Thermal and Spectral Imaging in a Clinical Environment

Károly-Géza Keresztes
(BSc)

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Space Research Centre
Department of Physics and Astronomy
University of Leicester
“Never surrender, never give up.”

– Jason Nesmith, Galaxy Quest (1999)
Abstract

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by Károly-Géza Keresztes

There is growing clinical interest in non-invasive techniques to diagnose disease - these include breath analysis, cardiovascular and imaging. The latter consisting of both Thermal infrared (TIR) imaging where the distribution of body heat at the skin can be measured, and spectral imaging where pigments within the skin can be detected. Recent developments in imaging technology are allowing both TIR and spectral imagers to achieve good spatial resolution.

Such techniques have been implemented at the Leicester Royal Infirmary (LRI) within the Diagnostics Development Unit (DDU). The majority of studies to date using these techniques have used a controlled laboratory environment. In this work the utility and practicality of both TIR imaging and hyperspectral imaging in a real clinical environment were investigated. Based upon previous work appropriate measurement and calibration protocols were developed. The clinical utility of using the available DDU imagers and protocols was investigated via ethically approved TIR studies of fever and sepsis patients and hyperspectral studies of kidney, liver and skin lesions patients. This work was conducted in the Accident and Emergency Department at the LRI and other appropriate clinical units within the University Hospitals of Leicester NHS trust.

TIR imaging studies of fever, sepsis patients showed an ability to detect significant temperature abnormalities both in terms of actual values and also in terms of their spatial distribution. This includes detection of mottling pattern which may be diagnostic of progression towards sepsis. Hyperspectral imaging showed some signatures associated with liver disease, particularly within the sclera of the eye. The possible confounding effect of tanning in hyperspectral imaging of the skin was investigated. It is clear that further development is required for practical utilisation of the hyperspectral technique within the clinical environment. The main output of this work has been to develop protocols for use of the two techniques.
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## Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>A&amp;E</td>
<td>Accident and Emergency</td>
</tr>
<tr>
<td>CAD</td>
<td>Computer aided design</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge coupled device</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CRF</td>
<td>Case report form</td>
</tr>
<tr>
<td>CSV</td>
<td>Comma separated values</td>
</tr>
<tr>
<td>DDU</td>
<td>Diagnostic Development Unit</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>EMCCD</td>
<td>Electron multiplier charge coupled device</td>
</tr>
<tr>
<td>FLIR</td>
<td>Forward looking infrared (also the name of an infrared imaging company)</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>FPA</td>
<td>Focal plane array</td>
</tr>
<tr>
<td>fps</td>
<td>frames per second</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width half maximum</td>
</tr>
<tr>
<td>IDL</td>
<td>Interactive data language</td>
</tr>
<tr>
<td>IFOV</td>
<td>Instantaneous field of view</td>
</tr>
<tr>
<td>II index</td>
<td>Intensity-intensity index</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>LRI</td>
<td>Leicester Royal Infirmary</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of diet in renal disease</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NETD</td>
<td>Noise equivalent temperature difference</td>
</tr>
<tr>
<td>NIR</td>
<td>Near infrared</td>
</tr>
<tr>
<td>OD</td>
<td>Optical depth</td>
</tr>
<tr>
<td>OO</td>
<td>Ocean Optics</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>Partial pressure of CO₂</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PGP</td>
<td>Prism-grating-prism</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SAM</td>
<td>Spectral angle mapper</td>
</tr>
<tr>
<td>SARS</td>
<td>Severe acute respiratory syndrome</td>
</tr>
<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>StO₂</td>
<td>Tissue oxygenation</td>
</tr>
<tr>
<td>SVM</td>
<td>Support vector machine</td>
</tr>
<tr>
<td>TIR</td>
<td>Thermal infrared</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>VIS</td>
<td>Visual</td>
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Publications

Conferences


Peer-reviewed publications

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Chapter 1  Introduction

1.1.  Aim

The aim of this thesis is to investigate the utility of thermal and hyperspectral imaging in a clinical environment.

The detailed aims of this work were to:

(1) Derive appropriate measurement protocols for use of hyperspectral and thermal infra-red measurements in real clinical environments namely the Accident and Emergency Department at Leicester Royal Infirmary and other appropriate clinical units within the University Hospitals of Leicester NHS Trust.
(2) Adapt standard protocols for instrument calibration for use in (1) above.
(3) Demonstrate the utility of instrumentation available and protocols via analysis of measurements with real patients with a variety of conditions.
(4) Draw conclusions and outline recommendations for future work.

For (3) fever and sepsis, kidney and liver conditions along with skin lesions were investigated. (3) also included obtaining ethics permission.

1.2.  Diagnostic Development Unit

Medical diagnosis is often a challenging task. Often the symptoms and signs are non-specific and the doctors tend to use a systematic diagnostic method to try to identify the cause of the problem. This is usually achieved by the process of deduction, elimination and testing that are based on either medical history or probabilities of a condition based on the circumstances. Many of today’s practices involve invasive methods for obtaining a diagnosis which can prove to be time consuming, can cause discomfort for the patient and can lead to complications e.g. infection. Consequently, use of non-invasive methods is preferred to get rid of the undesired effects of investigation(s).

In 2008, a collaboration between Medicine, Atmospheric Chemistry, Physics and Astronomy at University of Leicester and the University Hospitals of Leicester started with the aim of researching non-invasive methods for medical diagnosis. This partnership is called the Diagnostic Development Unit (DDU) (Sims et al., 2010).
The technique applied in the DDU are the same basic principles that have been used in medicine for hundreds of years where physicians use their sense of smell, touch and sight to give a diagnosis. For example in the 1800s they would smell the patient’s breath and wounds to determine the rate and direction of the healing progress. Similarly, a patient’s colour and pulse would inform about the disease state. Whilst these practices are still in use today, the difference is that the doctors are aided by state of the art technology that can emulate the doctor’s senses and go beyond the human sensitivity capabilities while keeping the procedures non-invasive.

In the DDU, the doctor’s sense of smell is aided by breath analysis techniques that look at the breath composition (volatile organic compounds) of the patient via mass spectrometry to establish potential presence of disease. The touch aspect of their diagnosis is aided by cardiovascular monitoring, looking for disease markers related to cardiovascular function. Finally, the author’s main focus is the doctor’s visual aid, their sight, or vision that is assisted via imaging of the patient, looking for both spectral and heat signatures associated with disease.

The Diagnostic Development Unit is currently running a pilot study of 500 patients, investigating the top 20 most common complaints in the Accident & Emergency Department of the Leicester Royal Infirmary (LRI) applying the abovementioned tools in a real time clinical setting.

1.3. The doctor’s sight
In the scenario of a doctor utilising imaging technology to establish the wellbeing of a patient, there are several key factors that will determine the outcome of this task. The first one is establishing the system that is under consideration. Is it a passive or active? An active system is referring to a system that emits electromagnetic radiation, whilst a passive system is in need of interaction with radiation to activate. In terms of a human body, the radiation of heat from the body can be regarded as an active system, compared to visually observing the colour and healing of a patient’s wound or skin that would need the presence of appropriate illumination to enable useful observation and hence is passive under these definitions.
Another factor is using appropriate tools and methods for making observations. With the patient and the doctor in mind, the tool for observing the thermal signature from the body is with a thermal camera and the spectral signature is via the use of a continuous, broad light source to illuminate the (body) surface whilst imaging with hyperspectral and multispectral cameras. The key is making sure that none of the image acquisition modes affect the results of the other whilst keeping the environmental effects to a minimum, along with suitable clinically compatible measurement protocols.

1.4. Physics of light, vision and imaging

Looking closer at the interaction between light and media, the following scenario is considered. When photons of wavelength $\lambda$ with energy $E$ (Tipler and Mosca, 2004), where

$$E = \frac{hc}{\lambda}, \tag{1}$$

$h$ is Planck’s constant and $c$ represents the speed of light, are illuminating a surface, whether it is a solid, liquid or gas, it undergoes one or more of the following processes;

- Reflection ($R$),
- Absorption ($\alpha$)
- Transmission ($T$),

where the three are related via Equation 2 (FLIR Systems, 2013a).

$$1 = T + R + \alpha \tag{2}$$

Each of these processes are important when trying to determine the composition of a material as they all are associated with the physical properties of the material and its surface. The material’s effectiveness in reflecting radiant energy is called reflectance. In most cases, light will hit the surface, scatter, depending on the wavelength of the light source, the size and structure of the surface. The penetration depth of a medium can be defined as $-\mu_a L$ as described in equations 3-4.
\[ T = e^{-\mu_a L} \]  

\[ \mu_a = -\frac{1}{L} \frac{\partial T}{\partial L} \]  

where \(-\mu_a\) is the absorption coefficient (cm\(^{-1}\)) and \(L\) is the mean free path. In other words, the signal in the medium falls off exponentially with each incremental path length of \(\partial L\) (Jacques, 2013).

The scattering processes in the skin are attributed to the skin layer’s structures, composition and properties and can thus vary from tissue to tissue (Jacques, 2013). The reduced scattering coefficient is a combination of scattering coefficient \(\mu_s'\) and the anisotropy \(g\) (directional dependence) of skin and the contribution of Rayleigh and Mie scattering in skin for different reduced scattering values of a generic tissue is shown in Figure 1.1 (Jacques, 2013).

![Figure 1.1: The reduced scattering coefficient \(\mu_s'\) for a generic tissue with variable contributions from \(\delta_{\text{Rayleigh}}\) and \(\delta_{\text{Mie}}\) scattering. The blue lines are contributions from Mie scattering only, while the red lines have varying contributions from Rayleigh scattering, as Mie scattering is fixed at 20 cm\(^{-1}\) (Jacques, 2013).](image)

Rayleigh scattering originates from particles and/or surfaces that are significantly smaller in size comparable to the wavelength of the incident light whilst Mie scattering is caused
by comparable or larger size particles/surfaces. The light measured from the illuminated skin is a result of scattering through the skin and absorption within the skin by compounds with particular absorption features (chromophores), see Section 1.5.2.

When light finally reaches the observer from having undergone the abovementioned processes, an intensity $I$ can be measured and compared to the original intensity $I_0$. This signal can be thought of as a spectrum representing the physical properties of the medium under observation, thus reflectance $\rho(\lambda)$ is expressed as,

$$\rho(\lambda) = \frac{I_0(\lambda)}{I(\lambda)}.$$  

In human vision, visual light, 400-780 nm, reaches the lens of the eye and is then focused onto the retina, where the signal is then converted by photoreceptors into neural signals that the brain processes as an image. The eyes spectral sensitivity, just like a camera sensors sensitivity varies with wavelength and peaks at 420.7 nm for blue (B), 530.3 nm for green (G) and 558.9 nm for red (R), with a large full width half maximum (FWHM) (Stockman and Sharpe, 2000). These three light cones are analogous to RGB filters in colour imaging, where filters are used to represent the cones, camera optics (a combination of focusing lenses) are used to focus the radiation onto the sensor that processes the signals.

The thermal spectral range for observing human body temperatures can be found by applying Wien’s displacement law (Tipler and Mosca, 2004), Equation 6.

$$\lambda_{max} = \frac{b}{T}$$

where, $T$ is temperature in Kelvin and $b$ is Wien’s displacement constant 2.898 × 10⁻³ mK. The law calculates the wavelength where a blackbody, a perfect absorber/emitter, that peaks according to Planck’s law (Tipler and Mosca, 2004), Equation 7.

$$B_\lambda(\lambda, T) = \frac{2hc^2}{\lambda^5} \frac{1}{e^{\frac{hc}{\lambda kT}} - 1}$$
where, $B_\lambda(\lambda, T)$ is the spectral radiance of a body, $k_B$ is Boltzmann’s constant. The total power emitted by the blackbody per unit area is given by Stefan-Boltzmann law (Tipler and Mosca, 2004), Equation 8.

$$P = \varepsilon \sigma T^4$$  \hspace{1cm} (8)

where, $\varepsilon$ is emissivity, in case of a blackbody $\varepsilon = 1$, $\sigma$ is Stefan-Boltzmann’s constant, $\sigma = 5.67 \times 10^{-8}$ Wm$^{-2}$K$^{-4}$.

The implication of equations 6-8 and measurements is that if the surface of the human body is 32°C, the peak emission occurs at 9.5 µm and the spectrum looks like a blackbody, with an emissivity of 0.98±1. Uncooled thermal cameras have a peak response between 7-14 µm, making them perfect tools for detecting human body surface temperatures (FLIR Systems, 2013a).

### 1.4.1. Hyperspectral and multispectral imaging

In remote sensing amongst other fields, cameras are used with many different type of filters or spectral dispersion in attempt to determine different object’s composition and thus reflectance. Each filter, having specific spectral width, records an intensity signal that is summed over a wavelength band. If the number of spectral bands in the camera are >100 and have small spectral width (<5 nm FWHM), they are usually referred to as hyperspectral cameras, whereas the definition of multispectral cameras could be any type of filter width, but usually in much smaller numbers and/or larger spectral width (>10 nm).

There are two main types of spectral imaging systems of which both have been used in this thesis. One that scans the spatial direction, pushbroom, collecting a full spectrum for each spatial pixel or one that collects a full spatial image but scans through a series of spectral bands, step-stare as seen in Figure 1.2.
Figure 1.2: Two of the most common types of spectral imaging systems are utilised in this thesis and are frequently used in remote sensing. They are the pushbroom (typically hyperspectral), spatial scanning, and the ‘step stare’ (typically multispectral), spectral scanning, imagers (Vagni, 2007).

In the pushbroom type of system the speed of the mirror determines the dwell time for each pixel, while the dwell time for the step-stare is determined by the exposure time. However, this also means that longer exposure times can be set, thus increasing the signal to noise ratio (Vagni, 2007).

1.4.2. Thermal imaging
Thermal imaging has recently gained a lot of popularity as the technology has improved and reduced significantly in cost and size (Diakides et al., 2012). There are two types of thermal imaging systems, cooled and uncooled. While the cooled systems are significantly more stable, than uncooled systems, the uncooled cameras are significantly cheaper and more portable. Recent years, uncooled systems have become available that are no larger than a mobile phone and cost only a few hundred pounds compared to 10 years ago where a similar type of system would be at least 10 times larger and would cost 100 times more.
1.4.3. Imaging patients

The aforementioned imaging tools hence would be of potential use to a doctor when examining a patient. As light interacts with chromophores in the skin, see Section 1.5.2, the doctor would be able to determine the skin chromophore composition via the use of spectral imaging systems. Similarly, the thermal signature of the skin surface can be measured with the help from a thermal camera by looking for thermal patterns.

1.5. Skin structure and functions

The imaging work described here views the outer surface of the body i.e. the skin. The characteristics of the skin along with the effects of some disease states are described below.

Skin can be thought of as a physical barrier or interface between the body and the environment (Lai-Cheong and McGrath, 2009; Venus et al., 2011; Houdas and Ring, 1982). It is the body’s means of keeping nutrients in and keeping it protected from outside interference. A basic structure of the skin and its functions can be found in Figure 1.3 below.

![Figure 1.3: Basic physiology of the skin as described by Venus et al. (2011) as a two layer medium.](image)
The skin can be thought of as being composed of two distinct layers, the epidermis and dermis. The epidermis, thickness varies across the body but typical values of ~100-150 µm thick (Young, 1997), contains melanocytes and keratinocytes that provide the protection of the skin as a barrier and via the production of melanosomes, but as a consequence are also responsible for the apparent skin colour (Tseng et al., 2008). The dermis, ~2-4 mm thick (Young, 1997), contains the vascular network that provides the tissue with nutrition and disposal of waste (Young, 1997; Venus et al., 2011; Angelopoulou, 2001). The consequence of blood transport via the vascular network is the temperature regulation in the body. Heat is produced in the body via different mechanisms and mediated via blood supply. Therefore another function of the skin is to act as a mediator for maintaining thermal homeostasis. In order for the body to maintain a constant core temperature of around 35.5-37.7°C (Jones and Plassmann, 2002) for a typical healthy person, heat generated needs to be balanced out by the heat lost. Consequently the skin temperature is determined by rate of heat exchange between the environment and the body (Jones, 1998). In a healthy body, heat is produced via metabolism, muscle activity and other mechanisms and is transported to the different parts of the body via the blood stream. The vascular network has its own thermoregulation and is easily fine tuned via vasodilation (expansion of vessels) or via vasoconstriction (tightening of the vessels). Other mechanisms that help maintaining heat is via goose pimples that are a consequence of hair raising up and trapping insulating layers of air.

1.5.1. Tissue oxygenation

Perfusion is a process that relies on the heart to generate a sufficient cardiac output to maintain the delivery of nutrients and oxygen to tissue (Wilson, 2013). Normal metabolism in a body relies on effective supply of oxygen to tissue via haemoglobin (McLellan and Walsh, 2004). Once the oxygen has been delivered in the capillaries, the deoxygenated haemoglobin returns back to the heart to restart the perfusion process.

1.5.2. Skin classification and colour

As previously described, skin colour is a consequence of light interacting with skin via absorption, reflection and remittance. The molecules responsible for the colours observed are known as chromophores. A skin classification was developed by Thomas B
Fitzpatrick in 1975 that differentiates between different skin colours and their response to sun exposure (Fitzpatrick, 1988).

The classification has over time expanded into its current form of six different skin types class I-VI as seen in Table 1 (Young, 1997). The skin types are based on a skin type score (Australian Radiation Protection and Nuclear Safety Agency, 2014) that takes the overall skin appearance into account, genetics such as hair and skin colour, but also environmental effects, such as sun exposure and tanning habits.

<table>
<thead>
<tr>
<th>Skin type</th>
<th>Susceptibility to sunburn</th>
<th>Constitutive skin colour</th>
<th>Tanning ability</th>
<th>Susceptibility to skin cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High</td>
<td>White</td>
<td>Very poor</td>
<td>High</td>
</tr>
<tr>
<td>II</td>
<td>High</td>
<td>White</td>
<td>Poor</td>
<td>High</td>
</tr>
<tr>
<td>III</td>
<td>Moderate</td>
<td>White</td>
<td>Good</td>
<td>Low</td>
</tr>
<tr>
<td>IV</td>
<td>Low</td>
<td>Olive</td>
<td>Very good</td>
<td>Low</td>
</tr>
<tr>
<td>V</td>
<td>Very low</td>
<td>Brown</td>
<td>Very good</td>
<td>Very low</td>
</tr>
<tr>
<td>VI</td>
<td>Very low</td>
<td>Black</td>
<td>Very good</td>
<td>Very low</td>
</tr>
</tbody>
</table>

Table 1: Fitzpatrick skin type classification based on the melanin pigmentation and skin's reactivity to ultraviolet radiation exposure (Young, 1997).

