Developing Terahertz Spectroscopy Techniques for \textit{In Vivo} Skin Evaluation

by

Xuefei Ding

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I want to dedicate this thesis to my grandmother Fengyu Li and my grandfather Yingxuan Xue.

I will always miss you.
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In P552, Physics Department, University of Warwick
Declarations

I hereby declare that this thesis is my original work and contains nothing that is the result of work done in collaboration with others, except as specified in the text and the acknowledgements. This work has not been submitted in full or in part to this or any other university for the award of another degree.

The material in some chapters has been published in the following papers and conference proceedings:

Chapter 4:


Chapter 5:


Chapter 7:

Abstract

Terahertz (THz) light is located between the infrared and the microwave radiation of the electromagnetic spectrum with a frequency range of 0.1-10 THz (1 THz = $10^{12}$ Hz). It is non-ionizing, non-destructive, sensitive to water and provides spectral fingerprints for biological molecules, which make THz spectroscopy techniques suitable to be utilized for biomedical applications.

This thesis focuses on developing THz characterization methods and exploring the potentials of THz time-domain spectroscopy (THz-TDS) for in vivo evaluation of skin. Biomedical samples are often small in volume or thickness, which are considered as thin-film samples and require high characterization sensitivity. Therefore, a sensitive thin-film characterization method is proposed in this thesis based on customising a multilayer structure to enhance the signal contrast induced by the sample. Then our investigations move forward to THz in vivo studies to take advantage of the non-ionizing nature of THz light. We evaluate the effect of transdermal drug delivery patches on skin with in vivo THz-TDS, starting by quantitative analysis on the normalised relative change in the THz response. Then with a skin modelling approach based on the stratified media model, the dynamic hydration profile of skin is extracted from the THz signal. The final study is based on a large-scale THz in vivo study that measured over 300 participants on their volar forearms with a portable THz handheld scanner in an out-of-the-lab environment. With data collected from the diverse subject group, we investigate the variation of subjects’ skin hydration profiles in relation to their biophysical factors and lifestyles.
Acronyms

ANOVA  Analysis of Variance.
ATR  Attenuated Total Reflection.
EMT  Effective Medium Theory.
FFT  Fast Fourier Transform.
Glu-Sols  Glucose Solutions.
LLL  Landau-Lifshitz-Looyenga.
MRI  Magnetic Resonance Imaging.
NMR  Nuclear Magnetic Resonance.
NRC  Normalised Relative Change.
OCT  Optical Coherence Tomography.
P2P  Peak-to-Peak.
PCAs  Photoconductive Antennas.
PET  polyethylene terephthalate.
PG  Propylene Glycol.
PP  Polypropylene.
RC  Relative Change.
SC  Stratum Corneum.
SEM  Standard Error of the Mean.
SNR  Signal-to-Noise Ratio.
**SRR**  Split-Ring Resonator.

**TDD**  Transdermal Drug Delivery.

**TEWL**  Transepidermal Water Loss.

**TF**  Thin-Film.

**THz**  Terahertz.

**THz-TDS**  Terahertz Time-Domain Spectroscopy.
List of Publications

Peer Reviewed Journals:

1. X. Ding, A. I. Hernandez-Serrano, J. Young and E. Pickwell-MacPherson, "Variation of Skin Hydration Profile with Biophysical Factors and Life Styles Revealed by In Vivo THz-TDS," (In Submission).


Conference Presentations and Proceedings:


Chapter 1

Introduction

1.1 THz Light and Spectroscopy

1.1.1 THz Light

Terahertz (THz) light refers to the electromagnetic radiation that locates in the frequency range of 0.1-10 THz, where 1 THz equals to $10^{12}$ Hz, and has a wavelength range of 3 mm to 30 µm. As shown in Figure 1.1, THz light is sandwiched between the microwaves and the infrared on the electromagnetic spectrum, which makes it an intermediate spectral regime bridging the electronics and the photonics fields. THz regime overlaps with the frequency range of low-frequency vibrations, the crystalline lattice vibrations, molecular rotations and the hydrogen-bond interactions, providing it with several advantageous features including spectral fingerprints for the identification of chemicals and biological molecules, and the strong sensitivity and absorption to water molecules. Its location on the electromagnetic spectrum also indicates that THz light has low photon energy which is well below the ionization limit. Other advantages of THz techniques include the capability to penetrate non-conducting materials such as plastics, a high signal-to-noise ratio ($> 60$ dB [1]) and good spatial resolution (typical spatial resolution of 250 µm laterally and 20 µm axially [2]), and the ability to acquire time-domain signal information.

The THz spectrum has not been widely explored and utilized until the late 20th century, due to the technological limitations in the generation and detection of THz light. In the 1960s Auston invented the Auston switch, a photoconductive switch operated with ultrafast laser pulses, which can be used to generate and detect THz pulses [3]. Then in the 1980s, Grischkowsky and
his colleagues first introduced THz time-domain spectroscopy (THz-TDS) [4]. Since then, along with the rapid development in THz generation and detection technology, the potential applications of THz light have been explored through different areas of study.

In the subsequent sections of this chapter, fundamental knowledge of the technology behind THz generation and detection will be explained in Section 1.1.2, followed by an introduction of the THz time-domain spectroscopy in Section 1.1.3, which is the main THz technique used in the studies of this thesis. Section 1.1.4 will briefly overview some of the main applications of THz techniques. Then Section 1.2 and Section 1.3 will review the state-of-the-art studies in the two specific topics this thesis mainly concerns- the characterization of thin-film samples and the \textit{in vivo} assessment of skin. Finally, Section 1.4 will present an overview of this thesis, including how it is structured and the content of each chapter.

1.1.2 THz Generation and Detection

Photoconductive switching is one of the most commonly used methods for the generation and detection of THz pulses. A photoconductive antenna typically consists of a semiconductor thin film (e.g. GaAs) with a metal dipole antenna patterned on it. As shown in Figure 1.2(a), for THz generation, a femtosecond (fs) laser pulse with frequency adapted to the band gap of the semiconductor is focused on the semiconductor substrate to excite electrons across the band gap and generate free charge carriers. The induced carriers are accelerated by the DC bias voltage added on the metal electrodes and form a short current pulse, and will then be recombined into the semiconductor band-structure on a picosecond (ps) timescale [5]. This transient photocurrent produces a THz electric field $E_{\text{THz}}$ proportional to the rate of acceleration of the photocurrent.
\[ I_{PC}: \]

\[ E_{THz} \propto \frac{dI_{PC}}{dt} \]  \hspace{1cm} (1.1)

The mechanism of THz detection is similar to that of THz generation. It can be seen from Figure 1.2(b) that the semiconductor substrate generates free charge carriers when excited by a femtosecond laser probe pulse. Instead of adding external DC bias, the received THz pulse \( E_{THz} \) accelerates the free carriers inducing a photocurrent \( J(t) \), which is related to the intensity of the received THz pulse through Equation 1.2. The induced photocurrent is then amplified and recorded as a digital output.

\[ J(t) = \int_{-\infty}^{t} E_{THz}(t') \sigma(t - t') dt' \]  \hspace{1cm} (1.2)

Other approaches for the generation of THz pulses include optical rectification, photo-ionization of air and optoelectronic continuous-wave sources; and for THz detection there are also electro-optic sampling, heterodyne receivers and thermal detectors [1]. Since the subsequent studies in this thesis are all based on THz-TDS systems with photoconductive antennas, the alternative techniques will not be introduced here.

Figure 1.2: A diagram showing the generation (a) and the detection (b) of THz pulses using the photoconductive switching technique.
1.1.3 THz Time-domain Spectroscopy

Terahertz time-domain spectroscopy (THz-TDS) is a spectroscopic technique probing the properties of materials using single-cycle THz pulses, where electric field waveforms are measured in the time domain and both the amplitude and the phase information are acquired simultaneously with a sub-picosecond time resolution [6]. Therefore THz-TDS can extract the real and imaginary parts of the dielectric and optical properties of the material without the use of the Kramers-Kronig relations. THz-TDS was first proposed by Martin van Exter at al. in 1989 to measure absorption lines of water vapor in the range of 0.2-1.45 THz [4]. Since then THz-TDS systems have been developed and commercialized, making THz spectroscopy more accessible to a wide range of applications.

Figure 1.3 shows the basic setups for THz-TDS in reflection geometry and transmission geometry. For both geometries, the key components include a femtosecond pulsed laser, a photoconductive THz emitter and detector, a delay stage, a beam splitter and some optical lenses.

For the reflection THz-TDS system, the femtosecond laser pulse is separated into two paths by the beam splitter, with one directed to the photoconductive emitter to excite free carriers in the semiconductor substrate for the generation of THz pulses, and the other one directed to the photoconductive detector via a delay stage to induce photocurrent from the detected THz electric field. The delay stage is composed of a set of mirrors that can be mechanically shifted to change the optical path difference and therefore change the arrival time of the pumping and the probing pulses at the detector, in which way the THz pulse can be measured at different optical delay times allowing a time-domain signal to be acquired. The mechanisms behind the photoconductive antennas used in the THz emitter and detector were explained in the previous Section 1.1.2. In reflection geometry, a quartz imaging window is normally used to position the sample in alignment with the THz beam, as shown in Figure 1.3(a). THz light is incident on the window with an angle of refraction, reflected from the air-window surface and the window-sample surface, and then received by the detector. More details about THz light propagation in the reflection geometry will be introduced in Section 2.4.

The transmission setup of THz-TDS, as shown in Figure 1.3(b), is similar to the reflection setup except the THz emitter and detector are aligned linearly and the sample is placed perpendicularly to the propagation direction of the THz.
Figure 1.3: Schematic diagrams of the THz time-domain spectroscopy set up in (a) reflection geometry and (b) transmission geometry.
light. In this geometry, the detector collects the THz signal transmitted through the sample, which requires the sample to be thinner than the THz penetration depth. More details about THz light propagation in the transmission geometry will be introduced in Section 2.3.

1.1.4 Applications of THz Techniques

THz spectroscopy and imaging techniques can extract information in both the time domain and frequency domain. The advantageous features of THz light, including the fact that it is non-ionizing, non-destructive, strongly absorptive by water and has the capability of providing spectral fingerprints, make it suitable for a wide range of application areas, as shown in Figure 1.4. In this section, a few representative applications will be introduced.

Figure 1.4: A summary showing some of the potential applications of THz light.

With the emission and absorption lines from the excited molecules in star-forming clouds lying mostly in the THz regime, THz light can be used in astronomy to study the chemical composition and physical conditions of the observed cosmic region [7]. It has also shown potential for security scanning on
human bodies given the non-ionizing nature of THz light. Researchers have demonstrated the ability of THz light to identify hidden weapons and the residues of explosives [8]. For wireless communications, the commercially used carrier frequencies have been using the lower frequency range of the THz region to achieve higher data transfer rates, increase physical security and reduce electromagnetic interference [9].

THz spectroscopic and imaging techniques have been extensively used for non-destructive testing in applications including material characterization and quality control and applied to a range of areas including the food production, polymer manufacturing, automotive and arts industries [10]. For food inspection, THz spectroscopy has been proven the capability of detecting potential contamination within the food matrix such as harmful compounds, antibiotics and micro-organisms, as well as assessing the moisture content in food for quality control [11]. THz-TDS is also a useful complementary evaluation method for the monitoring of polymer compounding processes and the detection of micro-scale leaks in plastic packaging materials [5]. With the ability to acquire time-domain signals, THz-TDS can reveal thickness information for samples with multiple layers by evaluating the separation of the pulses with different optical delays. This includes applications for the quality control of tablet coating thickness, the assessment of the consistency of car paint, and the identification of different layers on an ancient oil painting [12–14].

Finally, with low photon energy and sensitivity towards intermolecular interactions, THz spectroscopy and imaging are suitable for biomedical applications including ex vivo examination of excised biological tissues and in vivo assessment on human body. The studies in this thesis focus on the application of THz-TDS for the characterization of thin-film samples and the in vivo sensing on human skin, thus the review on these two specific fields will be elaborated in details in Section 1.2 and 1.3.

1.2 THz Spectroscopy Techniques for Thin-film Sensing

THz spectroscopy is a promising technique in sensing the characteristic features of many materials, because microscopic phenomena including molecular rotations, low-frequency bond vibrations, crystalline phonon vibrations and hydrogen-bonding stretches and torsions take place in the THz frequency band.
Thin-film samples in THz studies refer to those samples whose optical thickness are comparable to some fraction of the THz wavelength or that induce spectral overlapping between the main transmitted or reflected pulse and the internal reflected pulses. THz Thin-film sensing is necessary for situations including: when the sample is limited to a small volume or thickness, such as aqueous biological samples and toxic samples; when the sample is normally processed in a thin-film form, such as excised tissues and functionalized chemical monolayers; when the sample presents different or improved properties in the thin-film form compared to the bulk form. Therefore, thin-film sensing broadens the range of applications and is an important branch of THz sensing [15].

Due to the nature of thin-film samples, challenges arise in THz thin-film sensing. First of all, thin-film samples have very short interaction lengths with THz light, and as a result induce very little modification to the THz light passing through the samples. Minor interaction between the sample and the THz light means that the signal-to-noise ratio (SNR) of the THz system is crucial to successful sensing. However, the inherent instabilities in the femtosecond laser can induce fluctuations in the generated and detected THz pulses. Also, it is difficult to acquire the thickness of thin-film samples precisely, which can affect the extraction of the sample properties. Finally, a practical difficulty is that thin-film samples are tricky to operate during experiments.

In conclusion, thin-film sensing is hard to achieve when the influence of the sample on the interacted THz wave is smaller than the measurement uncertainty of the system caused by noise. The relationship between the measurement uncertainty and the thin film properties can be written as [16]

$$\frac{\omega l}{c} \Delta n(\omega) < \sqrt{\frac{2}{N}} S_{\text{arg}(E_{ref})}(\omega)$$  \hspace{1cm} (1.3)

where $l$ is the sample thickness, $\Delta n(\omega)$ is the difference in the refractive indices between the sample and the reference medium, $N$ is the number of sample measurements, and $S_{\text{arg}(E_{ref})}$ is the standard deviation of the phase of the reference signal.

In other words, a sample is thin when the phase change in the THz pulse transmitted through the sample is smaller than the scaled standard deviation of the reference signal. Therefore, the general strategies for thin-film sensing include either reducing the system noise or improving the signal contrast induced by the sample. Table 1.2 lists a number of studies approaching THz thin-film sensing with different strategies and characterized or detected different thin-film
An effective way to reduce the system noise is to measure the sample and reference signal simultaneously to cancel out the noise. This can be achieved by modifying transmission THz-TDS into THz time-domain interferometry. The mechanism is to induce destructive interference, which cancels uncertainty from laser fluctuations and results in a better SNR. Krishnamurthy et al. applied this method to characterize a free-standing polyester film with 2 µm thickness [17].

More studies focus on researching different ways to improve the signal contrast for sensitive THz thin-film sensing. It can be achieved by combining the thin-film sample with a substrate backing, in which way the reflection from

<table>
<thead>
<tr>
<th>Approach</th>
<th>Scheme</th>
<th>Thickness</th>
<th>Sample</th>
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<tbody>
<tr>
<td>THz interferometry</td>
<td>Characterization</td>
<td>2 µm</td>
<td>Polyester [16]</td>
</tr>
<tr>
<td>Substrate backing</td>
<td>Characterization</td>
<td>15 µm</td>
<td>Semiconductor layer [17]</td>
</tr>
<tr>
<td>Waveguides</td>
<td>Characterization</td>
<td>20 nm [18]</td>
<td>Water [18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 µm [19]</td>
<td>Explosive solids [19]</td>
</tr>
<tr>
<td>Surface waves</td>
<td>Detection</td>
<td>3.8 µm, 9 µm</td>
<td>Cyclotene [20]</td>
</tr>
<tr>
<td>Filters</td>
<td>Detection</td>
<td>40 – 80 nm</td>
<td>DNA samples [21]</td>
</tr>
<tr>
<td>Resonant cavities</td>
<td>Detection [22, 24]</td>
<td>10 nm [22]</td>
<td>DNA samples [22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 µm [24]</td>
<td>Breast cancer cells [24]</td>
</tr>
<tr>
<td>Metamaterials</td>
<td>Detection</td>
<td>50 nm [27]</td>
<td>Silicon nanosphere [27]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 nm [28]</td>
<td>Water, ethanol, CHCl3 [28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 µm [29]</td>
<td>IPA, BSA solutions [29]</td>
</tr>
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</table>

Table 1.1: A summary of studies exploring THz thin-film sensing.
the sample-substrate interface interferes with the reflection from the air-sample interface and therefore enhances the difference between the sample and reference THz signal. Hashimshony et al. utilized the substrate-backing method to measure the dielectric properties and thicknesses of thin semiconductor epitaxy layers with reflection THz-TDS. Epitaxy layers with thickness down to 15 µm were characterized [18].

Another strategy is based on confining the interaction field to two-dimensions by manipulating the THz wave to propagate along the thin-film sample instead of perpendicular to the film, in order to increase the wave-sample interaction length. This strategy is normally realized through waveguides or surface waves. Zhang et al. used a parallel-plate metal waveguide in reflection THz-TDS to measure the refractive index and absorption coefficient of thin-film water with 20 nm thickness [19]. Melinger et al. applied a parallel-plate metal waveguide to acquire the THz vibrational spectrum of explosive solids in polycrystalline thin-film form [20]. Saxler et al. proposed a method of adhering the thin-film dielectric samples to a gold plated surface and distinguished cyclotene films with thicknesses of 3.8 µm and 9 µm [21].

The interaction field can also be confined to be strong and localized at a small area with the use of resonant structures, where the presence of a foreign material will induce observable changes in the field. Nagel et al. used filter-loaded transmission lines in THz label-free sensing of DNA samples with only a small amount of 1.1 femtomol in a 40-80 nm thin-film solution [22]. Later on, the same group demonstrated the sensing sensitivity of a Bragg-structured resonant chip and sensed DNA sample down to 10 nm thick [23]. A metallic-grating resonator with the benefits of a simple structure that was easy to manufacture was proposed and utilized to measure the refractive indices of dielectric liquid samples including hexane, octane and decane in 10 µm thin films [24]. A more simple and cost-effective type of resonant cavity is silicon based microfluidic cell, which has been used for transmission THz-TDS in characterizing aqueous alcohol solutions with thickness of 50 µm [25] and detecting the viability of human breast cancer cells in a rapid, label-free and non-destructive manner [26].

A metamaterial sensor is formed with a matrix of sub-wavelength resonators on a substrate surface, and the frequency response can be tailored by changing the shape and/or dimensions of the resonators. It can be easily made resonant at THz frequencies and probed with conventional THz-TDS without changes to the system configuration. The most common type of metamaterial sensor
is the split-ring resonator (SRR), which was first proposed by Pendry et al. in 1999 [27]. As an early demonstration of utilizing SRR for THz thin-film sensing, Driscoll et al. sensed a silicon nanosphere with a diameter of 50 nm with a SRR sensor [28]. Then Sun et al. detected 50 nm thin liquid layers (double-distilled water, ethanol, and CHCl3) in reflection THz-TDS with a SRR, and redshifts in the resonant spectra were observed [29]. Later on, Zhang et al. proposed a multi-microfluidic-channel metamaterial biosensor with bow-tie shaped resonators and distinguished 15 µm isopropyl-alcohol–water mixtures and bovine serum albumin solutions with different concentrations [30].

1.3 THz Spectroscopy Techniques for In Vivo Assessment

As mentioned in previous sections, THz light shows potential to be utilized for biomedical applications owing to its advantageous characteristics. It has the capability of biomolecule identification for offering unique spectral fingerprints. The strong absorption of THz wave by water allows it for sensitive mapping of the water difference in soft tissues. The low photon energy means that THz light is non-invasive and non-ionizing, making it a safe medical imaging method to be used in vivo on human bodies [31].

Many studies exploring the biomedical applications of THz light are conducted ex vivo, which means that the biological tissues are removed from the living organisms for examination. This type of study can be performed in transmission THz-TDS if the excised tissues are thin enough, which will make signal processing easier. Ex vivo measurements also have higher repeatability with variables more easily controlled compared to measuring living subjects and they can be applied in some experiments that might not be ethically approved for testing on living subjects (e.g. the evaluation of transdermal drug delivery with active drugs involved [32]). Researchers have applied THz-TDS and THz imaging techniques on ex vivo studies of basal cell carcinoma [33, 34], colonic tissue [35], non-melanoma skin cancer [36], brain glioma tissue [37, 38], corneal tissue [39, 40] and breast cancer [41].

However, the necessary pretreatment and preservation procedures for ex vivo samples, such as dehydration and freezing of samples can change the biological structures of the samples. Also, the excised tissues may not be able to represent accurately the behaviours of the living tissues. Finally, removing the tissues
from human bodies makes THz examination an invasive method. Therefore, many researchers are thus interested in investigating in vivo THz studies.

