent of depression of a plunger in a weight-loaded device placed on the skin [11, 22]; and spherical indenters, which measure adhesion forces between the indenter and skin versus penetration depth by using force sensors and a motion controller to displace the indenter at a constant velocity [13]. Alternatively, some methods utilize dynamic loading. One example is the reviscometer, which couples sound waves to skin (in the frequency range 5-8 kHz) and records the transit time from transmitter to receiver, with stiffer and more dense tissues having a higher sound velocity [23]. There are also methods that apply in-plane deformations, such as the twistometer, which measures skin’s extensibility under
torsion using a torque sensor [24]; and the quasi-static extensometer, which uses strain gauges to record the required load and apply a known rate of extension to the skin between two adhesive tabs [19]. Additionally, suction and compression loading methods have been combined with ultrasound imaging to allow the thickness of the dermis and hypodermis to be simultaneously monitored [25-28].

Aside from the ultrasound-based approaches, these methods generally characterize the average properties of skin over regions with dimensions in the millimeter to centimeter range. Characterization of the local spatial variation of the mechanical properties of skin on the finer submillimeter scale has the potential to aid in the assessment of a number of skin pathologies, including skin cancers [29-32] and burn scars [6].

Optical coherence elastography (OCE) has been proposed as a means of providing high-resolution images of skin stiffness [33-38]. In OCE, skin deformation is measured using optical coherence tomography (OCT). The higher resolution and higher sensitivity to sample deformation of OCE, compared to the methods described above [11, 13, 21-23, 39-41], hold promise for detecting more subtle changes in stiffness. Several OCE approaches have been proposed for in vivo imaging of skin. In compression OCE, the local axial strain, defined as the rate of change of sample axial displacement versus depth, is measured in response to a compressive load on the skin surface. This technique can provide three-dimensional (3-D) strain elastograms of skin in vivo [35]. In surface acoustic wave OCE, the measured phase velocity is used to obtain the Young’s modulus of tissue directly and has been demonstrated to provide quantitative, two-dimensional (2-D) elastograms of skin in vivo [34, 36, 37].

In this paper, we present the first application of optical palpation, an OCT-based tactile imaging technique recently proposed by our group [42], to in vivo imaging of skin lesions. Optical palpation is a variant of OCE in which a translucent, compliant layer acting as a stress sensor is placed between the sample and a compressive loading element. 3-D-OCT images are acquired before and after loading. The strain in the compliant layer is estimated by measuring the layer thickness using OCT. Using prior knowledge of the stress-strain behavior of the sensor material, the surface stress at each lateral location is mapped into a 2-D image. This image represents the stress at the sample surface, analogous to the stress detected by manual palpation, but at a higher spatial resolution.

To enable optical palpation to be performed in vivo, we have developed a handheld imaging probe to apply a compressive load to the skin whilst simultaneously
performing OCT imaging from the same side. To assess the probe’s performance, we first performed optical palpation on skin-mimicking phantoms consisting of layers mimicking the epidermis, dermis and hypodermis. Stiff inclusions were embedded in the dermis layer to mimic a stiff lesion. Additionally, as many skin lesions feature irregular and raised surfaces, we performed optical palpation on phantoms with raised surface features to assess the impact of surface topology on measured stress. We subsequently performed optical palpation on various skin lesions from human volunteers, including: a nevus, a burn scar, a scar resulting from a sutured laceration, and a hypertrophic postsurgical scar. In each case, high mechanical contrast is observed. To validate the contrast obtained, optical palpation images are compared both to corresponding OCT en face images and photographs. In several instances, optical palpation reveals features not visible in the corresponding OCT image.

6.3.2 Materials and methods

(a) Imaging system

Scanning was performed using a portable swept-source OCT system (OCS1300SS, Thorlabs, USA) with a central wavelength of 1325 nm and a spectral bandwidth of 100 nm. The measured axial and transverse resolutions (full-width at half-maximum) of the system are 17 μm (in air) and 16 μm, respectively. Light illuminated the sample through an objective lens with a working distance of 25 mm, delivering a scanning beam with a numerical aperture of 0.03 and a measured optical power of 4.7 mW. The dimensions (xyz) of each OCT data volume are 8 × 8 × 3 mm. The system was operated at an A-scan rate of 14 kHz, and the 3-D data acquisition time was ~40 s. The OCT images presented here are normalized on a log-scale from 0-255 grayscale (with 0 corresponding to black and 255 corresponding to white).

(b) In vivo imaging probe

The handheld probe incorporates both OCT imaging and compressive loading from the same side of the sample and is an extension of OCE probes previously developed by our group [33, 35]. A schematic of the probe is shown in Figures 6.8(a) and (b), and a photograph of the probe during optical palpation imaging is shown in Figure 6.8(c). The compliant sensor is positioned between the probe and the skin. In the figure, \(l_0(x,y)\) and \(l(x,y)\) represent the sensor thickness before and after loading, respectively. These parameters are measured at each lateral position (x,y) by calculating the distance between the axial location of the upper and lower edges of the sensor. The edges are detected in each OCT B-scan, as the interfaces of the sensor and the bottom surface of
the imaging window and skin, respectively, using a Canny edge-detector [43]. The minimum measurable sensor thickness is \(\sim 12 \mu m\), limited by the axial resolution of the OCT system in silicone, and the maximum measurable thickness is \(\sim 2.14 \text{ mm}\), limited by the imaging range of the OCT system. The minimum change in sensor thickness that can be measured is determined by the axial pixel size, \(\sim 4 \mu m\) in our case. The deformation of the sensor is quantified by strain, \(\varepsilon\), as:

\[
\varepsilon(x, y) = \frac{l(x, y) - l_0(x, y)}{l_0(x, y)}.
\]

(6.2)

Figure 6.8. *In vivo* optical palpation imaging probe. (a) and (b) schematic diagrams of the optical palpation setup for skin imaging: (a) before; and (b) after compressive loading. \(L_c\) in (a) represents the length of the cylindrical head of the probe. \(l_0(x, y)\) in (a) and \(l(x, y)\) in (b) represent the local sensor thickness before and after loading, respectively. (c) Photograph of the probe, demonstrating optical palpation of skin *in vivo*. (d) Representative stress-strain curve of the sensor material, used to estimate the local stress from the measured local strain.