1.5.2.1. Melanin

The main chromophore that is responsible for the abovementioned classification, photoprotection and the darker skin tone, is melanin – produced in melanosomes. It is not necessarily the concentration of melanin that gives the darker skin tone, but rather the level of activity of the melanocytes (Karsten et al., 2013). There are two types of melanin; sulphur containing, reddish pigment called pheomelanin and sulphur free, black-brown pigment called eumelanin (Sardar et al., 2001; Angelopoulou, 2001). The most abundant of these is pheomelanin, which is present to varying concentrations between healthy individuals, while eumelanin is only present in those that have a certain genetic trait (Angelopoulou, 1999). Typical molar extinction spectra (absorption spectra) of pheomelanin and eumelanin are found in Figure 1.4.
1.4. Typical molar extinction spectra of the two types of melanin present in human skin are pheomelanin, a reddish sulphur containing pigment, and the sulphur free eumelanin (Prah, 2012c).

1.5.2.2. Haemoglobin

Haemoglobin is found in two forms; the oxygenated haemoglobin (HbO₂) present in the arteries with a bright red colour (Randeberg, 2005), and the dark red, blueish coloured deoxygenated haemoglobin (Hb) present in the veins. The molar extinction spectra for these are found in Figure 1.5. Since haemoglobin absorbs light, oxygen saturation levels can be found by the ratio of oxygenated blood to the total amount of blood present (Karsten et al., 2013) to give an indication of tissue perfusion and thus information about health.
1.5.2.3. Bilirubin and beta carotene

Other visible chromophores in the skin are bilirubin and beta carotene also contained in the dermis. Bilirubin is breakdown product (waste) of haemoglobin which occurs mainly in the liver (Randeberg, 2005) and has a yellow colour. Similarly, beta carotene also has a yellow colour, but is a residue from consumption of fruit. The molar extinction spectra for beta carotene and bilirubin are displayed below in Figure 1.6.
Figure 1.6: Typical molar extinction spectra of beta carotene and bilirubin (Prahl, 2012a,b).
1.5.2.4. Urea

While the liver is responsible for metabolising toxic substances, the kidneys are responsible for filtering these substances and one of them is urea. While urea is transparent in the visible part of the spectrum as reported by (Filutowicz et al., 2004) and seem to only possess absorption features below 200 nm for certain urea-water solutions. Dhinaa and Palanisamy (2010) suggests an absorption feature can be found in urea by adding different reagents. The urea was prepared using the Berthelot method. Urease enzymes were added to the urea-water solution and incubated to produce ammonia. Further to the solution, phenol and hypochlorite reagents were added and incubated to produce the spectrum found in Figure 1.7.

Figure 1.7: The visual absorption feature of urea with the presence of different reagents as found by Dhinaa and Palanisamy (2010).

1.5.3. Signs of disease

In disease, the body attempts to respond to the abnormality via repair functions. In some disease states, the effects of impaired function can be found by examining the absorption spectra of chromophores to see if their concentration can reveal anything about the body’s health state due to changes that may be induced by the disease state. Others can be found via abnormal temperature distributions.

1.5.3.1. Skin lesions and cancer

While skin has its own photoprotective mechanism against ultraviolet radiation (UVR), via activation and production of melanin, as seen from the strong absorption features in Figure 1.4, chronic, acute and repeated exposure UVR still penetrate beyond the
protection of melanin and causes damage to DNA (Bickers, 1982). Absorption of UVR in DNA causes structural changes in cells (Halliday et al., 2008; Young, 2006) that are either prohibited from replicating or attempted to be repaired to form photolesions. If cellular replication occurs before the repair, the result is a mutation of the original cell that can result in skin cancers (Young, 1997; Wolf et al., 2001; Young, 2006; Halliday et al., 2008; Sklar et al., 2013). Malignant melanoma tends to often be brown or black in colour. The reason behind this is that it begins in the melanocytes (ACS, 2013) that may still produce melanin. Colour variations may occur depending on the activity of the melanocyte. Other more common skin cancers begin in the keratinocytes (Young, 1997).

In 1985, an identification rule was devised to aid diagnosis and raise awareness for the public about melanoma (Rigel et al., 2010). This rule was initially called the ABCD rule; where A is for asymmetry, benign moles in general are considered spatially symetric in contrast to melanomas (Skincancer.org, 2015a); B stands for border, where benign moles have smooth and even borders, contrasted to melanomas that tend to have edges that are scalloped or notched (Skincancer.org, 2015b); C is for colour, while bening moles tend to have one colour, melanomas tend to be a mixture of different shades of brown, tan, black, red, white or even blue (Skincancer.org, 2015c); D is for diameter, as benign moles tend to be smaller than melanomas (<6 mm), although if the melanoma is detected at an early stage this rule would be violated (Skincancer.org, 2015d). It was later discovered that an addition of E for evolving needed to be added to the rule, as a change in size, shape, colour, symmetry and other factors could be an indicator for early detection of melanoma (Skincancer.org, 2015e; Rigel et al., 2010).

Cancerous lesions have also been found to be warmer than surrounding healthy skin (Gurjarpadhye et al., 2015) as they have increased metabolism and perfusion compared to healthy skin, providing necessary nutrition for the lesion to grow (Herman, 2013; Bonmarin and Le Gal, 2014).

1.5.3.2. Liver function
The liver plays a key role in metabolising carbohydrates, fats and proteins. When a liver disease is present, the metabolic functions are disrupted and as a consequence, the metabolic waste product, bilirubin, an end-product of haeme catabolism, is found
accumulating in blood and tissues (Higgins, 2010). Jaundice is the yellow discoloration of the sclera and skin caused by bilirubin (Dooley et al., 2011).

1.5.3.3. Kidney function

The main function of kidneys is filtration of blood and waste products. One of these products is urea, a waste product that is given off when the liver filters protein. A gold standard way of measuring kidney function is via estimated glomerular filtration rate (eGFR), obtained via blood sample (Cole et al., 2012; Zhang et al., 2013). One of the most common approaches for measuring eGFR is via modification of diet in renal disease (MDRD) method by looking at the creatinine levels and the patient characteristics. The eGFR estimated by MDRD (mL/min/1.73m²) is found by Equation 9.

\[
\text{Male: } 170 \times (sCr)^{-0.999} \times (\text{age})^{-0.176} \times (sU)^{-0.170} \times (sAlb)^{0.318} \\
\text{Black Male: } \text{MDRD} \times 1.180 \\
\text{Female: } \text{MDRD} \times 0.76 \\
\text{Black female: } \text{MDRD} \times 0.762 \times 1.180
\] (9)

Where sCr is serum creatinine, sU is serum uric acid, sAlb is serum albumin.

eGFR values categorises 5 different stages of chronic kidney disease (CKD) found in Table 2 below.

<table>
<thead>
<tr>
<th>CKD Stage</th>
<th>Description</th>
<th>GFR (mL/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Kidney damage with preserved GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Kidney damage with mildly decreased GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Moderately reduced GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Severely reduced GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Kidney failure/end-stage renal disease</td>
<td>&lt;15 (or dialysis)</td>
</tr>
</tbody>
</table>

Table 2: The different stages of chronic kidney disease as described by Cole et al. (2012).

1.5.3.4. Tissue oxygenation and thermal response

When blood supply or perfusion is impaired, cardiac output is decreased and the body’s response is to reduce blood flow via vasoconstriction (Wilson, 2013; Cheatham et al., 2008), resulting in peripheral cooling, but also in reduction in concentration of
oxygenated haemoglobin present in the outer extremities. Oxygen transport represents the balance between the oxygen delivery and the oxygen consumption. In states of malperfusion, oxygen demand is exceeded by the oxygen supplied and if the imbalance is sustained it will lead to organ dysfunction and failure (Cheatham et al., 2008).

1.5.3.5. Fever
Fever is an elevated temperature above the constant core temperature of around 35.5-37.7°C (Jones and Plassmann, 2002). It is caused by a disturbance in the body’s thermoregulatory system and is a net effect of pyrogenic (heat producing) substances present in blood that get released at the presence of a bacterial infection or inflammation of non-bacterial origin (Houdas and Ring, 1982).

1.5.3.6. Sepsis
Systemic inflammatory response syndrome (SIRS) is diagnosed when two or more of the four following criteria are met (Bone et al., 1992; Soong and Soni, 2012):

- Body temperature below 36°C or above 38°C
- Heart rate is above 90 beats per minute
- Respiratory rate is 20 per minute or PaCO₂ is less than 32mmHg
- An alteration in white blood cell count is greater than 12000/mm³ or count less than 4000/mm², or the presence of more than 10 percent immature neutrophils “bands”.

Sepsis is the response to a SIRS infectious process (Ortiz-Ruiz et al., 2004). Severe sepsis is associated with one or more signs of acute organ dysfunction, hyper perfusion or hypotension (Soong and Soni, 2012). Septic shock is the presence of systemic hypotension and impaired end-organ perfusion, even after adequate fluid resuscitation (Maggio, 2013; Ortiz-Ruiz et al., 2004; Bone et al., 1992).

1.5.3.7. Shutdown – “systemic vascular resistance”
Early stages of shock is manifested by decrease systemic vascular resistance and increased cardiac output, leading to a “warm shock” (Bone, 1991; Cheatham et al., 2008). At later stages a “cold shock” is present, where the cardiac output decreases and there is
inadequate blood flow to tissue leading to severe organ dysfunction and even death (Bone, 1991).

1.5.4. **Clinical environment vs lab environment**

When dealing with acutely ill patients, it is crucial that a protocol is devised patient centric that can maximise efficiency of the data acquisition at minimal risk and discomfort to the patient. On occasion, equipment can fail or other things can go wrong, but cannot be corrected in real time as it could delay patient treatment or cause discomfort to the already ill patient. Therefore, it is important to have a plan of action in case something does go wrong, but it also means that as the researcher, there are not many variables around the environment that can necessarily be controlled and can in some cases only be recorded for later consideration during the analysis stage.

One of the major problems with this is however the standardised illumination in hospitals. As of 2009 (Kanter, 2009; The Institution of Lighting Professionals, 2011) fluorescent lights are illuminating most offices and public areas, including hospitals. These lights have spectra that are not broad or continuous and would in most cases not make hyperspectral/multispectral imaging possible in the visible range, as they would over expose certain wavelength bands and could potentially interfere with thermal image acquisitions as well due to direct illumination, and indirect reflections that may cause heating. Such heating could be of the order 0.1-0.2°C as investigated at a distance of 1 meter of a fluorescent light tube (51.3°C) and the net result, depending on application, may be significant. Another illumination issue is associated with windows that would transmit sunshine through. This compromises spectral data because of the rapid variation in sunlight intensity and spectrum over the course of a day with introduction of clouds and other weather changes. These variables can however be controlled for in lab or semi lab environment.

1.6. **Clinical setting: imaging instruments**

For the patient data acquisition, described in this thesis, a hyperspectral, a multispectral and two thermal imagers were employed.
The hyperspectral imager is a pushbroom scanning mirror system. While the imaging spectrograph on the hyperspectral imager collects the full spectral information, the mirror scanner collects the spatial component as a function of time. The advantage of this system is that it has a broad spectrum, 400-1000 nm, but the limiting factor is the spatial resolution and the acquisition time, see Section 3.5.

The multispectral imager, SpectroCAM, is a step-stare type of imager with a filter wheel that can store up to 8 custom filters for the wavelength range of 400-1000 nm. The advantage of this is that the spatial resolution is higher than the hyperspectral imager; it can be focused in real time and allows for larger signal to noise measurements at the cost of spectral information and potential degradation in the spatial component if a movement is present.

The thermal infrared cameras, FLIR SC620 and FLIR T650sc, are uncooled microbolometer cameras. They are portable and have high spatial and thermal resolution that can detect temperature signatures from the human body surface. Their use is limited by the imaging environment, the set up and assumptions made of the patient.

1.7. Thesis organisation
Chapter 2 is a short introduction to thermal and spectral imaging in clinical environment and gives a brief introduction to the analysis techniques applied in this work. Chapter 3 discusses the imaging technologies, their calibration and the importance of illumination. Chapter 4 consists of protocol and analysis development for two thermal infrared imaging studies. Chapter 5 describes the protocol development and the results for the spectral imaging studies and the thesis finishes with conclusions and future work in chapter 6.
Chapter 2  Overview and examples of previous work

2.1.  Introduction

The theory of electromagnetism is a relatively modern concept. At the time of Newton, there was an ongoing argument in trying to establish the particle wave duality nature of light. Newton’s theory suggested that light was a particle that can get absorbed, reflected and transmitted. He found that if white light, approximately 400-700 nm (Tipler and Mosca, 2004), interacts with a prism, the light refracts into 7 groups of colours; red, orange, yellow, green, blue, indigo and violet (Newton et al., 1718). The rival theory was proposed by Huygens suggesting that light had a wave nature that travelled at finite speed. These theories were then combined by Fresnel (Huygens et al., 1900) with a mathematical formulation and supported by Young’s double slit experiment in 1803.

Three years before Young’s experiments, in 1800 the astronomer Sir William Herschel conducted an experiment that discovered infrared radiation. He was diffracting sunlight using a prism much like Newton had, but he had a thermometer monitoring the room temperature. He noticed that the thermometer’s temperature increased significantly over a period of time even though they were placed on a table with no visible light components illuminating it (Herschel, 1800). It is this infrared radiation that Professor Robert Wood, from John Hopkins University in 1910 used to create the first infrared photograph with the help from infrared sensitive film (Ring, 2010; Wood, 1910).

The initial development incentive of the infrared technology came with the rising military concerns of the Second World War. It was realised that tanks and missiles and other infantry could be detected without the aid of visual light by looking for the presence of thermal radiation. It was however not until the Vietnam War that the development flourished with the help from Common Modular FLIR (Forward Looking Infra-Red), led by the US Army Night Vision Laboratory (Kruse, 2001).

From the initial military applications that focused on thermal signatures of missiles, tanks and airplanes, thermal infrared imaging has been introduced in many different commercial applications (Peach, 2013). Other uses are manufacturing of different mechanical components of vehicles, monitoring cooling efficiency and chemical processes, building inspections and medical research.
2.2. Thermal infrared imaging medical application to date

In 1930s, James D Hardy conducted a series of experiments with the aim of determining different characteristics of human skin. He found (Hardy and Muschenheim, 1936; Hardy, 1934) that human skin has very similar emissive capabilities as those of a perfect blackbody and $0.95 \leq \varepsilon_{(\lambda)\text{skin}} \leq 0.99$. This value for white skin at 40°C has since been refined (Jones, 1998) to be $0.97\pm0.02$ at a wavelength range of 3-14 µm and for black skin to be $0.98\pm0.01$ for the wavelength range of 3-12 µm. By knowing the emissivity of the surface of the object being measured, it is possible to determine the surface radiation that is emitted by the object, as described by Equation 8. Thus, by knowing the emissivity of the human skin it became possible to determine the surface temperature of that skin and this application of thermal imagery is known as infrared thermography (Jones, 1998).

This technique has now been applied in a number of medical applications; see Table 3, taken from Diakides et al. (2012).

<table>
<thead>
<tr>
<th>Thermal infrared imaging applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncology (breast, skin, etc.)</td>
</tr>
<tr>
<td>Pain (management/control)</td>
</tr>
<tr>
<td>Vascular disorders (diabetes, deep vein thrombosis)</td>
</tr>
<tr>
<td>Arthritis/rheumatism</td>
</tr>
<tr>
<td>Neurology</td>
</tr>
<tr>
<td>Surgery (open heart, transplant, etc.)</td>
</tr>
<tr>
<td>Ophthalmic (cataract removal)</td>
</tr>
<tr>
<td>Tissue visibility (burns, etc.)</td>
</tr>
<tr>
<td>Dermatological disorders</td>
</tr>
<tr>
<td>Monitoring efficacy of drugs and therapies</td>
</tr>
<tr>
<td>Thyroid</td>
</tr>
<tr>
<td>Dentistry</td>
</tr>
<tr>
<td>Respiratory (allergies, SARS)</td>
</tr>
<tr>
<td>Sports and rehabilitation medicine</td>
</tr>
</tbody>
</table>

Table 3: Utility of thermal infrared imaging for medical applications as described by Diakides et al. (2012).
As seen from Table 3, thermal infrared imaging has been applied in a wide range of medical applications. Specific applications that are associated with the work in this thesis are those associated with fever and infection detection. The current protocols in place measuring fever or looking for infectious disease, like H1N1 flu virus or severe acute respiratory syndrome (SARS) (Diakides et al., 2012; Mercer and Ring, 2009; Ring, 2011; ISO, 2009) all rely on measurements of the inter canthus in the eyes, a region whose temperature is close to the core body temperature. In this work, in collaboration with Naseer (2013), a different region of interest (ROI) was investigated with the aim to detect a thermal signature pattern difference between patients with fever, infection and sepsis in a clinical environment.

Diakides et al. (2012), identified a number of important areas associated with thermal imaging. These are:

- IR camera systems designed for medical diagnostics
- Advanced image processing
- Image analysis techniques
- High-speed computers
- Computer-aided detection (CAD)
- Knowledge-based databases
- Telemedicine
- Effective clinical use
- Protocol-based image acquisition
- Image interpretation
- System operation and calibration
- Training
- Continued research in the pathophysiological nature of thermal signatures
- Quantification of clinical data

In this thesis, the author is addressing the effective clinical use, protocol based image acquisition, system operation and calibration, and image interpretation.
2.3. Thermal infrared imaging analysis

The data output from thermal infrared imagers are typically in form of colour coded images, where each pixel is associated with a temperature and is displayed as an intensity plot with n×m pixels. Analysis of these images is based around statistical analysis, correlation with physical features and where applicable image processing.

All statistical analysis was completed with GraphPad Prism version 6.04 for Windows, and the relevant statistical analysis techniques included are:

- Shapiro-Wilk test
- Paired and unpaired t-test
- Brown-Forsythe test
- Analysis of variance (ANOVA)

Shapiro-Wilk test relies on a null hypothesis stating that the data is sampled from a Gaussian distribution. If the p-value from the test is less than the threshold \( \alpha \), the null hypothesis is rejected (Goos and Meintrup, 2016). The \( \alpha \)-value can be thought of as the level of significance, and is normally, by convention, set to a value of 0.05, representing the 95% confidence interval and thus the equivalent level of 2\( \sigma \) values on a normal distribution.