<table>
<thead>
<tr>
<th>Application</th>
<th>Measurement</th>
<th>Subject</th>
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<tbody>
<tr>
<td>Skin burn</td>
<td>THz imaging [40]</td>
<td>Rat [40]</td>
</tr>
<tr>
<td></td>
<td>THz handheld scanner [41, 42]</td>
<td>Porcine [41, 42]</td>
</tr>
<tr>
<td>Cornea</td>
<td>Reflection THz-TDS [44]</td>
<td>Rabbit [43, 44]</td>
</tr>
<tr>
<td></td>
<td>THz imaging [43, 45]</td>
<td>Porcine [45]</td>
</tr>
<tr>
<td>Brain glioma</td>
<td>THz imaging</td>
<td>Mouse [46]</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>THz imaging</td>
<td>Human [32]</td>
</tr>
<tr>
<td>Pigmentary skin nevi</td>
<td>Reflection THz-TDS</td>
<td>Human [47]</td>
</tr>
<tr>
<td>Diabetic foot syndrome</td>
<td>THz imaging</td>
<td>Human [48, 49]</td>
</tr>
<tr>
<td>Scars</td>
<td>THz imaging</td>
<td>Human [50]</td>
</tr>
<tr>
<td>Variables affecting THz</td>
<td>Reflection THz-TDS [51-53]</td>
<td>Human [51-53]</td>
</tr>
<tr>
<td>in vivo measurements</td>
<td>THz imaging [51, 52]</td>
<td></td>
</tr>
<tr>
<td>Skin hydration</td>
<td>Reflection THz-TDS [55-57, 59]</td>
<td>Human [54-59]</td>
</tr>
<tr>
<td></td>
<td>THz imaging [54, 58]</td>
<td></td>
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Table 1.2: A summary of in vivo THz studies for biomedical applications.

Most THz in vivo studies take advantage of THz light’s strong sensitivity to water as a contrast mechanism to distinguish tissues with different hydration levels. This mechanism can be applied to several biomedical applications where the diseased or injured tissues possess different water content from the healthy tissues. Table 1.2 presents a summary of some of the main biomedical applications studied in vivo with THz techniques.
Evaluation of the severity of burn injuries during the early post-injury period is critical for deciding the subsequent treatment and monitoring the healing progress. Tewari et al. performed the first in vivo THz imaging study on burn wound assessment in a live rat model. High resolution THz images of deep and partial thickness burns were acquired and showed the formation and disappearance process of edema in and around the burn wound [42]. Then Osman et al. assessed the severity level of burn injuries in vivo on a porcine model, which is the closest animal model to human skin, with a commercial THz-TDS system in reflection mode mounted on a cantilevered c-arm. Later on the same group developed a THz handheld scanner, which they used to conduct two-dimensional hyperspectral THz imaging on a porcine scald model [43, 44].

Accurate in vivo assessment on corneal hydration change is important for clinical diagnosis of diseases and injuries in the cornea. Taylor et al. published the first THz images of corneas in vivo in a rabbit model. They dehydrated and then hyperhydrated healthy corneas and measured them with THz imaging and millimeter wave reflectometry. Next they spatially and temporally resolved the THz reflectivity maps of the corneas and were able to successfully measure the corneal water content and central corneal thickness [45]. Ke et al. also measured rabbit corneas in vivo with reflection THz-TDS and demonstrated the potential of THz techniques to reveal hydration information from different sublayers in the cornea [46]. Sung et al. proposed a novel THz corneal imaging system, which they used to image a corneal phantom and ex vivo porcine eyes to validate the reliability and sensitivity of the system. Then they performed non-contact imaging on a human cornea, and the acquired results from THz reflectometry well aligned with the predicted results from quasioptical theory [47].

THz examination of skin cancer is mostly conducted ex vivo for a better contrast between the cancerous and the healthy region after some pretreatment process and also controlling extra variables induced by living human subjects. Wallace et al. presented the first demonstration of the ability of THz imaging to detect skin cancer in vivo on human skin with a portable THz imaging system. The in vivo THz imaging of basal cell carcinoma was found to be capable of indicating the tumour’s depth, which was not visible to the naked eye [34]. Wu et al. conducted in vivo THz imaging of brain glioma in a mouse model and distinguished the cancerous region from the healthy region at 2.52THz. The THz images correlated well with the magnetic resonance, visual and hematoxylin and eosin stained images [48]. Zaytsev et al. measured and
analyzed the dielectric permittivity curves of human dysplastic skin *in vivo* with reflection THz-TDS, and significant differences were shown compared to non-dysplastic skin and healthy skin. Their study demonstrated the potential of THz-TDS for non-invasive early diagnosis of dysplastic nevi and melanomas of the skin [49].

*In vivo* THz imaging was also applied for evaluating diabetic foot syndrome and the healing process of scars. Hernandez *et al.* designed a THz imaging platform for mapping skin hydration on the foot soles on diabetic and non-diabetic subjects to examine feet deterioration in diabetic patients [50, 51]. Fan *et al.* monitored the healing process of a scar *in vivo* throughout a six-month period with THz imaging technique. The scar area was clearly distinguished from the surrounding tissues through differences in refractive index [52].

As THz *in vivo* studies for biomedical applications develop, researchers have been investigating the variables which could potentially affect the THz response during *in vivo* measurements, in order to achieve more repeatable and reliable studies with controlled variables. Sun *et al.* demonstrated the impact of occlusion effect on THz responses with reflection THz-TDS and THz imaging on *in vivo* human skin. The occlusion effect is caused by the living skin being in contact with the quartz imaging window, which will lead to water content accumulating to the surface of the skin. Their study showed that even 5 seconds of occlusion has notable influence on the THz response [53]. Then Wang *et al.* demonstrated how pressure between skin and the imaging window affected the THz response and the measured refractive index with reflection THz-TDS and THz imaging [54]. Based on the previous studies, Lindley-Hatcher *et al.* proposed a robust protocol for THz *in vivo* studies on skin. They introduced a pressure sensor to monitor the contact pressure and a way of data processing to account for the natural variations in skin [55].

Finally, skin hydration sensing is the most straightforward application for utilizing *in vivo* THz techniques for clinical purposes. In 2001, Cole *et al.* conducted the first THz *in vivo* study on human skin with THz imaging. They measured THz signals reflected from various sites on the arm and palm and acquired cross-section images showing the interface between the stratum corneum layer and the epidermis layer of skin [56]. Then Pickwell *et al.* measured the volar forearm and the palm skin *in vivo* with reflection THz-TDS and successfully simulated the THz response of skin with a finite difference time-domain model [57]. Zaytsev *et al.* proposed a method for extracting the refractive indices and absorption coefficients of samples measured *in vivo* with
THz-TDS and presented the properties of skin from various regions [58]. Later on, Sun et al. introduced the stratum corneum swelling model to describe the behaviour of skin during occlusion and estimated the water diffusivity in skin over a 20-minute occlusion process measured with reflection THz-TDS [59]. Researchers have also applied THz techniques for in vivo evaluation of skin hydration after the application of skin products. Wang et al. monitored the hydration change in skin induced by silicone gel sheeting, a scar treatment product, with in vivo THz imaging [60]. Lindley-Hatcher et al. tested the impact of different types of skin moisturizer samples (aqueous sample, anhydrous sample and water-oil emulsion) on skin with in vivo THz-TDS and compared the results with corneometer readings, which are the gold standard for quantitative measurement of skin hydration in the cosmetics industry [61].

1.4 Thesis Overview

This thesis focuses on the development of THz spectroscopy techniques for biomedical applications, especially focusing on the in vivo evaluation of human skin.

Chapter 2 introduces the theory that explains the behaviour of THz light propagating through media and the mathematical fundamentals for analyzing THz signals after interaction with samples. Equations are derived for data processing and parameter extraction of THz transmission and reflection measurements of both bulk and thin-film samples. A numerical characterization algorithm and the theoretical models of skin in THz frequencies are also outlined, which will be applied in subsequent studies. Then Chapter 3 introduces the THz systems and the complementary optical systems used in this thesis.

Chapter 4 contains a study of sensitive THz characterization of thin-film samples with a proposed method of customising multilayer structures to enhance the sensitivity compared to conventional THz measurement geometries. These can be utilized for ex vivo THz studies on biological solutions and excised tissues. Then Chapter 5, 6, 7 investigate into in vivo THz studies on human skin. In Chapter 5, a quantitative study is presented to evaluate the impact of transdermal drug delivery patches on the hydration level and occlusion process of skin with in vivo THz-TDS. Then Chapter 6 develops it further by introducing a skin modelling approach to retrieve the dynamic hydration profile of skin after the application of transdermal drug delivery patches. The final study presented in Chapter 7 involves a large scale THz in vivo study.
conducted with a portable THz handheld scanner, and the variation in skin hydration profiles caused by different biophysical factors (sex, age, dominant hand, ethnicity, skin tone) and lifestyles (water intake, coffee consumption, exercise) is investigated.
Chapter 2

Theory

2.1 Introduction

This chapter introduces the theoretical background that the data analysis process of the subsequent studies is based on. Section 2.2 starts by introducing the wave equations and the propagation of THz light at interfaces. Then Section 2.3 and 2.4 elaborate on the data processing procedures for THz transmission measurements and THz reflection measurements, including the cases where the sample is a bulk sample and a thin-film sample. Section 2.5 introduces a numerical characterization algorithm used for characterizing the optical properties of thin-film samples measured with the geometries presented in the previous sections. Finally, Section 2.6 introduces the theoretical models applied in this thesis for modelling the skin for THz in vivo studies.

2.2 Propagation of THz light in Media

THz light follows Maxwell’s electromagnetic theory and when it propagates through a homogeneous, isotropic medium without charges or currents, the electric field $E$ and the magnetic induction $B$ follow Eqs.2.1-2.4, where $\varepsilon$ and $\mu$ are the permittivity and permeability of the medium, respectively.

$$\nabla \cdot E = 0 \quad (2.1)$$

$$\nabla \times E = -\frac{\partial B}{\partial t} \quad (2.2)$$
∇ \cdot \mathbf{B} = 0 \quad (2.3)

∇ \times \mathbf{B} = \mu \varepsilon \frac{\partial \mathbf{E}}{\partial t} \quad (2.4)

The wave equation for THz light propagating in the x-axis direction can then be derived as Equation 2.5 and a solution of that can expressed by Equation 2.6 as a one-dimensional wave.

\[ \frac{\partial^2 \mathbf{E}}{\partial x^2} = \mu \varepsilon \frac{\partial^2 \mathbf{E}}{\partial t^2} \quad (2.5) \]

\[ \mathbf{E}(x, t) = E_0 e^{i(\omega t - Kx)} \quad (2.6) \]

Here \( E_0 \) is the wave amplitude, \( \omega \) is the angular frequency and \( K \) is the wavenumber given by \( K = \omega n / c \). Here the complex refractive index is defined as \( n = n - ik \), where \( n \) is the refractive index which represents the ratio between the wave propagation velocity in vacuum (the speed of light \( c \)) and the propagation velocity in the medium; and \( k \) is the extinction coefficient which describes the absorption of the wave in the medium. The complex refractive index is associated with the complex permittivity as Equation 2.7 since for THz radiation the assumption for the permeability is \( \mu = \mu_0 \) (\( \mu_0 \) is the permeability of vacuum), where \( \varepsilon_0 \) is the permittivity of vacuum and \( \varepsilon_r \) is the complex relative permittivity of the medium.

\[ n^2 = (n - ik)^2 = \varepsilon_r = \frac{\varepsilon}{\varepsilon_0} \quad (2.7) \]

Hence, the solution of the wave equation in Equation 2.6 can be rewritten as Equation 2.8. The absorption coefficient \( \alpha \) describes the attenuation of the wave amplitude in the vertical direction and is related to the extinction coefficient \( k \) through \( \alpha = \frac{2\omega}{c} k \).

\[ \mathbf{E}(x, t) = E_0 e^{-\alpha x} e^{i(\omega t - \omega nx / c)} \quad (2.8) \]

When THz light propagates from one medium to another medium with different optical properties, reflection and transmission will happen at the interface which are determined by the complex refractive indices of both media and the incident angle, as shown in Figure 2.1. The direction of the reflected light follows the law of reflection \( \theta_r = \theta_i \), where \( \theta_r \) and \( \theta_i \) are the angle of
the incident wave and the reflected wave respectively. When THz light enters another media at an oblique angle, the propagation direction will normally change due to the refraction of light. This phenomenon can be described by Snell’s law:

\[ n_i \sin \theta_i = n_t \sin \theta_t \]  

(2.9)

where \( n_i, \theta_i \) are the refractive index and the incident angle at the first medium and \( n_t, \theta_t \) are the refractive index and the angle of the transmitted wave at the second medium.

Figure 2.1: A schematic diagram of wave propagation at the interface of two media with different optical properties.

The polarization state of THz light also needs to be considered for propagation through an interface, which depends on the oscillatory direction of the electric field. The light is p-polarised when the electric field oscillates in the plane of incidence, and it is s-polarised when the electric field oscillates perpendicularly to the plane of incidence. Since the boundary conditions for electromagnetic waves require the \( E \) and \( B \) components parallel to the interface to be continuous at the interface, Fresnel equations can be derived, giving the complex reflection and transmission coefficients for p-polarised and s-polarised light waves:

\[ r_p = \frac{n_t \cos \theta_i - n_i \cos \theta_t}{n_t \cos \theta_i + n_i \cos \theta_t} \]  

(2.10)

\[ t_p = \frac{2n_i \cos \theta_i}{n_t \cos \theta_i + n_i \cos \theta_t} \]  

(2.11)
\[ r_s = \frac{n_i \cos \theta_i - n_t \cos \theta_t}{n_i \cos \theta_i + n_t \cos \theta_t} \]  

(2.12)

\[ t_p = \frac{2n_i \cos \theta_i}{n_i \cos \theta_i + n_t \cos \theta_t} \]  

(2.13)

2.3 Data Processing for THz Transmission Measurements

THz-TDS in a transmission geometry is often used for material characterization and it requires the sample to be either a thin-film sample or not very absorptive of THz light so that the light can penetrate through and be detected at the other side. A sample is considered a bulk sample when its optical thickness is larger than the THz wavelength, in which case the first transmitted pulse can be separated from the others (Figure 2.2(a)); otherwise it is defined as a thin-film sample that often requires to be sandwiched between two transparent solid substrates so that multiple transmitted pulses need to be taken into consideration (Figure 2.2(b)). Highly absorptive materials like water can only be measured in a thin-film fashion for transmission THz-TDS.

![Figure 2.2](image)

Figure 2.2: A schematic diagram of THz wave propagation in (a) a bulk sample and (b) a three-layer system for thin-film sample measurements in THz transmission geometry.

For bulk sample measurements, the THz signal detected without the sample (referred to as the air signal) is taken as the reference. The time-dependent
signals are transformed to the frequency domain through a Fourier transform,
and the ratio between the sample signal $E_{\text{sam}}(\omega)$ and the reference signal
$E_{\text{ref}}(\omega)$ is expressed as

$$M = \frac{E_{\text{sam}}(\omega)}{E_{\text{ref}}(\omega)} = t_{sa}t_{sa}e^{-\frac{\omega}{c}(n_s-n_a)d_s}$$

(2.14)

where the subscripts $a$ and $s$ represent the medium air and sample respectively,
$t_{xy}$ is the complex transmission coefficient from medium $x$ to medium $y$, $n_x$
and $n_x$ are the complex refractive index and the refractive index of medium $x$,
$\alpha_x$ is the absorption coefficient of medium $x$, and $d_x$ is the thickness of medium
$x$. As the phase change induced by the propagation within the sample is much
larger than that at the interfaces, the amplitude and the phase of $M$ can be
written as

$$|M| = t_{sa}t_{sa}e^{-\frac{\alpha_s}{c}d_s}$$

(2.15)

$$\phi_M = -\frac{\omega}{c}(n_s-n_a)d_s$$

(2.16)

and therefore the refractive index and the absorption coefficient of the sample
can be extracted:

$$n_s = 1 - \frac{c}{\omega d_1} \phi_M$$

(2.17)

$$\alpha_s = -\frac{2}{d} \ln \left( \frac{|M|}{4n_s/n_s + 1^2} \right)$$

(2.18)

For measurements of thin-film samples, multiple internal reflections need to
be considered. The reference signal is measured with air as the middle layer
with the same thickness as the sample. Therefore, the sample and the reference
signal can be derived as Equation 2.19 and Equation 2.20.

$$E_{\text{sam}} = E_0(t_{a1}t_{t1}t_{s2}t_{2a}P_1(d_1)P_s(d_s)P_2(d_2))$$
$$+ t_{a1}t_{t1}r_{s2}r_{s1}t_{s2}t_{2a}P_1(d_1)P_s^3(d_s)P_2(d_2) + ...$$
$$= E_0(t_{a1}t_{t1}t_{s2}t_{2a}P_1(d_1)P_s(d_s)P_2(d_2)(1 + r_{s2}r_{s1}P_s^2(d_s) + ...))$$
$$= E_0 \frac{t_{a1}t_{t1}t_{s2}t_{2a}P_1(d_1)P_s(d_s)P_2(d_2)}{1 + r_{s2}r_{s1}P_s^2(d_s)}$$

(2.19)
\[ E_{ref} = E_0 (t_1 a t_1 a t_2 a P_1(d_1) P_a(d_s) P_2(d_2) + t_1 a r_{a2} t_1 a t_2 a P_1(d_1) P_a^2(d_s) P_2(d_2) + ...) \]

\[ = E_0 \frac{t_1 a t_1 a t_2 a P_1(d_1) P_a(d_s) P_2(d_2)}{1 + r_{1a} r_{2a} P_a^2(d_s)} \] (2.20)

where \( E_0 \) is the incident THz signal, subscripts 1 and 2 represent substrate 1 and substrate 2 respectively, \( r_{xy} \) stands for the complex reflection coefficient from medium \( x \) to medium \( y \), and \( P_x(d) = e^{-ik_0 d n_x} \) is defined to describe the propagation of the light through medium \( x \) with a propagation distance of \( d \) and \( k_0 = \frac{2\pi f}{c} \). The other variables and subscripts are the same as previously defined.

The ratio between the sample and the reference signal is given by Equation 2.21. Here the multilayer system is too complicated to be solved analytically as in the bulk sample treatment, therefore an algorithm for numerical characterization is introduced in Section 2.5.

\[ M = \frac{E_{sam}(\omega)}{E_{ref}(\omega)} = \frac{t_1 s t_2 P_s(d_s) [1 + r_{1s} r_{2s} P_s^2(d_s)]}{t_1 a t_2 P_a(d_s) [1 + r_{1a} r_{2a} P_a^2(d_s)]} \] (2.21)

2.4 Data Processing for THz Reflection Measurements

2.4.1 Bulk Sample Measurements

Human skin contains a high concentration of water, which means that it is highly absorptive of THz light. Therefore, for THz in vivo studies of skin, they are normally conducted in a reflection geometry with the skin considered as a bulk sample. A quartz imaging window is placed in contact with the skin for aligning the THz light and flattening the skin surface, in which case two THz reflections will be recorded with one from the lower surface of the window and one from the window-skin interface, as shown in Figure 2.3(a,d). Theoretically, the first reflection should be identical for all reflection measurements when there is no variation from the incident wave and the window position, hence it is used for signal calibration and is referred to as the baseline signal. The reference signal is measured without the presence of the sample, as shown in Figure 2.3(b,e).
Figure 2.3: Schematic diagrams illustrating how to take measurements of (a) the sample signal, (b) the reference signal and (c) the baseline signal for data processing of bulk sample measurements in THz reflection geometry; and examples of (d) the sample signal, (e) the reference signal and (f) the baseline signal from a THz measurement.

Due to the ringing effect enduring after the main pulse, the baseline signal $E_{bl}$ needs to be subtracted from the sample signal $E_{sam}$ and the reference signal $E_{ref}$ to obtain precise results in the characterization of the sample properties [62]. The baseline signal can be measured with an identical quartz window in contact with the imaging window to eliminate the second reflection, as shown in Figure 2.3(c,f) [62], and the measured sample-reference ratio $M_{meas}$ is then calculated by Equation 2.22. Then the processed THz signal (or the impulse function) is acquired by taking the inverse Fourier transform of $M_{meas}$ after filtered with a double Gaussian window function to remove the low and high frequency noise, as given in Equation 2.23 [63]. The purpose of calculating the processed signal is to normalise the sample signal with the reference signal for fair comparison between measurements, and it is an important index used throughout this thesis.

$$M_{meas} = \frac{E_{sam}(\omega)}{E_{ref}(\omega)} = \frac{FFT(E_{sam}(t) - E_{bl}(t))}{FFT(E_{ref}(t) - E_{bl}(t))}$$ (2.22)

$$processed\ signal = iFFT\ (filter \times M_{meas})$$ (2.23)

The theoretical sample-reference ratio $M_{theo}$ for this measurement geometry is the ratio between the reflection coefficient of the quartz-sample interface
and that of the quartz-air interface, which can be given by Fresnel’s equations (Equation 2.24). Snell’s law is applied to reduce the number of unknown variables for analytically extracting the optical properties of the sample (Equation 2.25).

\[
M_{\text{theo}} = \frac{r_q}{r_q} = \frac{n_q \cos \theta_q - n_s \cos \theta_s}{n_q \cos \theta_q + n_s \cos \theta_s} \cdot \frac{n_q \cos \theta_q + n_a \cos \theta_q}{n_q \cos \theta_q - n_a \cos \theta_q} = M_{\text{meas}}
\]  

(2.24)

\[n_a \sin \theta_a = n_q \sin \theta_q = n_s \sin \theta_s\]

(2.25)

where \( n_x \) stands for the complex refractive index of medium \( x \), \( \theta_x \) is the incidence or the refractive angle of THz light in medium \( x \), and the subscripts \( a, q \) and \( s \) represent the air, the quartz window and the sample.