The stress at each lateral position is then estimated from the stress-strain curve of the compliant sensor, shown in Figure 6.8(d), which was independently measured using a standard compression tester (Instron, Norwood, MA, USA). The plate of the compression tester had a diameter of 40 mm and was used to test compliant sensors with thicknesses of 1 mm and 3 mm and with diameters of 20 mm and 50 mm,
respectively. Given that the minimum detectable change in sensor thickness is \( \sim 4 \, \mu m \), for sensors of thickness 1 mm, the minimum measurable strain is 0.004. Assuming a sensor preload of 30%, this corresponds to a minimum detectable change in stress of \( \sim 0.3 \, kPa \).

To provide uniform compression on the corrugated surface of the skin, the probe has a cylindrical head with an inner diameter of 10 mm and an outer diameter of 15 mm. A glass imaging window (2 mm thickness) was fixed to the base of the cylindrical head, providing optical access to the sample, as well as acting as a compression plate. The diameter of the window used in all scanning sessions, except in one, was 12.5 mm. In the session scanning the fourth skin lesion, this was replaced with a larger window of diameter 25 mm. As both of the imaging windows are larger than the area over which OCT imaging was performed (8 × 8 mm), and because the thickness of the compressed tissue was of the same order of magnitude as the diameter of the imaging plate, we assume that the tissue undergoes uniaxial compression, that no boundary conditions are present in the region scanned, and that no gradient in loading is introduced in the axial direction.

The length of the cylindrical head (13.5 mm), marked as \( L_C \) in Figure 6.8(a), was set to maximize the probe’s measurable displacement range and, thereby, also the achievable axial compression in skin. Maximizing the range of displacement is of particular importance for body locations containing a thick hypodermis, the soft layer of subcutaneous fat below the skin. In this case, a larger probe displacement is required to adequately compress the epidermis and dermis. The upper limit for the length of the cylindrical head was constrained by the working distance of the objective lens (25 mm) and was set to ensure that the skin remained in focus in both pre and postcompression scans (the distance between the objective lens and the bottom surface of the imaging window could be adjusted in the range 21-30 mm).

Optical palpation experiments were performed with two probe configurations: a handheld setup, where the operator held the imaging probe in both the pre and postcompression acquisitions; and a configuration in which the OCT probe was fixed to a translation stage to allow well-controlled compression of the sample. In the latter configuration, the translation stage was either fixed to a stand, as shown in Figure 6.8(c), or installed on an articulating arm, similar to one used by our group previously for OCT skin imaging [44].

(c) Stress sensor
The translucent stress sensors were fabricated with a thickness of 1 mm and a diameter of 50 mm using Elastosil® P7676 and AK50 Silicone Fluid (Wacker, Germany), as described in detail previously [45]. The mechanical properties of these compliant silicone materials can be controlled by altering the ratios of silicone catalyst, cross-linker and non-cross-linked silicone fluid.

Because optical palpation measures the sensor deformation in order to determine stress, a key requirement is that the sensor stiffness is such that it deforms when compressed against skin. The sensors used here have a Young’s modulus in the range 7-21 kPa. This range matches well with the range previously reported for skin stiffness [12, 14, 46, 47].

(d) Optical palpation of skin-mimicking phantoms
To assess the performance of our imaging setup, skin-mimicking phantoms were fabricated using combinations of silicone elastomers similar to those used in the fabrication of stress sensors and, additionally, Elastosil® RT601 (Wacker, Germany). The optical properties of the phantoms were controlled by adding titanium dioxide (TiO₂) particles to the silicone mixture prior to adding the silicone cross-linker [45]. The Young’s modulus of each material used in the phantoms was independently measured using a standard compression tester (Instron, Norwood, MA, USA). We performed optical palpation on phantoms with the probe fixed to a translation stage which was attached to a stand. The stress sensors used in these experiments have a Young’s modulus of 19 kPa.

Two 3-layer phantoms, illustrated in Figure 6.9, were fabricated with optical, mechanical and structural properties mimicking those of human skin. The superficial layer (Layer A) has Young’s modulus of 120 kPa and thickness of 200 μm, mimicking the epidermis. The thickness of this layer is in the range of the epidermal thickness of human skin, which varies from ~30-150 μm for thin skin up to ~500-800 μm for thick skin [48, 49]. The softer middle layer (Layer B) has Young’s modulus of 12 kPa and thickness of 1.7 mm, mimicking the dermis. This layer contains inclusions with Young’s modulus of 120 kPa and approximate dimensions (xyz) of 1 × 1 × 0.7 mm to mimic stiff lesions, such as intradermal lipomas [50]. The inclusions were embedded such that they extend in depth to ~0.5mm above the top of Layer C. The deepest, softest layer (Layer C) is 2 mm thick with Young’s modulus of 5 kPa, mimicking the hypodermis.
Figure 6.9. Schematic diagram of the structure of the skin-mimicking Phantoms 1 and 2. The three layers in the phantoms mimic the mechanical properties of the epidermis, dermis and hypodermis, respectively. The stiff inclusion in Phantom 1 has higher scattering than the surrounding layer, while in Phantom 2 it has scattering matched to that of the surrounding layer. The approximate dimensions (xyz) of the inclusions are 1 × 1 × 0.7 mm, and the thickness of the stress sensor and the layers of the phantom are marked at right.

In Phantom 1, the embedded inclusion has a higher concentration of TiO$_2$ scatterers (1.5 mg/mL) than in the substrate (0.5 mg/mL), providing optical contrast between the inclusion and the substrate. In Phantom 2, the inclusion has the same concentration of scatterers as in the substrate (0.5 mg/mL), resulting in negligible optical contrast between the inclusion and Layer B in the OCT image. The concentration of scatterers is 1.5 mg/mL in Layer A, making this layer distinguishable from Layer B. Optical palpation was performed on these phantoms by applying a 2-mm compression between scans.