Student t-test was used to test two normally distributed samples against each other to determine if they are from the same population (Mould, 1998). The null hypothesis assumes that the samples are from the same population, thus if the p-value at a given level of significance is statistically significant i.e. \( p-value < \alpha \)-value, the null hypothesis is rejected. An unpaired test is assuming independence between each measurement, while for the paired test the two samples are dependent.

The null hypothesis for the Brown-Forsythe test assumes that the compared group variances are equal. This assumption is crucial for the use of ANOVA, which is a multiple sample > 2 ‘t-test’ type, as it is relying on the samples being normally distributed and having the same group variance. If the p-value is statistically significant, the null
hypothesis must be rejected (Goos and Meintrup, 2016) and the use of ANOVA should be avoided.

(ANOVA) relies on F-statistics, where F-statistics is defined as between-group variance over the within-group variance (Winter, 2015). “One way ANOVA” determines how likely the F-values are, which is indicated by a p-value. If multiple comparisons are made, the p-values are adjusted based on the family comparisons made, reducing the type one error (incorrect rejection of the null hypothesis), but also makes it harder to reach a level of statistical significance. Just like for student t-test, the level of significance can be divided into groups. GraphPad Prism’s convention when denoting significance level is found in Table 4 below.

<table>
<thead>
<tr>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.05</td>
<td>Ns (not significant)</td>
</tr>
<tr>
<td>≤0.05</td>
<td>*</td>
</tr>
<tr>
<td>≤0.01</td>
<td>**</td>
</tr>
<tr>
<td>≤0.001</td>
<td>***</td>
</tr>
<tr>
<td>≤0.0001</td>
<td>****</td>
</tr>
</tbody>
</table>

Table 4: The convention that GraphPad Prism, version 6.04 for Microsoft Windows, applies when denoting significance level. Starting from not significant (ns) and finishing with four stars to denote extremely significant. Note however that the null hypothesis of the sample being from the same population is rejected at p≤0.05.

2.4. Spectral and hyperspectral medical applications

This technique has been rapidly developing within the timescale of this thesis work. A few examples of hyperspectral applications include studies on cancers, cardiac disease, ischemic tissue, skin burn, retinal disease, diabetes and kidney disease (Lu and Fei, 2014). More specific examples include application of multispectral/hyperspectral imaging and spectroscopy in: classifying people’s emotional state based on tissue oxygenation (StO2) (Chen, 2012); search and rescue, where algorithms were developed to find a characteristic skin signature, colour and model the sensor output (Nunez, 2009); skin lesion classification and detection of cancers (Lihacova et al., 2013; Ogorzałek et al., 2011); scleritis, where a differentiation can be made between different eye conditions (Bannister et al., 2014).
As eluded to above, a key parameter in health and hence used in diagnosis is tissue oxygenation. Haemoglobin (both oxygenated and deoxygenated) has strong spectral absorption peaks in the UV-visible band (Anderson and Parrish, 1981) which can be measured using point light sources and the level of oxygenation inferred from the absorption at the key wavelengths. The measurements currently used clinically are point measurements normally via some device clipped or stuck onto a finger or hand, for example the Inspectra StO2 monitor (InSpectra, 2015) or via probes measuring one spot only. Such devices, consisting of a light source, normally make a spectral measurement using a combination of appropriate narrow band filters. Deep tissue oxygenation measurement requires some separation between the light source and spectrometer input to ensure only deeply scattered light is detected. Hyperspectral imaging allows mapping of tissue oxygenation in terms of peripheral (near surface) oxygenation (as mentioned above). Various people for example (Meglinski and Matcher, 2003; Chen, 2012) have attempted to predict the characteristic absorption of haemoglobin in tissue and/or predict the measured oxygenation using Monte-Carlo modelling. Such models tend to be approximate either in assumptions made about the scattering and absorption processes, or in terms of short cuts taken to limit computation time by assuming “beams” of photons which undergo the processes simultaneously in concert, or in terms of simplifying the skin structure for example by assuming plane parallel structures. As mentioned in chapter 1, skin has a complicated 3D structure and hence currently it is difficult to match measurements made with hyperspectral or multispectral imagers with real clinical situations. More realistic simulations are necessary even though this may require more computer time and the possible use of supercomputer techniques. This is the subject of active research (Yuen, 2013; Sims, 2013) and is outside the scope of work described here. In the work reported here the haemoglobin absorption features are detected by hyperspectral imaging but are not interpreted in terms of actual tissue oxygenation measurements due to lack of time and the emphasis of the work is on developing appropriate measurement protocols.

2.5. Spectral analysis techniques

Principal Component Analysis (PCA) is a technique used for feature extraction and dimensionality reduction of data. Via the use of eigenvectors and its eigenvalues, it makes an orthogonal transformation (Schölkopf et al., 1998) of the coordinate system in which
the principal components (features), the highest ranked eigenvalues according to their significance, have the most information associated with the dataset (Smith, 2002). Once the principal components have been identified, the lesser significant components can be discarded (dimensionality reduction) as they do not contribute to the overall covariance of the data.

A technique for determining similarities between spectra is spectral angle mapper (SAM), which treats each spectrum as a vector and finds the angle between them in a multidimensional space (Navalgund and Ray, 2011). This technique is useful when there is a library of known spectra that a measured spectrum can be compared to.

Supervised spectral unmixing is a hyperspectral tool that makes use of prior knowledge of the spectral composition of the signal (Keshava and Mustard, 2002), usually a library of spectra, and decomposes it into distinct spectra known as endmembers. Another type of unmixing method is the unsupervised spectral unmixing that aims to detect endmembers directly without user interaction. These methods are commonly used in conjunction with SAMs.

Another method is classification of spectra via machine learning. Support vector machines (SVM) (Cristianini and Shawe-Taylor, 2000) are classifiers that are commonly used when a large sample size is considered that are not normally distributed. Two types of datasets are needed for support vector machines to work: training data and sample data. Training data can be from the population of the sample data, but the more training data is fed into the algorithm, the more accurately can the SVM classify and distinguish the different data. It should be noted that large numbers >100-1000s or more are required, which is impractical for proof of concept work.

None of the abovementioned tools are applied within the scope of this thesis. These methods are, as alluded to above, usually applied to large samples of data where there is an interest to determine the composition of the different spectra and compare them to known spectra, values or simulations. In this thesis, the interest is mainly in determining if the different protocols and imaging techniques are viable in a clinical environment, rather than performing a detailed diagnosis itself. The current protocols are a first step and are still in need of improvement for use of such analysis techniques. In future work,
once the protocols have been finalised and the set-up has been improved beyond a sample of 10 patients in each category, the analysis techniques above could be utilised to look for spectral compositions, feature extractions and in conjunction with SVMs classify the different spectra.

In this work, spatially defined regions of interest are chosen and spectral classification is based upon those regions of interest. They are then compared by looking at the variability of spectral reflectance/absorbance compared within the small sample groups. Due to the imaging acquisition issues and the resultant spectral reflectance of different regions of interests, there is an intensity difference between different ROI. These issues include reflectance correction, patient movement, noise, contamination or other effects, therefore, the spectra have been converted into mean centered spectra, by normalising each spectrum to its own mean.
Chapter 3  **Calibration and characterisation**

When dealing with any type of measurement, the first questions that should arise are related to how the measurement was made, what was measured, what the significance of it is, how accurate it may be and if it is reproducible. The only question out of these, that is not necessarily associated with calibration, is the significance of the measurement. Therefore it is vital to first understand the type of measurement an instrument would make, how well that instrument can reproduce that measurement and the accuracy of the reading before trying to understand the significance of the phenomenon measured.

This chapter is divided into three distinct parts. The first part is associated with thermal imaging equipment and their calibration. The second part is calibration of spectral imaging equipment and finally the third part is evaluation of measurement protocols.

### 3.1.  Thermal imaging equipment

Three different thermal cameras, FLIR SC620, FLIR T640 and FLIR T650sc, have been used in this thesis. A variety of cameras were used due to technical problems with the initial FLIR SC620 camera, which was replaced eventually with the FLIR T650sc. The major focus is however on the latter, FLIR T650sc, as it was utilised for the majority of the patient data collection. Each camera was calibrated using the methods in 3.3.1. The common features of these cameras are that they are all uncooled microbolometer cameras. Their full specification is found in Table 5 below.
Table 5: Camera specifications for the FLIR T650sc, T640 and SC620 (FLIR Systems, 2012; 2014; 2015). The accuracy of the detectors are quoted as ±2°C or ±2% (whichever is the largest) except for the FLIR T650sc, where within the temperature range of 15-30°C the accuracy is higher and is quoted as the largest of ±1°C or ±1%.

A clarification is needed at this point with regards to “IR resolution”. It is the terminology that the manufacturer uses for the total number of pixels in the focal plane array that produces the image.

### 3.2. Comparison of thermal imaging equipment

FLIR T650sc is a research instrument specifically designed to be operated in a room temperature environment with a higher accuracy window in the 15-30°C range compared to most uncooled infrared (IR) cameras that operate with a measurement accuracy of ±2°C or ±2%, whichever is largest. Unfortunately, the higher accuracy range does not fully cover the human body temperature range of 20-40°C, but is still an improvement compared to most competitors. The importance of this becomes apparent when considering that ±2°C or ±2% on the human temperature range in Kelvin, 293-313 K, has a significantly higher relative error than ±1°C or ±1%.

<table>
<thead>
<tr>
<th></th>
<th>FLIR T650sc</th>
<th>FLIR T640</th>
<th>FLIR SC620</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IR resolution</strong></td>
<td>640×480 pixels</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thermal sensitivity/NETD</strong></td>
<td>&lt; 30 mK @ +30°C</td>
<td>&lt;40mK @ +30°C</td>
<td></td>
</tr>
<tr>
<td><strong>Field of view (FOV)</strong></td>
<td>25° × 19°</td>
<td>24° × 18°</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum focus distance</strong></td>
<td>0.25 m</td>
<td>0.3 m</td>
<td></td>
</tr>
<tr>
<td><strong>Focal length</strong></td>
<td>25 mm</td>
<td>38 mm</td>
<td></td>
</tr>
<tr>
<td><strong>Spatial resolution (IFOV)</strong></td>
<td>0.69 mrad</td>
<td>0.68 mrad</td>
<td>0.65 mrad</td>
</tr>
<tr>
<td><strong>F-number</strong></td>
<td>1.0</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td><strong>Image frequency</strong></td>
<td>30 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Detector type</strong></td>
<td>Focal Plane Array (FPA), uncooled microbolometer</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Spectral range</strong></td>
<td>7.5–14 µm</td>
<td>7.5–13 µm</td>
<td></td>
</tr>
<tr>
<td><strong>Detector pitch</strong></td>
<td>17 µm</td>
<td>25 µm</td>
<td></td>
</tr>
<tr>
<td><strong>Object temperature range</strong></td>
<td>-40°C to +2000°C</td>
<td>-40°C to +500°C</td>
<td></td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>±1°C or ±1% for 15-30°C, otherwise ±2°C or ±2%</td>
<td>±2°C or ±2%</td>
<td></td>
</tr>
</tbody>
</table>
As can be seen from Table 5 the newer cameras, T650sc and T640, have better overall spatial resolution and thermal sensitivity than the older SC620. This is the result of improvement in the microbolometer technology and the mechanics used to maintain a high signal image by the reduction of noise or time dependent temperature drift that often is caused by a temperature change in the sensor and/or the circuit.

Another comparison can be made to equipment used by other researchers, Table 6 from (Vardasca, 2011) shows the improvement of three IR cameras over time. While the table does not contain the same level of detail as Table 5, the development of the microbolometer technology can be evaluated as the cooled sensor in FLIR SC7000 has the same level of accuracy as the uncooled bolometer in FLIR T650sc. Furthermore, the image resolution evolution can be seen, much like the improvement in sensitivity of the sensors.

<table>
<thead>
<tr>
<th>FLIR B2 Portable</th>
<th>FLIR A40 Thermovision</th>
<th>FLIR SC7000 Titanium</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR resolution</td>
<td>120×120 pixels</td>
<td>320×256 pixels</td>
</tr>
<tr>
<td>Thermal sensitivity/NEDT</td>
<td>1K</td>
<td>0.08K @ +30°C</td>
</tr>
<tr>
<td>Detector type</td>
<td>Uncooled microbolometer</td>
<td>Cooled</td>
</tr>
<tr>
<td>Accuracy</td>
<td>±2°C or ±2%</td>
<td>±1°C or ±1%</td>
</tr>
</tbody>
</table>

Table 6: The camera characteristics of three different thermal infrared imaging systems (Vardasca, 2011).

### 3.3. Calibration

Thermal cameras, much like any measurement tool need to be calibrated to a known standard to make sure that the measurements taken can be reproduced and validated (Simpson et al., 2006). Therefore, part of the manufacturer process is that each camera is calibrated before delivered to the customer. The recommended rate of calibration for thermal imaging equipment is once a year as the internal mechanics of the shutter, responsible for the internal non-uniformity calibration, with respect to the camera system can change over time and provide offset readings, the circuits or sensor can degrade and other mechanical errors can occur that lead to uncertainty in measurement. While it is necessary for the manufacturer to make sure that the camera is properly maintained, there are some in-house calibrations that can be performed to characterise the camera system and to make sure that the accuracy of the equipment is still within the specifications given.
3.3.1. Quality assurance in thermography

The University of Glamorgan and the National Physical Laboratory (Plassmann et al., 2007) have developed a series of experiments and protocols that would give a first order of accuracy of the thermal equipment in use and indicate if a manufacturer calibration is necessary; outside or within the recommended one year calibration window. This protocol also provides insights to what type of measurements could and should not be made. Their investigated areas were: camera stability, human body thermal range test, uniformity tests, scene test, spatial resolution test and thermal resolution test. These tests were also made with easy to access materials making them affordable. The following sections, 3.3.1.1 to 3.3.1.5 are all based on the experimental procedures described by (Plassmann et al., 2007; Plassmann et al., 2011) unless otherwise stated. As a result of the work here, some modifications of these test protocols have been developed and are specified in the sections below.

3.3.1.1. Camera stability

When a camera is first initiated, there is a period of time that it needs to heat up and reach internal thermal equilibrium. Since the cameras used are uncooled microbolometer based imagers, they rely on measurement of resistances on the FPA which it converts into temperatures. If there are internal fluctuations of temperature, the measured image might drift, even with the activation of the internal shutter calibration. FLIR camera manual suggests waiting 5 minutes between powering on the equipment and acquiring the first measurement (FLIR Systems, 2011; 2013b). The suggested protocol for measuring the camera stability proposes to image an ice-water bath at the melting point of ice.

The protocol specified preparations are as follows:

- Set up the experiment in a room with stable temperature
- Set the camera emissivity to that of ice, $\varepsilon = 0.99$
- Set the target distance to approximately 1m (the target is an insulated water container with 50% ice and 50% water)
- Let the camera acclimatise to the room temperature before turning it on. The acclimatisation phase should be at least one hour.
This acclimatisation phase is merely to ensure that the air inside the camera and camera components are acclimatised to room temperature.

Protocol specified method:
- Set the camera perpendicular to the water/ice container and switch the camera on.
- Stir the mixture frequently and acquire an image every five minutes, the first 30 minutes, and then one every 10 minutes for the remainder of 2 hours.
- Measure the values from a 10×10 pixel block.

The 10×10 pixel block results from this protocol have been plotted, in red, in Figure 3.1 and the full field of view of the surface have been included, in blue, as a reference. The error bars are associated with the spread of values covered in $3\sigma$, as it represents 99.7% of the data values (Walck, 2007).

![Graph showing measured ice temperature vs time](image)

**Figure 3.1:** The mean of the 10×10 pixels (red), and the mean of the full field of view has been included for reference (blue). Each error bar (I) represents the $3\sigma$, as it covers 99.7% of the data values present in the ROI. The mean of the full 2h, $\mu$ has been included to show the overall offset of the measurements and the $\mu_{(30-120)}$ shows the mean offset of the last 90 minutes.
Method revision 1:
The author found that acquiring more data points within the 2 hours would be beneficial, thus an image was acquired every 5 minutes for the duration of 2h and 5 minutes. Furthermore, the author found it more useful to make use of the full field of view (FOV) rather than 10×10 pixels in order to see the variation in the ice.

The results from the method revision 1 of the stability experiment are shown in Figure 3.2.

![Measured Ice Temperature vs Time](image)

Figure 3.2: The response of the cameras FLIR SC620 (green), T640 (red) and T650sc (blue) is shown as a function of time, when imaging the surface of ice-water mixture for 2h and 5 minutes as specified in method revision 1. The error bars, 1, plotted are showing the range of values for 3σ, μ is the mean and μ(80−125) is the mean for the range 80-125 minutes.

It is evident that the newer cameras take less time to reach stability than the SC620. T650sc and T640 seem to reach stability after the first 5 minutes compared to SC620 that
takes approximately 80 minutes. From the graph, the different averages, $\mu$, were found for the full duration of the experiment and for the range 80-125 minutes ($\mu_{(80-125)}$). These are quoted in Figure 3.2 accordingly.

Method revision 2:
While ice-water mixture is a cheap way of looking at an object with a relatively constant temperature, the author found, from this experiment and other experiments in this suite, that water does not provide a good uniform medium for any other temperature. Even the ice-water mixture becomes difficult to deal with as the relative ratios of ice and water become uneven as the room temperature heating effects start becoming noticeable. Furthermore, the experiment requires supervision and interaction from the experimenter and would be limited to a few hours at maximum before ice is fully melted, caused by the temperature gradient between the ice and the room temperature. Therefore, the author decided to redo the stability with the help from a Hyperion R, model 982 blackbody target ($\epsilon = 0.995$, $\pm 0.1^\circ$C) set to a temperature of 30.0°C, which is the middle of the expected range of human body skin temperature. The acquisition parameters were set to obtain an image every minute for 73 h and see how the stability varies as a function of time that imager has been operating for to determine if there are any negative effects of leaving the camera on for a prolonged period of time. The results are shown in Figure 3.3.
Figure 3.3: The top panel shows the thermal variation recorded every minute by the FLIR T650sc for the first 15 minutes of the 0th, 1st, 2nd and 4th hour. The bottom panel shows the first 15 minutes after 24, 48 and 72 hours. The error bar I represents the range of values at $3\sigma$ and $\mu$ is the mean for the displayed range.

From Figure 3.3 it can be concluded that the stability of the camera does not drop a significant amount from operating over a longer period of time, even at the rate of capturing one picture every minute for 73 hours. Also, the higher time resolution suggests that the T650sc reaches stability already after the first 2 minutes from powering on.