Derived from Equation 2.24 and Equation 2.25, the unknown variables of the sample \( X = n_s \cos \theta_s \) can be represented by the known variables and the measured sample-reference ratio \( M_{\text{meas}} \) as given in Equation 2.26. Then with the application of Snell’s law, the complex refractive index of the sample can be extracted (Equation 2.27), which is associated with other optical and dielectric properties through Equation 2.7.

\[
X = n_s \cos \theta_s
\]

\[
= \frac{n_s^2 \cos^2 \theta_q(1 - M_{\text{meas}}) + n_a n_q \cos \theta_q \cos \theta_q(1 + M_{\text{meas}})}{n_q \cos \theta_q(1 + M_{\text{meas}}) + n_a \cos \theta_q(1 - M_{\text{meas}})}
\]

(2.26)

\[
n_s = \sqrt{X^2 + n_q^2 \sin^2 \theta_q}
\]

(2.27)

### 2.4.2 Thin-film Sample Measurements

Similar to the thin-film sample measurement in transmission geometry as introduced in Section 2.3, for reflection measurements, the thin-film sample is often sandwiched between a top substrate and the quartz window, and the multiple internal reflections inside the sample layer need to be considered.
Figure 2.4: A schematic diagram of THz wave propagation in a three-layer system for thin-film sample measurements in THz reflection geometry.

Figure 2.4 presents a schematic diagram of the THz wave propagation in this geometry. The reference signal is measured with air as the middle layer with the same thickness as the sample. The theoretical representation of the sample signal $E_{sam}$ and the reference signal $E_{ref}$ can be derived as

\[
E_{sam} = E_0 t_{aq} t_{qa} P_q^2 (d_q \cos \theta_q) \left[ r_{qs} + t_{qs} t_{st} t_{sa} P_s^2 (d_s \cos \theta_s) + \ldots \right] (2.28)
= E_0 t_{aq} t_{qa} r_{qs} + r_{st} P_s^2 (d_s \cos \theta_s) \left[ 1 + r_{qs} r_{st} P_s^2 (d_s \cos \theta_s) \right]
\]

\[
E_{ref} = E_0 t_{aq} t_{qa} P_q^2 (d_q \cos \theta_q) \left[ r_{qa} + t_{qa} r_{at} t_{aq} P_a^2 (d_s \cos \theta_a) + \ldots \right] (2.29)
= E_0 t_{aq} t_{qa} r_{qa} + r_{at} P_a^2 (d_s \cos \theta_a) \left[ 1 + r_{qa} r_{at} P_a^2 (d_s \cos \theta_a) \right]
\]

where $E_0$ is the incident THz signal, $t_{xy}$ and $r_{xy}$ are the complex transmission coefficient and the complex reflection coefficient from medium $x$ to medium $y$. $d_x$ and $\theta_x$ are the layer thickness and the incident or refraction angle in medium $x$. $P_x(d) = e^{-i k_0 d n_x}$ where $k_0 = \frac{2\pi f}{c}$ describes the phase propagation and amplitude attenuation in medium $x$ with a travelling distance of $d$. The subscripts $a$, $q$, $s$ and $t$ represent the medium air, quartz window, sample and the top substrate, respectively.

The theoretical sample-reference ratio for measuring thin-film samples in THz reflection geometry is then derived as Equation 2.30. Analytical solutions
are difficult to find for Equation 2.30 as well as Equation 2.21 due to the complexity of the optical geometries. Therefore, a numerical characterization algorithm is introduced in the next section for extracting the optical properties of the sample measured in these complex optical geometries.

\[
M = \frac{E_{sam}(\omega)}{E_{ref}(\omega)} = \left[ r_{qs} + r_{st}P_2^2(d_s \cos \theta_s) \right] \left[ 1 + r_{qa}r_{at}P_2^2(d_a \cos \theta_a) \right]^{-1}
\]

(2.30)

2.5 Numerical Characterization Algorithm

As discussed in Section 2.3 and 2.4, when measuring thin-film samples in THz transmission and reflection geometries, three-layer optical systems are established with the thin-film sample sandwiched between two bulk substrates. Due to the complexity of Equation 2.21 and Equation 2.30, the optical properties of three-layer systems can hardly be extracted with analytical methods. Therefore, a numerical characterization algorithm, developed by a previous member of our group Dr Xuequan Chen in [64], is applied in this thesis for searching the optical properties of such complex optical systems.

Figure 2.5 presents a flow chart of the numerical characterization algorithm. Input parameters include the sample-reference ratio, the frequency range, the searching accuracy and the optical structures for measuring the sample signal and the reference signal. \( Ns_1, Ns_2 \) and \( Ns_3 \) represent the complex refractive index of the first, second and third layer respectively of the three-layer system for sample signal measurement; for the sample layer, the complex refractive index is input as a range (e.g. \([1 - 0i, 4 - 3i]\), which is a range that can cover the complex refractive index of most dielectric materials). \( ds_2 \) is the thickness of the middle layer and \( ps \) stands for the polarization state for sample signal measurement. Similarly, \( Nr_1, Nr_2, Nr_3, dr \) and \( pr \) are the parameters defining a three-layer system for the measurement of reference signal. \( rt \) indicates the measurement geometry and \( \theta_i \) is the incident angle. The main part of the algorithm consists of two loops, with the inner loop searching for the refractive index \( (n) \) and the extinction coefficient \( (k) \) at a certain frequency and the outer loop repeating the searching process for all frequency points in the input frequency range.

The searching process is mainly proceeded by finding the best optical properties that make the theoretical sample-reference ratio close to the measured ratio so that the difference between them is smaller than the input accuracy. An
initial 2D matrix of \( n \) and \( k \) is created based on the input range, and when the searching time has not exceeded the maximum searching number, the relative difference between the theoretical and the measured sample-reference ratio is calculated as 

\[
Diff = \left| \frac{E_s}{E_r} - \text{ratio} \right| / |\text{ratio}|
\]

for every point in the matrix and the best result \((n_{\text{best}}, k_{\text{best}})\) is found at the minimum of \( Diff \). The next searching matrix would be either generated around the last \((n_{\text{best}}, k_{\text{best}})\) with a decreased size to achieve better accuracy, or shifted without shrinking and centred at the last \((n_{\text{best}}, k_{\text{best}})\) if the point is found close to the boundary, in which case the last searching matrix may not have covered the global minimum.

The inner loop will repeat the searching process and updating the searching matrix until \( Diff \) is smaller than the input accuracy, and the outer loop will repeat until finishing searching at the last frequency point.

Figure 2.5: A flow chart demonstrating the numerical characterization algorithm for extracting the optical properties of multilayer optical systems.
2.6 Theoretical Models for THz Biomedical Applications

Previous sections introduced the optical models for THz light-matter interactions under some basic measurement geometries and discussed how to extract the optical properties. However, for THz biomedical applications focusing on the measurements of *in vivo* skin or *ex vivo* tissue sections, it would be useful to extract the hydration profile of the sample in addition to the optical properties. Therefore, this section will introduce some commonly used modelling approaches for THz biomedical applications.

### 2.6.1 Dielectric Models

Dielectric relaxation is the relaxation response of a dielectric medium to an external, oscillating electric field, where the permittivity of the medium presents a negative correlation with the frequency. The double Debye model is commonly used to describe the dielectric relaxation response of liquid water with a slow relaxation term and a fast relaxation term, as given in Equation 2.31.

\[
\varepsilon_r(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_2}{1 + i\omega\tau_1} + \frac{\varepsilon_2 - \varepsilon_\infty}{1 + i\omega\tau_2}
\]

where \(\varepsilon_r(\omega)\) is the complex permittivity of water, \(\varepsilon_\infty\) is the limiting permittivity at high frequencies, \(\varepsilon_s\) is the static permittivity at low frequencies and \(\varepsilon_2\) is the transitional permittivity at intermediate frequencies. \(\tau_1\) and \(\tau_2\) are the relaxation time constants of the slow and fast relaxation process during the breaking and reorientation of hydrogen bonds. \(\varepsilon_\infty, \varepsilon_s, \varepsilon_2, \tau_1\) and \(\tau_2\) are the five double Debye parameters, which determine the permittivity of water across desired frequency through the double Debye model. It has also been employed to describe the THz dielectric response of human skin, due to the high water concentration in skin and the strong THz light absorption of water [65, 66]. However, for tissues or skin regions with relatively low water content, the accuracy of this model remains questionable.

Effective medium theory (EMT) is used for the approximation of the effective macroscopic properties of composite material systems. Compared to the double Debye model, EMT models biological tissues into a binary composite system with water content and dry biological background, and calculates the effective permittivity of the whole system based on the permittivity and the volume fraction of each component. The dielectric permittivity of water can be described
with the double Debye model and that of the dry biological background can be approximated by the measured permittivity of dehydrated tissues [67]. Based on different assumptions on the shape of the particles, the volume fraction of the components and the permittivity contrast between components, there are different EMT models. The Bruggeman model (Equation 2.32) is based on spherical approximation of the particles and allows a large contrast between the permittivities of the components, while the Landau-Lifshitz-Looyenga (LLL) model (Equation 2.33) makes no assumption on the particle shape and requires the permittivity contrast to be small [68]. For THz measurement of biological tissues, there is high contrast in permittivity between the water and the biological background and the particles of the background tissues are irregularly shaped. Although the assumptions of the two EMT models are not entirely fulfilled in this scenario, Hernandez-Cardoso et al. have demonstrated empirically that the two models can fit the experimental data adequately and are suitable for modelling the dielectric response of biological tissues in the THz range [69].

\[
\eta_1 \frac{\varepsilon_1 - \varepsilon_{\text{eff}}}{\varepsilon_1 + 2\varepsilon_{\text{eff}}} + \eta_2 \frac{\varepsilon_2 - \varepsilon_{\text{eff}}}{\varepsilon_2 + 2\varepsilon_{\text{eff}}} = 0
\]  

Equation 2.32

\[
\sqrt[3]{\varepsilon_{\text{eff}}} = \eta_1 \sqrt[3]{\varepsilon_1} + \eta_2 \sqrt[3]{\varepsilon_2}
\]  

Equation 2.33

Here \(\varepsilon_1\) and \(\varepsilon_2\) are the dielectric permittivity of the water content and the dry biological background, \(\eta_1\) and \(\eta_2\) are the volume fraction of the two aforementioned components, where \(\eta_1 + \eta_2 = 1\), and \(\varepsilon_{\text{eff}}\) is the effective permittivity of the whole composite system. Therefore, given the permittivity of the water content and the dry biological background, the effective permittivity of the skin system is directly associated with the water concentration.

### 2.6.2 Stratified Media Model

The biological structure of human skin contains three main layers including the stratum corneum (SC), the epidermis and the dermis. Previous studies on the interaction of THz light with skin have treated the skin as a homogeneous semi-infinite single layer for the simplicity of extracting the optical properties [53, 54], or modelled the skin with two homogeneous layers to separate the SC and the epidermis based on the clear difference of water concentration in the two layers [60, 65]. However, results from the in vivo skin measurement with
confocal Raman spectroscopy suggest that the water concentration in skin is depth-dependent, which can be approximated by a parabolic function in the SC layer, a positive linear function in the epidermis layer and a constant value in the dermis layer, as shown in Figure 2.6 [70]. Therefore, simplifying the skin into a single-layer or a two-layer model may affect the accuracy in the extraction of the skin hydration profile, and a stratified media model has been proposed to account for the depth dependency of skin hydration [71].

![Figure 2.6: A diagram showing the water concentration in skin as a function of the depth into the skin. The three skin layers, stratum corneum (SC), epidermis and dermis, are labelled.](image)

In general, this model simulates the interaction of THz light with the skin as a plane wave reflecting from an effective medium that has stratified dielectric permittivity. The continuous change of water concentration in skin is approximated by multiple thin layers, each of which has an effective permittivity related to the water concentration level through the Bruggeman EMT model. Then according to the stratified media theory, when the electromagnetic wave is incident on a stack of thin dielectric layers with an incident angle $\theta$, the wave propagation in each layer can be described with a longitudinal propagation constant $k_m$ and a characteristic impedance $\zeta_{s,p}^m$:

$$k_m = \omega \sqrt{\varepsilon_0 \mu_0} \sqrt{\varepsilon_m - \sin^2 \theta} \quad (2.34)$$
where $\varepsilon_m$ and $\mu_m$ are the effective permittivity and permeability of each layer, $\varepsilon_0$ and $\mu_0$ are the permittivity and permeability constant of vacuum. $\zeta_s^m$ and $\zeta_p^m$ are the characteristic impedance of s-polarised and p-polarised wave respectively.

Then due to the interface conditions of electromagnetic waves that require continuity at interfaces between layers, the wave reflection coefficient of the $m$-th layer is derived as

$$
\Gamma_s^m = \frac{Z_{m+1} - \zeta_s^m e^{-2jk_m z_{m+1}}}{Z_{m+1} + \zeta_s^m} \tag{2.37}
$$

$$
\Gamma_p^m = \frac{\zeta_p^m - Z_{m+1} e^{-2jk_m z_{m+1}}}{\zeta_p^m + Z_{m+1}} \tag{2.38}
$$

where $z_m$ indicates the depth into the stack from the surface. $\Gamma_s^m$ and $\Gamma_p^m$ are the wave reflection coefficient of s-polarised and p-polarised wave respectively. The effective impedance of the whole stack underneath the $m$-th layer is then calculated as

$$
Z_m = \zeta_m \frac{Z_{m+1} + i\zeta_m \tan(k_m t_m)}{\zeta_m + iZ_{m+1} \tan(k_m t_m)} \tag{2.39}
$$

where $t_m$ is the layer thickness. For the bottom layer (the $M$-th layer), the impedance is its own characteristic impedance: $Z_M = \zeta_s^M$. By computing Equation 2.39 in a recursive fashion from the bottom layer to the top, the effective impedance of the entire stack ($Z_1$) can be acquired. The reflection coefficient from the surface of the entire stack is then given by

$$
\Gamma_s^0 = \frac{Z_1 - \zeta_s^0}{Z_1 + \zeta_s^0} \tag{2.40}
$$

$$
\Gamma_p^0 = \frac{\zeta_p^0 - Z_1}{\zeta_p^0 + Z_1} \tag{2.41}
$$

A detailed derivation of the wave reflection coefficient and the effective impedance of each layer in the stratified dielectric stack can be found in [71].
2.7 Summary

This chapter introduced the fundamental theories needed for the processing and analysis of THz data. The optical equations for sample characterization in THz transmission and reflection geometries were derived and a numerical characterization algorithm was introduced for extracting the properties from complex optical structures. Finally, the modelling approaches used for THz in vivo studies in the subsequent chapters were introduced, including double Debye model for modelling the dielectric properties of liquid water, effective medium theory for modelling the effective permittivity of the skin, and stratified media model for modelling the depth-dependent hydration profile of skin.
Chapter 3

Experimental Setups

3.1 Introduction

This chapter introduces the main experimental setups that have been used for the studies included in this thesis. It involves two commercial THz systems (the TeraView TPS spectra 3000 THz spectrometer and the Menlo TERA K15 THz spectrometer), a customised THz handheld scanner and a 3D imaging camera.

3.2 THz Systems

3.2.1 Commercial THz Systems

In Chapter 3, the THz transmission and reflection measurements were conducted on the TPS spectra 3000 THz spectrometer developed by TeraView Ltd. Figure 3.1(a) shows a schematic diagram of the system, with a Ti:sapphire femtosecond laser, a photoconductive emitter and detector, an optical delay unit, a sample chamber, parabolic mirrors and optical lenses, all of which are integrated in a sealed box providing mechanical stability. This system generates THz light in the range of 60 GHz to 3 THz. Measurements can be recorded in either rapid-scan mode or step-scan mode: during rapid scan, THz pulses are recorded at a rate of 30 Hz with a spectral resolution of 32 GHz; during step scan, the rate depends on the resolution and the highest spectral resolution is 7.5 GHz. Figure 3.1(b) shows the photo of the sample chamber inside the system with a transmission module for THz measurements in transmission geometry, and Figure 3.1(c) is the photo of a specular reflectance module installed in the chamber for THz reflection measurements with a fixed incident angle of 45°.
Figure 3.1: (a) A schematic diagram of the TeraView TPS spectra 3000 system. (a) A transmission module installed in the sample chamber inside the system for THz transmission measurements. (b) A specular reflectance module installed to the chamber for THz reflection measurements.

A TERA K15 fibre-coupled THz spectrometer system (Menlo Systems GmbH) is used for \textit{in vivo} THz point-scan measurements in reflection setup and THz imaging measurements in Chapter 4. The system is available for broadband THz spectroscopy with a bandwidth over 6 THz and a dynamic range over 100 dB. The fast delay line offers a scan range up to 1700 ps with a spectral resolution of 0.6 GHz. Figure 3.2(a) shows the system in a free-space reflection setup for THz point-scan measurements, where a fibre-coupled THz emitter and detector, a quartz window and two pressure sensors are included along with the optical lenses. In Figure 3.2(b), the TERA K15 reflection setup is attached to x-y translational stages for THz imaging measurements.
3.2.2 THz Handheld Scanner

A customised THz handheld scanner was developed by a previous member of our group, Dr Arturo Hernandez-Serrano, and was used for more flexible \textit{in vivo} THz measurements in Chapter 5 and 6. The scanner is based on the TeraSmart spectrometer from Menlo Systems GmbH, with a fibre-coupled THz emitter and detector fixed in reflection setup with a 30° incident angle. The TeraSmart spectrometer has the capability of recording signals at an acquisition rate of 15 Hz within a time window of 50 ps. However, an in-house software was built to compute the real-time reflectance, impulse function and optical properties during measurement, which limits the handheld scanner to an acquisition rate of 4 Hz. THz pulses delivered by this handheld scanner are typically in the bandwidth of 0.1 to 4 THz, with a dynamic range of approximately 75 dB and a central frequency at 0.7 THz. As shown in Figure 3.3, the THz handheld scanner is arranged in a compact and portable fashion, with a quartz window ($n = 1.95 - 0.0048i$) which is 2 cm in diameter and two pressure sensors to control the contact pressure on skin during \textit{in vivo} measurements \cite{55}. This portable handheld scanner can be used in \textit{in vivo} THz studies that require measurements on anatomical sites that are inaccessible for conventional THz systems and also for the studies to be conducted in an out-of-the-lab environment \cite{72}. 

![Figure 3.2: (a) The TERA K15 system for THz point-scan measurements in reflection geometry. (b) THz imaging setup with the TERA K15 system attached to translational stages.](image-url)
In order to validate the reliability of the THz handheld scanner, it was used to measure the volar forearm region of one participant in comparison with two other commercial THz systems which are robust but bulky. The results of the extracted refractive indices and absorption coefficients given by the three systems are presented in Figure 3.4, where the error bars represent the standard deviation of the mean of 20 THz scans. The optical properties display good agreement between the three systems and the uncertainty (error bars) is relatively small for the handheld scanner, which is due to the enhanced stability of the setup preventing mechanical deformations when the window is in contact with the skin. More information of the THz handheld scanner can be found in [72].
Figure 3.4: The refractive index (a) and absorption coefficient (b) of the volar forearm of a participant measured with the THz handheld scanner and two commercial systems. The error bars represent the standard deviation of 20 THz scans.

3.3 Complementary Optical Systems

3.3.1 3D Imaging Camera

In Chapter 4, in order to measure the surface roughness change of the skin, a high-precision surfaceCONTROL 3D 3500 snapshot sensor was applied for 3D imaging of the skin surface. Figure 3.5 shows a photo of the device. The sensor is compact with high measurement accuracy and fast data processing. It has an x-y resolution of 40 µm and a vertical resolution of 1 µm, which is defined as the smallest distance between two 3D points at the x-y plane and the vertical direction respectively; at the same time, it can acquire up to 2.2 million 3D points per second [73].
3.3.2 Portable Microscopic Camera

In the out-of-the-lab THz in vivo skin study presented in Chapter 7, a portable microscopic camera was used to take optical images of the skin surface before THz-TDS measurement, in order to record subjects’ skin tone and surface roughness as complementary information, as shown in Figure 3.6. The microscopic camera is Rotek Wireless Digital Microscope, which provides image resolution of $1920 \times 1080$P. The device has a 2.0MP camera with HD colour CMOS sensor, and has the magnification power from 50x to 1000x.
Figure 3.6: A photo of the Rotek Wireless Digital Microscope (a) and it being applied to image skin \textit{in vivo} (b).

3.4 Summary

This chapter introduced the THz systems and other complementary systems that have been used for the research in the thesis. A TeraView TPS spectra 3000 THz spectrometer was used in Chapter 3 for THz point-scan measurements in transmission and reflection geometry. A Menlo TERA K15 THz spectrometer was used in Chapter 4 for THz single-point reflection measurements and THz imaging. A THz handheld scanner was also presented, which was customised specifically for THz \textit{in vivo} studies applied to use in Chapter 5 and 6. A high precision surfaceCONTROL 3D 3500 sensor was used as a complementary technique in Chapter 4 for 3D imaging of the skin surface in order to monitor the change in the surface roughness.
Chapter 4

Customized Multilayer Structure for Sensitive THz Characterization of Thin-film Samples

4.1 Introduction

This chapter focuses on THz ex vivo measurements of thin-film samples and proposes a method of customising tri-layer structures for sensitive characterization of thin-film glucose solutions with small concentration differences. Simulations are conducted based on the theoretical sample-reference ratios of different THz geometries to compare and find out the most sensitive tri-layer structure. Then the proposed structure is utilised for characterizing thin-film glucose solutions with concentrations between 0-20% (with a 5% interval), and results are compared with those measured with the conventional transmission geometry. Experimental results are in line with the simulation results, both demonstrating that the proposed tri-layer structure can enhance the characterization sensitivity of THz-TDS measurements of thin-film glucose solutions.