To assess the performance of optical palpation on uneven surfaces, we fabricated two silicone phantoms with raised surface features. In Phantom 3, we fabricated a raised feature with higher stiffness (300 kPa) than the substrate (18 kPa). In Phantom 4, we fabricated a raised feature with the same stiffness (18 kPa) as the substrate. The raised features are cylindrical, with diameter of 1 mm and length of >5 mm and are parallel to and partially embedded, 200 μm beneath the surface of the phantom, creating a rounded ridge on the surface, as seen in Figures 6.12(a) and (b). In both phantoms, the thickness of the substrate, marked as $L$ in Figures 6.12(a) and (b), is ~2.6 mm and the concentration of TiO$_2$ scatterers is 0.8 mg/mL throughout. Optical palpation was performed by bringing the probe into full contact with the phantom surface. A minimal preload was applied to the sensor over the raised features, just sufficient to ensure that the probe was in full contact with the sensor in the precompression scans. The preload was set by observing, using OCT imaging, the contact between the window of the probe and the sensor immediately prior to loading and image acquisition. The postcompression scans were acquired after applying an additional displacement of
1.1 mm, which corresponds to 30% bulk strain on the system, including the 1-mm sensor and the phantom.

(e) Optical palpation of skin lesions

Optical palpation was carried out in vivo on volunteers (Caucasian, 2 males and 1 female, mean age 32) with skin lesions on the dorsal forearm, the wrist, the dorsal hand and the ventral arm. The lesions were a nevus and three scars resulting from a burn, a sutured laceration and a surgical excision, respectively. In each case and prior to imaging, photographs of the imaging location were taken and the skin lesion and the adjacent normal skin were marked for 3-D-OCT imaging. Hair on the imaging location was trimmed using an electric shaver prior to scanning to reduce shadowing artifacts in OCT imaging. To minimize friction, the skin and both sides of the stress sensor were lubricated with silicone fluid, before positioning the sensor on the skin surface. After positioning the sensor, the imaging probe was brought into full contact with the sensor. Prior to 3-D-OCT acquisition in the unloaded case, it was verified by visual inspection of OCT B-scans that potential sources of artifact, such as compression at an angle or trapped oil, were not present. To minimize compression and motion artifacts, the limb being scanned was supported using sandbags. After acquiring the unloaded 3D-OCT dataset, the imaging probe was lowered to increase the compression in both the sensor and the skin. In the loaded case, the same procedure was adopted: several OCT B-scans were visually inspected, to verify the absence of artifacts. In the processing routine, if a noticeable lateral shift between the OCT scans before and after compression was observed, a landmark in the image, such as a hair follicle, was used to laterally co-register the scans prior to estimating the axial strain in the sensor. In the results presented, the depth of the en face OCT image plane beneath the sample surface is reported as a physical length assuming an average group refractive index of 1.4 for silicone and 1.43 for skin [51].

6.3.3 Results

(a) Tissue phantoms

(i) Phantoms 1 and 2 – three layers with “dermal” inclusions

Figure 6.10 shows the results of optical palpation on a portion of Phantom 1 that contains a stiff inclusion in the “dermis” layer. Figures 6.10(a) and (b) show representative OCT B-scan images before and after loading, respectively. In these B-scans, the specular reflection close to the top of the image is caused by the bottom surface of the imaging window. The stress sensor lies below this surface, appearing as a
region of very low scattering between the imaging window and the phantom. The superficial layer of Phantom 1, Layer A, appears as a high scattering layer above the less scattering middle layer in which the inclusions are embedded. In the unloaded image, the penetration depth of OCT does not reach the location of the inclusion [Figure 6.10(a)], but this inclusion comes fully into the B-scan field-of-view after compression [Figure 6.10(b)]. Also in Figure 6.10(b), Layer C comes slightly into the field-of-view, appearing as a dark band below the moderately scattering Layer B [indicated by the arrow in Figure 6.10(b)].

![Figure 6.10. Phantom 1. (a), (b) OCT B-scans acquired from the central region of the phantom before and after compression, respectively. The arrow in (b) indicates the top section of Layer C. (c) En face OCT image after compression, 580 μm beyond the interface of the sensor and Layer A. (d) Corresponding stress map.](image)

As seen in Figure 6.10(b), the stress sensor is compressed more over the stiff inclusion (mean strain ~43%) than over the rest of the substrate (mean strain ~34%). The 2-D *en face* map of stress is shown in Figure 6.10(d), illustrating mechanical contrast between the inclusion and substrate. The mean stress above the inclusion is ~22 kPa, compared to ~14 kPa in the rest of the phantom. This result demonstrates the ability of optical palpation setup to detect mechanical contrast in a phantom mimicking a stiff lesion in the dermis. Figure 6.10(c) shows an *en face* OCT image, taken from a physical depth of 580 μm beyond the interface of the sensor and Layer A. Comparing the OCT image with the stress map [Figure 6.10(d)] confirms that the variation in mechanical contrast matches the apparent lateral location of the stiff inclusion. The spatial resolution of optical palpation will be discussed below in Section 6.3.4.

To demonstrate the independence of mechanical and optical contrast, Figure 6.11 shows the results of optical palpation on Phantom 2, which has the same structural and mechanical properties as Phantom 1 but contains an inclusion with optical
properties matched to those of Layer B. Thus, the inclusion is not visible in the OCT image. Figures 6.11(a) and (b) show representative OCT B-scan images before and after compression, respectively.

![Figure 6.11](image)

Figure 6.11. Phantom 2, in which the optical properties of the embedded stiff inclusion match the surrounding silicone matrix. (a), (b) OCT B-scans acquired from the central region of the phantom before and after compression, respectively. The arrow in (b) indicates the top section of Layer C. (c) *En face* OCT image after compression, 840 µm beyond the interface between the sensor and the phantom surface, cutting through the center of the stiff inclusion. (d) Corresponding stress map.

The response of the stress sensor in Figure 6.11(b) is similar to that in Figure 6.10(b); a higher axial deformation (~45% mean strain) is observed above the stiff inclusion compared to the soft surrounding material (~38% mean strain). Figure 6.11(c) shows an *en face* OCT image of Phantom 2 after compression at a physical distance 840 µm beyond the interface of the sensor and the phantom surface. This *en face* plane cuts through the center of the stiff inclusion; however, the inclusion is not visible as it has the same optical properties as the surrounding layer. The stress map, shown in Figure 6.11(d), provides high contrast between the inclusion (~30 kPa mean stress) and the soft surrounding material (~17 kPa mean stress). This experiment demonstrates that optical palpation can detect features independently of the optical contrast of the sample. This result also suggests that optical palpation could provide contrast complementary to that provided by OCT if the variations in optical and mechanical contrast were to be different for a given feature.