Comparing the T650sc results from the ice bath in Figure 3.2, and the blackbody in Figure 3.3, the results are very similar and it can be concluded that the ice-water mixture is a good reference source for measurements at melting point of ice. The benefit of using the ice-water bath is that it is cheap and can cover a large FOV. Compared to a blackbody, it is however labour intensive and can only be performed for as long as the ice-bath temperature can be maintained at $-0.0^\circ$C.
3.3.1.2. Human body thermal range test

The thermal range test is devised around the human body temperature range 20.0–40.0°C. The aim is to measure how accurately the thermal camera can reproduce the temperatures of the human body temperature range compared to a ITS90 standard thermometer and what the offset is for each measurement. The experimental results could act as a way of interpreting any temperature dependent local bias by the lens-sensor system and the ability to reproduce temperatures in the specific range.

Protocol specified preparation:

- Same initial set up as for the stability test, Section 3.3.1.1, except that the water bath is 40°C.
- Calibrated platinum resistance thermometer

Protocol specified method:

- Stir the bath for at least 1 minute (the author decided to stir continuously)
- Acquire simultaneous image and thermometer readings every 0.5°C
- Add cold water to speed up the cooling (the author added icy water)

The results from the experiment are found in Figure 3.4. When looking at the data points themselves, it is not as evident what the limiting factor of this experiment is. However, when considering the spread of values of each point found by including the 3σ, it is clear that the variation in recorded temperature is more influenced by the experimental design than the thermal camera hardware. This conclusion can also be drawn by comparing the error bars of each measurement in Figure 3.4 to those in Figure 3.2. Due to heat lost to environment, it is difficult to maintain a uniform water temperature as cooling is caused by not only convection but also conduction and evaporation.
Figure 3.4: The average values of the full FOV of the human body temperature range test have been included for each apparent 0.5°C temperature increment, as measured by an external thermometer, between 20.0-40.0°C as imaged by the FLIR T640. The error bars of the measurements represent the $3\sigma$ range (99.7%), as it demonstrates the variability of the values. This experiment does not seem to measure the camera’s ability to reproduce temperature (suggested by the large variations of values) within the specified range, instead it seems to show the variation of the water surface. To investigate this further, a revision needs to be made.

Method revision:
The author found the process of cooling water from 40.0-20.0°C takes a long time and the cooling is not uniform. Even with continuous stirring of the water, the variation of temperature was significant as measured by a platinum resistance thermometer. Therefore, the author decided to replace the water by the previously used blackbody radiator. This experiment was performed for both the T650sc and SC620. The results are found in Figure 3.5, where the T640 results are included as a reference.
Figure 3.5: The revised version of the protocol, imaging a blackbody target, for the two cameras FLIR SC620 (green) and T650sc (blue) show an increase of precision compared to the initial protocol for the T640 (red), where water surface was imaged. The level of accuracy of each depend on the camera calibration. The error bars of the measurements still represent the $3\sigma$ range (99.7% of the values).

As can be seen in Figure 3.5, the spread of values from T640 are significantly higher to those of T650sc and SC620. Not much can be concluded from the T640 results other than the efficiency of the experimental method; however the results for the T650sc suggest a high level of accuracy and precision, while the results for the SC620 suggest a high level of precision, but lower accuracy, namely an average offset of 1.28°C over the human body range. This is an important conclusion as it suggests to the level of calibration and reproducibility for the application for the human body temperature range.

3.3.1.3. Uniformity test
The aim behind the uniformity test is to look at the lens-sensor system and map how uniform the signal is. The resultant image can form a reference for flat fielding as part of...
data processing; removing artifacts, faulty pixels, optical defects or fixed pattern noise (Krupiński et al., 2013).

Protocol specified preparation:
- Same as the initial set up for the stability test, Section 3.3.1.1, except that the water bath is 23°C.
- Make sure that the water covers the full field of view.

Protocol specified method:
- Defocus the image to a maximum
- Stir the water for 30 seconds
- Acquire image of the water surface

Since no orientation specification was given in Plassmann et al. (2011) for the maximum defocusing, the author decided to defocus the image in both extremes to see the effect and make three measurements for each defocusing direction. The results were plotted as 3D images to emphasize the map structure, see Figure 3.6 for the +ve maps and Figure 3.7 for the –ve ones.

It is once again important to realise that the temperature maps obtained are influenced more from the temperature distribution of water than the sensor-lens system itself. It is also evident from Figure 3.7 that the extreme defocusing of the lens is exhibiting vignetting (Heavens and Ditchburn, 1991). Furthermore, even though the temperature was measured to be of the order 23°C, using a thermometer, the water surface and the cooling is not uniform, especially when the medium is of different temperature than the overall room temperature. This effect was also clearly noticeable in the human body temperature range experiment in Figure 3.4.

Method revision 1:
Repeat the experiment using room temperature water, which would reduce the heat loss effects from the environment and reduce the variation in the temperature map. These results are found in Figure 3.8 and Figure 3.9.
Figure 3.6: The datasets (a-c) for the maximum +ve defocused uniformity experiment, imaging surface water temperature of approximately 23°C, as specified by the first protocol show a lot of variation associated with the surface.
Figure 3.7: The datasets (e-f) for the maximum -ve defocused uniformity experiment, imaging surface water temperature of approximately 23°C, as specified by the first protocol show a strong vignetting effect.
Figure 3.8: The datasets (a-c) for the maximum +ve defocused uniformity experiment, imaging surface water temperature of approximately room temperature, still shows a large variation of the water surface.
Figure 3.9: The datasets (e-f) for the maximum -ve defocused uniformity experiment, imaging surface water temperature of approximately room temperature, still shows a strong vignetting effect.
While the amplitude of the variation has reduced for all the datasets, there are still fluctuations caused by uneven heat distribution and strong effects from the optics in its extreme settings. Therefore, a final modification will be made to the method.

Method revision 2:
Instead of relying on different medium of a certain temperature, the author decided to make use of the lens cap of the camera. Due to the short distances that are involved between the lens cap and the sensor, there was no need for defocusing as the lens cap is significantly below the minimum focusing distance and the sensor would not pick up any astray radiation that is caused by defocusing the lens into any of the extremes, thus the lens could be set into normal focus mode. Furthermore, the lens cap covers the full FOV of the lens and is already at equilibrium with the room temperature. After attaching the lens cap, the system was left to acclimatisate for 15 minutes and, the non-uniformity calibration was performed and an image was acquired, Figure 3.10. A second image was acquired by disconnecting the cameras ability to perform automatic non-uniformity calibration, and was left for 15 minutes before acquiring the image for Figure 3.11. Finally, a third image was collected straight after performing the internal non-uniformity calibration, Figure 3.12.

Figure 3.10: Uniformity temperature map after its initial acclimatisation phase of 15 minutes with the lens cap on and after non-uniformity calibration.
The gradient in Figure 3.10 could be caused by a structural non-uniformity in the lens cap, a temperature gradient present in the cap, a potential reflection from the internal mechanism or the lens-sensor system itself. This bias present is of the same order as the $3\sigma$ in the previous tests in sections 3.3.1.1 and 3.3.1.2.

![Uniformity test: (Lens cap)](image)

Figure 3.11: Uniformity temperature map of the sensor-lens system via imaging the lens cap after not calibrating the camera for 15 minutes shows an increase in temperature, but retains the same gradient as just after calibration.

While a temperature increase is present in Figure 3.11, with respect to Figure 3.10, the overall gradient seems to be kept, even if it is more pronounced.
Comparing Figure 3.12 to Figure 3.10, there are similarities and differences present. The temperature gradient across the field of view is more pronounced in the last figure and there is a slight overall temperature increase present. The reason behind the temperature increase is caused by trapped air between the lens and the cap that is heated via the operation of the camera. A possible explanation for the increase in temperature gradient might be that the trapped air is altering slightly the acclimatised environment, which in turn could lead to heating of the internal circuits. This explanation could be tested by performing the same experiments on a cooled camera, but is beyond the scope of this thesis. Other explanations could be that the lens cap is not uniform or there is a misalignment/tilt of the sensor with respect to the optics.

Comparing the two different methods for obtaining a map of the uniformity in the lens-sensor system, the revised version, of imaging the lens cap, is preferred as it has smaller environmental variability. The temperature map, in Figure 3.12, is also taken under normal camera optics operation, i.e. not defocused into the extremes. This is a necessity when maintaining integrity of datasets as defocused images not only show incorrect intensity values, Figure 3.6-Figure 3.9, but also introduces optical effects that in normal circumstances would not be present. A flat fielding correction could be performed at this point, but the author has decided not to do so, as the variability in the measurement is of
less than 0.1°C and the exact mechanism for what is causing the gradient in the experiment is not precisely known. Due to no obvious artifacts or faulty pixels being present, a flat fielding at this point would introduce a larger error than it would help reducing and is left to be investigated further outside the scope of this thesis.

3.3.1.4. Scene test

Some infrared cameras have a tendency to introduce a systematic error caused by the presence of large temperature gradients across the field of view of the camera. Thus, if there are two objects with radically different temperatures, especially uncooled thermal infrared cameras tend to be unable to read the correct temperature due to the thermal flooding from the scene (Plassmann et al., 2007). It is therefore important to check how the camera behaves within the human body temperature range.

Protocol specified preparation:

- Set up the experiment in a room with a stable temperature
- Set the camera emissivity to $\varepsilon = 0.99$
- Set the distance to $\approx 1$ m from the target (a container with room temperature water)

Protocol specified method:

- Set the camera perpendicular to the container
- Stir the water in the container and acquire image
- Add a container to the field of view with 38°C water and stir both containers
- Acquire image with both in the field of view

The results from the abovementioned method are found in Figure 3.13. The mean of the room temperature water surface ~21.7°C exhibited no significant change with the inclusion of a 38°C water container into the field of view. Therefore, the author decided to investigate this further by altering procedure slightly.
Figure 3.13: Left image shows the results from the room temperature water in a container. Right image shows the room temperature water with the inclusion of 38°C water container in the field of view.

Method revision:
A third, significantly hotter, container is introduced, first positioned on the right of the second container. It is then moved adjacent to the original container. An image is recorded for each position and a measurement is made of the first container for each step.

Figure 3.14: Left image shows the results from including a third, significantly hotter container of water. The right image shows the results from moving the third container adjacent to the room temperature water container.
Figure 3.15: The surface temperature of the water of the initial container is measured (step 1) and the range of values with the use of error bars, of the same surface, are shown for each step of modifying the scene.

While the average values for each of the measurement steps are very similar, it is evident from Figure 3.15 that the range of the values are extended, with the introduction of a much hotter object into the field of view, and this range is not the same effect seen in the uniformity test. A possible explanation for this difference is that the hotter object is able to affect the intermediate air between the object and the camera. So while the camera is assuming the surrounding air temperature and humidity to be constant, convection or evaporation effects may play a role in the process and the measured temperature variation would rather be an effect from the experimental set up rather than the camera sensor being flooded. A way to eliminate this effect would be to change the angle of view and distance to the object so that the camera is not affected by the local environment that the different temperature water containers may affect. Another possible effect would be reflection from the hotter surfaces that could alter the readout of the initial surface. However, these investigations are outside the scope of this thesis.
3.3.1.5. Spatial resolution test

The aim of this experiment is to understand how much an edge can affect temperature readouts and what minimum resolvable distance is as a function of viewing distance.

Protocol specified preparation:

- Set the camera emissivity to $\varepsilon = 0.99$
- Use a cardboard with a triangular cut-out of 10×2 cm, marked out with aluminium makers.
- Set the distance to 30 cm from the target (a container of ~40°C)

Protocol specified method:

- Set the camera perpendicular to the container
- Stir the water in the container and put the triangular shape over the container
- Capture the image

Rather than using aluminium markers and cardboard, the target was cut from a thin sheet of aluminium and then laminated between two sheets of paper. Furthermore, the distance was set to 80 cm, as it was more representative of generic intended data acquisition (of the human body), and the water temperature was set to 38°C (~body temperature).

The triangle construction is seen in Figure 3.16, where $h$ is the base, $l$ is the side, and $l_{cs}$ is the cross sectional length of the triangle.

Plassmann et al. (2011) analysis procedure for this experiment is to draw several line profiles across the triangle and determine at what point the profile has a significant temperature drop with respect to the other profiles.

![Figure 3.16: IR image of the triangular target used for the edge test.](image)

The relationship between the sides of the triangle is given in Equation 10.
\[ h_{\text{min}} = \frac{h \times l_{\text{min}}}{l} \]  

where \( h_{\text{min}} \) is defined as the minimum width of the triangle at \( l_{\text{min}} \) and \( l_{\text{min}} \) is the minimum length of the triangle still measuring the ‘correct’ temperature (Plassmann et al., 2011).

The author found this approach rather vague, as it does not specify what a reasonable ‘correctness’ or significant temperature drop is defined as. Therefore, in order to justify the measurements, a cross sectional profile of \( l_{cs} \), of normalised temperature, is, displayed in Figure 3.17. This shows the trend of the temperature signal drop.

![Signal drop as function of cross sectional distance \( l_{cs} \)](image)

**Figure 3.17:** The cross sectional profile of \( l_{cs} \) of the triangle shows the temperature drop as a function of triangle width, \( h \), and thus is an indirect measure of the spatial resolution of the camera.

It is assumed that the cross section profile is a function of the width of the triangle, \( h \), and at a certain width, the spatial resolution of the camera is unable to resolve the temperature of the underlying water properly and thus result in a temperature drop. On the basis of
this and the use of Equation 10, the relationship between the cross section intensity and the width can be found, and is displayed in Figure 3.18.

![Graph showing width and temperature drop](image)

**Figure 3.18:** The relationship between the width of the triangle and the temperature drop as a function of cross sectional distance along the profile of the triangle.

Before interpreting the results, it is important to consider what level of signal loss is acceptable within a ‘correct’ measurement of temperature. This threshold is defined by the author as 0.5°C difference, and in the range of 38°C, the change would correspond to 0.998% (as the scale is derived in absolute temperature).

Two key results can be obtained from Figure 3.18. First, for a threshold of 0.5°C at a distance of 80 cm, the spatial resolution (where the intensity is 0.998) under the presence of a triangular shape, reads ~5.7 mm. Second, the edges themselves have a different temperature readings compared to the water, as can be seen in Figure 3.16 from the difference in colours, and in Figure 3.18 towards the end of the line. The temperature difference between the edge and the maximum water temperature is 8.5°C. This large temperature difference could be caused by the emissivity difference between the
laminated material and the aluminium (at the edge), but a conclusion is still that edge measurements should be avoided at all cost, as the camera is not sensitive enough to resolve sharp edges.

Another inexpensive way of measuring the spatial resolution of optical systems is making use of the 1951 US Air Force target (Fischer et al., 2000) and for thermal imaging adapting it to radiate thermally (Ring, 1984). This was however not performed by the author.

3.3.1.6. Thermal resolution test

The thermal resolution is characterised by “noise + digitisation step error” (Plassmann et al., 2011). A simple experiment can be conducted by looking at the thermal variation of a maximally defocused target (noise) and use the digitisation step (digitisation error). A digitisation step is temperature divided by the bit depth. The thermal resolution can be found for each pixel via the relationship in Equation 11.

\[ T_{Res} = \sqrt{(\text{digStep})^2 + (2\sigma)^2} \]  \hspace{1cm} (11)

where \( T_{Res} \) is the thermal resolution of a pixel, \( \text{digStep} \) is the digitisation step and \( \sigma \) is the standard deviation of the sample.

Protocol specified preparation:

- Same initial set up as for the uniformity test except that the water bath is of ~30°C.

Protocol specified method:

- Defocus the image to a maximum (+ve)
- Stir the water for 30 seconds
- Take an image of the water surface

As have been concluded in the previous experiments above, making use of water surface temperature, other than at around 0°C (ice bath) and room temperature, provides a large temperature variation associated with the measurement and makes it difficult to differentiate between errors caused by the equipment measuring the surface temperature.
of the water or the dynamic surface that is constantly moving and cooling. Similarly, while a defocused image gives a relatively smooth distribution, the measurement would be made of a combination of random and systematic errors. Therefore, it would be better to observe a surface with minimum influence of random errors, a blackbody of known temperature, as used in the previous experiments. The blackbody target was set to 30.0±0.1°C, to keep the temperature within normal human body surface temperature range, and the imager recording bit depth is 24, as specified in the radiometric JPEG images.

For FLIR T650sc, the magnitude of one digitisation step (recorded temperature over bit depth) at 30°C was found to be, \[ \frac{303.06K}{2^{24}} = 1.81 \times 10^{-6} K, \] thus the thermal resolution per pixel at a 95% confidence interval is:

\[
T_{Res(95\%)} = \sqrt{\left(\frac{303.06K}{2^{24}} \times 0.95\right)^2 + (2 \times 0.022K)^2} = \pm 44 \text{ mK}
\]

Comparing this result with the camera specifications given in Table 5, where the thermal sensitivity/noise equivalent temperature difference (NETD) is quoted as <30 mK, the results are of the same order of magnitude. NETD is a measure of the microbolometer’s ability to yield a 1:1 signal to noise ratio as a response to change in temperature (Kruse, 2001). Thus a magnitude of 44 mK at a confidence interval of 95% is an acceptable value, where some of the noise would be from the experimental set up and some from the imaging system as a whole. Another comparison is from Plassmann et al. (2011), where their camera (model not stated) has a measured thermal resolution of ±0.3°C, suggesting that the T650sc camera can produce higher signal to noise images.

3.3.1.7. Implications of the University of Glamorgan quality assurance protocol

The University of Glamorgan quality assurance experiments give an idea of the overall camera performance. It provides a method of estimating the overall error for each measurement, but also gives a good indicator for things to be considering when creating a measurement protocol for any study. The overall conclusions to take away from the protocol and the modified measurements are:
• Determine the stability of the equipment and ensure that the study protocol includes procedures to ensure that the camera has been turned on for long enough to produce stable and reliable measurements. The FLIR T650sc imager seems to reach stability after about 2 minutes when its conditions can be regarded as new, and the radiometric certificate is still valid. However, good practice is to turn on the camera at least 30 minutes prior use (FLIR Systems, 2012). No performance issue has been found by operating the camera for a longer period of time (73h) and the average variation in amplitude for this region was found to be 0.14°C below the actual temperature.

• Make sure that the imager measurements are is still within the calibration certificate specification for the specific temperature range of interest. The FLIR T650sc shows a good agreement with its calibration certificate and compares well with a blackbody of known temperature. The overall variation in the human body temperature range was found to be -0.12°C.