4.2 THz Thin-film Characterization

Due to the high water-sensitivity and non-ionizing photon energies of THz light, THz-TDS has been widely used in biomedical research [74]. Biomedical samples,
including biological solutions and paraffin-embedded tissues, are often small in volume or thickness leading to high requirements on the characterization sensitivity. This is further compounded by the long THz wavelengths (3 mm - 300 µm), when compared to visible light, because what is an optically thick sample is likely to be considered a thin-film (TF) sample for THz frequencies. In TF samples the sample thickness is comparable or smaller than the wavelength which results in very short interaction length between the sample and electromagnetic wave. For time-domain pulsed measurements there is an overlap between pulses during sample measurement resulting in reduced characterization sensitivity. This problem can be alleviated by either reducing system noise or improving the signal contrast induced by the TF sample [15].

Transmission THz-TDS is normally used to measure solid TF samples, however conductive samples can be difficult to characterise accurately because of the insignificant absorption of THz signal amplitude and the negligible phase shift [75, 76]. Attenuated total reflection (ATR) spectroscopy is an efficient way to measure liquid samples, but it requires the sample thickness to be larger than the penetration depth of the evanescent wave [77, 78]. Metamaterial sensing can be used to trace small concentration changes in liquid TF samples, however the fabrication process is often complex and uneconomical [79, 80]. Some researchers have been trying to utilise microfluidic techniques for TF liquid measurements [30, 81, 82], where the liquid flows through a micro-sized channel whilst being measured by THz waves; and others like Soltani et al. have been researching on manufacturing small-volume liquid sensor based on electromagnetic resonance [24]. Finally, Sun et al. proposed a trilayer structure to enhance the THz characterization sensitivity of aqueous solutions and also paraffin-embedded tissues [83].

4.3 THz-TDS for Glucose Studies

Glucose is an essential monosaccharide in the human body and using THz-TDS to study aqueous glucose solutions has aroused researchers’ interest [84–86]. Furthermore, with the increasing number of diabetics, methods for blood glucose monitoring have been developed, including vibrational spectroscopic techniques like Raman spectroscopy, mid-infrared and near-infrared spectroscopy [87, 88]. In recent years, THz-TDS has been demonstrated to be a powerful tool in measuring diabetic blood plasma ex vivo, and correlations have been seen between the optical properties and blood glucose concentration [89–91]. The
aforementioned research has been conducted in bulk reflection geometry or transmission geometry; the former geometry requires large amount of liquid sample and the latter geometry can hardly detect any subtle concentration changes in TF solutions. To measure blood plasma more sensitively without strong THz absorption from water, Lykina et al. pretreated the human blood plasma samples by lyophilization and pressing the powder into pellets before measuring with transmission THz-TDS, however the pretreatment process can be time-consuming and solid-state plasma pellets may have different features compared with real liquid plasma [92].

To overcome the problems outlined above, this paper proposes an optimised multilayer structure to improve the sensitivity of THz characterization of TF glucose solutions with a reflection measurement geometry. In the multilayer structure, the TF liquid sample is sandwiched between a top substrate and an imaging window with a fixed slot thickness. Theoretical simulations are carried out for structural optimisation, and this study finds the most sensitive multilayer structure customised for our samples. TF glucose solutions with different concentrations are then measured, and this study shows that the signal contrast between different glucose concentrations is enhanced with the proposed structure compared to an ordinary transmission geometry. Our work provides a new method for characterizing TF samples, and results presented here can be used as a reference for future research on THz sensing of blood glucose.

4.4 Methods

4.4.1 Data Processing

When a TF sample is measured in reflection THz-TDS, the sample is placed on an imaging window, and an additional top substrate could be put on top of the sample and thus form a sandwich structure. In this case, unlike the reflection geometry for bulk sample measurements, the multiple internal reflections inside the TF sample must be considered. As illustrated in Figure 4.1, when the THz light is directed onto a TF (Medium 2) there are reflections from both interfaces between the sample and the substrates, therefore the measured reflected and transmitted responses from the structure contain all the reflections from within the TF layer (Fabry Perot reflections) [93, 94].
Figure 4.1: A schematic diagram of the numerical treatment for thin-film sample (medium 2) in reflection geometry, \( d_2 \) is the TF thickness.

Thus, the reflection coefficient of such a tri-layer TF geometry is acquired through the summation of the internal reflections. The theoretical sample-reference ratio of geometries commonly used in THz characterization of TF liquid samples are given [95–97]:

\[
M_{\text{trans}} = \frac{T_{\text{sam}}}{T_{\text{ref}}} = \frac{t_{1-\text{sam}} - 2}{t_{1-\text{air}} - 2} = \frac{t_{1-\text{sam}} t_{0-\text{sam}} - 2 P_{\text{sam}} (d_{\text{sam}}) \left( 1 + r_{1-\text{air}} r_{\text{air}} - 2 P_{\text{air}} (d_{\text{air}}) \right)}{t_{1-\text{air}} t_{0-\text{air}} - 2 P_{\text{air}} (d_{\text{air}}) \left( 1 + r_{1-\text{sam}} r_{\text{sam}} - 2 P_{\text{sam}}^2 (d_{\text{sam}}) \right)}
\]

\[
M_{\text{TF}} = \frac{R_{\text{sam}}}{R_{\text{ref}}} = \frac{r_{1-\text{sam}} - 2}{r_{1-\text{air}} - 2} = \frac{r_{1-\text{sam}} + r_{\text{sam}} - 2 P_{\text{sam}}^2 (d_{\text{sam}} \cos \theta_{\text{sam}})}{(1 + r_{1-\text{sam}} r_{\text{sam}} - 2 P_{\text{sam}}^2 (d_{\text{sam}} \cos \theta_{\text{sam}})) (r_{1-\text{air}} + r_{\text{air}} - 2 P_{\text{air}}^2 (d_{\text{air}} \cos \theta_{\text{air}}))}
\]

\[
M_{\text{br}} = \frac{R_{\text{sam}}}{R_{\text{ref}}} = \frac{r_{1-\text{sam}}}{r_{1-\text{air}}}
\]

where \( M_{\text{trans}} \) is the ratio of the TF liquid measured in transmission geometry, and the sample is sandwiched between two substrates. \( T_{\text{sam}} \) and \( T_{\text{ref}} \) are the
transmitted signals from the sample and reference, respectively, where the reference is taken with air as “Medium 2” in the middle layer. \(d_{sam}\) and \(d_{air}\) are the thicknesses of the middle layer for sample and reference. The written form of \(t_{1-2-3}\) and \(r_{1-2-3}\) represent the transmission and reflection coefficient of a tri layer TF geometry formed by “Medium 1”, “Medium 2” and “Medium 3” sequentially; \(t_{1-2}\) and \(r_{1-2}\) represent the transmission and reflection coefficients from “Medium 1” to “Medium 2”. \(P_2(d_2) = \exp(-i\omega\tilde{n}_2d_2/c)\) is the phase propagation and amplitude attenuation in “Medium 2” for a distance of \(d_2\), and \(\tilde{n}_2 = n_2 - ik_2\) is the complex refractive index. The subscripts “1”, “2”, “sam” and “air” stand for “Medium 1”, “Medium 2”, sample and air respectively. Similarly, \(M_{TF}\) is the ratio of the TF liquid measured in a tri-layer reflection geometry, where the sample is sandwiched by an imaging window as “Medium 1” and a top substrate as “Medium 2". \(R_{sam}\) and \(R_{ref}\) represent the sample and reference signal, and reference would be the reflected signal from this tri-layer system with air as “Medium 2”. \(\theta_{sam}\) and \(\theta_{air}\) are the refractive angles in the middle layer of the sample and air respectively. Finally, \(M_{br}\) is the ratio of bulk sample measured in reflection geometry, where sample would be placed on an imaging window; the reference signal is taken using the bare window.

In THz material characterization, the optical properties of a sample can be extracted by fitting the experimentally measured sample-reference ratio to the theoretically calculated ratio \(M\) (Eqs. 4.1, 4.2, 4.3). When measuring a group of samples with similar optical properties, the values of \(M\) are often close for every sample, leading to systematic errors in the classification of those samples. Therefore, when the measurement geometry for THz-TDS (i.e. transmission, bulk sample reflection, thin-film reflection, etc.) can produce larger variations in the sample-reference ratios of different samples, it has higher sensitivity in measuring or distinguishing such samples. Thus the characterization sensitivity of a geometry can be defined by the relative change rate (RC) of \(M\):

\[
RC_{abs(M)} = \left| \frac{\text{abs}(M) - \text{abs}(M_{0\%})}{\text{abs}(M_{0\%})} \right| \times 100\%
\]

where \(M_{0\%}\) is the base value by definition (i.e., when measuring aqueous solution samples with different concentrations, it can be the theoretical ratio of pure water).
4.4.2 customisation of the Multilayer Structure

Sun et al. proposed a prism-sample-quartz tri-layer sandwich structure and proved that it has higher characterisation sensitivity compared to traditional transmission or reflection geometry by utilising it to characterise aqueous ethanol solutions and paraffin-embedded oral cancer tissue [83]. This study further investigates into this idea by considering the top substrate refractive index and the slot thickness of the sandwich structure as two variables and optimise them to find out the most sensitive structure, in other words it has the largest relative change in $M$ for the samples to be measured. Here, the samples are aqueous glucose solutions (Glu-Sols) with different mass fractions. Simulations are conducted in MATLAB by calculating Equation 4.1, and the theoretical complex refractive indices of the mixtures are estimated by Landau-Lifshitz-Looyenga Effective Medium Theory [98].

The top substrates are categorised into four types according to their refractive index ($n$) compared to that of the imaging window (made of quartz, $n_{\text{win}} = 2.1$), and the ratio is defined as $N = n/n_{\text{win}}$. Therefore, the four categories are when $N < 1$, $N = 1$, $N > 1$ and $N \gg 1$. For simulation purposes, we chose the top substrate to be polypropylene (PP, $n = 1.5$) for the $N < 1$ group, quartz ($n = 2.1$) for the $N = 1$ group, Germanium (Ge, $n = 4$) for the $N > 1$ group, and mirror (approximated as a highly-reflective metal, $n = 300$) for the $N \gg 1$ group. The slot thickness is varied in the range of 25-75 $\mu$m which is below the wavelength of 1 THz. As shown in Figure 4.2(a-b), when the top substrate has a smaller ($N < 1$) or similar ($N \approx 1$) refractive index to the window, the curve of relative change in $\abs(M)$ has a steeper rise as the slot thickness $d$ decreases, and thus the structure is most sensitive when $d = 25\mu$m. The high sensitivity comes from the matching of the impedance of the top and bottom layer, therefore a thinner sample layer produces higher sensitivity. While in Figure 4.2(c-d), when the top substrate has a larger ($N > 1$) or far larger ($N \gg 1$) refractive index, there is an opposite trend as the $\RCabs(M)$ curve grows steeper when the slot thickness increases, and then reaches the highest slope at $d = 75\mu$m. Here the high sensitivity is considered to be related to the addition of pulses reflected from the lower and upper interface of sample, and when the slot thickness increases to a certain value, the overlapping between pulses introduces high contrast in $M$. 

45
Figure 4.2: Simulation of the $R_{\text{abs}(M)}$ for different ‘sandwich’ structures at 0.6 THz: (a) top substrate: polypropylene (PP, $n = 1.5, N < 1$) [99], slot thickness: 25-75 µm; (b) top substrate: quartz ($n = 2.1, N = 1$), slot thickness: 25-75 µm; (c) top substrate: Germanium (Ge, $n = 4, N > 1$) [100], slot thickness: 25-75 µm; (d) top substrate: mirror (assumed to have the same refractive index as highly-reflective metal, $n = 300, N \gg 1$) [101], slot thickness: 25-75 µm.

Altogether, Figure 4.2 shows that the sandwich structure with mirror as the top substrate and 75 µm slot thickness displays the most significant contrast in THz responsivity as the Glu-Sol concentration changes. The new 75 µm window sample-mirror (win-sam-mirr) structure is then compared with the 25 µm prism-sample-quartz (psm-sam-qz) structure proposed in [83]. Figure 4.3 displays the $R_{\text{abs}(M)}$ for different THz measurement geometries at 0.5, 0.6, 0.7 THz, including sandwich structures in reflection geometry: 75 µm window-sample-mirror (refle, win-sam-mirr), 75 µm prism-sample-mirror (refle, psm-sam-mirr), 25 µm window-sample-quartz (refle, win-sam-qz), 25 µm prism-sample-quartz (refle, psm-sam-qz); and ordinary bulk reflection geometry (bulk refle) and transmission geometry with 75 µm thickness (trans). The figure indicates that the 75 µm win-sam-mirr structure has the largest variation in $R_{\text{abs}(M)}$ with gradient approximately 3 times of other geometries at 0.5 THz and 10 times
of others at 0.6 and 0.7 THz. Simulations have also been conducted at other frequency points and the 75 µm win-sam-mirr structure gives better sensitivity in the range of 0.4-0.8 THz.

Figure 4.3: Comparison of the $RC_{\text{abs}(M)}$ for different THz measurement geometries at 0.5, 0.6, 0.7 THz.

4.4.3 Experimental Setup and Protocol

In this study, a TeraView TPS spectra 3000 spectrometer was used for measurements; for reflection geometry, the incident angle is 45°, and the incident THz wave is s-polarised. Figure 4.4 shows the design of the 75 µm win-sam-mirr structure, where the top substrate is a silver-plated reflecting mirror, the bottom substrate is the quartz imaging window from the THz system, and they are spaced by two 75 µm thick Teflon spacers. Glu-Sol samples with mass fraction in the range of 0-20% at 5% intervals ($\Delta = 5\%$) were made from dehydrated D-glucose powder (Fisher Chemical) and distilled water; Glu-Sol concentrations up to 20% were chosen for experiments due to the practicality of sample preparation and storage. Higher glucose concentrations could be used but they are more difficult to prepare and store due to the solubility of D-glucose at room temperature. The technique could also be extended to measure other solutions including protein solutions.

For the experimental procedure of the win-sam-mirr structure, it started with the acquisition of the slot thickness, which is a crucial step for the subsequent properties extraction process. First of all, THz reflection from the window-air interface (win-air) of the bare imaging window was measured as a reference signal (ref1); then spacers, quartz and a weight were stacked sequentially on the bare window to form a window-air-quartz structure (win-air-qz) and the reflected signal from the middle layer was measured as the sample signal (sam1).
By fitting the experimental ratio $R_{sam1}/R_{ref1}$ to the theoretical ratio, the slot thickness could be acquired. The win-air-qz structure instead of the win-air-mirr structure was used for thickness acquisition because the former structure is more sensitive to the change in slot thickness. Here the combination of the spacers and weight is for thickness control, so that no change in the slot thickness is expected during the measurements of different samples.

In the sample measurement process, the top substrate quartz was replaced with the mirror and the reflected signal from the window-air-mirror structure (win-air-mirr) was measured as reference (ref2). Then after taking away the weight and mirror, an appropriate amount of the aqueous sample was dropped on the window, then the mirror and weight were placed on sequentially again, and reflection from the window-sample-mirror layer (win-sam-mirr) was measured as our sample signal (sam2). Optical properties of the sample were then extracted by fitting the experimental ratio $R_{sam2}/R_{ref2}$ to the theoretical ratio as given in Equation 4.2.

Measurements were also conducted in bulk reflection and transmission geometries: for the former geometry, bulk liquid was held within a 2.5 mm-thick polymer ring placed on the imaging window; for the latter geometry, a liquid sample cell with two polymer wafers ($n=1.454$) and a 75 $\mu$m Teflon spacer was used to hold the liquid.

![Figure 4.4: A schematic diagram of the win-sam-mirr structure.](image)
4.5 Results and Discussion

4.5.1 Validation of the Multilayer Structure

Thin-film water was first measured in the proposed win-sam-mirr structure in order to validate the results, the extracted optical properties were compared with those measured in bulk reflection and transmission geometries and also the results from references [102, 103]. As shown in Figure 4.5, the refractive index and absorption coefficient match well with the results from other geometries and references. Some variations between the different literature values are reasonable due to the fact that the measurements were taken under different lab environments. Results from win-sam-mirr and transmission agree very well with the relative difference at each frequency point less than 2%. The error bars on the win-sam-mirr results are the standard deviation from 3 repeat measurements and the narrow span shows good robustness and repeatability.

Figure 4.5: (a) The refractive index and (b) absorption coefficients of water measured with the win-sam-mirr structure and the bulk reflection and transmission geometries, compared to literature results [102, 103].

4.5.2 Characterization of Thin-film Glucose Solutions

Glu-Sol samples with concentrations in the range of 0-20% at 5% intervals were measured with the 75 µm win-sam-mirr structure; and samples with 0, 10% and 20% concentrations were measured with transmission geometry for comparison. Figure 4.6(a-b) show the THz time-domain signals measured with the win-sam-mirr and transmission geometries respectively; where the reference pulses have been multiplied by a factor of 0.3 and 0.5 respectively. In the results from the win-sam-mirr structure, there is an overlap of multiple reflected THz pulses which creates a notable peak distinguishing different Glu-
Sols as shown in Figure 4.6(a). Figure 4.6(c) shows the frequency domain signals of both geometries, which are obtained by performing the fast Fourier transform (FFT) on the time domain signals. A peak is also visible in the win-sam-mirr signals at 0.6-0.7 THz, leading to high contrast in the experimental sample over reference ratios of different Glu-Sols as shown in Figure 4.6(e). The shape of each resonance is related to the refractive index of the liquid sample. In particular, the depth of the resonance is related to the Fresnel coefficients, and the frequency position is related to the relative delay between the echoes. Figure 4.6(d) illustrates the relative change in FFT integral as glucose concentration increases from 0% to 20%; due to the introduced peak, the change in the FFT integral of the win-sam-mirr signals is much larger compared to the transmission signals, with the gradient approximately 5 times of the latter.

Compared to the results acquired by transmission geometry, the win-sam-mirr structure has a better capability for enhancing the contrast between different concentrations of Glu-Sol samples by introducing a notable peak in both the time- and frequency-domain signals. The relative change in the experimental ratio of Glu-Sols measured by win-sam-mirr is also enlarged around the peak, which agrees with the large variation in $RC_{abs(M)}$ in the previous simulation results.
Using the slot thickness acquired in the previous process, the optical properties of the Glu-Sol samples measured with the win-sam-mirr structure were extracted and compared to those extracted from measurements performed in the transmission geometry. As illustrated in Figure 4.7, the refractive indices and absorption coefficients measured by both geometries match well for Glu-Sols with 10% concentration difference. Furthermore, the proposed win-sam-mirr structure can reliably detect samples with 5% concentration difference: this is below the sensitivity that can be reliably detected using transmission geometry. In Figure 4.7(a), refractive indices of Glu-Sols with 5% concentration difference are close to each other, yet they can still be clearly distinguished in the range of 0.3-0.5 THz without the error bars overlapping (as shown in the inset figure).

In Figure 4.7(b), the absorption coefficients of different Glu-Sols measured using the sandwich structure are distinguishable throughout the measured frequency
range with no overlapping of error bars for most frequencies. However, in transmission geometry, the error bars are much larger and as seen in the inset of Figure 4.7(b), they already start to overlap in the range 0.3-0.5 THz even when the change in concentration is 10%. This highlights the lower sensitivity achievable in transmission geometry.

Figure 4.7: (a) The refractive indices and (b) absorption coefficients of 0-20% Glu-Sols measured by win-sam-mirr structure and transmission geometry. The inset in (a) shows the error bars for the win-sam-mirr structure. The inset in (b) shows the error bars for the transmission data. The error bars are the standard deviation from three measurements.

Figure 4.8 plots the correlation between the optical properties and glucose concentration. The refractive index and absorption coefficient of Glu-Sols are reported to have a linear relationship with the concentration of the solute, so a first order polynomial is fitted to each of the experimental data curves [103, 104]. The fitted polynomials and the Pearson correlation coefficients $r$ are annotated in the figures. In general, a decreasing trend can be seen in both the refractive index and absorption coefficient, and according to the correlation coefficients, both properties show very strong linear correlation with the mass fraction of glucose with $|r| > 0.98$ for all the three frequencies. For refractive index, the slope of the fitted lines decreases as the frequency increases from 0.3 THz to 0.5 THz, and the change rate of the refractive index is 30%, 47% and 73% per percentage concentration for 0.3, 0.4 and 0.5 THz; whereas for absorption coefficient the slope increases with frequency, and the change rate is between 174, 177 and 190 per percentage concentration for 0.3, 0.4 and 0.5 THz. The correlation between the optical properties and the solute concentration shows
potential in estimating the concentration of an unknown Glu-Sol by measuring its refractive index and/or absorption coefficient.