(ii) **Phantoms 3 and 4 – phantoms with raised surface features**

Figure 6.12(c) shows the result of optical palpation on a portion of Phantom 3 containing a stiff, raised feature. This stress map provides high contrast between the region above the raised feature (~22 kPa mean stress) and the rest of the phantom.
(~13 kPa mean stress). To validate that the higher stress above the raised feature resulted from its higher stiffness and not as an artifact of its raised topology, Figure 6.12(d) shows the result of optical palpation on a portion of Phantom 4, containing the raised feature with the same stiffness as the rest of the phantom. In Figure 6.12(d), the mean stress of the raised feature (~9 kPa) is close to that of the remainder of the phantom (~9.8 kPa).

The results of optical palpation on Phantoms 3 and 4 indicate that, firstly, mechanical contrast between a stiff raised feature at the surface and the rest of a sample is readily observable and that, secondly, the raised topology of surface features does not lead to overestimation of the stress over a raised feature.

In the stress maps presented in Figures 6.12(c) and (d), two parallel regions of lower stress are visible at the left and right sides of the raised features. These regions appear due to the incompressibility of the sensor material, which links the axial compression with lateral expansion in order to preserve volume. The lateral expansion of the sensor in the regions adjacent to the raised features leads to lower axial strain and, therefore, lower stress than in regions far from the raised features.
(b) Optical palpation of skin lesions
In this section, we demonstrate the contrast provided by \textit{in vivo} optical palpation on skin lesions from volunteer subjects. In each case, a photograph, \textit{en face} OCT image and stress map are presented. The representative \textit{en face} OCT image was chosen from the 3-D volume so as to maximize the contrast between the lesion and the surrounding skin.

(i) Subject 1: nevus
To demonstrate optical palpation \textit{in vivo} on a clearly delineated feature, we scanned a nevus (diameter \~{}2 mm) on the dorsal forearm of a volunteer. The nevus, shown in the photograph in Figure 6.13(a), is dark, slightly raised, and under manual palpation felt stiffer than the surrounding tissue. Figure 6.13(b) shows an \textit{en face} OCT image in the unloaded case, 200 \textmu{}m beyond the interface of the sensor and the skin surface, in which the nevus appears as a slightly darker region (lower signal), most likely caused by melanin. The dark, oval-shaped regions around the nevus of diameter <0.3 mm are hair follicles in cross-section.

![Figure 6.13. Subject 1: Nevus on the dorsal forearm. (a) Photograph of the region imaged. The red rectangle corresponds to the field-of-view in (b) and (c). (b) \textit{En face} OCT image before compression, 200 \textmu{}m beyond the interface of the sensor and the skin surface. (c) Corresponding stress map of the skin lesion.](image)

Optical palpation was performed on this nevus in a handheld configuration in which the operator held the probe over the imaging location. For stability, the arm of the operator was supported on a cushion and located close to the imaging location. Using a stress sensor with Young’s modulus of 21 kPa, the stress map in Figure 6.13(c) is obtained, in which high mechanical contrast is provided between the lesion (~12 kPa mean stress) and the surrounding skin (~4 kPa mean stress). Comparing Figures 6.13(b) and (c), we note that the border of the nevus is more clearly delineated in the stress map than in the \textit{en face} OCT image.

(ii) Subject 2: burn scar
In Figure 6.14, we demonstrate optical palpation of a burn scar resulting from a scald with hot oil on the wrist above the radius bone. This lesion, shown in Figure 6.14(a), is...
mature (>10-years old), slightly raised and stiffer to the touch than the surrounding tissue. The lesion was imaged with the probe fixed to a translation stage which was affixed to a stand. Figure 6.14(b) shows an en face OCT image in the unloaded configuration, 200 μm beyond the interface of the sensor and the skin surface. The OCT intensity is slightly higher in the scarred region. A displacement of 2.6 mm was applied between the unloaded and loaded scans. The acquired stress map in Figure 6.14(c) shows: higher stress over the burn scar than over the surrounding skin; clearer delineation of the scar than the corresponding en face OCT image [Figure 6.14(b)]; and heterogeneity of mechanical properties distributed throughout the lesion.

Figure 6.14. Subject 2: A burn scar on the wrist. (a) Photograph of the region imaged. The dashed green line delineates the scar. The red rectangle corresponds to the field-of-view in (b) and (c). (b) En face OCT image before compression, 200 μm beyond the interface of the sensor and the skin surface. (c) Corresponding stress map.

(iii) Subject 3: suture scar

To demonstrate optical palpation on a flat lesion, we imaged a portion of a mature scar (>10-years old) resulting from a sutured laceration, shown in Figure 6.15(a), on the dorsal hand of a volunteer. Notably, variations in mechanical properties between the scar and surrounding tissue were sufficiently subtle that, by palpating the lesion manually with a fingertip, it was not possible to distinguish the scar from the

![Figure 6.15](image-url)
surrounding skin. To acquire this scan, we used an articulating arm to facilitate uniaxial compression of the imaging region and applied a 1.5 mm probe displacement between the unloaded and loaded scans. Figure 6.15(c) shows the stress map, which readily differentiates the scar region from the surrounding skin. The stress map corresponds well to the photograph in Figure 6.15(a), in delineating the region of scarring. Such contrast is not present in the *en face* OCT image, shown in Figure 6.15(b), which corresponds to a depth of 330 μm beyond the interface of the sensor and the skin surface.

(iv) **Subject 4: hypertrophic scar due to surgical excision**

Figure 6.16 shows results from a mature (~3-year-old) hypertrophic scar on the ventral arm of a volunteer, formed as a result of surgical excision. The scarred region was stiff and raised, due to an overproduction of collagen characteristic of hypertrophic scarring. Microvasculature imaging, performed using a speckle decorrelation technique [44], revealed a prolific network of blood vessels present, which is also characteristic of hypertrophic scarring.