• The uniformity test provide thermal map of the lens-sensor system, and would suggest bias in the system that can be accounted for if a high fidelity image is sought for. The FLIR T650sc has a small temperature gradient across its field of view of the order ±0.1°C.

• Be aware of the scene in field of view and thus avoid potential bias caused by the presence of large temperature variation that uncooled microbolometers may have difficulties processing due to the flooding of much larger temperature signals than from the region of interest. The mean values extracted from this experiment for the FLIR T650sc are however only affected by a 0.05°C, despite a larger range being measured.

• Avoid edges as the spatial resolution of the system is limited to a certain factor and may give misleading temperature values.

• Know the thermal resolution of the camera, and make sure to avoid point measurements, as larger number of pixels would reduce the error associated with the measurement. The FLIR T650sc has digitisation step of 24 bits and this by making measurements of a blackbody target of a known temperature, the thermal resolution at a 95% confidence intervals if found to be ±44 mK.

Combining the errors from sections 3.3.1.1 - 3.3.1.6 using Equation 12 below,
\[ Z_{\text{combined}} = \sqrt{(a)^2 + (b)^2 + (c)^2} \ldots \] (12)

yields an error of \( \sim \pm 0.20 \) K for measurements done with the FLIR T650sc within the human body temperature range. This is considerably lower than the manufacturer quoted value of \( \pm 1^\circ \text{C} \) or \( \pm 1\% \) for 15-30°C, otherwise \( \pm 2^\circ \text{C} \) or \( \pm 2\% \) and could give a better estimate of the values associated with the human body temperature range. Otherwise, the quoted \( \pm \% \) range would always be considered as the highest error, since 15-30°C is equivalent to 288-303 K and at 1\%, the minimum error is \( \pm 2.88 \) K to 3.03 K, while for the window of 30-40°C, 303-313 K, it would be quoted as \( \pm 3.03 \) K to 6.26 K. This error is detrimental for the human body temperature range and would not yield any useable measurements.

3.3.2. Imaging equipment calibration frame

The author constructed a calibration frame in order to investigate the different imaging systems employed. The idea behind the frame is to be able to apply it to any type of imager and obtain information about the operational characteristics of the equipment. An alternative was to employ the gold standard 1951 US Air Force target, but the author found it insufficient and lacking flexibility to employ for the characterisation processes that this frame was designed for.

The frame is composed of 3 main parts. The first part is the fixed camera mount, where the cameras are mounted. The second part is a movable perforated aluminium sheet with 1.6 mm diameter holes, with a square pitch of 5 mm. This sheet is black anodised to reduce reflections. Behind the aluminium is a white blank sheet to provide a high contrast, both visually and thermally as it has different properties (optical, reflectivity and emissivity). The third part is the rail that enables movement of the second part. While ideally, it would be best to move the camera with respect to the field, rather than the field with respect to the camera, it has been constructed this way to eliminate the issues associated with cables/leads and connectivity to a computer. An add-on to the calibration frame is a light source diffused by a shoot through umbrella. It is located behind the white sheet and is illuminating the white sheet, diffusing the light even further. See Figure 3.19 for the whole calibration frame set up.
Figure 3.19: Calibration frame and its set-up, designed by the author. Part (1) is the camera mount, (2) is the perforated sheet with a white sheet in the background providing contrast, (3) is the rail and finally an-add on is a shoot through umbrella with a light source.

This setup can be used for both thermal and visual calibration, but the author decided to exclude the light source from the thermal measurements as the differences between the perforated sheet and the white sheet were large enough to be detected thermally. The different tests that can be performed with this frame are:

- Field of view and distortion
- Spatial resolution
- Depth of field

When designing an experiment, these three different imaging system characteristics need to be taken into account, as they will determine the fidelity of the data and in some cases be the limiting parameters for image acquisition.

3.3.2.1. Field of view and distortion

The Field of view (FOV) can be calculated by using the Equation 14 and 17, both derived from Figure 3.20.
Figure 3.20: The distance relationship in one dimension when imaging an object with a sensor.

The sensor distance $d_{\text{sensor}} = N \times P_p$, where $N$ is number of pixels and $P_p$ is the pixel pitch (distance between the center of two pixels).

From Figure 3.20, when the image is in focus; $f = s_2$, where $f$ is the focal length of the lens, $d_{\text{obj}_x} = \text{FOV}_x$, $d_{\text{obj}_y} = \text{FOV}_y$, $d_{\text{sensor}_x} = N_x \times P_{px}$ and $d_{\text{sensor}_y} = N_y \times P_{py}$ the following relationship is true:

$$\frac{N_x P_{px}}{\text{FOV}_x} = \frac{N_y P_{py}}{\text{FOV}_y} = \frac{f}{s_1} \quad (13)$$

Solving Equation 13 for $\text{FOV}_x$ and $\text{FOV}_y$, yield Equation 14.

$$\text{FOV}_x = \frac{s_1 \times N_x P_{px}}{f}, \text{FOV}_y = \frac{s_1 \times N_y P_{py}}{f} \quad (14)$$

The field of view expressions from Figure 3.20 can also be expressed in terms of trigonometric relationships as shown in equations 15 and 16.

$$\text{FOV}_x = s_1 \sin(\alpha_x), \text{FOV}_y = s_1 \sin(\alpha_y) \quad (15)$$
\[ \alpha_x = 2 \tan^{-1} \left( \frac{N_x P_x}{2f} \right), \alpha_y = 2 \tan^{-1} \left( \frac{N_y P_y}{2f} \right) \]  \hspace{1cm} (16)

Combining equations 15 and 16 give a full trigonometric expression for the field of view in both x and y directions and is expressed in Equation 17 below.

\[ \text{FOV}_x = s_1 \times 2 \tan^{-1} \left( \frac{N_x P_x}{2f} \right), \text{FOV}_y = s_1 \times 2 \tan^{-1} \left( \frac{N_y P_y}{2f} \right) \]  \hspace{1cm} (17)

When performing the FOV test on the calibration frame, the camera is attached on the camera mount and the perforated sheet is moved to a distance \( s_1 \), which has been specified by the researcher, and an image is acquired. Several distances can be chosen, based on the size of the ROIs that the researcher is interested in. These FOVs are then analysed and compared to the results given in Equation 17.

Figure 3.21: Field of view of FLIR T650sc as a function of observing distance \( s_1 \). The error bars have been excluded as they are too small.
No visible distortion effects were found in the field of view measurements of the FLIR T650sc, which is also quantifiable from the results in Figure 3.21. The minimal difference between the theoretical value and the measured value is more likely caused by misalignment of the camera with respect to the perforated sheet, misjudgement of the distance or a combination of the two. The differences are at maximum 1 cm at a distance of 2 meters.

3.3.2.2. Spatial resolution

Spatial resolution has already been covered in Section 3.3.1.5, however another interpretation of spatial resolution is spatial size that can be resolved by a pixel at a specified distance. This measurement is useful when considering how large spatial distances can be resolved in a ROI at the imaging distance and gives an indication of what size of information can be resolved in the analysis.

![FLIR T650sc Pixel size](image)

Figure 3.22: The pixel size of FLIR T650sc as a function of observing distance s₁. The uncertainty of the measurement is below the level that can be displayed.
According to Figure 3.22, at a distance of 2 meters, the largest size one pixel can resolve is 1.33 mm in the horizontal direction and 1.38 mm in the vertical direction of the camera. For the thermal imaging applications in this thesis, this result is only important when considering skin lesions, as they can be rather small. However, when imaging skin lesions, the imaging distance $s_1$ was reduced to ~0.5m, where the horizontal size is 0.33 mm and the vertical is 0.34 mm. If a temperature difference is present and the thermal signature is not found within this limit, then technique is not sensitive enough to detect any temperature differences between the lesion and the surrounding skin.

There is a limit associated with minimum resolvable detail known as the diffraction limit or Rayleigh criterion (Ostlie and Carroll, 2007). Equation 18, of Rayleigh’s criterion for circular apertures, is included for completeness, but is not regarded in this thesis, as this limit is beyond the experimental conditions and accuracy employed.

$$\theta_{\text{min}} = 1.22 \left( \frac{\lambda}{D} \right)$$

(18)

where $\theta_{\text{min}}$ is the distance in radians, $\lambda$ is wavelength and $D$ is the aperture diameter.

### 3.3.2.3. Depth of field

Depth of field is a concept describing the amount of spatial variation that is in focus at a distance from the observer. In order to detect this, the calibration frame was set to an imaging distance and focused. After focus, the perforated sheet is allowed systematic movement away and towards the imager and a recording is made of that movement without refocusing the imaging system. If the movement was significant with respect to the aperture of the lens at the initial focusing distance, the resultant image will be outside focus. Thus by tracking the distance offsets that create the out of focus images, the depth of field can be found for the lens at the given focusing distance.

For the experiments performed within the scope of this thesis, the author decided to keep a ±5 cm offset, with 1 cm increments, as a limit for the imaging distances of 0.5 m, 1 m, 1.5 m and 2 m. Within this range, if at any point the image is out of focus, that offset distance determines the boundary for the depth of field. While there was a minimal blur at the offset of the two extremes for the imaging distance of 0.5 m, no particular effect
was found at the other distances and it was concluded that imaging objects with large spatial variation, ±5 cm, along the line of sight with the imager should be avoided at a distance of 0.5 m.

3.4. Hyperspectral camera
The hyperspectral camera is a commercially available unit assembled by Gilden Photonics. It is composed of an EMCCD Andor Luca R sensor, a SPECIM ImpSpector V10E spectrograph, a Schneider Xenoplan F1.4/f = 23 mm lens, a SPECIM scanning mirror and the unit as a whole is operated via a software called SpectraSENS.

3.4.1. Andor Luca R EMCCD sensor
The Andor Luca R sensor is a cooled electron multiplier charged coupled device (EMCCD) made up of a two dimensional array of silicon photo-sensors. This silicon-based semiconductor chip is similar to a conventional charged coupled device (CCD), but with the added multiplication register before the signal output on the readout sequence. The multiplication operates via the process called “impact ionisation”, which relies on a probability that a charge will have sufficient amount of energy to create another electron-hole pair and free an electron in the conduction band of the semiconductor resulting in an amplification (Andor, 2009). This kind of CCD is used for low light intensity applications.

It has 1004×1002 pixels of pixel pitch 8 µm and a spectral sensitivity between 400-1000 nm and a peak quantum efficiency of 65% as seen in Figure 3.23.
The on chip binning capabilities allow for higher signal to noise ratio, as the charge in the read out sequence get summed up over the binned up pixels before reaching the output register, but resulting in larger effective pixels size and thus a coarser image.

The maximum frame rate of the sensor is a function of the sensor that is activated for operation and the mode of its operation. The frame rate for the sensor is found in Table 7 below.

<table>
<thead>
<tr>
<th>Binning</th>
<th>Full Frame (fps)</th>
<th>512×512 (fps)</th>
<th>256×256 (fps)</th>
<th>128×128 (fps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x1</td>
<td>12.4</td>
<td>23.8</td>
<td>45.8</td>
<td>85.0</td>
</tr>
<tr>
<td>2x2</td>
<td>24.2</td>
<td>45.5</td>
<td>84.6</td>
<td>148.2</td>
</tr>
<tr>
<td>4x4</td>
<td>46.1</td>
<td>83.8</td>
<td>146.8</td>
<td>235.9</td>
</tr>
</tbody>
</table>

Table 7: Andor Luca R frame rates as a function of binning and active sensor array size (Andor, 2009).

### 3.4.2. Imaging spectrograph

SPECIM ImSpector V10E is the imaging spectrograph present in the hyperspectral camera. Figure 3.24 displays a schematic of the spectrograph. The target in this case is light-/image reflected from the scanning mirror, Section 3.4.3, which enters via a Schneider Xenoplan lens of transmission of around 90% between 400-1000 nm (Schneider, 2009). This radiation then passes through a slit of 30 µm (SPECIM, 2013).
and gets dispersed by the ImSpector optics, which is of prism-grating-prism (PGP) design. This collection of optical elements disperses the information onto the sensor, where the spatial information is dispersed horizontally and the spectral information is dispersed vertically, creating an \((x, \lambda)\) image. The second spatial dimension is obtained via a scanning mirror, creating an image cube of the dimensions \((x, \lambda, y)\). The overall transmission of the spectrograph is found in Figure 3.25.

Figure 3.24: ImSpector spectrograph (Photonics, 2015) and the way light is dispersed via the optical elements onto the sensor.

![ImSpector spectrograph](image)

Figure 3.25: The simulated transmission curve of the ImSpector V10E spectrograph (Marmion, 2013).
3.4.3. Scanning mirror

As mentioned above, in order to create a two dimensional spatial image, a mirror scanner is utilised. The scanner is composed of a motor, connected via rs232 port and powered via an external power adapter. It has a mirror component and a glass component protecting the mirror from the outside environment. The window, or glass, is made of a 3 mm thick BK7. Due to no detailed specification for the mirror scanner being available, a generic transmittance for a 3 mm BK7 was researched. BK7 transmittance (Polyanskiy, 2015) for 3 mm thickness has a transmittance of 0.999 for the wavelength range of 400-1000 nm for internal transmission only. If considering Fresnel reflection losses caused by a difference in refractive index between the media of propagation the transmittance drops to around 0.92.

Similarly, the mirror component is also a BK7 material, but has an Ag coating with $R_{ave}$ (average reflection) > 93% within the aforementioned wavelength range (Marmion, 2013).

The total throughput of the hyperspectral imager is thus less than 23% (based on the peak performances of each component).

3.5. HSI Calibration and characterisation rationale

At the experimental protocol construction stage, the author determined the type of imaging that would be suitable and tailored the calibration and characterisation of the hyperspectral unit accordingly. The region of interest would be the size of a human head 14.5×19.1 cm, which was based on head anthropometry values (Ahlstrom and Longo, 2009). The head was chosen as a ROI as it is readily visible on a patient and does not require undressing.

Other regions of interest would be the size of moles, as part of the work on skin lesions, but since the imager does not enable easy access to the lens in order to refocus the image and is running a prime (i.e. fixed) lens - a “one setup fits all” setting was developed based upon:
• Size of ROI
• Highest effective spatial resolution
• Highest effective spectral resolution
• Shortest imaging time (limited by the exposure time, ROI size, distance from the observer, data size, processing speed, illumination, sensor sensitivity, geometry of the setup, efficiency of the system)

One of the main problems associated imaging is the image acquisition time. Data acquisition time is crucial for the setup; as the subjects are acutely ill patients whose condition is a priority and they cannot remain still for a longer period of time. Because the observer-to-object distance is small, any movement would reduce the spatial resolution significantly and would alter the integrity of the spectral reflectance measurements as well.

Therefore the following calibration and characterisations were completed/considered:
• Imaging time for two different exposure times with different on chip binning settings as a function of observer to object distance
• Field of view and spatial distortion as a function of scanning distance and as a function of observer to object distance
• Spectral calibration of the spectrograph using a mercury lamp
• Light source characterisation in search for a broad, stable and featureless spectral illumination source.

3.5.1. Region of interest size calibration
As the data acquisition time is crucial for the experiments, it is of interest to reduce the amount of data collected, both dimensionally and storage wise, as data transfer and storage requires high amount of computer resources and storage, which in turn takes time to process. The following measurements were made, as seen in Figure 3.26-Figure 3.28 below to investigate imager collection time as a function of FOV and the effect of binning.

For the first measurement, the observer to object distance was set to 20 cm with a fixed exposure time of 50 ms. A scan was performed for a field of view of 5, 10, 15, 20 cm and
the image acquisition time for each was recorded. Similarly, another measurement was made for an exposure time of 20 ms to check whether this change would alter the total image acquisition time. The difference was found to be negligible, as seen in Figure 3.26, as the image acquisition time is defined primarily by mirror scan time. Similarly, the dark frames of each exposure time were investigated, but the noise levels remained constant at <4% of the maximum readout. Therefore, the remaining measurements were made using 50 ms only. The FOV measurements were repeated for the observer to object distances of 30 cm and 40 cm. Similarly, all the measurements were repeated for binning values of 2×2, Figure 3.27, and 4×4, Figure 3.28.

Figure 3.26: The time to acquire a full frame image of different FOV at different observer to object distances.
Figure 3.27: The time to acquire a 2×2 binning image of different FOV at different observer to object distances.

Figure 3.28: The time to acquire a 4×4 binning image of different FOV at different observer to object distances.
In a patient environment, especially with patients that are poorly, one of the main criteria is to keep the image acquisition time to a minimum. It should not be expected from a human subject to remain seated completely still and without blinking for more than 60-90 seconds. Thus, it can be concluded from the above graphs that full frame measurements of no binning are not worthwhile as they exceed any reasonable time that a patient can remain still for and the disk space required to store the data exceeds 8 GB per image. If the other end of the binning spectrum is considered, 4×4 binning would not be suitable either. While the acquisition time from the 4×4 imaging is desirable, the resolution reduction caused by the binning is too severe and would leave few pixels to make up an image and resolve features in the face. Therefore, from data acquisition time graphs, data size and image resolution, the author decided to follow through the remaining calibration by applying 2×2 binning.

3.5.2. **Spatial data calibration: focusing the camera**

In order to make any measurements with the camera, the camera needs to be focused. Due to the system configuration, the mirror needs to be removed to access the focusing element of the lens. This new system was set up at a distance of 20 cm away from the lens and was focused on an A4 sheet of graph paper marking every 1 mm and 1 cm. The author found this more effective than focusing on an edge of a standard checker board pattern and would also provide a size indicator for \( FOV_x \). \( FOV_x \) would then be replicated when the mirror was attached and would give the effective distance between the mirror and the object. The distance difference was found to be 11 cm. This procedure was completed every time an observer to object distance was altered.

3.5.3. **Spatial data calibration: frame calibration**

For every fixed observer to object distance, an image was obtained for every increment of 1 cm for the range of ±5 cm, using the calibration frame described earlier, in order to determine the depth of field in each direction at each distance. Furthermore, these images would give an indication of the distortion pattern that is introduced by the mirror scanner as a function of scanning distance and observer to object distance.

While the depth of field images did not show any significant blur caused by the distance offset, it was observed that the imager control software does not reliably reproduce; the
expected mirror speeds or expected \( \text{FOV}_y \). As a result, the image is marginally elongated. Furthermore, on occasion, the image acquisition would loop indefinitely and would only stop by quitting the software. Another issue that was found was that the software would occasionally lose track of the mirror speed preferences and would default to a speed of 10 mm/s. Whilst a custom mirror speed could be defined, due to software instability and lack of knowledge and understanding of the annotated fields in the software, the author decided to postpone such investigation for future work in order to maintain control of data acquisition parameters and collect the necessary data within the allocated time. Other future work includes investigation of uneven \( x \)-\( \lambda \) binning and EMCCD settings and their response.