Figure 4.8: The correlation of (a) refractive index, (b) absorption coefficients and glucose concentration at 0.3 THz, 0.4 THz and 0.5 THz measured by win-sam-mirr structure. Dashed lines are the first order polynomials fitted to the experimental data; \( r \) is the Pearson correlation coefficient.

Glu-Sols with mass fraction of 0-20% contain a high proportion of water, and the 5% concentration difference leads to small differences in the refractive indices; hence, limited by the experimental system, sensitive characterization of Glu-Sols could be difficult, while absorption coefficients are more acquirable than refractive indices. The peak in THz time- and frequency-domain signals introduced by our geometry could be used in the application of THz sensing of Glu-Sols with little concentration differences, and the linear correlation between the optical properties and the glucose concentration could be used in mapping Glu-Sol concentration to our THz-TDS technique.

4.6 Summary

In this chapter, an optimised sandwich structure (75 \( \mu \text{m} \) win-sam-mirr) was proposed for sensitive THz characterization of thin-film aqueous glucose solutions. Theoretical simulations were conducted to quantify the characterization sensitivity of the sandwich structure with different top substrates and slot thicknesses, and the sensitivity of the proposed structure was compared with other THz geometries to show its advantage in thin-film characterization. The proposed win-sam-mirr structure was then utilised to measure thin-film liquids: thin-film water was measured for validation, and thin-film Glu-Sols with mass
fractions between 0% and 20% were measured. The results were compared to those acquired by the conventional transmission geometry. A notable peak was observed in both time-domain and frequency-domain signals, which was introduced by the use of the proposed geometry and enhanced the contrast between the sample-reference ratios of different Glu-Sols. Experimental results were in line with the theoretical simulations and indicated that the high sensitivity came from the overlapping effect of multiple reflected THz signals. Optical properties of 0-20% Glu-Sols were also extracted and linear correlation was found between the refractive index, absorption coefficient and glucose concentration. This study provides a new train of thought for THz thin-film characterization: with the proposed approach of utilizing theoretical simulation to optimise multilayer structures, such a structure could be customised for sensitive measurements of specific thin-film samples. The observed spectral feature at 0.6-0.7 THz introduced by the proposed structure shows potential for THz sensing of Glu-Sols.
Chapter 5

Quantitative Evaluation of Transdermal Drug Delivery Patches on Skin with *In Vivo* THz-TDS

5.1 Introduction

Chapter 4 presented a study on characterizing thin-film aqueous glucose solutions and proposed a simulation method of customising tri-layer sandwich structure for sensitive characterization of thin-film samples. The proposed window-sample-mirror structure has increased the characterization sensitivity of measuring thin-film glucose solutions in 0.3-1 THz compared to the conventional THz transmission geometry. In this chapter, the investigation into the applications of THz measurements has moved forward to *in vivo* measurements of human skin.

In this chapter, *in vivo* THz spectroscopy and imaging are conducted for monitoring human skin condition after treatment of transdermal drug delivery patches with different backing materials and excipient concentrations. Changes in the skin hydration level and skin’s response to occlusion effect induced by the patches are investigated and compared, with features given by the processed THz time-domain signals. A 3D imaging camera is also used as a complementary technique to study the changes on the skin surface. Results given here show potential of THz technique being utilised for *in vivo*, non-invasive and label-free
evaluation of the influence of topical skin treatment methods on skin hydration status.

5.2 Transdermal Drug Delivery Patches

Transdermal drug delivery (TDD) has become a preferable alternative to conventional methods such as oral delivery and hypodermic injection due to being non-invasive, self-administered, inexpensive and reducing over-dose situations [105, 106]. So far, TDD has been utilised in medical treatments for delivering drugs including nicotine, fentanyl and scopolamine [107].

When designing the patches for TDD, the choice of excipient composition and backing material are influential factors to consider. Permeation enhancers are often added to TDD patches to enhance the penetration of the drug through the skin and temporarily decrease the resistance of the skin barrier [108]. Propylene glycol (PG) is a commonly chosen permeation enhancer which also serves as humectant and co-solvent for poorly soluble chemicals, and it has been reported to have stronger ability in improving the transdermal flux for topical drug delivery than other widely used enhancers [109, 110]. Backing materials can alter the drug delivery efficacy and the response of skin to the patch through different levels of occlusive features. When the drug contained in the patch is highly potent and toxic, it is essential to prevent molecular exchange with the environment by using a fully occlusive backing [111]. However, long-term occlusion of the skin can cause over-accumulation of water at the skin surface without evaporation and lead to skin irritation, therefore a partially occlusive backing is preferred under certain circumstances [112].

One of the most preferable advantages of TDD patches is that they allow a controlled amount of drugs to be delivered into the human skin at a relatively consistent rate. However, studies have noted that the hydration of the skin area to be treated is an important factor affecting the drug delivery rate, with the permeability of skin increasing significantly with the growth in hydration [113]. The stratum corneum (SC) is the outermost layer of skin, which has a thickness of approximately 10-15 $\mu$m in the dry state and can swell up-to 40 $\mu$m when hydrated; by increasing the hydration level of SC, the barrier function of skin can be reduced [107]. Therefore, it is essential to study the influence of TDD patches on skin hydration in order to have better control and understanding of the drug delivery rate. Additionally, analysing the recovery of skin after removing the patches can provide supporting information on the frequency of
applying patches and switching the application area [114].

5.3 THz Techniques for Evaluation of Transdermal Drug Delivery

THz spectroscopic and imaging techniques have recently been utilised as non-destructive, label-free methods for evaluation of transdermal drug delivery. Kim et al. have demonstrated the ability of THz dynamic imaging in visualizing the spatial distribution and penetration of a topical drug on excised mouse skin [115]. Later on, Wang et al. applied ex vivo THz imaging to compare the efficacy between different TDD methods including the use of microneedles and nanoneedles [32]. Lee et al. reported a new way of quantifying drug delivery rate with THz sensing by measuring nicotine patches before and after application [116]. The above-mentioned studies are either conducted ex vivo on excised skin tissues or verified indirectly by measuring the changes on the patches. To acquire a more comparable result to the actual dynamic process in skin during TDD, in vivo THz measurements on human skin need to be studied. Lindley-Hatcher et al. have conducted in vivo THz point scan on volar forearm to investigate the changes induced in the skin by TDD patches with different backing materials and PG concentrations [114]. The study focused on how different types of patches can influence the drug delivery rate through the changes in skin hydration and skin’s response to occlusion. Their work provided a proof of concept with a small scale of participants and measured with THz point scan observing only a single location on the skin.

In this study, we developed the work further by increasing the number of subjects measured and within a larger scale we were able to categorise them into different skin groups according to their initial skin condition. We have investigated the effect of different types of patches on the skin hydration level and skin occlusion process; trends were studied in skin type groups as well as in general for all subjects, offering a deeper understanding of the skin’s response to the patches. In addition to the in vivo THz point scan, we also conducted in vivo THz imaging and 3D camera imaging on skin to visualise the results. THz imaging provided spatial information of the skin hydration, while the 3D camera imaging showed changes in the roughness of skin before and after patch application. Both techniques provided supplementary information to THz-TDS revealing the mechanism of the changes induced in the skin by TDD patches.
5.4 Methods

5.4.1 Materials and Experimental Protocol

In this study, *in vivo* THz skin measurements were acquired with a Menlo TERA K15 THz Time-Domain system, as mentioned previously in Section 3.2.1. To measure the THz response of living skin, each participant was asked to rest their volar forearm on top of the quartz imaging window for one minute. During that time the THz system recorded approximately 280 reflected THz pulses from a single point on the skin. For the *in vivo* THz imaging, the K15 spectrometer was connected to an identical reflection setup on a motorised x-y imaging stage for raster scanning the skin. Each image has a size of 17 mm × 17 mm covering the full area where the patches were applied, taking approximately 3 minutes to image the area with a resolution of 1 mm in both x-y directions. A high-precision surfaceCONTROL 3D 3500 by Micro-Epsilon camera was used to take the 3D images of the skin (section 3.3.1), providing information about the skin surface roughness.

Ethical approval was obtained for this study from the Biomedical Scientific Research Ethics Committee, BSREC, (REGO-2018-2273 AM03). The 19 subjects participating in this study were in the age group of 23 to 33 and possessed healthy skin in the regions of interest. Prior to the start of the study, all subjects were informed of the experiments and gave their signed consent. To provide further insight into the significance of the observations in the previous work [114] on the effects of the patches on the skin, woven and film based patches were applied on the volar forearms of the 19 subjects.

The purpose of this study was to explore the changes that transdermal drug delivery patches generate in the skin via *in vivo* THz spectroscopy. As previously stated, in this study patches with two types of backing materials were used: the fully occlusive polyethylene terephthalate (PET) film-backed patches and the partially occlusive woven-backed patches; and for each type of patch, an excipient with propylene glycol added at a concentration of 0%, 3% and 6% was tested. These are commonly used for the enhancement of TDD rate in such patches, and 10% transcutol was added in all patches as co-solvent. No drugs were present in the patches to allow singling out the changes induced on the skin to be a consequence of the patch material and of the excipient concentration.

Figure 5.1(a) shows three woven-backed patches and three film-backed patches applied to the right and left volar forearm respectively; a control area
was also marked on each arm to take into account the natural variation of skin occurring between each set of measurements. All of the 8 areas were marked with surgical skin marking pen in advance to keep track of the areas treated with patches. The measurements were taken in identical time frames: before the application of the patches; then 0 minutes, 30 minutes and 4 hours after removing the patches, which have been applied on the skin for 24 hours. Patches were applied and removed in a fixed order to allow each patch being applied on the skin for 24 hours. The ‘0 minutes’ measurements were immediately conducted after removing each patch, and then the skin areas were measured at 30 minutes and 4 hours after the time of removal in the same order as patches were removed. The control areas were measured each time along with the treated areas.

To further assure repeatability in the measurements, an established protocol for THz in vivo skin measurements was followed, and the pressure of skin contact and environment condition factors were taken into consideration [55]. Previous studies have found that the pressure between the skin and the imaging window affects the THz response of the sample [54]. To mitigate against this effect, pressure sensors were applied on each side of the quartz window to assure the pressure applied on the window was kept constantly in a range of 1.5-2.5 N/cm². All participants were subjected to a 20-minute period of acclimatization in the lab before starting measurements. Other external factors that might affect the skin properties of each individual were accounted for through data processing which will be described in the next section.

Figure 5.1: (a) Photo of the areas of interest indicating the locations of patches on the volar forearms. (b) Schematic diagram of the reflected signals of bulk sample in reflection geometry.
5.4.2 Data Processing

_In vivo_ THz measurements of volar forearm are conducted in reflection geometry with an imaging window in contact with the skin. In this case the detected sample signal $E_{\text{sam}}$ contains two main reflections (Figure 5.1(b)): the first is the reflection from the air-window interface which is defined as the baseline $E_{\text{bsl}}$; and the second is the actual reflection from the window-sample interface. The reflection from the bare window is also measured as the reference signal $E_{\text{ref}}$. $E_{\text{sam}}$, $E_{\text{bsl}}$ and $E_{\text{ref}}$ are all functions of time, $t$, which is the optical delay in picoseconds (ps). We measured the baseline signal from a very thick window so that there is no second reflection. However, due to the ringing effect, the baseline reflection has an enduring component that needs to be subtracted from the sample and reference signals. This is done by aligning the first reflection in the baseline with the first reflection in the sample (or reference) in the time domain and then subtracting the baseline from the sample (or reference). More details are available in [63, 97]. The reference and the baseline were measured on each day during the study to eliminate the subtle variation in system signals. The processed signal is then calculated according to Equation 5.1, where a double Gaussian filter is applied to remove noise. For the THz imaging data, the processed waveform is calculated individually for each point to account for any inhomogeneity in the imaging window.

$$\text{processed signal} = i\text{FFT} \left( \text{filter} \times \frac{\text{FFT}(E_{\text{sam}}(t) - E_{\text{bsl}}(t))}{\text{FFT}(E_{\text{ref}}(t) - E_{\text{bsl}}(t))} \right)$$

(5.1)

Figure 5.2(a) presents the processed THz signals throughout a measurement of untreated skin occluded for 60 seconds in which the signals were shifted horizontally for clarity. The peak-to-peak (P2P) variable is defined as the change between the highest and lowest amplitude of the processed signal. A decrease in P2P is observed for the 60 seconds process which was proposed in a previous study as the occlusion effect: when skin is in contact with the quartz imaging window, the surface of skin is occluded leading to accumulation of water, which will then reduce the reflection at quartz-skin interface [53]. Figure 5.2(b) is the occlusion curve showing how the P2P changes with occlusion time and the data points are fitted with a biexponential function. Here we take a sampled point on the biexponential fit at 52 seconds into occlusion when the curve tends to be stable, and the $\Delta$P2P variable is defined as the difference.
between the first point into occlusion and the sampled point.

Figure 5.2: (a) An example of the processed THz signals measured from untreated skin for 60 seconds of occlusion time. The pulses are plotted every half second for the first two seconds and every five second for the rest; they have been shifted horizontally for clarity. Peak-to-peak (P2P) index is defined. (b) The correlation between the P2P of processed signal and the occlusion time. A biexponential function is fitted to the measured data. A sampled point is taken at 52 seconds into occlusion and the definition of \( \Delta P2P \) is illustrated.

In this study, we measure the skin at different time points throughout a rather long scale of time (28 hours in total), so it is necessary to consider the natural variation of skin while investigating the effects induced by the patch application. Therefore, the normalised relative change (NRC) is calculated to isolate the change of the THz response of skin that is caused by the patches [55]:

\[
NRC(\%) = \left( \frac{X_{Tt} - X_{T0}}{X_{Ct} - X_{C0}} \right) \times 100 \tag{5.2}
\]

where \( X_{Tt} \) and \( X_{Ct} \) are the chosen variables of THz responses measured from the treated (T) area and control area (C) of skin at a specific time (t) point after removal of patches, and \( X_{T0} \) and \( X_{C0} \) are the variables measured from those two areas before patch application.

Trans-epidermal water loss is relatively constant in the longitudinal direction of the volar forearm [117]. Furthermore since we are calculating the NRC of variables, we are able to make meaningful comparisons between the patches in the different positions within the volar forearm.
5.5 Quantitative Evaluation of the Effect of TDD Patches on Skin

5.5.1 Variation on the Initial Skin Properties

Among the 19 subjects measured, variations can be seen in their initial skin hydration profile without patch application. As illustrated in Figure 5.3, the largest P2P difference between subjects is approximately 3-5 times the $\Delta P2P$ of one subject throughout the occlusion process for the control area on the dominant arm. As the P2P value is associated with the hydration level of skin in the way that lower P2P represents higher water content in skin, this indicates that individuals have notable variations in their natural skin hydration even without any skin treatment. Therefore, it is of our interest to categorise the subjects into different groups according to their initial skin hydration given by the control areas, in order to study the effect of the TDD patches on people with different skin hydration level. Here we categorise them into three groups: subject 1-6 as the ‘Dry’ group; subject 7-13 as the ‘Average’ group; and subject 14-19 as the ‘Hydrated’ group. Figure 5.3 is a rough indication of the grouping, whereas the actual categorization is based on a comprehensive analysis of the hydration state of the control area of different subjects at several time points (before, 0 minutes, 30 minutes and 4 hours) and also their individual skin response to the patch treatments. The categorization is customised for the group of subjects in this study so that we can study the influence of the patches on skin with different initial hydration levels, in addition to analysing the effect of the patches on all the subjects as a whole. Further research needs to be conducted to give a more robust method for the categorization of skin hydration.

In the following sections, we will be looking at the statistical trends for each skin hydration group as well as for all the subjects in general.
5.5.2 Effect on Skin Hydration Level

NRC of the sampled P2P is calculated for all subjects at 0 minutes, 30 minutes and 4 hours after the removal of the different patches as according to Equation 5.2. A negative NRC value of P2P represents an increase in skin hydration level compared to the initial state before patch application and a positive value implies a decrease; the hydration level is inversely proportional to the P2P NRC.

The average P2P NRC of all subjects is shown in Figure 5.4(a) with error bars indicating the standard error of the mean. In general, an increase in skin hydration associated with the decrease in P2P is observed for all patch application areas at 0 minutes after the patches were removed after being applied for 24 hours. The changes show a declining trend with time indicating the recovery process of the skin, however the hydration effect persists even 4 hours after removal. Changes induced by the film patches are slightly larger than the woven patches and last longer in time. Different excipient compositions are observed to have some influences on both patches, and a higher PG concentration results in more hydrated skin.
Figure 5.4: The average normalised relative change (NRC) of the sampled P2P in (a) all 19 subjects, (b) the ‘Average’ group, (c) the ‘Dry’ group and (d) the ‘Hydrated’ group measured at 0 minutes, 30 minutes and 4 hours after the removal of different patches (woven patches and film patches with 0%, 3% and 6% propylene glycol). Error bars are acquired by the standard error on the mean.

Compared to the ‘All subjects’ group, results given by the ‘Average’ group in Figure 5.4(b) show similar trend; more notable differences are observed for skin hydration changes induced by film and woven patches, with areas covered by the film patches being more hydrated than woven patches after 0 minutes, 30 minutes and 4 hours of removal respectively. For this skin group, we can still see a positive correlation between PG concentration and skin hydration level for both patches. For subjects with ‘Dry’ skin group, both film and woven patches have smaller impact on the THz response of skin compared to the ‘Average’ group; on the other hand, the skin recovery rate increases in general and some areas appear to be even dryer than the initial state after 30 minutes following the removal of the patches, as shown in Figure 5.4(c). When the
initial skin state of the subjects is more hydrated than average group, we can observe from Figure 5.4(d) that film patches induce smaller changes on skin than the woven patches; and the recovery rate of the skin strongly decreases for all areas, leading to the skin staying at a highly hydrated state even 4 hours after removing the patches. A possible explanation is that when the initial skin is already hydrated to a certain extent, occlusion effect caused by the fully occlusive film patches is no longer dominant in altering the skin hydration level, while the partially occlusive woven patches allow moisture exchange between skin and air and have more effect on further hydrating the skin. The impact induced by different PG concentrations for the ‘Dry’ group and ‘Hydrated’ group shows similar trend as observed in all subjects.

5.5.3 Effect on Skin Occlusion Process

The study shown in the last section provides an idea on how patch application interacts with the general hydration level in skin. Due to the increased hydration levels of the skin after the application of the patches, it is natural to ask if the skin’s response to occlusion has also been affected. As mentioned in Figure 5.2, the occlusion process happens on untreated skin once it is in contact with the imaging window, blocking the exchange of water with the environment during the one-minute measurement. This process is also observed as a decrease in the P2P of the THz waveform which can be modelled by a biexponential curve. However, if the patch application disrupts the normal water distribution in skin, changes in the occlusion curve would be expected and the defined ∆P2P can be used as a variable for quantification.

Figure 5.5 presents the NRC of ∆P2P in a box plot, where the red/blue lines inside the boxes indicate the median response and the upper and lower limits show the upper and lower quartiles. A negative NRC in ∆P2P suggests a decline in the variation of P2P during the occlusion process and that the occlusion curve flattens, which can imply that the skin is in a comparable condition of already being occluded and that further occlusion has less impact on it. While a positive NRC in ∆P2P is correlated with an increment in the change of P2P and the occlusion curve steepens.

As shown in Figure 5.5(a-b), the ‘Average’ group presents similar results as taking all subjects into account, where looking at the film patches at 0 minutes after removal we can see ∆P2P NRC far below the zero-line indicating flattened occlusion curves. This observation further demonstrates that the impact on skin
hydration by film patches is through the fully-occlusive feature. Woven patches, on the other hand, seem to have different effect on skin occlusion for different PG concentrations. After 4 hours of removing the patches, ΔP2P NRC for all excipient concentrations goes back to the state before treatment, revealing a recovery process for the water distribution in skin. For the ‘Dry’ group in Figure 5.5(c), the impact of the film patches on skin occlusion decreases compared to the ‘Average’ group and barely any impact is seen for woven patches. In Figure 5.5(d), we can still observe clear occlusive effect for all the film patches; this indicates that for skin that is already much hydrated, film patches still have an occlusive effect on it although they do not increase the skin hydration level as much (see Figure 5.4(d)).

Figure 5.5: Box plots showing the NRC of ΔP2P in (a) all 19 subjects, (b) the ‘Average’ group, (c) the ‘Dry’ group and (d) the ‘Hydrated’ group measured at 0 minutes and 4 hours after the removal of different patches. The red/blue lines inside the boxes represent the median response; the upper and lower edges of the boxes are the upper and lower quartiles of the responses.
5.5.4 Statistical Significance

In addition to the data analysis in the previous sections, the statistical significance of the changes in skin hydration level and skin occlusion process induced by patch application is tested with the one-way analysis of variance (ANOVA) test and the post hoc Dunnett’s test among all the 19 subjects. The NRC of P2P measured at 0 minutes and 4 hours after removal of 6 types of patches is tested along with the control group which has an NRC value of 0 to check if there is significant difference after treatment. According to the one-way ANOVA test there is a statistically significant difference between groups for both measurement times, and Figure 5.6(a) illustrates the results from the Dunnett’s test where the shaded bars represent the 95% confidence intervals. It is observed from Figure 5.6(a) that all patches result in a significant change in the P2P of skin at 0 minutes, while the changes induced by 3% and 6% film patches persist significant after 4 hours. The NRC of ∆P2P is tested in a similar pattern and results are presented in Figure 5.6(b). The one-way ANOVA test reveals statistically significant difference between groups for those measured at 0 minutes; from the Dunnett’s test, the 3% and 6% film patches and the 3% woven patch have significant impact on the ∆P2P of skin immediately after removing the patches, and all changes lose their significance after 4 hours.