As this nodular scar, shown in Figure 6.16(a), had a diameter comparable to the probe head, it was challenging to place the probe such that both the scar and adjacent skin were compressed. To overcome this, the 12.5 mm-diameter imaging window was replaced by a larger window of diameter 25 mm. To perform imaging, we used a softer stress sensor (Young’s modulus 14 kPa) to account for the thicker layer of subcutaneous fat at this imaging location. Using the articulating arm, the compression angle of the probe was adjusted to be parallel to the plane of the skin-scar surface and the probe was displaced by ~12 mm. Figure 6.16(b) shows an *en face* OCT image in the compressed case, 270 μm beyond the interface of the sensor and the skin surface. As the scar was elevated, there was a lateral shift between the OCT scans acquired before and after
compression: a 1.4 mm and 1.54 mm shift away from the fast and slow scanning directions, respectively, shown in Figure 6.16(b) as \( x \) and \( y \) arrows. The pre and postcompression OCT scans were manually co-registered in the lateral direction prior to calculating the strain.

The stress map is shown in Figure 6.16(c) and demonstrates high mechanical contrast between the scar region in the top left of the image and the adjacent skin. The region of high stress in Figure 6.16(c) corresponds well with the scar region in the photograph in Figure 6.16(a) and the \textit{en face} OCT image in Figure 6.16(b), in which the scar appears as a region with slightly higher OCT signal in the top left.

### 6.3.4 Discussion

The results presented here demonstrate the ability of optical palpation to provide mechanical contrast between lesions and the surrounding skin, which exceeds that available from OCT imaging alone. An advantage of optical palpation over many OCE techniques for imaging mechanical contrast \textit{in vivo} is that phase-sensitive detection is not required, placing less stringent requirements on the OCT system. Additionally, as optical palpation is derived from the OCT signal in the stress sensor, it can be used to map the mechanical properties of even very opaque tissue.

The use of a compliant layer enables this technique to provide useful measurements when the skin surface is uneven, as its conformity to the surface topography allows sufficiently uniform stress to be applied across the sample surface. This feature is highlighted by the results on the phantoms with raised features and in three of the \textit{in vivo} results, where the imaged lesions had raised and irregular surfaces.

In this study, we aimed to probe mechanical contrast produced within the epidermis and dermis. As the tissue beneath the dermis, such as the hypodermis and bone, also influences the contrast in optical palpation, several steps were taken to minimize its influence. First, we found that the presence of a thick layer of hypodermis required significantly higher displacements of the imaging probe before the mechanical contrast in the superficial layers of skin could be observed. If the hypodermis was insufficiently loaded, we observed bulk motion of the sensor during loading, as the hypodermis compressed in preference to the stiffer superficial layers, precluding our ability to measure stress. To achieve mechanical contrast from the dermis also required this layer to compress in the loaded state. To meet these two demands, we fabricated sensors with stiffness intermediate between that expected of the dermis and hypodermis, allowing both layers to be compressed. Underlying bone also influences the contrast in...
optical palpation. In particular, an uneven distribution of bone within the region under compression introduces a gradient in stress that is largely independent of the mechanical properties of the superficial skin layers. To mitigate this effect, we chose the smallest probe size that provided both sufficiently uniform loading and an adequate imaging field-of-view. Gradients in stress also result from the probe and the underlying bone not being parallel. Consequently, an important aspect of the imaging protocol involved careful alignment of the probe to the skin surface, as described in Section 6.3.2(e).

As part of our imaging protocol, whilst applying the probe displacement and while performing imaging, volunteers were asked to report any pain or discomfort. Additionally, the operator used real-time feedback of the sensor compression observed in OCT B-scans as an indicator to avoid overloading the tissue. During image acquisition, we utilized the minimum probe displacement necessary to produce strain contrast in the sensor. For cases in which very high skin compression is needed to produce contrast, the stress observed in the stress-strain curves of the sensors at 70% strain (the highest strain level characterized) is less than 205 kPa. This level of stress is below the reported pain threshold for humans (0.4 MPa) [52] and far below the reported pain tolerance values (~0.5-1.1 MPa) [22, 52-55]. In the results presented here, the measured stress was always <20 kPa.

The lateral resolution of optical palpation has previously been reported to be 180 μm [42]. This measurement was obtained using a phantom comprising a column of soft silicone, with Young’s modulus 20 kPa, adjacent to a column of stiffer silicone, with Young’s modulus of 4 MPa. While this measurement is to some extent representative of the lateral resolution in optical palpation imaging of skin in vivo, it is important to note that the resolution is dependent not only on that of the optical imaging system, but also on the structural and mechanical heterogeneity within the sample and, consequently, is likely to vary for different skin regions. For the phantoms used in this study, using the technique reported previously [42], we measured lateral resolutions in the range 160-390 μm. The measured values correspond to the 10% to 90% spatial “step” response of the stress at the interface of the inclusion and the adjacent phantom matrix. Insight into the variation in lateral resolution can be gained by considering the different geometries and mechanical contrasts in the phantoms reported here. In particular, phantoms 1 and 2 demonstrate how feature geometry can impact the lateral resolution in optical palpation. In these phantoms, the axial thickness of the inclusions is small compared to the overall phantom thickness (Figure 6.9), and consequently, the edges of the inclusions are blurred in the stress maps [Figures 6.10(d) and 6.11(d)].
resolution of optical palpation is also linked with the compressibility of the material used in the stress sensor. We have used soft silicone sensors with Poisson’s ratio of ~0.5 (nearly incompressible), which signifies axial compression in the sensor is accompanied by lateral expansion, which also contributes to blurring of the feature boundaries detected. Further studies incorporating models of skin deformation are required to rigorously assess the variation in resolution caused by the presence of heterogeneity within skin.

The lateral resolution reported here (160-390 μm) for optical palpation of skin-mimicking phantoms is much higher than that of methods which record the mechanical behavior of skin by averaging it over the region being probed: including the cutometer (2 mm) [21], tonometer (1 mm) [22], twistometer (3 mm) [24], and indentation testers (2–6 mm) [13, 56]. There is scope to improve the lateral resolution towards that of the underlying OCT resolution by using inverse methods. Such methods have been proposed in related tactile imaging techniques [57].