An example of the FOV as observed in a 2×2 binning setting at a distance of 40 cm from the target, scanning over a distance of 20 cm (rotated 90 degrees) is shown in Figure 3.29 below. Due to the large discrepancy between the scanned distance and the set scan distance, the author attempted to find a standard setting that would give a reasonable FOV closely related to the desired ROI of 19.5×14.5 cm. A compromise was reached at a scan distance of 10 cm that resulted in an effective scan of about 16.5×14.0 cm, with minimal distortion in the image as seen in Figure 3.31.

![Figure 3.29](image)

Figure 3.29: The field of view acquired with the hyperspectral imager at a distance of 40 cm from the calibration frame, with a 2×2 binning setting, and a scan distance of 20 cm as specified in the camera control software. Each hole has a pitch of 5 mm and the overall image sizes are given in the image as a measure of the FOV. The image has been rotated by 90°.
The distortion of the FOV in Figure 3.29 and Figure 3.31 was found by Lapington (2016) and is displayed in Figure 3.30 and Figure 3.32 below.

Figure 3.30: The distortion vector map of Figure 3.29 shows how the center (in the line of sight of the camera) is best suited for imaging, and how the larger field of view, at the given distance, produces pincushion effects and barrel distortion.

Figure 3.31: The line of sight of the hyperspectral camera, set 40 cm from the calibration frame, with a 2×2 binning setting, and a scan distance of 10 cm as specified in the camera control software. The image has been rotated by 90°.
3.5.4. Spectral calibration

Another important calibration that was performed was that of spectral calibration. The procedure was to acquire hyperspectral images of a Mercury lamp. The SpectraSENS software that operates the hyperspectral camera has a build in mode of operation that allows the operator to choose the 436 nm and the 546 nm lines of the Mercury and it makes a calculation that relates these lines to specific positions on the sensor, see Figure 3.33. The top three graphs represent the raw data of the Mercury 436 nm, 546 nm and 577 nm lines. The middle graph shows a fitted Gaussian to each line with the known values overlaid in a brighter colour. The bottom three show the normalised Gaussian for each line, with the measured FWHM, the central point of the Gaussians and the known value of Mercury to indicate accuracy of spectral calibration.
Figure 3.33: The mercury lines used to calibrate the hyperspectral imaging system. The top three graphs show the spectral response of the calibrated hyperspectral camera for the mercury features at 436, 546 and 577 nm. The middle plot (4th plot) shows the overview of the whole spectral range with the three mercury lines over plotted for reference. The bottom three graphs show the Gaussian fit, its FWHM, the central peak and the reference mercury lines for each of the three main spectral lines.

3.6. Multispectral camera: SpectroCAM

The multispectral camera is a filter wheel based step-stare imager and can operate with 8 interchangeable wide or narrowband filters. The sensor is silicon based CCD and has 1392×1040 pixels with square pixel pitch of 6.45µm (Pixelteq, 2015). The sensor sensitivity is in the wavelength range of 400-1000 nm as shown in Figure 3.34.
Figure 3.34: The SpectroCAM CCD relative spectral response (Pixelteq, 2015).

The available filters for the camera are shown in Figure 3.35 and have been measured using an ocean optics spectrometer, described in Section 3.7.

Figure 3.35: Filter specifications available for the SpectroCAM as measured by an ocean optics USB 2000+ spectrometer. The legend name is based on [wavelength peak FWHM].

The SpectroCAM has a standard Nikon F-mount and uses a coastal UV-VIS-IR 60 mm focal length lens with a transmission of ~80% over the range of 400-1000 nm and its largest aperture is F/4.
The field of view of the multispectral imaging system is found using the calibration frame as described previously and is displayed in Figure 3.36. The distance was set to 180 cm and the theoretical FOV was calculated using Equation 17. The calculated FOV is 26.79×20.06 cm compared to the measured FOV of ~26.9×20.5 cm. This discrepancy could be due to errors in the measurement of the observer to object distance, rather than the system itself.

While data was recorded using the SpectroCAM, there was insufficient time to analyse the multispectral results, so they will not be discussed further. However, the data is stored in a database for future analysis.

Figure 3.36: The field of view of the SpectroCAM when imaging the calibration from at a distance of 180 cm. The dimensions are ~26.9×20.5 cm, which compares very well with the theoretical values of such a system at that distance, as the values are identical within a few millimeters 26.79×20.06 cm.

The vector distortion map of Figure 3.36 was found by Lapington (2016) and is displayed in Figure 3.37 below.
Figure 3.37: Distortion vector map of Figure 3.36 shows no significant distortion caused by the SpectroCAM optics.

3.7. **Illumination**

Ideally, only one source of illumination should be present, thus allowing the experimenter to control the illumination, avoid unnecessary shadowing, and spectral contamination i.e. light source emission that may interfere and block spectral features of interest. The ideal source should be spectrally broad, reasonably featureless (as flat as possible) and stable. A blackbody type of illuminator would be preferred. It should also have a long lifetime and should not change over the patient examination time (the length is depending on study, but ideally a minimum of 15 minutes), either from a spectral or an intensity point of view. It should also not heat up the surrounding environment or subject. The source should portable, diffused evenly over the region of interest (ROI), and finally; it should be as cheap as possible, given the repeated power switching during the use in a clinical environment and typical lifetime of a bulbs being 100-1000 hours.

The main important characteristics that were considered when making the choice of light source were that the light source covers the wavelength range of interest, is stable, reasonably flexible and affordable. With this in mind, the author examined a number of
3.7.1. Characterising light sources

A simple characterisation of available light sources in the wavelength region 400-1000 nm was obtained by VIS/NIR spectroscopy. For the measurements an Ocean Optics (OO) USB 2000+ spectrometer was used with a XR-1, Extended Range Grating, sensitive from 200 nm to 1025 nm, see Figure 3.38 below for the quantum efficiency of the device.

![Quantum Efficiency of Ocean Optics XR-1 Grating](image)

Figure 3.38: The quantum efficiency graph of Ocean Optics XR-1 Grating (Optics, 2010) used in the USB 2000+ spectrometer.

The experimental method was to illuminate a spectralon surface, a surface of reflectance close to 100% over the wavelength range from 200-2200 nm, with the appropriate light source and collect the spectra with the spectrometer using an optical fiber input. The data was collected via the Ocean Optics SpectraSuite software using the relative irradiance measurement mode. In order to make relative irradiance measurements a reference blackbody spectrum was collected of an LS-1 2800 K standard reference light source and a dark measurement is collected to account for the noise of the equipment.
3.7.2. Types of Light Source

The ideal illumination for the visual range would be to make use of a light source that has a 5778 K blackbody spectrum, like the Sun, as seen in Figure 3.44. The light is broad and has high intensity over the VIS range. The reason for why the natural light is excluded and blocked out is because of the variation of light reaching the surface over time. Some of the solar radiation is absorbed in the atmosphere and most of the light reaching our target of interest is already remitted light that is a distortion of the original spectral shape. The intensity, colour changes over time and the results are therefore not reproducible.

Consequently artificial light sources were investigated, namely:

- Fluorescent Tubes
- LED Lighting Technology
- Halogen Lights

The results of measuring these, their advantages and disadvantages are discussed below.

3.7.2.1. Fluorescent light tubes

While fluorescent light tubes are the most common type of illumination present in most office spaces and hospitals as they are efficient and cheap to operate, they have very strong peaks, as seen in Figure 3.39, throughout the visual spectrum, making them unsuitable for the medical applications in this thesis.
3.7.2.2. LED technology

The typical visible spectrum of white light emitting diodes (LED) is shown in Figure 3.40. While this type of spectrum is unsuitable for very broad light applications, they are cold and stable light sources that are perfect for narrow wavelength measurements as they can be customised to emit light in only certain parts of the spectrum.
3.8. **Halogen Lights**

The choice of light source for the hyperspectral studies is the Osram 3100 K tungsten halogen light source, see Figure 3.41 for its spectrum.

![Graph](attachment:Osram_3100K.png)

**Figure 3.41: Osram halogen 3100K lights measured with OO USB2000+.**

Tungsten halogen lamps are incandescent lamps with a tungsten filament and the filling gas contains traces of halogens, typically iodine or bromine. (Technologies, 2014; Osram, 2014a) The reason for the tungsten filament is twofold: one is to generate light and the second is to heat the bulb to high enough temperature to be able to make use of the halogen traces present in the gas. As the filament heats up, tungsten is evaporated from the filament, migrating towards the wall of the bulb. At sufficiently high temperatures, above 2500°C, the halogens present can react with the tungsten atoms to form tungsten halide, preventing the blackening of the bulb walls but also brings back the tungsten particles to the filament. As the tungsten halide reaches the coil, they dissociate due to the high temperatures and the tungsten gets deposited in the cooler part of the coil, while the halogen is freed up to repeat its cycle. This cycle helps maintaining a steady light output without considerable change in colour temperature or flux fluctuation. It however does require a heat up period in order for the filament to reach the temperatures necessary to start the cycle. Once these temperatures have been reached, the cycle will usually clean up the potential blackening caused by the burning of the filament during the heating up period (Technologies, 2014). Furthermore, the particular bulbs used are Osram’s "GLF
Osram 54460 230v 235w Lowel Pro Light lamp”, containing Osram’s UV filtering technology. The bulb glass is made up of quartz glass doped UV filters, removing harmful UV radiation from the bulb output (Osram, 2014b).

The bulb has an average lifetime of 100 h (Osram, 2016) and has a small fluctuation in intensity and colour temperature from powering on, Figure 3.42. The first 20 minutes has the largest variation, but after this heating up phase the output seems cyclic and could be the halogen cycle referred to above. The wavelengths compared are chosen as they are of interest in Chapter 5.

![Figure 3.42: Time dependence of the Osram 3100 K light source measured with the OO USB2000+. The time dependent variation has a magnitude of about 5%, of which most is caused by the spectrometer itself. The error bars have been omitted so that the cycle can be viewed easier.](image)

While the light source is broad and stable, it heats up to rather high temperatures and can cause discomfort to patients if they are illuminated for a longer period of time, thus it is
important to make sure that the illumination is well diffused and that the imaging procedures are kept to a minimum.

3.9. Diffusing the illumination

The diffusion process occurs in two parts. The light from the bulbs were first diffused by the lamp’s own diffuser and again via utilising a shoot-through white umbrella. The dual diffusion was necessary in order to maximally diffuse the light, in order to reduce intensity variations across the field of view caused by short distances between the subject and the source of the illumination. The spectral shapes of; a calculated blackbody of temperature 3100 K, using Planck’s law Equation 7, the Osram 3100 K measured without diffuser and with diffuser are found in Figure 3.43.

![Light Source Spectral Shapes](image)

Figure 3.43: Spectral shapes of Osram 3100 K with and without a diffusing umbrella compared to a calculated 3100 K blackbody.

While Osram 3100 K produces a broad spectrum over the visible range of the spectrum, it needs to be noted that due to the colour temperature, the diffusers present and the technologies in the lamp, the shorter wavelength end of the spectrum has relatively low intensities. This would make measurements in this part of the spectrum difficult, as longer exposure times would be needed to produce a high signal to noise ratio image. Ideally, for imaging in the VIS/NIR part of the spectrum, a blackbody spectrum of 5778 K (solar colour temperature) or 4700 K should be sought after, with filters preventing the patient...
from being exposed to too much UV radiation. For reference, common blackbody spectral shapes are displayed in Figure 3.44.

Figure 3.44: The top panel shows the spectral radiance of the common blackbody spectra, while the bottom panel is normalised to the peak to show the range differences between the different blackbody spectra. The solar 5778 K or the 4700 K sources would be ideal for work in the VIS/NIR region, as they are much brighter in the desired range, but the Osram + diffuser at 3100 K would still suffice for the research at hand as it illuminates the full visual spectral range.

3.10. Environmental reflections

Whilst light is diffused and softened when illuminating the region of interest, care has to be taken so that there are minimal reflections from surrounding materials. A dummy head was used to investigate the effect of having coloured materials in the vicinity of the ROI. Three different coloured cloths were examined, bright red Figure 3.45a;b, bright blue Figure 3.45c;d and black Figure 3.45e;f. First (a,c,e) the cloth was placed 10 cm in front of the dummy head, second it was rearranged to be 10 cm on the right side of the dummy head (b,d,f). Below each image there are spectra showing the difference the cloths make. In each case, shadows are being cast on the dummy head and the conclusion from these
is that coloured materials should be avoided around the region of interest, or if present, they should be covered by a white gown/sheet.
Figure 3.45: A dummy head was imaged in two settings. Setting one, with a cloth 10 cm in front of the dummy head (a,c,e), and setting two, with the cloth moved 10 cm to the right side of the dummy head (b,d,f). The same regions of interest were chosen for each setting and they were the nose, chin, left cheek and right cheek. It is evident from the spectra that coloured fabrics, whether they are clothes or gowns, should be avoided as they reflect light from the surroundings to the object of interest and thus alter their reflectance spectrum.
3.11. Data organisation principles

When dealing with a vast amount of data, it is important to have data storage and organisation principles in place to enable data navigation and easy interpretation. The naming conventions and organisation is an adaptation of Derek Pullan’s suggestions to the DDU (Pullan, 2012). The idea is to create a string name representative of data setting and content, see Figure 3.46 below.

Figure 3.46: The external database structure for the Diagnostic Development Unit by (Pullan, 2012).

Since the different studies represented in this thesis is a sub study under the diagnostic development unit; the author has categorised the different type of imaging studies to differentiate between the DDU patients and their numbers, and the relevant study patients and their respective numbers. The DDU tag was replaced by IMG[#], # = 1, represents the thermal infrared feasibility study, # = 2, is the imaging in fever and sepsis, # = 3, is makeup/fake tan/volunteer study and # = 4, is the spectral imaging study dealing with renal failure, liver disease and skin lesions. Furthermore, the patient identifier, i5 was reduced to i3.
Another alteration was the inclusion of relevant camera settings to the file name, tagging each file with the appropriate settings rather than a label file to enable easy access during analysis. Similarly, displacement of patient was also recorded this way and was included as a distance in cm at the end of the file name, making calibration easier.
Chapter 4  Thermal infrared results

This chapter is divided into two main parts. The first one is the volunteer study, where a protocol is devised for a control environment (laboratory) and is tested on 20 volunteers. The learning outcomes from the volunteer data create the backbone for the protocol to be used for the second study on patients in an uncontrolled (clinical) environment. The second study was on patients with fever and sepsis in the Accident & Emergency (A&E) Department, Leicester Royal Infirmary (LRI).

The volunteer study is being submitted for peer review and is a collaboration between Naseer and the author over the period of October-December 2012 in the Jarvis building, LRI. It was published as part of a thesis by Naseer in fulfilment of her intercalated medical BSc degree (Naseer, 2013)

Similarly, the fever and sepsis (patient study) was also published in the same BSc thesis by Naseer, but was a collaboration between her, the author and Dexter. The patient recruitment was performed by Naseer, over the period of January to April 2013, while the data extraction and analysis was a joint effort between the collaborators and formed the basis of Dexter’s MPhys project (Dexter, 2013).

The author also investigated the upper arm as a region of interest to look for variation and determine if it can be used on its own as an indicator for disease.

4.1. Volunteer study protocol

The imaging protocol was designed around the arm as a region of interest as it is relatively easy to image in a large variety of patients. While there are standardised protocols and laboratory settings for infrared thermography (Jones and Plassmann, 2002; Ammer, 2008; ISO, 2009) the author needed to design a protocol that would still maintain the same level of integrity and reproducibility as such standardised protocols, but for an environment that is not necessarily as controlled and for a variety of different type of arm orientations, beyond the predefined ROIs given in the Glamorgan protocol (Ammer, 2008). These predefined ROIs are based on anatomical features/areas rather than body heat distribution which is dictated by blood flow and muscle mass.
The lab environment, in which the volunteer study was conducted, was controlled as far as possible to abide by the recommendations by the abovementioned standardised techniques. The humidity was controlled to be as low as possible with a median of 38% while the room temperature was in the range of 21.5-24.9°C (median of 23.8°C) and significant convective airflow was avoided. Furthermore, lighting conditions were carefully considered in order to reduce reflections or irradiation from external sources, heating surfaces within the field of view or the subject. The background was also considered, and was chosen to be devoid of any heat sources and kept as constant as possible. These factors need to be taken into account due to the sensitivity of modern thermal infrared imagers.

The camera was positioned perpendicular to the subject at a fixed distance of 2 meters via the careful use of a tripod. While tripods are not recommended for use, as they can cause angles between the measurements, the tripod was carefully set up and kept at a fixed angle with respect to the subject. The subject was given instructions on the seating arrangement to present the different ROIs and each of the positions were marked out with tape on the floor in order to enable reproducibility and consistency between datasets. While ISO (2009) suggests having an external blackbody source in the background of each image, the author found this not to be possible so the imager’s temperature calibration was checked with a reference blackbody target before and after each measurement to ensure camera stability.

The camera employed was a FLIR SC620 uncooled camera and was initiated a minimum of 2 hours before image acquisition to ensure camera stability, Section 3.3.1.1.

Each imaging session lasted 40 minutes and included a 15 minute acclimatisation of the exposed fingers, hands and arms. During this period, a brief questionnaire was completed and pre-cut adhesive tape markers were attached to predefined anatomical areas (acromioclavicular joint, antecubital fossa and at the wrist), see Figure 4.1. These markers were used as reference points during the analysis as no visual camera image acquisition option was available to overlay with the thermal images.
Figure 4.1: The different marker locations on the anterior (a) and the posterior (p) arm surface in thermal infrared images. 1: Wrist, 2: antecubital fossa, 3: acromioclavicular joint.

The anterior arm positions imaged in the study are found below in Figure 4.2 and the posterior are found in Figure 4.3.

**Right arm (anterior)**

Position 1  Position 2  Position 3

**Left arm (anterior)**

Position 1  Position 2  Position 3

Figure 4.2: The top panel shows the three thermal imaging positions for the anterior of the right arm, and the bottom panel shows the three anterior positions of the left arm.
As can be seen from position 1 from each of orientations, the arm is resting on a stand in order to maintain the position of the arm with respect to the camera. The session finished with 2 minutes of video of each arm in position 1. These videos would give a measure of the variation that could be expected from holding out an arm for a longer period of time.

At the end of the session, the anatomical markers were removed, the subjects were able to re-dress and the data was securely stored and backed up using the file naming convention as in Figure 3.46 and study number \# = 1.