![Figure 5.6](image)

Figure 5.6: Results of performing the one-way analysis of variance test on (a) the NRC of sampled P2P and (b) the NRC of ∆P2P for all subjects measured at 0 minutes and 4 hours after the removal of patches. The cross/plus markers indicate the estimated mean of distribution; the shaded bars show the 95% confidence intervals calculated with the Dunnett’s test. The control group is represented by the red dashed line at the value of 0.
5.6 Visualization of Skin Hydration and Skin Surface Roughness

5.6.1 THz Imaging

THz imaging is performed for better visualization of the hydration change in the entire region of skin covered by patches. Figure 5.7 shows the imaging results of the skin areas of one subject from the ‘Average’ group at different times after removing the 6% film patch (Figure 5.7(a-c)) and the 6% woven patch (Figure 5.7(d-f)). In general, blue areas indicate negative NRC in P2P, associated with an increase in the skin hydration level. We can conclude from the figures that the film patches compared with the woven patches have greater impact on the skin with a larger hydrated area and a deeper hydration level. The skin area applied with film patches stay hydrated for at least 4 hours with the hydration level only decreasing slightly. This imaging result serves as a complement to the quantitative results in Figure 5.4, showing the impact of patches on the entire treated area of skin with more spatial information.

![THz Imaging Results](image)

Figure 5.7: THz imaging results of the skin areas of one subject measured at 0h, 2h and 4 hours after the removal of (a-c) 6% film patch and (d-f) 6% woven patch.

5.6.2 3D Camera Imaging

The 3D camera is able to take images of an object obtaining the height information in the z-axis along an x-y surface, which in this study can be used to
measure the roughness of the skin surface as shown in Figure 5.8. Our previous discussions indicate that film patches hydrate the skin through occlusion effect, and according to Figure 5.7 the hydration is more spatially uniform and persistent than that induced by woven patches. Therefore we used the 3D camera to measure the skin area of one subject from the 'Average' group before and after treatment with the 6% film patch and the 6% woven patch. In Figure 5.8(a-c), we can directly see the change in skin roughness before and after the application of the film patch; the skin surface is clearly smoother immediately after the patch removal, and its roughness recovers partially after 4 hours yet remains less bumpy than before. To quantify the change in the surface roughness, height information along the white dashed line in each 3D image is acquired and the standard deviation is calculated, where smaller standard deviation is correlated with a smoother surface. From Figure 5.8(d) we can see that before treatment $\sigma = 31 \mu m$, while 0 minutes after removing the film patch $\sigma$ decreases to 17 $\mu m$, and then after 4 hours it goes back to 24 $\mu m$. In contrary, the skin treated with the woven patch does not have such a visible difference in the surface roughness before and after treatment, as shown in Figure 5.8(e-g). The quantitative evaluation of the roughness in Figure 5.8(h) indicates that the standard deviation $\sigma$ changes from 29 $\mu m$ to 20 $\mu m$ immediately after removing the woven patch and then increases to 23 $\mu m$ after 4 hours.

3D images can display the roughness changes in skin surface before and after patch application with a straightforward visualization. Results show that occlusion induced by film patches changes the skin surface profile by smoothing the surface and reduces the depth of furrows, while accumulating water in the stratum corneum.
Figure 5.8: 3D camera images of the skin area of one subject taken before patch application, 0 minutes and 4 hours after removing (a-c) the 6% film patch and (d-f) the 6% woven patch. Roughness of the skin surface at different times with the application of (g) the 6% film patch and (h) the 6% woven patch. Roughness is acquired along the black dashed lines on 3D images. Standard deviation $\sigma$ is calculated.

### 5.7 Summary

In this chapter, *in vivo* THz spectroscopy was used for quantifying the changes in human skin induced by transdermal drug delivery patches with different backing materials and propylene glycol concentrations. We found that the impact of TDD patches on skin hydration and skin’s response to occlusion...
depends on the initial skin hydration of the participant as well as the backing material and excipients of the patches. Patches with film backings increased the skin hydration level in general, except for some participants with more hydrated skin; and this impact from the backing material dominated over the impact induced by the different concentration levels of propylene glycol in the patches. Patches with woven backings did not increase the skin hydration as much, and the effect of the different propylene glycol concentrations was noticeable across most subjects. THz imaging and 3D camera imaging complemented the results from THz spectroscopy by showing the spatial distribution of skin hydration change and the change in skin roughness caused by TDD patches. This study shows how THz sensing can be used to evaluate the impact on human skin hydration induced by transdermal drug delivery patches in an \textit{in vivo}, non-invasive style. This study provides further insights into the impact of different types of TDD patches on skin and the mechanism behind it with a larger group of participants and more detailed analysis compared to the previous study. Studying the effect of TDD patches on skin hydration is useful for controlling the drug delivery rate when designing the patches. This study demonstrates the potential of using \textit{in vivo} THz sensing for evaluating human skin hydration non-invasively and aiding the design of transdermal drug patches.
Chapter 6

Retrieving the Dynamic Hydration Profile of Skin with \textit{In Vivo} THz-TDS

6.1 Introduction

Chapter 5 focused on the \textit{in vivo} quantitative evaluation of human skin hydration after the treatment of transdermal drug delivery (TDD) patches with the use of a commercial reflection THz-TDS system. By analyzing the peak-to-peak features of the measured THz pulses, the relative changes in skin hydration level and skin’s response of occlusion have been investigated quantitatively over 19 participants, who have been categorised into three groups according to their initial skin hydration. TDD patches with different backing materials and propylene glycol concentrations were applied on the volar forearm region and their impact on skin have been compared. THz imaging and 3D camera imaging have also been utilised as complementary techniques for mapping the hydration distribution of the skin area and analyzing the roughness change on the skin surface, both induced by the TDD patches.

In this chapter, with the use of a portable THz handheld scanner, the upper arm region is measured after the application of TDD patches, which is a more common skin region for patch treatment compared to the volar forearm region. The skin hydration profile, which includes the depth-dependent water concentration in the skin and the stratum corneum thickness, has been extracted through a modelling approach. Compared to the previous chapter which studied the relative change of skin hydration given by the peak-to-peak
features of the detected THz signals, this chapter presents estimated values of skin hydration and thickness extracted from the detected THz signals through a modelling approach. This study presents more straightforward skin hydration results for evaluating the effect of TDD patches on skin with in vivo THz-TDS. It also provides a demonstration of retrieving skin hydration profile from THz spectrum and broadens the potential use of in vivo THz sensing for non-invasive evaluation of skin hydration.

6.2 In Vivo Sensing of Skin Hydration

The outermost layer of skin, the stratum corneum (SC), maintains the skin’s barrier function, plasticity and normal process of desquamation. It is essential that the SC has an appropriate water content to perform this role as disrupted skin hydration may result in several dermatologic conditions, for example eczema and atopic dermatitis [118, 119]. Additionally, evidence from magnetic resonance imaging (MRI) studies has shown that tumour areas have increased water content compared to healthy ones [120, 121]. Therefore, there is a need for non-invasive, accurate and real-time monitoring of skin hydration. Existing techniques for monitoring skin hydration include evaporimetry for the measurement of transepidermal water loss (TEWL), electrical-based methods like corneometry, optical-based methods such as optical coherence tomography (OCT) and nuclear magnetic resonance (NMR) [122–125]. Limitations of the evaporimetry and corneometry are that they are easily influenced by intrinsic and extrinsic factors, for example variables such as room temperature and ambient humidity can alter the TEWL results and substances other than water in the skin may have an effect on the skin impedance. OCT and NMR can provide clear images of skin layers and acquire multiple properties including SC thickness and water concentration gradient, but the instrumentation is often large and expensive and only able to measure a limited range of locations on the human body.

Terahertz (THz) radiation is non-ionizing and very sensitive to changes in water content due to strong hydrogen bond absorption in the THz region. This has motivated research into utilizing THz sensing for various biomedical applications including the diagnosis of skin cancer [126], evaluation of burn wounds [127], sensing the SC hydration profile [63, 128, 129] and monitoring transdermal drug delivery (TDD) processes [130–133]. As a label-free, non-destructive sensing method for TDD, Kim et al. conducted ex vivo THz imaging
to visualise the penetration of topical drug on excised mouse skin [130] and Wang et al. compared the efficacy of different TDD methods with similar THz techniques [131]. More recently, Lindley-Hatcher et al. have conducted in vivo THz measurements on human skin treated with TDD patches and demonstrated the potential of using in vivo THz sensing to quantify the response of skin to the application of TDD patches with different backing materials [133]. They conducted single point measurements in a pilot study to serve as a proof of concept. As a further development, the study in Chapter 5 of this thesis increased the experimental scale and analysed the response of skin to the patches with volunteers classified into different groups according to their original state of skin hydration.

Researchers have been trying to develop the THz evaluation of TDD efficacy from ex vivo to in vivo to examine the response of living skin to the patches. However, the aforementioned in vivo TDD studies quantified skin hydration in an indirect way by analyzing variables such as the peak-to-peak amplitude of reflected waveform, instead of directly determining the hydration profile and thickness change in the skin. Another issue in these pilot studies was that the patches can only be applied on the volar forearms of the participants due to the restricted configuration of the THz systems. In this work, a portable THz handheld scanner was used to measure the upper arm skin after the treatment of TDD patches as it is the most common area to apply TDD patches on for clinical applications. Patches with two different backings and three concentration levels of propylene glycol were studied on 10 subjects for a total duration of 28 hours (24 hours of patch treatment and 4 hours of measurement). A skin modelling approach was proposed based on the combination of the stratified media model and the effective medium theory to extract the dynamic hydration profile within the skin after treated with the TDD patches, which provides information of the skin hydration as a function of depth into the skin and the occlusion time.

6.3 Methods

6.3.1 Skin Modelling Approach

For the purpose of THz applications, human skin can be seen as mainly containing three layers: stratum corneum, epidermis and dermis, as illustrated in Figure 6.1. Water content is one of the main components of skin, normally
ranging from 20% to 70%, other than which skin also consists of collagen, elastin and other proteins [134]. Many THz in vivo studies looking at the skin hydration change have applied a single-layer or a two-layer model to simulate the skin structure [53, 54, 60]. The former model simplifies the skin as a homogeneous semi-infinite material, which aids the THz optical properties characterization to be easier but also assumes that the water content is uniform in the depth level of skin that can be detected by THz; whereas the latter model separates the skin into the SC layer and the epidermis layer with each layer having a constant water concentration, since the hydration difference between the two layers is evident. However, it is observed by confocal Raman spectroscopy that the water concentration in skin is depth dependent, where it is similar to a parabolic function in the SC layer, an increasing linear function in the epidermis layer and is constant in the dermis [70, 135], as shown in Figure 6.1.

Figure 6.1: A diagram showing the skin layers (stratum corneum (SC), epidermis and dermis) and the skin hydration change as a function of depth. The skin hydration change follows a parabolic trend in the SC layer, a linear increase in the epidermis, and a constant hydration in the dermis. The red dot indicates the SC surface Hydration ($H_0$), and the red arrow indicates the SC thickness ($d$).

Therefore, a stratified media model has been proposed to simulate the reflected THz beam from skin with a more realistic hydration profile similar to the one observed in Raman spectroscopy [71]. This composite model describes
the THz light interacting with living skin as a plane wave reflecting from an effective medium that has stratified dielectric permittivity. This model separates the skin into multiple thin layers to approximate the continuous water concentration change. The composition of skin is seen as a binary mixture of water content and dry biological background, therefore in each stratified layer the effective permittivity is associated with the water concentration through the Bruggeman EMT model, written as

$$\eta_1 \frac{\varepsilon_1 - \varepsilon_{eff}}{\varepsilon_1 + 2\varepsilon_{eff}} + \eta_2 \frac{\varepsilon_2 - \varepsilon_{eff}}{\varepsilon_2 + 2\varepsilon_{eff}} = 0 \quad (6.1)$$

where $\eta_1$ and $\varepsilon_1$ are the volume concentration and permittivity of the water content, $\eta_2$ and $\varepsilon_2$ are the volume concentration and permittivity of the dry biological background, and $\eta_1 + \eta_2 = 1$. $\varepsilon_{eff}$ is the effective permittivity of the whole composite system. The permittivity of water is described by a double Debye model with the parameters given by literature [103] and the permittivity of the dry biological background is approximated by the measured result of dehydrated porcine skin ($n = 1.20$) [71]. The effective permittivity of the skin system is therefore directly associated with the volume fraction of the water content.

Given the effective permittivity of each layer, the propagation of the wave in the $m$-th layer is characterised by a longitudinal propagation constant ($k_m$) and a characteristic impedance ($\zeta_m$) by Eqs.6.2, 6.3. Then the effective impedance looking down at the top of the $m$-th layer ($Z_m$) can be calculated in a recursive fashion as Equation 6.4.

$$k_m = \omega \sqrt{\varepsilon_0 \mu_0} \sqrt{\varepsilon_m - \sin^2 \theta} \quad (6.2)$$

$$\zeta_m = \frac{\omega \mu_m}{k_m} \quad (6.3)$$

$$Z_m = \frac{\zeta_m Z_{m+1} + i \zeta_m \tan(k_m t_m)}{\zeta_m + i Z_{m+1} \tan(k_m t_m)} \quad (6.4)$$

Here $\varepsilon_m$ and $\mu_m$ represent the effective permittivity and permeability of each layer. $t_m$ stands for the layer thickness. Through iteration of the former equations, reflections from this multilayer system can be summed up from the deepest layer all the way to the surface, resulting in the effective reflection coefficient seen from the top of the system ($\Gamma_0$) given by Equation 6.5. More
details regarding the double Debye model, the effective medium theory and the stratified media model are given in Section 2.6.

\[ \Gamma_0 = \frac{Z_1 - \zeta_0}{Z_1 + \zeta_0} \]  

(6.5)

Results from the confocal Raman spectroscopy reveal that when the skin is hydrated, the main changes appear in the SC surface hydration \( H_0 \) and SC thickness \( d \) [70]. Therefore, the water gradient inside the skin can be represented by a depth dependent function with \( H_0 \) and \( d \) as unknown variables, which can then determine the calculated reflectivity of the skin with the stratified media model and the Bruggeman EMT model. The measured THz reflectivity from in vivo skin measurement can be acquired according to the bulk-sample treatment for THz reflection geometry as described in Section 2.4. By fitting the calculated reflectivity to the measured reflectivity, the SC surface hydration and SC thickness can be extracted. The whole process is summarised in Figure 6.2.

![Figure 6.2: A flow chart summarizing the data processing procedure.](image)

### 6.3.2 Simulation and Model Fitting

In order to understand the influence on THz reflection induced by different skin properties, simulations have been conducted on the reflectivity change
according to different SC surface hydration levels and different SC thicknesses. The hydration level is set to be 75% and 90% at the SC-epidermis interface and the epidermis-dermis interface respectively, while the epidermis and dermis thickness is set to be 80 µm and 100 µm [70]. The SC thickness is kept at 20 µm when looking at the impact of SC surface hydration, and the surface hydration level is kept at 30% during the variation of the SC thickness. Simulations are conducted in the frequency range of 0.3-0.8 THz, which is the same frequency range for processing the experimental results of skin measured with the THz handheld scanner to avoid large scattering effect in the higher frequencies and low signal-to-noise ratio in the lower frequencies. According to Figure 6.3, the reflectivity curve shifts down when the SC surface hydration increases from 10% to 40%, whereas it rises upwards along with the increase of the SC thickness from 10 µm to 40 µm. The influence strength of the two variables seems to be comparable given the similar amount of variation in the reflectivity curve induced by a unit change in each variable.

![Figure 6.3](image)

Figure 6.3: Simulation of the reflectivity changing with (a) the SC hydration and (b) the SC thickness. The epidermis surface hydration is set to be 75% and the epidermis thickness is 80 µm; the dermis hydration is at 90% and the dermis thickness is 100 µm. (a) The SC thickness is kept at 20 µm while the SC surface hydration changes from 10% to 40%. (b) The SC surface hydration is kept at 30% while the SC thickness changes from 10 µm to 40 µm.

Figure 6.4 presents an example of fitting the calculated reflectivity (solid lines) to the measured reflectivity (circles) of a 60-second measurement in the range of 0.3 to 0.8 THz. The figure shows the accuracy of fitting to the measured reflectivity with the skin modelling approach described above, and also the variation of the reflectivity curve due to occlusion. As expected, when the skin hydration increases along with the occlusion time, the measured reflectivity
curve moves down vertically and the aforementioned skin modelling approach is capable of fitting each reflectivity curve precisely.

Figure 6.4: An example of fitting the calculated reflectivity to the measured reflectivity. Dots represent the measured reflectivity and solid lines represent the fitted reflectivity. Different colours indicate the reflectivity of skin measured at different time into occlusion, with red, yellow and blue standing for 2 seconds, 30 seconds and 60 seconds respectively.

6.3.3 Experimental Setup and Protocols

The transdermal drug delivery patches used for this study are identical to those described in Chapter 5 with the same backing materials and excipients, but in a smaller size so that they can be applied to a wider range of locations on human body. No active drug ingredients are added in the patches as well. As shown in Figure 6.5(a,b), woven patches with propylene glycol (PG) concentrations of 0%, 3% and 6% (W1, W2, W3) were applied sequentially on the right upper arm of the participant followed by a control area (C1); PET film patches with 0%, 3% and 6% (F1, F2, F3) PG were applied on the left upper arm of the participant successively also followed by a control area (C2). The experimental protocols are also the same as Chapter 5, where the 8 regions of interest are measured before the application of the patches, and after applying the patches and leaving them on the skin for 24 hours, measured 0 minutes, 30 minutes and 4 hours after removing the patches. Ethical approval was obtained from the Biomedical Scientific Research Ethics Committee, BSREC, (REGO-2018-2273 AM03). 10 participants were measured in total within the age range of 23-33 and with healthy skin in the regions of interest. Participants were informed of
the protocols and other related information of the study and gave their signed consent before the study. The same robust protocol for in vivo THz study was followed with consideration of contact pressure control and acclimatization of skin [55].

As an improvement from Chapter 5, TDD patches in this study were applied on the upper arms of the participants instead of the volar forearms, which are the more common and practical body locations for TDD treatment. This is achieved with the use of the portable THz handheld scanner as shown in Figure 6.5(C), which has been described in detail in Section 3.2.2. The handheld scanner is set up in reflection geometry with an incident angle of 30 degrees. It has a quartz window for beam alignment and flattening the skin surface; two pressure sensors are attached next to the window for controlling the contact pressure. Each measurement was taken for 60 seconds with continuous contact and 4 THz scans were recorded per second.

Figure 6.5: (a-b) An indication of the skin areas on both upper arms treated with different patches and the control regions. W1, W2, W3 stand for 0%, 3%, 6% woven patches and F1, F2, F3 stand for 0%, 3%, 6% film patches, respectively. C1 and C2 are the marked control regions with one on each arm. (c) A photo of the THz handheld scanner being held against a participant’s upper arm to measure within a skin region.

6.4 Results

6.4.1 The Dynamic Hydration Profiles of Skin

With the skin modelling approach and data fitting procedures mentioned above, the SC surface hydration ($H_0$) and SC thickness ($d$) can be extracted at every single measurement. Therefore, by fitting $H_0$ and $d$ into the stratified media model of skin, the skin hydration change as a function of the depth into the skin
and the measurement time (occlusion time) can be acquired, which is referred to as the dynamic hydration profile of skin in this study.

Figure 6.6 shows an example of the dynamic hydration profiles of the skin regions of one participant after treated with a 3% film patch and a 3% woven patch for 24 hours. The x-axis is the occlusion time ranging from 0 to 60 seconds and the y-axis is the depth of the skin from the skin surface (0 µm) to 40 µm into the skin. The colourmap indicates the water concentration within a range of 15%-70%, where some precise values are also given by the contour lines in black solid lines. The white dashed lines represent the boundary of SC and epidermis, which also indicates the change in SC thickness during the occlusion.

In general, it is observed that both skin regions are most hydrated immediately after peeling off the patches, and the overall hydration decreases over time (30 minutes and 4 hours after). However, the region treated with the film patch displays a much higher initial surface hydration than the woven patch region at 0 minutes, with nearly 35% water content compared to 25%. In addition, the film patch has an evident impact on skin’s response to occlusion, in a way that the skin seems to be saturated and thus the skin hydration profile remains nearly consistent during the 60-second measurement at 0 minutes after patch removal. In comparison, the skin region treated with the woven patch presents a clear image of occlusion at 0 minutes, with water accumulating in the skin surface over the measurement time. This is due to the different occlusive nature of the two backing materials, with PET film being fully-occlusive and woven being partially-occlusive. Also, the film patch region shows a slower decreasing rate of skin hydration, as the surface hydration remains to be higher than that of the woven patch region after 30 minutes and 4 hours of removing the patches. The SC thicknesses are similar for the two regions, with a slight increase after 30 minutes and 4 hours as the skin gets drier.