In the 2-D stress map in Figure 6.13(c), we note additional structures caused by the skin microrelief. Skin microrelief refers to the fine intersecting lines on the outermost layer of skin, which are visible in Figure 6.13(a). This contrast does not arise from higher or lower stress in the sensor; rather, it arises from a limitation of our edge-detection algorithm in accurately detecting the bottom surface of the sensor. The skin microrelief restricts the stress sensor from making full contact with the skin. As the OCT signal from the interface of air and skin is stronger than that from the interface of the sensor and air, our algorithm detects the former interface rather than the latter. One means to remove this small artifact could be to add a thin, high-scattering layer of silicone to the sensor surface that is in contact with the sample and to estimate the thickness of the sensor as that of the low-scattering region. Using spin coating techniques, it may be possible to make very thin (<10 μm) layers for this purpose.

The results on the phantoms presented here demonstrate that optical palpation can tolerate surface unevenness comparable to that found in skin. Further studies are required to establish the degree of unevenness that may be tolerated. Additionally, optical palpation of skin in vivo could potentially be extended to provide quantitative measurements of elasticity by calculating the strain in the tissue, i.e., by combining optical palpation with compression OCE [39, 42].

6.3.5 Conclusion
Assessing the mechanical properties of skin at submillimeter spatial resolution has great potential to aid in the assessment of a number of skin pathologies. In this paper, we presented the first demonstration of optical palpation on human skin in vivo, which probes the mechanical properties of skin by measuring the local stress imparted to a compliant sensor placed on the skin surface. Our in vivo imaging probe was first validated on skin-mimicking phantoms, demonstrating mechanical contrast of a lesion embedded in the artificial dermis layer, and confirming that stress maps provide useful mechanical contrast even in the presence of irregular surface topology. Our in vivo results on a range of human skin lesions demonstrate the ability of this technique to provide high spatial resolution and mechanical contrast, enabling submillimeter delineation of the borders of stiff lesions and providing additional contrast compared with the corresponding 3-D-OCT image.

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6.3.7 References


6.4 Chapter summary

This chapter demonstrated first the application of the vasculature assessment method developed in Chapter 3 for assessing the laser fractionation treatment of burn scars. In addition to the vasculature assessment method, we developed a scanning protocol to enable repeat imaging of a specific scar location over a time period of weeks to months, using physical landmarks on the scar surface. Aided by this protocol, we compared the longitudinal vasculature images to assess the acute changes, such as the occlusion of vessels, and the long-term changes in the quantified vessel area density. The results from a series of case studies elucidated the distinct long-term vascular response with a mean 23% decrease and 22% increase of the relative area vessel density (post-treatment area density divided by pre-treatment area density) in immature and mature scars,
respectively. This study demonstrated the promise of the vasculature assessment method for assessing scar treatment for improved patient management.

While changes in vascularity provide a good, indicative measure of scar change, we have previously noted that this only reveals one aspect of the wound healing process. Scars also undergo changes in their collagen content, modifying both their optical and mechanical properties. The importance of these changes is indicated by their inclusion in common scar scales, such as the Vancouver Scar Scale, where both appearance (optical) and pliability (mechanical) are assessed. In previous chapters, we have described fully automated methods to quantify the optical properties, specifically attenuation coefficient (Chapter 4) and birefringence (Chapter 5). In this current chapter, we have extended this work to propose a method to produce images that assess the mechanical properties of the scar. The optical palpation technique described in Section 6.3 illustrates that OCT has the potential to provide multi-faceted insight into tissue structure through innovative adoption of the imaging protocols, such as the inclusion of a translucent, compliant stress sensor. This section, published as a journal paper, is indicative of future work to be undertaken in OCT assessment of burns scars. It also indicates the potential of OCT to underlie a family of assessment techniques, both optical and mechanical, that could provide a comprehensive, objective characterisation of burns scars, and their longitudinal response to treatment.
Chapter 7

Conclusion

7.1 Significance of research outcomes

The research presented in this thesis has focused on the development of OCT as a tool for non-invasive clinical scar assessment. We have explored and demonstrated methods for the objective, quantitative assessment of the characteristic vasculature and, separately, the collagen network in scars. The results show great potential for the use of OCT in scar assessment.

The first OCT scar assessment method presented in this thesis assessed the scar vascularity by imaging and quantifying the blood vessels (Chapter 3). Whilst OCT vasculature imaging has been widely investigated, its application for clinical imaging of scars has not been specifically explored, and there has been a lack of a validated clinical protocol for its use. The research in Chapter 3 presented an imaging and data post-processing protocol to enhance the feasibility of OCT vasculature imaging for clinical usage. The imaging protocol incorporated solutions, aided by data post-processing, to mitigate imaging and motion artefacts. Automatic quantification of the blood vessel diameter and area density was developed to score the vascularity. Compared with assessment of scar vascularity using the observed colour (i.e., normal, pink, red and purple), our proposed method has the advantage of providing the clinicians with the
microscopic structure of the vasculature for visualisation, and objective scores (i.e., vessel diameter and area density) for quantitative assessment. Using this method, we performed pilot clinical studies on burn scar patients and observed a larger average vessel diameter and area density in hypertrophic scars than was found in normal skin. The results provide an in vivo validation of the prolific vasculature in hypertrophic scars at the microscopic level.

The OCT scar assessment method then focused on assessing the non-vascular tissue, primarily the collagen network, in Chapters 4 and 5. As most clinical OCT systems are not able to resolve individual collagen fibrils or fibres, we developed methods to quantify their optical properties, which are strongly influenced by the structure and organisation of the collagen. Two measures were presented. The first was the optical attenuation coefficient, calculated by fitting the corrected OCT A-scans to a single-scattering model (Chapter 4). This is the first measurement of the attenuation coefficient of scar tissue with OCT, enriching our understanding of the optical properties of scars. We proposed a novel vascular masking technique to remove vasculature-induced artefacts in the attenuation coefficient estimates, caused by the strong optical scattering in the blood vessels. The burn scars assessed by our method showed lower attenuation coefficients than the normal skin, indicating that attenuation coefficient estimates are influenced by the structure of scar tissue. To explore the sources for this contrast, simulation of the optical properties was performed, which indicated that the scars’ abnormal water content might play an important role. We believe that this finding could provide helpful inputs into future simulation studies on the optical properties of scar tissue.