4.1.1. Data files and information extraction

When the thermal infrared camera is connected to a laptop and the imaging is controlled remotely via FLIR’s operational software ResearcherIR, no visual images can be stored in this configuration in comparison to standalone configuration where a reference visual
image is accompanying the radiometric infrared files. There are however many advantages of remotely operating the camera. Other than the self-explanatory advantage of being able to remotely control the camera parameters and acquisition, it is also advantageous to have full control over the storage location, file naming, manual non-uniformity correction and many more. Another advantage is the ability to record video, which is not supported in a standalone configuration. ResearcherIR is software that is a necessary interface in order to extract information from the radiometric images that the camera collects. The camera creates encrypted radiometric JPEG files that can only be extracted via the usage of the software and its data export functions. While the software can be used to display data, apply filters and give the option to extract point, line or area information, the author found that the software is too unreliable for precise measurements and has limited freedom. Therefore, an attempt was made to write a suite of software in interactive data language (IDL) to enable reliable feature extraction and more freedom to manipulate data than the constraints of the ResearcherIR.

The first necessary software that was written in IDL was a conversion program that would convert the extracted radiometric CSV files, (semicolon separated files for older versions of the software and comma separated files for the newer) into space separated files. This was a necessary product as other imaging software’s import functions expected space or comma delimited files, rather than the uncommon semicolon delimited image text files. Therefore, once an image was extracted via the ResearcherIR software, the CSV files were converted into space delimited files and renamed into Dat files for consistency, still abiding by the data organisation principles. These files were then ready for manipulation.

A second software program was written by the author, which later on was modified by Dexter and Winkworth (summer project student). The purpose of this software was to open the image Dat file, visualise the image, adjust the temperature levels to remove background noise, applying standard edge detection techniques to see the region of interest clearly and draw a line across the arm (Naseer, 2013; Dexter, 2013). The temperature levels were set to room temperature to reduce variations in the field and to aid visualisation of the patient and the edge detection. The line was drawn by identifying the tip of the middle finger, the base of the middle finger, the midpoint of the anatomical markers on the wrist, at the antecubital fossa and midpoint between the acromioclavicular joint and the armpit, as seen in Figure 4.4.
The information from this line would contain the temperature variation along the arm. The software also stores the line information for each line segment, where the line segments are defined as finger, hand, forearm and upper arm.

The author decided to investigate the upper arm further following study data collection and wrote a series of macros and programs to extract the relevant information from the upper arm. This decision was made due to a number of interesting features seen in images, from the fever and sepsis study, which will be described later. Since the statistical analysis was performed in GraphPad Prism 6, the author decided to write a program in IDL that would create macros for use in ImageJ/FIJI, image analysis software that the author found easy to use, to load in all the images and define the region of interest from which the relevant data was extracted. Since each image data product was standardised, the information could then easily be read in by a different program that would with the help from the case report from (CRF) classify each of the patients based on complaint, age group, gender and arm of interest. Since the input file for the program was the CRF that would link to the different data products, any number of patients could be added without any need for software modification. The data output from the software was a series of excel data tables and GraphPad tables ready to be analysed.

4.1.2. Results
Over the period October-November 2012, 20 healthy volunteers were recruited from LRI and University staff with a mean age of 27 years (standard deviation 10 years) with a female to male ratio of 1:1. The volunteers were predominantly right handed 85%.
A line was drawn across each arm (as described in the protocol above) and was divided into four different sections (finger, hand, forearm and upper arm). For each section a
Shapiro-Wilk test was performed to check for normality and for each case, the null hypothesis was rejected as the data across the lines were not normally distributed. Therefore, the median value was chosen to represent the section of interest. These were then grouped up for each patient to enable inter-patient comparisons and another Shapiro-Wilk test was performed for the inter-patient data. Normality was found for each of the cases enabling mean value representation of the median data.

4.1.2.1. Anterior vs posterior

Due to the vast amount of data gathered, it is important reduce the data to a manageable analysis. Therefore, the first order of analysis is to look at which positions would yield the most informative data. Position 1 was chosen from all the different orientations Figure 4.2 as it shows the largest exposed surface area. The second reduction was made by looking at the anterior and the posterior positions, see Figure 4.5 for temperature distribution of anterior and posterior measurements.

Figure 4.5: The first frame of the video recording data of the anterior and posterior views of the volunteer study. The temperature is established by taking the median of a line drawn from the finger to the upper arm as specified in Figure 4.4.
As seen from Figure 4.5, there is a significant difference in temperatures between the anterior and the posterior views. However, the blood vessel distribution varies significantly between the two regions. The posterior surface has blood vessels that are deep below the muscle layers, reducing the heat signatures more than compared to the anterior surface, where the blood vessels are more superficial (Naseer, 2013). When considering the effects of blood circulation and variations in systemic vascular resistance at the presence of disease; there should be more pattern based information in the anterior view than the posterior, while the posterior view could give a quicker indication of reduced blood flow to the extremities. While both would be of interest, it would be difficult to image both views in an emergency setting. Therefore, the anterior view is more preferred over the posterior, as it may give a better thermal pattern associated with disease than the absence of temperature. Another parameter that has to be taken into account at this point is the background temperature. In environments such as the Leicester Royal Infirmary the ambient temperature cannot be controlled, only measured. ISO (2009) suggests to keep the ambient temperature below 24°C, while Ring et al. (2011) performed their data collection in ambient temperature ranging between 22-23°C. Since the ambient temperature in LRI cannot be controlled for and the median of the ambient temperature was 23.8°C, the author decided to only take data into consideration within the temperature window of 23.0-25.0±0.2°C. This value was determined based on the mean skin temperature change as a response to different ambient temperatures as investigated by Olesen (1982). The error in the measurement is based on the equipment uncertainty used to measure the ambient temperature. For the volunteer study, this reduces the patient numbers to 16 males and 20 females.

4.1.2.2. Male vs female

The temperature variations between the two genders are found in Figure 4.6 and show a difference of 0.67 ± 0.17°C. This was obtained via an unpaired t-test, which appears to show that the result is statistically significant in a two tailed normal distribution at an alpha value of 0.05, as the p-value is 0.0004.
Figure 4.6: The thermal variation between female and male volunteers using the median of the temperature along the arm.

In research by Mackowiak et al. (1992), temperature from women and men were measured orally and female were found to have an average of 0.5°C higher temperature than men. Similarly, in research by Nguyen et al. (2010), when comparing three different thermal imaging systems at three different sites, and comparing oral measurements to thermal infrared imaging measurements of the face, they found that oral temperatures were found to be higher in women, while thermal infrared imaging temperatures were found to be higher in men. Their results are displayed in Table 8.
Table 8: Temperature measurement of two different regions of interest (oral and face), taken at three different sites with three different imaging systems by Nguyen et al. (2010). The different imaging systems are, OptoTherm Thermoscreen, FLIR ThermoVision A20M and Wahl Fever Alert Imager HSI2000S. They established that women have higher oral temperature measurements, while men have higher thermal infrared response from the face. (σ is standard deviation)

Comparing the results from the volunteer study with the research produced by Nguyen et al. (2010), there is support for why the males arms should have a higher temperature than the females, but at the same time, the variation is much larger. One reason for the variation would be the region of interest (arm vs face), and another is the sample size. The volunteer study has \( n_{vol}=36 \) data points (after data reduction), compared to \( n_{Nguyen}>2000 \). Thus, whilst there is a statistically significant difference between women and men at \( n_{vol}=36 \), and with a mean difference of \( 0.67 \pm 0.17°C \), it is inconclusive to say that this mean difference will remain the same at larger sample sizes. Furthermore, variables that have not been considered, which might change the variability (and are beyond the scope of this thesis), are: ethnicity, body mass index, smoking, exercise, time of day of imaging and consumption of a meal or beverage.

Therefore, the gender specific information will not be considered at this stage, but will be stored for future projects once the sample size has increased significantly to check for potential age and gender variations. This is however outside the scope of this thesis.

4.1.2.3. Right vs left arm

Both arms of each volunteer were examined and the line distribution is displayed in Figure 4.7. A two tailed unpaired t-test gives the difference between the mean temperature of right and the left arm to be \(-0.49 \pm 0.19°C\) and is regarded as statistically significant.
with a p-value of 0.02 at an alpha of 0.05. It was not possible to attribute this difference to left or right handedness as it was not possible to quantify how the handedness would affect the temperature distributions with only 3/20 patients being left handed.

Figure 4.7: The median temperature distribution of the right and left arm data for the volunteer study.

A further investigation finds that the significant temperature difference is attributed to the upper arm region, as seen in Figure 4.8 with a difference of 0.54°C.
Figure 4.8: The mean temperature difference between the right and the left arm. One sigma is included for variation reference and the statistical p-value from the t-test show that only the upper arm has a large enough variation to cause this significance.

In order to be able to determine how significant this difference is, it is important to consider the whole upper arm region, rather than a line segment. The temperature distribution for the upper arm region is displayed in Figure 4.9.

Figure 4.9: The whole upper arm temperature distribution for the volunteer study.
The mean value of the line temperature in Figure 4.7 seems very representative of the whole upper arm region as the difference between the line and the whole region is 0.06 ± 0.16°C for the right arm and 0.05 ± 0.21°C for the left. If both arms are considered for both genders, the temperature difference becomes marginally different, as seen in Figure 4.10.

![Volunteer study: female - male right - left arm data (line along the arm)](image)

**Figure 4.10:** Gender specific differentiation between right and left arms of healthy volunteers.

As a consequence, even if the temperature difference seems small between the arms for the line, the whole upper arm region suggests a larger underlying difference. Therefore recording of which arm was imaged is necessary and will in future give a better estimate of the effect the left and the right arm may have. For small sample size as this, the effects may be amplified, but could potentially still be significant.

From a clinical point of view, the difference could be due to a difference in blood supply to different arms. The left arm blood supply arises directly from the arch of the aorta compared to the right arm where it arrives from a branch connected to the aorta (Naseer, 2013).

When considering the line along the arm, see Figure 4.11, the line suggests a progressive cooling as a function of distance away from the center of the body where also the largest
temperature variations are found. These variations are attributed to individual differences in blood circulation or differences in systemic vascular resistance.

Figure 4.11: The mean temperature and standard deviation along each of the four sections on along the right arm for the volunteer study (with one standard deviation error plotted).

4.1.2.4. Time effects on extended arm

From the temperature effects on the arm of extending the arm for 2 minutes, shown in Figure 4.12, and from the results it can be concluded that holding the arm extended for a period of 2 minutes will not affect the temperature of the arm sufficiently to make a statistical or clinical difference and therefore was not be considered further in this work.
4.1.3. Conclusions

Different views of healthy volunteer arms have been imaged to establish a measurement protocol to be used in a clinical setting. While a significant difference is found between the anterior and posterior surfaces in a healthy subject’s arm, the most practical surface to image in a clinical setting would be the anterior arm as it is easiest to access and would provide thermal information about the near skin surface blood vessels indicating blood supply to the arm. A temperature difference was found along the anterior of the arm when comparing genders and left vs right arm however a larger sample size is needed to confirm this variation. The largest significant temperature distributions seem to be localised in the upper arm. It was also not possible to determine how handedness would affect the temperature distribution along the arm, and cannot be attributed to the large variation.

4.2. Fever and sepsis (Patient) study protocol

While the volunteer study protocol was conducted in a controlled lab environment, the patient protocol, for looking at fever and sepsis, needed a design that could be carried out anywhere in the accident and emergency department. The primary learning outcomes from the volunteer study was that the orientation and area of interest for imaging is the anterior of the arm. Due to time constraints, space and health constraints, to avoid
interrupting clinical flow, it was decided that only one arm was to be imaged, and the arm in question is a selection on the basis of which is available or least affected by other factors; like patient ID wrist band or other objects from either other types of monitoring or invasive procedures that could affect the temperature distribution of the arm and along it.

Since the imaging location could be anywhere in the A&E, the researcher was relying on camera mobility, thus the camera could not be operated via the use of a laptop (as in the volunteer study). It also implied that the camera needed to be sufficiently charged at all times, as the camera is not regarded as medical grade equipment and would need to be connected to a medical grade isolation transformer in order to be used, as set by the regulation (BSEN, 2000) and enforced by medical physics. Having equipment connected to a power outlet would potentially pose a risk in terms of a tripping hazard, which was also assessed as part of the medical physics risk assessment in the research and development application stage of the project. Furthermore, no prior seating arrangements were possible and displacement with respect to the patient could vary, even if an attempt was made to keep it standard, patient cubical sizes were not constant. Other limitations included patient immobility or inability to move their arm into position due to illness. Therefore, a flexible protocol was constructed keeping the fundamentals constant.

The accident and emergency department at the LRI tries to maintain a regulated temperature ($T_{\text{median}} = 23.5^\circ\text{C}, \sigma = 0.9^\circ\text{C}$) and humidity ($H_{\text{median}} = 41.9\%, \sigma = 9.2\%$). via air conditioning. It was therefore assumed that the camera acclimatisation phase could be neglected as long as the camera was not moved outside of the department. When not in use, the camera was charged and stored in a secure, well-ventilated area (DDU).

When the patient was approached by the researcher, the patient was given information and 10 minutes to make a decision to participate in the study. If the patient gave consent for participation, the patient’s arms were exposed to the environment for an acclimatisation phase of 10 minutes. During this time the adhesive markers were attached, just like in the volunteer study and an image was acquired of the anterior of an outstretched arm (position 1) Figure 4.2.
At the end of the session, the anatomical markers were removed and the patient was allowed to re-dress. If the patient was diagnosed with fever, a temperature measurement was acquired via the use of a tympanic ear thermometer for reference. Patients diagnosed with fever had their thermal infrared images taken before being treated with any drugs. At the end of each day, the data was stored and backed up securely using the same file naming conventions as described for the volunteer study, but with a study number \( # = 2 \).

In recent years it has been established that the most efficient way of screening for fever is via imaging the face of the patient using a thermal infrared camera and measure the temperature of the inner canthus (at least 9 clear pixels) (Ring, 2011). In some cases with lower fever levels, the fever detection becomes gradually difficult, and other regions of interest are in addition measured simultaneously (forehead and axilla) (Mercer and Ring, 2009; Ring et al., 2011; Ring, 2011; Ring and Ammer, 2012). For the patient study however, this was not considered in order to avoid person identifiable information. The DDU project, Section 1.2, on the other hand takes this into consideration. For the thermal infrared part of the DDU project a face image is acquired and an anterior image is taken of both arms, in contrast to only one image taken of the anterior of an arm.

4.2.1. Data extraction

The data extraction process proved to be a challenge for the patient study and in some cases would not provide clinically useful information. Since the protocol is difficult to strictly abide by in a clinical environment, mistakes are bound to happen and data corruption could occur without the researcher realising it. Therefore, some patient data could not be processed. In a standalone configuration of the camera, during image acquisition, the researcher can tag the images with an identifier for later analysis. Each patient was tagged with a data table containing the study specific patient identifier, the arm that was imaged, the temperature and the humidity. The extraction of this information is rather complicated and involves access to FLIR TOOLS, software that enables viewing the tags associated with each image, but only a manual extraction of the tags are possible, leaving room for human error. The images then have to go through the same processing as mentioned in the volunteer study. The patient data is then added to a digital master CRF associated with the study.
4.2.2. Results
Over the period of 3 months, 68 patients were successfully recruited, however due to technical faults (camera freeze or crash), human error (images out of focus or insufficient field of view), only 56 patients data were successfully extracted and analysed. Out of the 56 patients, 34 were control patients, 17 had fever and 5 were septic recruits. Septic patients were the most challenging group to recruit, as patients in the early stages of infection could be in a confusion state and could be very ill towards the later stages of the illness (Naseer, 2013). The temperature variations from the three groups are found in Figure 4.13.
Three main groups of patients were recruited for the fever and sepsis study (control, fever, sepsis). A line was drawn along the arm of each patient, and the median value was used to represent the skin surface temperature. The fever group is subdivided into two groups as 11 out of the 17 presented with fever also had an infectious disease. Each group was also subdivided based on the ambient temperature where the data collection was made. The mean value ($\mu$) and standard deviation ($\sigma$) for each sub-group is presented. NaN is the result of only having one data point present.
From the restricted background temperature range, $23.0 \leq T_B \leq 25.0 \pm 0.2 \degree C$, the number of data points get reduced to $n_{\text{control}}=19$, $n_{\text{Fever}}=10$ (of which $n_{\text{Infection}}=7$), $n_{\text{Sepsis}}=4$. These sample sizes are too small to be able to infer any statistically significant information from them. Thus, only the control group successfully passed the Shapiro-Wilk normality test.

In order to increase the sample size, the author decided to combine data from the DDU project, section 1.2 and 4.2, sharing a very similar thermal infrared protocol to investigate the top 20 most common complaints in A&E at LRI, with the volunteer data, the fever and sepsis data.

The combination of datasets, within the background temperature range, increases the total number of patients in each category to $n_{\text{control}}=57$, $n_{\text{Fever}}=15$ (of which $n_{\text{Infection}}=7$), $n_{\text{Sepsis}}=5$. The differences between each category is found in Table 9, and the distribution of the new groups is displayed in Figure 4.14 below.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference (°C)</th>
<th>95% CI of diff (°C)</th>
<th>Significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs Fever</td>
<td>-0.71 ± 0.25</td>
<td>-1.20 to -0.21</td>
<td>Yes (**)</td>
<td>0.0063</td>
</tr>
<tr>
<td>Control vs Sepsis</td>
<td>-0.49 ± 0.43</td>
<td>-1.35 to 0.38</td>
<td>Ns</td>
<td>0.3766</td>
</tr>
<tr>
<td>Fever vs Sepsis</td>
<td>0.22 ± 0.65</td>
<td>-1.15 to 1.58</td>
<td>Ns</td>
<td>0.7431</td>
</tr>
</tbody>
</table>

Table 9: Student t-test show the differences between the patient types based on the whole arm temperature distributions. The measure of significance is based on the p-value, where the significance levels are found in Table 4.
The results in Table 9 suggest that a statistically significant difference can be found between controls and patients with fever from the temperature variation along line of the patient’s arm. No statistical difference was found from comparing control to sepsis or fever to sepsis, as the sample size is too small. Furthermore, from the p-values, it can also be concluded that the mean temperature along the arm of a fever and sepsis patient is very similar, as the p-value is not far from 1 and the mean difference between the two populations is very small.

The temperature distribution difference for finger, hand, forearm and upper arm for the different groups are displayed in Figure 4.15 below.
Figure 4.15: The finger, hand, forearm and upper arm temperature distribution difference as the line goes along the arm for the different patient groups. The error is the standard deviation of each region.