The dynamic hydration profile contains meaningful information of the changes in skin hydration that can be compared over both the measurement time and the depth in skin. By visualizing it as a colourmap, it is clear and intuitive to compare the overall skin hydration between measurements.
Figure 6.6: Colourmaps of the dynamic hydration profiles of skin of one participant after the application of (a-c) a 3% film patch and (d-f) a 3% woven patch. Three columns of the colourmaps show the results from 0 minutes (a and d), 30 minutes (b and e) and 4 hours (c and f) after the removal of patches respectively. The colourmaps indicate the skin hydration value from the skin surface (0 µm) to 40 µm into the skin at different occlusion times ranging from 0 to 60 seconds. The colour bar has a hydration range of 15% to 70%. The solid black lines are the contour lines of the hydration, while the dashed white lines indicate the boundary between SC and epidermis.

6.4.2 Impact of TDD Patches on Skin Properties

In order to quantitatively compare the impact of the different TDD patches on skin properties, here the results of the SC surface hydration and SC thickness are sampled at 55 seconds into occlusion to acquire a single value for each measurement.

Figure 6.7 presents the overall trends of SC hydration affected by different TDD patches amongst the 10 participants. The bar heights represent the mean value and the error bars are the standard error of the mean. The colour blue, red and yellow indicate the measurement at 0 minutes, 30 minutes and 4 hours after the removal of patches; and grey bars are the results from the control regions measured at 0 minutes. In general, SC hydration increases for all the skin regions previously treated with patches as compared to the control region, and the hydration gradually decreases after 30 minutes and 4 hours of removing the patches.
Figure 6.7: The extracted SC hydration of all participants after the application of 0%, 3% and 6% (a) film patches and (b) woven patches. The bar plots show the average value among all participants with error bars indicating the standard error of the mean. Blue, red and yellow bars exhibit results acquired from 0 minutes, 30 minutes and 4 hours after removing the patches respectively; while grey bars are the results from the control regions measured at 0 minutes. Results here are sampled at 55 seconds into occlusion.

In Figure 6.7(a), immediately after the patch removal, the SC hydration rises up to around 32%, 33% and 30% respectively for the areas applied with 0%, 3% and 6% film patches, compared to 23% SC hydration for the control area. Then after exposing the treated skin to air for some duration of time, the skin displays a recovery phenomenon where the SC hydration gradually decreases to 25%, 28% and 26%, tending towards the hydration level of the control region. On the other hand, as shown in Figure 6.7(b), the areas treated with woven patches have less increase of hydration at 0 minutes, where the hydration level is around 27%, 28% and 26% after the application of 0%, 3% and 6% woven patches respectively, while the control region has around 23% of water concentration. Then the three skin areas display different trends after 30 minutes and 4 hours of the removal of patches. The hydration decreases gradually over time for both the 0% and 3% woven patch areas, whereas for the former area it gets drier than the control region after 4 hours; and for the 6% woven patch area, there seems to be no clear trend of the SC hydration change, however as it is changing within the range of the error bars, it can be seen as the natural fluctuation in skin hydration over a period of time. In conclusion, the different backing materials alter the skin hydration differently, with the fully-occlusive PET film rises the hydration by over 5% more than the partially-occlusive woven material, and the occlusive impact from the former material persists longer than the latter even after 4 hours of removing the
patches. However, there is no significant difference between the impact induced by different excipient concentrations in patches.

**Figure 6.8:** The extracted SC thickness of all participants after the application of 0%, 3% and 6% (a) film patches and (b) woven patches. The bar plots show the average value among all participants with error bars indicating the standard error of the mean. Blue, red and yellow bars exhibit results acquired from 0 minutes, 30 minutes and 4 hours after removing the patches respectively; while grey bars are the results from the control region measured at 0 minutes. Results here are sampled at 55 seconds into occlusion.

Figure 6.8 presents the quantitative results of the extracted SC thickness affected by the different TDD patches in a similar fashion as Figure 6.7. In Figure 6.8(a), it can be seen that the SC thicknesses increase significantly in the three skin regions after removing the 0%, 3% and 6% film patches immediately after the 24 hours of treatment, from around 22 μm (the control region) to 24 μm, 27 μm and 27 μm respectively. Relating this observation to the notable increase in SC hydration at 0 minutes in Figure 6.7(a), this can be explained by the SC swelling motion happening when the skin surface is highly hydrated, which has also been confirmed by previous experiments with *in vivo* confocal Raman spectroscopy [135, 136]. Then after 30 minutes of exposing the skin to air, as the SC hydration level drops, the SC thicknesses decrease rapidly to 19 μm, 20 μm and 20 μm, which are even smaller than the control region thickness; and after 4 hours of removing the patches, the SC thicknesses of the three regions rise back to become similar to that of the control region. This observed trend in the SC thickness change induced by the film patches further confirms the dominant impact on skin hydration is caused by the fully-occlusive nature of the PET film backing material, which has strong occlusion effect on the skin after 24 hours of application. Whereas in Figure 6.8(b), there is
no significant change in SC thicknesses for the areas treated with 0% and 6% woven patches as compared with the control area, with some small fluctuations in SC thicknesses at different measurement times which are within the range of the error bars. For the skin area treated with 3% woven patch, the SC thickness appears to decrease from approximately 23 µm to around 20 µm after 0 minutes and 30 minutes of the removal of the patch, and then increase back to 23 µm after 4 hours. This could be due to the skin being compressed when pressed against the imaging window, since the SC becomes softer and more deformable when hydrated [137]. In general, compared to the PET film patches, woven patches don’t seem to have significant impact on the SC thickness. Similar to Figure 6.7, it is not conclusive how the different excipient concentrations affect the SC thickness change differently.

In Figure 6.7 and Figure 6.8, the results of the control regions are only presented with those measured at 0 minutes after removing the patches in comparison with the treated regions measured at 0 minutes, 30 minutes and 4 hours afterwards. Therefore in order to validate the constancy of the SC properties of the control regions during the long period of time, Figure 6.9 shows the extracted SC hydration and thicknesses of the control regions on both upper arms (left arm for film patches, right arm for woven patches) measured before the patch application and 0 minutes, 30 minutes and 4 hours after the removal of patches. The figure shows that in general the SC hydration and thicknesses for the two control regions remain relatively stable throughout the time, with some differences in the mean values that are still within the error bars. For the control region on the left upper arm, the SC thickness is smaller at 0 minutes after peeling off the patches compared to before the patch application. This is due to the natural variation in skin between the 24-hour interval of the two measurements, which is also the reason why the results of the treated skin regions are compared to the control region measured on the same day instead of the before treatment results in Figure 6.7 and Figure 6.8.
Figure 6.9: The extracted SC hydration (a-b) and SC thickness (c-d) of the control regions on both arms measured before patches and 0 minutes, 30 minutes and 4 hours following the removal of patches. Results here are sampled at 55 seconds into occlusion.

6.5 Summary

In this chapter, the effect of different types of transdermal drug delivery patches on skin hydration and thickness has been studied \textit{in vivo} with a portable THz handheld scanner. The TDD patches used in this study are similar to those in Chapter 5 with two different backing materials (woven and PET film) and three propylene glycol concentrations (0%, 3% and 6%); but they are in a decreased size and have been applied to the upper arm region, which is a more common skin region for TDD treatment in clinical applications. As a further development to the study in Chapter 5, the skin hydration profile and stratum corneum thickness have been extracted through data analysis procedure involving skin modelling and model fitting. Consequently, we have achieved extracting the direct information of skin hydration from the THz spectroscopic signal, instead of analyzing the relative change in the THz signal associated with the skin
hydration in an indirect manner. This study reveals the influence of different TDD patches on skin hydration and thickness, which are important factors to consider when designing TDD patches, with the use of THz spectroscopy in an *in vivo*, non-invasive way. It also explores the association between THz spectroscopic signal and skin hydration profile and further expands the potential of *in vivo* THz sensing for non-invasive evaluation of human skin.
Chapter 7

Variation of Skin Hydration Profile with Biophysical Factors and Lifestyles Revealed by THz In Vivo Study

7.1 Introduction

Chapter 6 studied the effect induced by transdermal drug delivery patches on upper arm skin \textit{in vivo} with a THz handheld scanner, and retrieved the dynamic hydration profile of skin with a skin modelling approach. The stratum corneum hydration and thickness have been extracted and compared between the skin treated with different types of TDD patches. Chapter 6 has introduced the skin modelling method which can associate the reflected THz signal with the skin hydration change, and also presented an application of \textit{in vivo} THz skin measurement with the portable THz handheld scanner.

In this chapter, the portable THz handheld scanner is utilised for a THz \textit{in vivo} skin study and skin hydration profiles have been extracted with the aforementioned approach. This study has measured over 300 participants on their volar forearm skin in an out-of-the-lab environment with the THz handheld scanner and is the largest scale \textit{in vivo} THz study hitherto. Within the large and diverse subject group, participants have been classified through different biophysical factors and lifestyles, and correlations between each of the factor and the SC surface hydration and SC thickness have been investigated. This
study has demonstrated the robustness and portability of the THz handheld scanner and also investigated into the potential factors that may have impact on individual’s initial skin conditions. This large scale THz \textit{in vivo} study paves the way for future clinical applications of non-invasive THz sensing for the evaluation of dry skin conditions or early diagnosis of skin cancer.

7.2 Portable THz Handheld Scanner

In recent years THz techniques have been employed in many biomedical studies due to the unique features of THz light [138]. Studies have revealed the potential of utilizing THz spectroscopy and imaging for \textit{ex vivo} investigations on excised biological tissues [139–142] and recently progress has also been made in \textit{in vivo} THz spectroscopy and imaging on living human body [143–148]. These studies mostly employed conventional THz time-domain spectroscopy (THz-TDS) system set up in reflection geometry [149–151]. One limit of conventional reflection THz-TDS systems is that they are bulky in size and difficult to be moved around, so they are often laboratory based. Their fixed reflection configuration restricts the region of interest that can be investigated by a particular system, whereas in real-life applications, living tissues with irregular shapes, rough surface and inconvenient positions are required to be measured [152]. Furthermore, if doing contact measurements, the contact pressure between the skin and the imaging window highly depends on the participants, who need to be trained beforehand [55]. Therefore, the compactness, portability and flexibility of THz systems needs to be improved to support the development of THz \textit{in vivo} applications [152].

Solutions of the aforementioned problems have been proposed through the development of handheld THz probes, which are designed from photoconductive antennas (PCAs) with fiber coupled lasers. TeraView designed a THz handheld ‘wand’ in 2004 with six photoconductive detectors surrounding one emitter all incorporated in the head unit [153]; later on they developed another handheld probe with oscillating mirrors so that it can scan an area of $15 \times 2 \ mm^2$ and applied the probe to the THz measurements of excised breast cancer tissues [154]. In 2011, Zomega presented battery-powered commercial handheld systems Micro-Z [155] and Mini-Z [156], which are sealed in a single box and can perform normal-incidence reflection measurement; they used them to measure biological liquid samples and excised porcine tissues in 0.1-1.6 THz. Recently Harris \textit{et al}. developed a THz portable handheld spectral reflection (PHASR)
scanner for fast THz imaging in 0.25-1.25 THz with a field of view of $12 \times 19 \, \text{mm}^2$ and it was applied in the assessment of burn wounds on a porcine model [157, 158].

THz handheld probes have the advantages of compactness and portability, and moreover, they have great potential to be utilised in medical areas for real-time and point-of-care assessment of living skin. Various regions on human body can be measured with a handheld probe, and the level of contact and the applied pressure are controlled by the operator instead of the subject. Consequently, the robustness and accuracy of THz handheld systems applied to in vivo skin measurements still require further improvements before they can be used by a clinical operator. Additionally, the application of THz sensing for in vivo skin assessment in a large and diverse population has not been studied. Therefore in this study, we presented a homemade handheld THz scanner, suitable for in vivo point-scan measurements on human skin, and utilised it to conduct the largest hitherto in vivo THz study (over 300 individuals) in an out-of-the-lab environment.

7.3 Biophysical Factors and Lifestyles

The properties and condition of the skin of an individual can be altered by various inherent biophysical factors and lifestyles, including the ethnicity, the Fitzpatrick skin type, sex, age, lifestyles and the anatomical body site [159].

Ethnicity mainly affects individual skin condition through the difference in skin tone induced by different levels of chromophores [160]. It has been reported that the natural skin hydration and the thickness of the stratum corneum are varied among ethnic groups, where in general the White and the Black ethnic group possess drier skin than the Chinese due to lower natural moisturizing factors in the SC [161, 162]. Also, darker skin tends to have thicker SC and more cell layers in the SC, which make it more resistant to damage [163]; but at the same time darker skin shows higher transepidermal water loss (TEWL) due to larger gland pore size [164]. The Fitzpatrick scale divides people into six different skin types based on their skin’s reaction to the sun, as shown in Table 7.1. This can be used as an effective indicator for differences in skin tone, since people from the same ethnic group may have variations in skin types [165, 166].
Table 7.1: A table showing the brief definition of the Fitzpatrick skin types, with information of the skin colour, eye colour, hair colour and the reaction to sun [165].

<table>
<thead>
<tr>
<th>Skin Type</th>
<th>Skin Colour</th>
<th>Eye Colour</th>
<th>Hair Colour</th>
<th>Reaction to Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Light Pale white</td>
<td>Light blue</td>
<td>Red</td>
<td>Always burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light green</td>
<td>Light blonde</td>
<td>Never tans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Light grey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>White Fair</td>
<td>Blue Green Grey</td>
<td>Blonde</td>
<td>Usually burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tans with difficulty</td>
</tr>
<tr>
<td>III</td>
<td>Medium White to olive</td>
<td>Hazel Light brown</td>
<td>Chestnut Dark blonde</td>
<td>Sometimes burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tans gradually</td>
</tr>
<tr>
<td>IV</td>
<td>Beige olive Moderate brown</td>
<td>Brown</td>
<td>Light to medium brown</td>
<td>Rarely burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tans easily</td>
</tr>
<tr>
<td>V</td>
<td>Brown Dark brown</td>
<td>Medium to dark brown</td>
<td>Dark brown</td>
<td>Very rarely burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tans very easily</td>
</tr>
<tr>
<td>VI</td>
<td>Very dark brown to black</td>
<td>Dark brown</td>
<td>Black</td>
<td>Never burns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Always tans</td>
</tr>
</tbody>
</table>

The correlation between sex and skin hydration and SC thickness is not as clear, where the impact on skin induced by this factor is often outweighed by other individual variations [167–169]. Though some researchers found that males have higher TEWL, which is explained by their skin being more damaged from their exercise habit and lack of skin care [167].

Some studies reported that the skin hydration level has a negative correlation with age as a result of the decreasing natural moisturizing factors in skin [167, 168], while others presented opposite results [170], due to diverse ways of grouping the participants into different age groups. For example, Man et al. showed that the forearm skin hydration for males between the age of 0-50 years old increased with age, with the highest skin hydration observed in the age group of 36-50 years old, and then the hydration decreased significantly for
males over 70 years old [168]. While in [170], their subjects were divided into two groups with the younger group in the age of 20-24 years old and the elder group in the age of 60-68 old years, and they found slightly higher epidermal hydration in the elder group compared to the younger group.

The volume of daily fluid consumption is a main parameter to look at when studying the relationship between lifestyle and skin condition. Boelsma et al. reported a weak positive correlation between daily fluid intake and the SC hydration level [171]. Some studies showed that an additional water intake on top of individuals’ regular diet on a daily basis for over 30 days would result in an increase in the SC hydration [172, 173].

7.4 Methods

7.4.1 Experimental Setup and Protocols

Ethical approval has been acquired from the Biomedical Scientific Ethics Committee, BSREC, (REGO-2018-2273 AM03) prior to the study. Participants were asked to roll up their sleeves upon arrival to let their volar forearm acclimatize to the ambient room conditions. Then they were provided with an information sheet (including details of the protocol of the study) and their consent of participation was acquired. Participants were then asked to fill out the questionnaire, which included questions regarding their biophysical factors, lifestyle and details of any relevant skin conditions or medical conditions. Table 7.2 presents a list of the main categories involved in the questionnaire regarding the participants’ biophysical factors and lifestyles along with the number of participants in each category. 19 participants who failed to input their correct measurement IDs in the questionnaires have been excluded from this study, since their THz measurements can not be associated with their personal information without the correct measurement IDs. After the aforementioned procedures, a microscopic image was taken on the region of interest on the participant’s right volar forearm with a portable microscopic camera to keep record of the skin surface condition, for example the skin tone and visible dryness, of the region of interest, which can be complementary to the information acquired from the questionnaire. The entire study was conducted over four days at the same location and it is possible that some participants have been measured repeatedly on different days.
<table>
<thead>
<tr>
<th>Category</th>
<th>No. of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>103</td>
</tr>
<tr>
<td>Male</td>
<td>192</td>
</tr>
<tr>
<td>Prefer not to tell</td>
<td>3</td>
</tr>
<tr>
<td><strong>Year of Birth</strong></td>
<td></td>
</tr>
<tr>
<td>2000 – 2004</td>
<td>118</td>
</tr>
<tr>
<td>1990 – 1999</td>
<td>123</td>
</tr>
<tr>
<td>1980 – 1989</td>
<td>26</td>
</tr>
<tr>
<td>1970 – 1979</td>
<td>17</td>
</tr>
<tr>
<td>1956 – 1967</td>
<td>14</td>
</tr>
<tr>
<td><strong>Dominant Hand</strong></td>
<td></td>
</tr>
<tr>
<td>Right-handed</td>
<td>266</td>
</tr>
<tr>
<td>Left-handed</td>
<td>32</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>214</td>
</tr>
<tr>
<td>Chinese</td>
<td>20</td>
</tr>
<tr>
<td>Black</td>
<td>11</td>
</tr>
<tr>
<td>Other Asian</td>
<td>34</td>
</tr>
<tr>
<td>Other</td>
<td>19</td>
</tr>
<tr>
<td>Type I</td>
<td>19</td>
</tr>
<tr>
<td>Type II</td>
<td>59</td>
</tr>
<tr>
<td><strong>Fitzpatrick Skin Type</strong></td>
<td></td>
</tr>
<tr>
<td>Type III</td>
<td>132</td>
</tr>
<tr>
<td>Type IV</td>
<td>62</td>
</tr>
<tr>
<td>Type V</td>
<td>18</td>
</tr>
<tr>
<td>Type VI</td>
<td>8</td>
</tr>
<tr>
<td>Healthy skin w/o moisturizer</td>
<td>224</td>
</tr>
<tr>
<td>Healthy skin w/ moisturizer</td>
<td>18</td>
</tr>
<tr>
<td><strong>Skin Condition</strong></td>
<td></td>
</tr>
<tr>
<td>Dry skin w/o moisturizer</td>
<td>43</td>
</tr>
<tr>
<td>Dry skin w/ moisturizer</td>
<td>10</td>
</tr>
<tr>
<td>Other skin conditions</td>
<td>3</td>
</tr>
<tr>
<td>Less than 1L</td>
<td>40</td>
</tr>
<tr>
<td>1L to 2L</td>
<td>121</td>
</tr>
<tr>
<td>2L to 3L</td>
<td>114</td>
</tr>
<tr>
<td>More than 3L</td>
<td>23</td>
</tr>
<tr>
<td><strong>Water Consumption</strong></td>
<td></td>
</tr>
<tr>
<td>No or more than 2hrs ago</td>
<td>201</td>
</tr>
<tr>
<td>Within the last 2hrs</td>
<td>97</td>
</tr>
<tr>
<td><strong>Coffee Consumption</strong></td>
<td></td>
</tr>
<tr>
<td>No or more than 2hrs ago</td>
<td>267</td>
</tr>
<tr>
<td>Within the last 2hrs</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 7.2: Main categories in the questionnaire and the number of participants in each category.
THz measurement was then taken on the same area for a continuous 60 seconds with the THz handheld scanner described previously in Section 3.2.2. As shown in Figure 7.1(a-b), the handheld scanner is built with a fibre coupled THz emitter and a detector from the TeraSmart THz-TDS spectrometer and arranged in a reflection setup with a 30° incident angle. A quartz sampling window serves to both align the THz light to the skin surface, and also to flatten the skin during a measurement. Two pressure sensors are attached next to the quartz window to ensure a steady and consistent force application to the skin during measurements [54, 55]. As shown in Section 3.2.2, the handheld scanner measures the dielectric properties of skin in excellent agreement with commercial THz systems, while also having enhanced stability due to its superior mechanical rigidity. This allows consistent alignment of the quartz window. The start of the measurement was triggered by the pressure sensors when the skin was in good contact with the quartz window, after which 240 THz scans were recorded during the 60-second measurement, which would allow us to see clear occlusion effect on skin [53]. In addition, a single measurement of air was taken as the reference signal and a single measurement of an identical quartz on top of the quartz window was taken as the baseline signal, as shown in Fig.2.3 in Section 2.4.1. Figure 7.1 shows a scene of the THz measurement during the in vivo study, where the researcher is using the handheld THz scanner to measure the participant’s volar forearm and the in-house software is displaying the real-time reflected THz signal, the contact pressure and the occlusion curve on the monitor.
7.5 Results

7.5.1 Individual Variations in Terahertz Response of Skin

In Chapter 5, the existence of individual variations in the initial skin condition have been noticed through the vertical distribution of the occlusion curves. Then in Chapter 6, a data analysis procedure has been proposed to extract the SC hydration and thickness from raw THz data. Therefore in this chapter, the study utilised the same method presented in Chapter 6 to extract the SC hydration and thicknesses of all the participants in the in vivo study and compared with their occlusion curve features (P2P and ΔP2P), as shown in Figure 7.2.