Birefringence is the second optical property that we explored for burn scar assessment. We quantified and visualised the scar birefringence by measuring the rate of change of the phase retardation from PS-OCT scans in Chapter 5. The quantification algorithm was adapted specifically for skin, with care taken to reliably quantify the skin regions with low birefringence. Such a technique could potentially be utilised by other applications in dermatology. The observed characteristic higher birefringence of the in vivo scars, in comparison to normal skin, agrees with the higher degree of parallel orientation of the collagen proposed in the literature [42]. We identified a correspondence of birefringence with scar type, suggesting that birefringence may be indicative of scar type and has promise as a clinically viable scar assessment means.

The clinical monitoring of skin tissue was investigated in Chapter 6 for two applications. The first application extended our blood vessel assessment method in
Chapter 3 to the longitudinal monitoring of scars following fractional laser treatment using a CO₂ laser. One challenge of OCT for longitudinal assessment of tissue is the small field of view, making it challenging to accurately scan the same tissue at multiple time points. Failure to scan the same area may introduce bias due to the tissue heterogeneity. To overcome this challenge, we developed a clinical protocol to successfully track the same scar location. We imaged and quantified the scar vasculature longitudinally to evaluate the short-term and long-term changes caused by fractional CO₂ laser treatment. Although the use of CO₂ laser fractional treatment of scarring is becoming more common, there is a lack of understanding of the scar vascular responses to treatment. The results showed occlusion of individual vessels in the short term and the different long-term responses to treatment of the immature (a decrease in vessel area density) and mature scars (an increase in vessel area density). The reported vascular changes in Chapter 6 contribute to a better understanding of this treatment. The second application used an OCT-based optical palpation technique to image scars, based on their mechanical stiffness. We developed a method utilising a handheld imaging probe to perform in vivo imaging and quantification of the surface stress of scar tissue with OCT. An en face map of stress at the scar surface was generated from the tactile imaging with OCT optical palpation, showing higher stress than in the adjacent normal skin. In comparison to the scoring of the scar’s pliability by VSS (i.e., normal, supple, yielding, firm, ropes and contracture), our method provides an objective quantification with the spatial differentiation of the scars’ mechanical properties. This capability could aid clinicians not only in assessment of the scar mechanical properties, but also in the delineation of scar boundaries for assessing the total body surface area of scarring.

### 7.2 Study limitations and future work

The research presented in this thesis developed objective, quantitative OCT scar assessment methods to assess the vasculature and collagen in scar tissue, with encouraging results. However, before the translation of this method to clinical usage, several limitations still need to be addressed. In this section, we list several key limitations, suggest possible solutions, and recommend future work.

**OCT imaging**

OCT is a promising modality for imaging scars, with a relatively suitable trade-off between the imaging resolution and depth. However, it has several technical limitations for scar assessment:
(1) OCT has a small imaging depth, which only allows the visualisation and assessment of the tissue up to ~1 mm into skin.

(2) OCT FOV is commonly smaller (≤ 10 × 10 mm) than the whole area of the scar.

(3) In the OCT system utilised in this thesis, OCT data acquisition typically takes 1-4 min for a C-scan. While tolerable for patients, such lengthy acquisitions mean the scans will be subject to motion artefacts.

The small imaging depth and FOV may reduce how representative the scanned area is of the entire scar region, due to the tissue heterogeneity in the scar. To mitigate these effects, the imaging depth may be able to be extended using techniques such as the extended-focus OCT through Bessel beam illumination [23] and multi-beam OCT [116], although such improvements based on present knowledge are expected to be incremental. The FOV can be improved by acquiring multiple C-scans from the adjacent skin areas and stitching them together to form a large effective FOV, which has been recently shown to be highly feasible by colleagues in our laboratory developing similar approaches based on elastography, up to 50 mm scans.

Motion artefacts are a major barrier to the successful implementation of our method for clinical application. We identified that motion artefact increased with increasing scan time, with the patients finding it increasingly difficult to remain stationary during the entire scan. There is large body of work on improving the imaging speed of OCT [91-93], and these techniques could be readily applied to shorten the data acquisition time to reduce the motion artefact for future work.

Vasculature imaging

The vasculature imaging and quantification method based on the analysis OCT speckle decorrelation shows promise as a means to assess scar vascularity. There are, however, several limitations associated with this method:

(1) The speckle decorrelation technique is subject to significant shadowing artefact, leading to the reliable visualisation and quantification of only the 2-D en face projection of the vasculature.

(2) This method is only capable of visualising the structure of the vasculature, not quantifying the blood flow velocities.

(3) The OCT imaging interface is based on contacting the skin surface, which may induce changes to the vasculature by the contact pressure.
(4) The current implementation of the data post-processing of the collected OCT scans to image the vasculature is relatively time-consuming (~1-2 hours using a MATLAB implementation for a single C-scan).

(5) There is a lack of a validation study of this method by comparison with other alternative techniques.

The shadowing artefact in the speckle decorrelation method manifests as high decorrelation in the tissue regions beneath a vessel. For this reason, the projection of the vasculature for visualisation is usually taken along the depth direction to reduce the impact of this artefact. Our quantification of the vessel diameter and area density was based on such 2-D projection images of the vasculature. The quantified results mainly represent the vessels in the lateral plane within a certain depth range, neglecting the depth spread of these vessels, which may lead to unreliable assessment, depending on the organisation of the vessels. One possible solution to address this limitation is to perform the quantification of the vasculature in the 3-D image by, first suppressing the shadowing artefact [7] and segmenting the 3-D vasculature, and then extending our quantification techniques to a 3-D geometry.

The blood flow velocity is an important physiological parameter of biological tissue and may be helpful for understanding pathological processes in scar tissue. In contrast to Doppler OCT techniques, the blood flow velocity is not directly accessible with the speckle decorrelation method. However, recent work by Uribe-Patarroyo et al. demonstrated the feasibility of extracting the flow velocities from the OCT speckle decorrelation [127]. Their method has the potential to be adapted and added to our proposed scar vasculature imaging method to investigate the properties of the scar blood flow velocity.