Figure 7.2(a) is a 3D scatter plot showing the distribution of the P2P, SC thicknesses and SC hydration among all the participants, and the correlation between each two of the three properties. These values are all sampled at 55 seconds into occlusion: The x-axis is the SC hydration in a range of 10%-40%; the y-axis is the SC thickness in a range of 10-30 µm; and the z-axis is the P2P. The projections on the three surfaces illustrate the correlation between each
two properties. Here the sampled P2P value appears to have a strong linear correlation with the SC hydration and the SC thickness, where smaller P2P associates with higher SC hydration and smaller SC thickness. The correlation between the P2P and the SC hydration is in line with the theory and our observations in the previous chapters. Another observation is the negative correlation between the SC hydration and the SC thickness, which shows that within the entire cohort we measured, participants with higher SC hydration tend to have smaller SC thickness.

Figure 7.2(b) presents the distribution of $\Delta P2P$ among individuals with varied SC hydration and thicknesses. The value of $\Delta P2P$ is calculated by the difference of the P2P at 2 seconds and at 55 seconds into occlusion. Similar to P2P, $\Delta P2P$ also exhibits a negative linear correlation with SC hydration and SC thickness. However, the strength of this correlation with $\Delta P2P$ is weaker than the correlation with P2P, and this could be due to the uncertainty in the starting time of the occlusion for each individual.

![Figure 7.2: 3D scatter plots showing the distribution and correlation of (a) the P2P versus the SC thickness and hydration and (b) the $\Delta P2P$ versus the SC thickness and hydration for all the participants. The P2P, SC hydration and SC thickness are sampled at 55 seconds into occlusion.](image)

### 7.5.2 Impact of Sex, Age and Dominant Hand on Skin Properties

As introduced in the previous Section 7.3, SC surface hydration and SC thickness can be influenced by various biophysical factors and lifestyles. This section studies the influence induced by sex, age and dominant hand.

Figure 7.3 presents the extracted SC hydration and thicknesses as a function
of the measurement time (2-60 seconds) for all the female participants (103 people) and all the male participants (192 people). From Figure 7.3 it can be observed that although the male participants group shows a slightly higher mean value in the SC hydration and SC thickness compared to the female participants group, the error bars of the two groups largely overlap with each other during all the measurement time. Student’s t-tests were performed and results showed that $p > 0.05$ for SC hydration and SC thickness throughout the entire measurement time, therefore it can not be confirmed that there is any significant difference in the skin properties induced by the different sexes. Similar results have also been reported in [159], which stated that the correlation between skin hydration and sex is usually outweighed by individual variations.

Figure 7.3: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between female participants (103 people) and male participants (192 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

Figure 7.4 shows the extracted SC hydration and thicknesses as a function of the measurement time for participants who were 18-22 years old (born in 2000-2004), 23-32 years old (born in 1990-1999) and the rest of the participants who were 33-66 years old (born in 1956-1989). There are 118, 123 and 57 participants in the aforementioned age groups respectively. It is observed from Figure 7.4(a) that in general older participants show higher SC hydration during the entire measurement, where the group of participants aged 33-66 years old have higher mean SC hydration than the group aged 23-32 years old and the group aged 18-22 years old have the lowest mean SC hydration. In
Figure 7.4(a), the error bars of the standard error of the mean (SEM) show clear separation between the 18-22 years old group and the other two groups, with slight overlapping between the 23-33 years old group and the 33-66 years old group. In Figure 7.4(b), there are some differences in the mean SC thickness between the three age groups, but the SEMs are all overlapping with each other. One-way analysis of variance (ANOVA) tests followed by Tukey’s Honest Significant Difference tests were performed, where $p > 0.05$ for all the pairwise comparisons of SC thickness and the results for SC hydration are presented in Figure 7.5.

Figure 7.4: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between participants aged 18-22 years old (118 people), 23-32 years old (123 people) and 33-66 years old (57 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

Figure 7.5 shows that there are significant pairwise differences ($p < 0.05$) between the age groups of 18-22 years old and 33-66 years old during most of the measurement time up to 40 seconds; significant pairwise differences ($p < 0.05$) only occur between the 18-22 years old group and the 23-32 years old group at 8 time points around 5, 14 and 22 seconds; and there are no significant pairwise differences ($p > 0.05$) between the 23-32 years old group and the 33-66 years old group. Therefore it can be concluded that participants aged 33-66 years old show significantly higher SC hydration that participants aged 18-22 years old during the first 40 seconds of measurement. Man et al. reported in [168] that within a group of 328 male participants in the age of 0.5-94 years old, the SC hydration on their forearm measured by a corneometer showed that participants within the age groups of 36-50 and 51-70 years old have higher SC.
hydration compared to those in 13-35 and 0-12 years old.

Figure 7.5: Results of performing one-way ANOVA tests and Tukey’s Honest Significant Difference tests on the SC hydration results of the different age groups. Here y-axis is the p value and x-axis is the measurement time. The solid lines represent the pairwise comparisons between each two groups as shown in the label. The black dashed line indicates where $p = 0.05$.

Results of the extracted SC hydration and thicknesses for participants with different dominant hands are presented in Figure 7.6, with the left-handed group (32 participants) shown in red solid lines and patches and the right-handed group (266 participants) in blue. It can be observed from Figure 7.6(a) that the two groups have similar mean SC hydration with the SEM error bars fully overlapping with each other. In Figure 7.6(b), the left-handed group show a larger mean SC thickness compared to the right-handed group during the entire measurement, but the SEM error bars are strongly overlapping between the two groups. Furthermore, Student’s t-tests showed that $p > 0.05$ for SC hydration and SC thickness between the two groups during the entire measurement time, indicating no significant impact on the skin properties is induced by different dominant hands.
Figure 7.6: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between the left-handed group (32 people) and the right-handed group (266 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

7.5.3 Impact of Ethnicity and Skin Tone on Skin Properties

This section studies the correlation between individuals’ initial skin properties and their ethnicity and skin tone. Here three main ethnic groups within our recruited participants are investigated: the Black ethnic group (11 participants), the Chinese ethnic group (20 participants) and the White ethnic group (214 participants). From Figure 7.7(a) it can be seen that the White ethnic group have comparable mean SC hydration with the Chinese ethnic group with fully overlapping SEM error bars, while the Black ethnic group show the lowest mean SC hydration with the error bars slightly overlapping with the other two groups. In Figure 7.7(b), the White ethnic group and the Black ethnic group show identical mean SC thickness with fully overlapping error bars, while the Chinese ethnic group appear to have smaller mean SC thickness with the error bars strongly overlapping with the Black ethnic group and slightly overlapping with the White ethnic group. Results from one-way ANOVA tests and Tukey’s Honest Significant Difference tests showed that $p > 0.05$ for all the pairwise comparisons, which means that there are no significant differences in skin properties between the three ethnic groups.
Fitzpatrick skin type is a recognized tool for numerically categorizing human skin tone and skin’s response to the sun [165]. Under this scheme, participants in the White ethnic group with different skin tones were divided into several skin types including type I, II, III, IV. For further investigation, 78 participants in the White ethnic group with type I and II skin are compared to the other 136 participants with type III and IV skin. It is then observed in Figure 7.8 that the type I-II group have slightly higher mean SC hydration and smaller mean SC thickness compared to the type III-IV group, with strong overlapping in the SEM error bars for the SC hydration and slight overlapping for the SC thickness. Results from Student’s t-tests suggested that $p > 0.05$ for SC hydration during the entire measurement and SC thickness for most of the measurement time except for 3 time points around 6 and 25 seconds into the measurement where $p < 0.05$. Therefore, we can not draw a conclusion on whether different Fitzpatrick skin types (or different skin tones) have an impact on the initial skin properties. However, this shows that even within the same ethnic group, people might have different Fitzpatrick skin types (or different skin tones) which might lead to some possible variations in the SC thickness.
Figure 7.8: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between participants in the White ethnic group who have type I or type II skin (78 people) and those who have type III or type IV skin (136 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

Within the entire cohort, there are 78 participants with type I-II skin, 194 participants with type III-IV skin and 26 participants with type V-VI skin. As shown in Figure 7.9, the three groups with different skin types have overall identical mean SC hydration with fully overlapping SEM error bars, but their SC thicknesses are different with the type I-II group having the largest mean SC thickness and the type V-VI group having the smallest. One-way ANOVA tests and the Tukey’s Honest Significant Difference tests were then performed on the SC thickness and significant pairwise differences ($p < 0.05$) were only found at 25 seconds into measurement for type I-II and type III-IV groups, and at 18 and 25 seconds into measurement for type I-II and type V-VI groups. Therefore, the statistical significance of the impact of Fitzpatrick skin type (or skin tone) on individual’s skin properties is still unclear.
Figure 7.9: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between participants who have type I or type II skin (78 people), type III or type IV skin (194 people) and those who have type V or type VI skin (26 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

7.5.4 Impact of Lifestyle on Skin Properties

Another aspect of factors we are interested in is participants’ lifestyles, including their daily water consumption, coffee consumption and exercise routine, and how these factors affect the participants’ initial skin hydration and thickness.

In our questionnaire, the question regarding participants’ daily water consumption asks the typical amount of water the participant consumes in one day with options of several volume ranges. Within the entire cohort, there are 40 participants who drink less than 1 litre of water per day, 121 participants who drink 1 to 2 litres, 114 participants who drink 2 to 3 litres, and 23 participants who drink more than 3 litres of water per day. As shown in Figure 7.10(a), the groups that drink 1 to 2 litres and 2 to 3 litres of water per day show identical and also the highest mean SC hydration compared to the other two groups, and the group that drink more than 3 litres have the lowest mean SC hydration. In Figure 7.10(b), the group that drink more than 3 litres of water per day have the largest mean SC thickness, while the other three groups show similar mean SC thickness. However, according to the results from One-way ANOVA tests and the Tukey’s Honest Significant Difference tests, there are no significant pairwise differences ($p > 0.05$) between any two groups for both the SC hydration and SC thickness.
Figure 7.10: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between participants who drink less than 1 litre of water per day (40 people), 1-2 litres of water per day (121 people), 2-3 litres of water per day (114 people) and those who drink more than 3 litres water per day (23 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

Participants have also been categorised according to whether they have consumed coffee or participated in exercises prior to the experiment. There are 97 participants who have drunk coffee within 2 hours before being measured, and 201 participants who have drunk coffee more than 2 hours before the measurement or haven’t drunk coffee at all. As shown in Figure 7.11, the group of participants that have drunk coffee within 2 hours prior to the experiment show higher mean SC hydration and smaller mean SC thickness compared to the other group, with clear separations between their SEM error bars. Therefore, Student’s t-tests were performed on the SC hydration and SC thickness of the two groups, and results in Figure 7.12 show that there are significant differences ($p < 0.05$) in the SC hydration and SC thickness between the two groups for most of the measurement time (84% and 83% of the measurement time for SC hydration and SC thickness respectively). This indicates that coffee consumption is likely to be a factor that affects participants’ initial skin hydration and thickness.
Figure 7.11: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between participants who have drunk coffee within 2 hours prior to the experiment (97 people) and those who haven’t drunk coffee or have drunk coffee more than 2 hours prior to the experiment (201 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.

Figure 7.12: Results of performing the Student’s t-tests on the (a) SC hydration and (b) SC thickness results of the different coffee drinking groups. Here y-axis is the p value and x-axis is the measurement time. The red solid line represents the pairwise comparisons between the two groups as shown in the label. The black dashed line indicates where $p = 0.05$.

Similarly for participants’ exercise routine, there are 31 participants who have exercised within 2 hours before the measurement, and 267 participants who have exercised more than 2 hours before the measurement or haven’t exercised. Figure 7.13 shows that the group of participants who have exercised within 2
hours before being measured have higher mean SC hydration and smaller mean SC thickness compared to the other group, with clear separations between their SEM error bars. As shown in Figure 7.14, $p < 0.05$ for SC hydration during the entire measurement and $p < 0.05$ for SC thickness for 67% of the measurement time. Therefore, it can be concluded that exercising has a significant impact on participants’ initial skin hydration and also possible impact on the stratum corneum thickness.

![Figure 7.13: The extracted (a) SC hydration and (b) SC thicknesses as a function of the measurement time compared between participants who have exercised within 2 hours prior to the experiment (31 people) and those who haven’t exercised or have exercised more than 2 hours prior to the experiment (267 people). The solid lines indicate the mean values and the coloured patches show the standard errors of the mean among all the individual results in each category.](image-url)
Figure 7.14: Results of performing the Student’s t-tests on the (a) SC hydration and (b) SC thickness results of the different exercise groups. Here y-axis is the p value and x-axis is the measurement time. The red solid line represents the pairwise comparisons between the two groups as shown in the label. The black dashed line indicates where $p = 0.05$.

7.5.5 Dynamic Hydration Profiles of Dermatitis Skin

Some of the recruited participants are reported to have dry skin conditions including eczema, dermatitis and psoriasis. For participants with dermatitis skin, some of them claimed in the questionnaire to have put moisturisers on their volar forearms (region of interest) prior to the experiment while some hadn’t. Therefore, this section aims at using the dynamic hydration profile introduced in Chapter 6 to visualise the skin hydration change in occlusion process for individuals with dermatitis skin compared with those with healthy skin.

Figure 7.15 shows the colourmaps of the dynamic hydration profiles of 3 participants with eczema conditions and hadn’t moisturised their volar forearm skin before experiment (Figure 7.15(a, d, g)), 3 participants with eczema conditions and had moisturised their volar forearm skin (Figure 7.15(b, e, h)) and 3 participants with healthy skin and unmoisturised (Figure 7.15(e, f, i)). According to the impact of different biophysical factors and lifestyles to individual’s initial skin conditions investigated in the previous sections, the 9 participants selected for analysis fall within the criteria of born in the year of 1990-2004, categorised as Fitzpatrick type IV, V or VI, drinking 1 to 2 litres of water daily and hadn’t drunk coffee or done exercises before the experiment.
Figure 7.15: The colourmaps of the dynamic hydration profiles of individuals who have dry skin conditions and didn’t apply moisturiser on skin prior to the experiment (a, d, g), individuals with dry skin conditions but applied moisturiser on skin prior to the experiment (b, e, h) and individuals who have healthy skin (no dry skin conditions) (c, f, i). Colours ranging from light yellow to dark blue represent the hydration range of 10% to 65%. The solid black lines are the contour lines of the hydration, and the dashed white lines indicate the boundary between SC and epidermis.

The colourmaps in Figure 7.15 visually display that participants with dermatitis and unmoisturised skin have the lowest initial hydration level at the skin surface (10-12%), and during the occlusion process the surface dryness tends to preserve indicating a low water accumulation rate when the skin is occluded. Those who had put moisturisers on their dry skin before the experiment display slightly higher SC surface hydration at the start of occlusion (13-15%) and the rate of water accumulation also slightly increases. Compared to the previous two groups, participants with healthy skin show the highest surface hydration level at 0 seconds into occlusion (19-20%) with the highest water accumulation rate where the SC surface hydration increases to around
30% after 60 seconds of occlusion. The SC thicknesses for the three groups are identical at the start of the measurement ($\approx 30 \mu m$), however the decrease in SC thickness due to the compression against the imaging window is relatively small for the group with unmoisturised dermatitis skin ($\Delta d=1-4 \mu m$) compared to the group with moisturised dermatitis skin ($\Delta d=7-8 \mu m$) and the group with healthy skin ($\Delta d=8-10 \mu m$). This is because for dermatitis skin, the epidermis is thickened and the water content in SC is low, resulting in a less deformable material [174].

7.6 Summary

In this chapter, the portable THz handheld scanner has been applied in a large scale THz in vivo study which has measured over 300 participants in an out-of-the-lab environment. With personal information acquired by the questionnaires, the diverse set of participants have been categorised into groups according to their different biophysical factors and lifestyles, including sex, age, dominant hand, ethnicity, Fitzpatrick skin type, water intake, coffee consumption and exercise routine. The impact of these biophysical factors and lifestyles on participants’ stratum corneum hydration and thickness has been studied. Results show that age difference, drinking coffee and exercising have significant influences on individual’s initial skin properties, while the influences from other factors are not as clear. Dynamic hydration profiles of dermatitis skin with and without moisturisers have also been retrieved and compared with healthy skin. This study is the largest hitherto in vivo THz study which has collected a large data set of THz response in a diverse population and has demonstrated the prospective application of in vivo THz sensing for fast diagnosis of skin conditions. By investigating into the variation of skin properties with individuals’ different biophysical factors and lifestyles, this study provides insight and experimental support on the types of factors that need to be considered for future studies on human skin hydration with in vivo THz-TDS.
Chapter 8

Summary and Future Work

In this thesis, THz spectroscopy techniques are investigated and applied for biomedical applications including THz thin-film sensing and THz in vivo evaluation on skin. This thesis mainly focuses on developing THz characterization methods as well as exploring the potentials of THz-TDS for in vivo skin evaluation. This chapter will provide a summary of the key content and findings of each chapter and present some possible ideas for future work building on the thesis.

8.1 Summary of Thesis

Chapter 1 introduced the background of THz light, THz generation and detection and THz spectroscopy. Main applications utilizing THz techniques were summarised, among which THz techniques for thin-film sensing and in vivo assessment for biomedical applications were reviewed in detail.

Then in Chapter 2, the fundamental theory of THz light propagating through media and the mathematical solutions for THz optical geometries were described. Parameter extraction methods for THz-TDS measurements in transmission and reflection geometries with bulk and thin-film samples were deduced, followed with introductions on a numerical characterization algorithm and the theoretical models of skin for THz in vivo studies.

Chapter 3 presented the experimental setups used in the studies of this thesis, including different THz systems and complementary optical systems.

In Chapter 4, the study proposed an approach of customising multilayer structures for sensitive THz thin-film characterization. A sandwich structure was chosen based on simulation results and utilised for characterizing thin-film
aqueous glucose solutions with different concentrations. Experimental results showed enhanced contrast in the sample-reference ratios which results in higher sensitivity compared to conventional THz measurement geometries.

Chapter 5 started to investigate in applying THz-TDS for in vivo monitoring of human skin condition after the treatment of transdermal drug delivery (TDD) patches. Changes in the hydration level and occlusion effect of skin were quantified by the normalised relative change in the spectral features given by THz pulses. THz imaging and 3D camera imaging were also performed to visualise the spatial distribution of skin hydration and surface roughness of the skin.

Chapter 6 was also based on THz in vivo skin assessment of the effect induced by TDD patches, but the dynamic hydration profile of skin was provided instead of the normalised relative change. The absolute values of the stratum corneum surface hydration and thickness were extracted through a skin modelling approach, which involved effective medium theory combined with a stratified media model of the skin. This study developed THz in vivo skin evaluation further by successfully associating skin hydration profile with THz spectroscopic data.

Finally, Chapter 7 involved a large-scale THz in vivo skin study conducted in an out-of-the-lab environment with a portable THz handheld scanner, and this chapter researched into the variation of individuals’ skin hydration profiles with their biophysical factors and lifestyles. Results showed that biophysical factors and lifestyle including age, coffee consumption and exercise had significant impact on the skin hydration profile, while influences from sex, dominant hand, ethnicity, skin tone and water intake were not as clear.

8.2 Future work

8.2.1 Multimodal Study on Skin Hydration

In Chapter 6, the dynamic hydration profile of skin was extracted through a skin modelling approach with the use of a stratified media model. The model for skin hydration with dependency of the depth in skin used in the stratified media model was adopted from the results given by in vivo confocal Raman spectroscopy [70]. The skin hydration profile acquired with THz-TDS was retrieved through model fitting, which was an indirect method and dependent on the accuracy of the model as well as the fitting procedure. Therefore, it
is meaningful to involve other techniques that can measure skin hydration in vivo into the THz in vivo study, such as confocal Raman spectroscopy, in order to compare the skin hydration results with those acquired by the modelling approach from the THz spectroscopic signal. In this way, we can validate the skin modelling approach and improve the accuracy of the model.

8.2.2 THz Non-contact Skin Measurement

As introduced in Chapter 1 and then presented in the study in Chapter 5, the occlusion effect of skin is an inevitable phenomenon as long as the living skin is in contact with the imaging window during THz in vivo measurement. In this thesis, in vivo studies were performed with the occlusion effect taken into consideration through data processing approach. However, if researchers want to remove the occlusion effect from the experimental stage, then THz in vivo measurement of skin needs to be developed into a non-contact manner.

THz non-contact measurements have been achieved in measuring static non-living samples or samples that have a uniform shape [47, 175]. However, when measuring skin in vivo on a living subject, there will be challenges including difficulties in aligning the THz beam. Without an imaging window to place and flatten the skin region as well as aligning the THz beam, it is difficult to control the movement of a living subject and even a tiny movement can affect the reflected THz signal notably; the irregular shapes of different regions of the skin also make THz alignment challenging. Therefore, in order to develop THz non-contact measurements for in vivo skin evaluation, there should be a more advanced platform to accurately control the position of the THz spectrometer for the THz beam to be aligned on the skin, as well as an algorithm to track the tiny motions of the living subject in order to update the position of the THz spectrometer.
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