The scanning mode of OCT imaging in our scar assessment, involving direct contact with the skin surface, was designed to optimise the imaging quality by reducing both imaging and motion artefacts. When the scanning was performed, care was taken to apply a similar amount of pressure on the skin, especially during the longitudinal studies. However, there is possible bias in the vasculature data caused by the variation in this pressure. Choi et al. applied external pressure to the nailfold region and observed a decrease of the skin vascular perfusion in response [159]. This suggests that the impact of pressure should be further considered. In future work, developing a sensor to measure the contact pressure and correct its impact on the vasculature could further enhance the reliability of our vasculature assessment.
The data processing codes for imaging and quantifying scar vasculature were developed in MATLAB® (vR2012a, MathWorks, Inc., USA). The time required for data processing becomes a limitation when processing a large number of C-scans, as required for the longitudinal studies. Optimising the efficiency of the computation with MATLAB or re-coding the data processing method using a different programming language, such as C++, should reduce the current limiting computation time.

In addition, the speckle decorrelation method has been widely applied for various areas, but the thorough validation of this method is still lacking. Comparison with other vasculature imaging modalities by imaging the same subject is still needed to understand the performance of the speckle decorrelation method for assessing blood vessels.

**Attenuation coefficient and birefringence imaging**

The parametric imaging of optical properties of scar tissue provides a means to quantify tissue composition, complementing the assessment of the vasculature. The encouraging results presented in this thesis demonstrated the feasibility of this method for scar assessment. However, several limitations remain with this method, including:

1. The vascular masking technique does not take the vessel depth into account and tends to overestimate the regions for masking.
2. The attenuation coefficient imaging techniques are subject to errors caused by the presence of tissue heterogeneity, primarily in the axial direction.
3. The birefringence imaging method has not been thoroughly validated and our PS-OCT system does not provide the full polarisation information.

The vascular mask for removing the vasculature-induced artefact in the quantified optical properties was generated by thresholding the speckle decorrelation MIP image. This approach may remove additional (non-vascular) regions since some vessels are located completely below the depth window for calculating the optical properties and will not lead to artefact. As our method is not capable of detecting the exact depths of the vessels, we chose to remove all the vascular regions. Future work could encompass the development of a method to perform the 3-D segmentation of the vasculature network and incorporate the vessel depth into the masking to mitigate this limitation.

The single-scattering model, integral to the method used to calculate the attenuation coefficient, assumes homogeneity of the tissue within a small spatial window used for computations, typically a few hundred µm in the axial direction. This
assumption is invalidated at the boundary of tissue types and anatomical layers. The vascular masking method removes one significant source of heterogeneity. However, there are remaining sources of heterogeneity caused by other skin structures, such as hair follicles, sweat glands and sebaceous glands, which can lead to artefacts in the attenuation coefficient. The removal of such remaining artefacts can be realised by segmenting these structures with imaging processing techniques [160] and masking them from the scans. Alternatively, a more intelligent way for choosing the depth for calculation could be developed to confine the calculation to homogeneous regions.

The OCT scanner for assessing scar birefringence was a fibre-based PS-OCT system with a single polarisation state for imaging, which presents additional challenges for reliable quantification of birefringence. Optimisation of the polarisation state of the imaging light is necessary for reliable birefringence measurements. Due to the current technical difficulty in manufacturing a phantom with precise birefringence, we were unable to perform a quantitative calibration of the polarisation state. Instead, we used a plastic phantom to validate the polarisation state qualitatively by maximising the dynamic range of the phase retardation signal, which may result in errors in the measured birefringence. Future work could be performed with a PS-OCT system with improved performance, one which incorporates multiple polarisation states [156], for more reliable measurement and to provide more polarisation information, such as the orientation of the optic axis.

Building on from the work presented here, we would suggest that the assessment of the scar’s mechanical properties should be further investigated. Although optical palpation based on OCT shows the contrast of the scars’ mechanical properties, it remains a qualitative method, so a more robust method is still required. A method based on optical coherence elastography [145], which is a variant of OCT for measuring tissue mechanical properties, is currently being developed in our research group for reliable quantification of the scar mechanical properties. It is intended to augment the qualitative description of the scar pliability, such as that used by VSS, with absolute quantities.

Finally, scar OCT scans were acquired using different systems in this thesis with different data formats, impeding the extraction of three OCT assessment parameters for the same scar. Future studies would naturally seek to combine the assessment of the vasculature and the optical properties for each scar to explore the integration of these parameters into a unified assessment system. More scars subjects are needed to further demonstrate the reliability of the objective, quantitative OCT scar assessment methods. A comparison of the assessed results with other scar assessment methods, such as the
VSS, is also a necessary step in evaluating the performance of the OCT scar assessment methods reported here.

7.3 Summary of contributions

In summary, the novel contributions of this thesis include:

(1) Demonstration of the first clinical assessment of scar vasculature integrating methods to mitigate motion artefact. Scars were automatically imaged and quantified by measuring vessel diameter and area density to show the prolific larger vessels in hypertrophic scars as compared with normal skin.

(2) Development and application of a parametric attenuation coefficient imaging method for objective, quantitative scar assessment. Vascular masking was incorporated into this method, thereby improving the reliability of parametric imaging. Clinical assessment of scars demonstrated lower scar attenuation coefficient values than for normal skin.

(3) Development and application of a parametric birefringence imaging method for objective, quantitative scar assessment. We demonstrated strong correspondence of the magnitude of the birefringence with scar type, with hypertrophic scars showing a much higher birefringence than normal skin.

(4) Application of the vasculature assessment for longitudinal investigation of fractional CO\textsubscript{2} laser treatment of burn scars. We demonstrated acute responses, such as the occlusion of vessels, and different long-term responses of immature and mature scars, with a decrease and increase of vessel area density, respectively.

(5) Demonstration of OCT-based optical palpation for the assessment of a scar’s mechanical stiffness. We applied this method to scars and demonstrated the higher stress in scar tissue than in the adjacent normal skin.

7.4 Final remarks

The assessment of scars is an important aspect of the treatment of scarring, providing the important element of feedback to guide treatment over time. The research in this thesis is dedicated to the development of objective, quantitative scar assessment methods based on OCT. The presented methods provide a quantitative assessment of the significant vasculature and collagen network in scars with encouraging initial results. With further improvement, such objective, quantitative methods may provide clinicians with more reliable assessments of scars and their responses to treatment, thereby contributing to better scar management for the benefit of burn scar patients.
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