An overall elevated temperature is found in people with fever and the largest temperature difference is found in the extremities, possibly caused by peripheral vasodilation associated with the presence of fever.

While there is an overall increase in temperature between control and sepsis along the arm, comparing the sepsis patients to those that have fever shows a different story. The more distal regions in the sepsis patients are cooler than in those with fever, suggesting that this is possibly caused by a lower blood supply as a response to vasoconstriction or an increase in systemic vascular resistance that is associated with sepsis. The second part of the arm increases in temperature, suggesting more of the blood supply is concentrated towards the center of the body, maintaining blood supply to the more vital organs.

Examples of the different type of thermal distributions found among patients are displayed in Figure 4.16.
The temperature distribution associated with a healthy arm is found to be smooth, examples of these are found in both the volunteer study Figure 4.1 and from the control patients left image in Figure 4.16. In some patients with fever, a mottling pattern in the thermal distribution was found. The reason for this is speculative. Mottling skin patterns have previously been found in the visible part of the spectrum as a consequence of low tissue perfusion and the presence of vasomotor livedo reticularis and acts as an early warning sign in sepsis (Lemyze and Favory, 2010). In sepsis, right image in Figure 4.16, a cooling away from the midline is found and could be a potential sign of peripheral shutdown and a thermal visualisation of systemic vascular resistance to maintain blood pressure (Naseer, 2013).

While the extremities have the largest temperature difference that could be the case of the presence of fever, or absence of blood flow caused by vasoconstriction or other phenomena. The upper arm temperature seems to be relatively similar between the different patient groups. Therefore, the author decided to see if the upper arm region contains indicators based on the temperature distribution that could differentiate between control, fever and sepsis patients.

For each patient the upper arm was segmented using FIJI and read into IDL software written by the author as described earlier.

Each group passed the Shapiro-Wilk normality test (except the sepsis group with too small sample size, but normality was assumed) and the Brown-Forsythe test of variances, allowing a multiple comparison ANOVA to be applied to determine how the groups
compare. The differences in each of the groups and their statistical significance are displayed in Table 10.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference (°C)</th>
<th>95% CI of diff (°C)</th>
<th>Significance</th>
<th>Adjusted p-value</th>
</tr>
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<tbody>
<tr>
<td>Control vs Fever</td>
<td>-1.06 ±0.23</td>
<td>-1.62 to -0.51</td>
<td>Yes (****)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Control vs Sepsis</td>
<td>-0.74 ±0.36</td>
<td>-1.63 to 0.16</td>
<td>No</td>
<td>0.1284</td>
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<tr>
<td>Fever vs Sepsis</td>
<td>0.33 ±0.48</td>
<td>0.66 to 1.32</td>
<td>No</td>
<td>0.7044</td>
</tr>
</tbody>
</table>

Table 10: Results from a one way multiple comparison ANOVA test of the upper arm region for the different patient groups. Much like for the line along the arm, the control group is significantly different from the fever group.

From the statistical analysis, it can be concluded that the upper arm region of the control group is significantly different from the patients with fever. The fever and sepsis patients’ upper arm populations seem to have commonalities, and there seem to be minor common features between the control and the septic patients. The upper arm distribution is displayed in Figure 4.17 below.

Figure 4.17: The upper arm temperature distribution for the different patient groups. Note how the variation in each group has decreased, suggesting the upper arm is a better region of interest when comparing patients’ arms than a whole line along the arm.
In order to investigate the common parts and the dissimilarities between the patients’ upper arm region, the upper arm was divided into 9 segments as seen in Figure 4.18. The segmentation was initially performed by an IDL program, which in most cases would automatically detect the 9 regions, based on the previously defined line along the upper arm and then tracing the data in the vertical direction until the pointer reached the edge of the arm. The software was initiated by the author, then developed further by (Dexter, 2013) and Winkworth. It was however abandoned, as the pointer failed on occasion to find the edges of the arm. Therefore, the segments were manually divided up by calculating the upper arm area in pixels and the segments were extracted via a polygon fitting in FIJI. The results from the polygon fitting were then read in by an IDL program, classified and output in Graphpad XML format. The author decided to only compare the segments 2, 5 and 8, as all the other segments could be affected by armpit temperature or by other hotspots.

![Figure 4.18: The segmentation of the upper arm.](image)

Each of the segments were tested for normality (except for the sepsis group with too small sample size, but normality was assumed) and variance, and passed successfully. They were then compared using a one way multiple comparison ANOVA and the results are found in Table 11 below.
<table>
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<th>Comparison</th>
<th>Mean Difference (°C)</th>
<th>95% CI of diff. (°C)</th>
<th>Significance</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control seg2 vs Fever seg2</td>
<td>-0.99±0.28</td>
<td>-1.879 to -0.09915</td>
<td>Yes (*)</td>
<td>0.0171</td>
</tr>
<tr>
<td>Control seg2 vs Sepsis seg2</td>
<td>-0.37±0.46</td>
<td>-1.800 to 1.061</td>
<td>Ns</td>
<td>0.9965</td>
</tr>
<tr>
<td>Control seg2 vs Control seg5</td>
<td>-0.37±0.18</td>
<td>-0.9472 to 0.2019</td>
<td>Ns</td>
<td>0.523</td>
</tr>
<tr>
<td>Control seg2 vs Fever seg5</td>
<td>-1.92±0.28</td>
<td>-2.814 to -1.034</td>
<td>Yes (*****)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Control seg2 vs Sepsis seg5</td>
<td>-1.78±0.46</td>
<td>-3.206 to -0.3453</td>
<td>Yes (**)</td>
<td>0.0042</td>
</tr>
<tr>
<td>Control seg2 vs Control seg8</td>
<td>-0.10±0.18</td>
<td>-0.6698 to 0.4793</td>
<td>Ns</td>
<td>0.9999</td>
</tr>
<tr>
<td>Control seg2 vs Fever seg8</td>
<td>-1.61±0.28</td>
<td>-2.503 to -0.7225</td>
<td>Yes (*****)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Control seg2 vs Sepsis seg8</td>
<td>-1.75±0.46</td>
<td>-3.178 to -0.3173</td>
<td>Yes (**)</td>
<td>0.0052</td>
</tr>
<tr>
<td>Fever seg2 vs Sepsis seg2</td>
<td>0.62±0.51</td>
<td>-0.9646 to 2.203</td>
<td>Ns</td>
<td>0.9503</td>
</tr>
<tr>
<td>Fever seg2 vs Control seg5</td>
<td>0.62±0.28</td>
<td>-0.2735 to 1.507</td>
<td>Ns</td>
<td>0.4289</td>
</tr>
<tr>
<td>Fever seg2 vs Fever seg5</td>
<td>-0.93±0.36</td>
<td>-2.055 to 0.1853</td>
<td>Ns</td>
<td>0.1866</td>
</tr>
<tr>
<td>Fever seg2 vs Sepsis seg5</td>
<td>-0.79±0.51</td>
<td>-2.371 to 0.7972</td>
<td>Ns</td>
<td>0.8273</td>
</tr>
<tr>
<td>Fever seg2 vs Control seg8</td>
<td>0.89±0.28</td>
<td>0.003885 to 1.784</td>
<td>Yes (*)</td>
<td>0.0481</td>
</tr>
<tr>
<td>Fever seg2 vs Fever seg8</td>
<td>-0.62±0.36</td>
<td>-1.743 to 0.4967</td>
<td>Ns</td>
<td>0.7189</td>
</tr>
<tr>
<td>Fever seg2 vs Sepsis seg8</td>
<td>-0.76±0.51</td>
<td>-2.343 to 0.8252</td>
<td>Ns</td>
<td>0.8544</td>
</tr>
<tr>
<td>Sepsis seg2 vs Control seg5</td>
<td>0.00±0.46</td>
<td>-1.433 to 1.428</td>
<td>Ns</td>
<td>&gt; 0.9999</td>
</tr>
<tr>
<td>Sepsis seg2 vs Fever seg5</td>
<td>-1.55±0.51</td>
<td>-3.138 to 0.02991</td>
<td>Ns</td>
<td>0.0591</td>
</tr>
<tr>
<td>Sepsis seg2 vs Sepsis seg5</td>
<td>-1.41±0.62</td>
<td>-3.346 to 0.5339</td>
<td>Ns</td>
<td>0.3646</td>
</tr>
<tr>
<td>Sepsis seg2 vs Control seg8</td>
<td>0.27±0.46</td>
<td>-1.156 to 1.705</td>
<td>Ns</td>
<td>0.9996</td>
</tr>
<tr>
<td>Sepsis seg2 vs Fever seg8</td>
<td>-1.24±0.51</td>
<td>-2.827 to 0.3412</td>
<td>Ns</td>
<td>0.2587</td>
</tr>
<tr>
<td>Sepsis seg2 vs Sepsis seg8</td>
<td>-1.38±0.62</td>
<td>-3.318 to 0.5619</td>
<td>Ns</td>
<td>0.393</td>
</tr>
<tr>
<td>Control seg5 vs Fever seg5</td>
<td>-1.55±0.28</td>
<td>-2.441 to -0.6612</td>
<td>Yes (*****)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Control seg5 vs Sepsis seg5</td>
<td>-1.40±0.46</td>
<td>-2.834 to 0.02734</td>
<td>Ns</td>
<td>0.0592</td>
</tr>
<tr>
<td>Control seg5 vs Control seg8</td>
<td>0.28±0.18</td>
<td>-0.2972 to 0.8519</td>
<td>Ns</td>
<td>0.8488</td>
</tr>
<tr>
<td>Control seg5 vs Fever seg8</td>
<td>-1.24±0.28</td>
<td>-2.130 to -0.3498</td>
<td>Yes (**)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Control seg5 vs Sepsis seg8</td>
<td>-1.38±0.46</td>
<td>-2.806 to 0.05534</td>
<td>Ns</td>
<td>0.0702</td>
</tr>
<tr>
<td>Fever seg5 vs Sepsis seg5</td>
<td>0.15±0.51</td>
<td>-1.436 to 1.732</td>
<td>Ns</td>
<td>&gt; 0.9999</td>
</tr>
<tr>
<td>Fever seg5 vs Control seg8</td>
<td>1.83±0.28</td>
<td>0.9386 to 2.719</td>
<td>Yes (*****)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Fever seg5 vs Fever seg8</td>
<td>0.31±0.36</td>
<td>-0.8087 to 1.431</td>
<td>Ns</td>
<td>0.9942</td>
</tr>
<tr>
<td>Fever seg5 vs Sepsis seg8</td>
<td>0.18±0.51</td>
<td>-1.408 to 1.760</td>
<td>Ns</td>
<td>&gt; 0.9999</td>
</tr>
<tr>
<td>Sepsis seg5 vs Control seg8</td>
<td>1.68±0.46</td>
<td>0.2500 to 3.111</td>
<td>Yes (**)</td>
<td>0.0088</td>
</tr>
<tr>
<td>Sepsis seg5 vs Fever seg8</td>
<td>0.16±0.51</td>
<td>-1.421 to 1.747</td>
<td>Ns</td>
<td>&gt; 0.9999</td>
</tr>
<tr>
<td>Sepsis seg5 vs Sepsis seg8</td>
<td>0.03±0.62</td>
<td>-1.912 to 1.968</td>
<td>Ns</td>
<td>&gt; 0.9999</td>
</tr>
<tr>
<td>Control seg8 vs Fever seg8</td>
<td>-1.52±0.28</td>
<td>-2.407 to -0.6272</td>
<td>Yes (*****)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Control seg8 vs Sepsis seg8</td>
<td>-1.65±0.46</td>
<td>-3.083 to -0.2220</td>
<td>Yes (*)</td>
<td>0.0108</td>
</tr>
<tr>
<td>Fever seg8 vs Sepsis seg8</td>
<td>-0.14±0.51</td>
<td>-1.719 to 1.449</td>
<td>Ns</td>
<td>&gt; 0.9999</td>
</tr>
</tbody>
</table>

Table 11: The multiple comparison test between the upper arm segments 2, 5, 8 for the different patient groups.

While the segmentation of the upper arm did not provide any new significant diagnostic information associated with the different disease groups. It does however show consistency in the thermal distributions associated with the different groups compared to the whole upper arm and the line along the arm. The only potential inconsistent result is the comparison of sepsis segment 2 to control, where the statistical analysis shows that the segments in question are most likely of the same distribution. Nevertheless, the reasoning behind choosing to compare the middle segments 2, 5 and 8 is to reduce the effects from contamination from other hot spots that are not necessarily related to the...
disease at hand. Segment 2 and 8 would potentially act as indicators for a thermal peripheral cooling as indicated in the right image of the sepsis patient in Figure 4.16 compared to segment 5, which should represent the midline heating. While this is not indicated by the results found in Table 11, a reason for this could be that the sample size, \( n_{\text{sepsis}} = 5, n_{\text{fever}} = 15, n_{\text{control}} = 57 \), is too small to detect a significant cooling. The cooling would only occur in the later stages of sepsis as a response to a cold shock, rather than warm shock, the first phase, where the systemic vascular resistance is low as a response to high cardiac output (Wahl, 1989). With more data, it would be interesting to see the different temperature distributions for each segment. Perhaps they would say something more about the overall temperature map of each disease type. Whilst this investigation could be performed using a thermometer, it is crucial that the measurements are as quick as possible and are non-contact, which a thermal imaging device excels at.

### 4.2.3. Conclusions

The newly developed protocols for obtaining thermal images of patients in the accident and emergency department, based on standardised imaging of the arm, successfully produced reproducible data. Differences between fever and control patients have been found using different methods. By drawing a line along the midsection of the arm, the effects of peripheral cooling can be seen. In fever, there is an elevation of temperature along the arm and in some cases a mottling type of patterns can be found, which could potentially be an indicator of poor perfusion. In sepsis, the effects of systemic vascular resistance can be seen in terms of cooling away from the midline of the arm, while the upper to lower arm gradient is higher than for that of fever. The largest statistical significance has been found to be attributed to the upper arm temperature variation and was investigated further by using the whole upper arm region rather than a line. Both the upper arm and the upper arm segmentation temperature distributions are successfully able to differentiate between fever and control patients. While they are not sensitive enough to detect sepsis, it could be due to the low sample sizes and the stage of sepsis the patients may be in.
Chapter 5  Spectral results

This chapter is divided up into three main parts. The first part deals with the protocol design and development for 5 spectral studies. The second part discusses the data extraction and results and the final section discusses the learnt outcomes.

The studies in question are:

- Dialysis study: Looking at the pre and post dialysis effects - if the filtration of toxic wastes from the body are detectable in the visual part of the spectrum.
- Renal study: Renal failure patients have chronic kidney disease, but are not yet poorly enough to be on dialysis. If toxic wastes have visual components, the likelihood of detecting them in these patients would be the largest, as they do not have the same level of aided filtration as the dialysis patients have.
- Tan study: Looking for spectral signatures associated with fake tan and natural tanning – what is their effect on the visual spectrum. This study was undertaken as this was seen as a confounding effect in spectral studies.
- Liver study: Looking for an elevated level of bilirubin that is associated with liver disease.
- Skin lesion: Looking to determine if the spatial and spectral resolution of the imagers are sensitive enough to detect signs of malignant melanoma.

An important additional factor is ethnicity, as darker skin (skin types IV-VI) has different activated levels of melanin and may respond differently when exposed to light than paler skin (skin types I-III). However, this was not a formal part of the study due to the expected low study subjects, but when applicable, the limitations it may cause will be discussed for all of the abovementioned studies.

5.1. Protocol development

Although this is a study of imaging in the clinical environment, it was found necessary to image patients in a controlled environment. Such an environment was established in the vicinity of each relevant clinic to avoid patients having to move significant distances.

There are many difficulties associated with spectral image acquisition on live subjects in a close subject setting, as the measurements are highly environment and subject
dependent. The first step in the design process was to establish what region of interest can be imaged with the equipment at hand that would give a clinically significant and reproducible result. Since the subjects are human patients, that in many cases are acutely ill, their physical conditions and needs need to be taken into account when determining a region of interest to be imaged.

The suite of imagers used for the spectral imaging studies are:

- Hyperspectral imager (400-1000 nm, pushbroom type)
- Nikon D5000 (VIS) used for context
- Nikon D70s, (NIR converted)
- FLIR T650sc (thermal camera)
- SpectroCAM VIS-NIR (400-1000 nm, multispectral imager)

The data from the latter, although recorded, was not analysed due to time restrictions. The impression of the data is that there are problems with the exposure times that’s caused by the step stare type of imaging as it is recording information from breathing subjects. A general observation is that spectral bands that require exposure times larger than 100 ms are likely to have a motion blur that reduces texture information from that data. Otherwise, the resolution increase, compared to the hyperspectral imager, gives much sharper and clearer texture images for the ROI. The data is however stored for future analysis.

The protocol development will be discussed from the perspective of the hyperspectral camera, as it is the imager of main interest and had the largest restrictions. The same principles apply for all the other cameras, unless otherwise stated, but the distances to the ROI vary.

The hyperspectral camera due to its construction, is significantly limited by its spatial resolution, field of view, mirror scan speed (acquisition time) and illumination. It was therefore concluded that the most significant region of interest that could be imaged, for different type of patients, would be the face. A spatial calibration was performed on the equipment to optimise the measurement distances and to ensure that the optimal settings were used during image acquisition.
5.1.1. Controlling the environment

The imaging room was set to be devoid of stray light, thus windows were blacked-out to minimise external lighting contamination. From the characterisation of light sources in Section 3.7.1, Osram 3100 K tungsten halogen light bulbs were used to illuminate the region of interest. The light from the bulbs were first diffused by the lamp’s own diffuser and again via utilising a shoot-through white umbrella. The dual diffusion was necessary in order to maximally diffuse the light, in order to reduce intensity variations across the field of view caused by short distances between the subject and the source of the illumination. An example of the set-up is found in Figure 5.1.

![Diagram of illumination setup](image)

Figure 5.1: The illumination set-up with respect to the patient and the patient position with respect to the hyperspectral imager.

The patient was seated 29 cm away from the hyperspectral imager and was flooded with diffused, broad spectrum of light for the duration of the image acquisition. Therefore, it was also important that the acquisition time was kept to a minimum as the lights radiate a lot of thermal heat. As a result, it is crucial that the room is well ventilated, especially if the patients are poorly.
If the room is sufficiently large, a curtain should be hung up, preferably in front of the imaging equipment, as seen in Figure 5.1 to provide the patient with privacy while redressing, and restrict the heat flow from the illumination onto the surface of the patient outside of spectral image acquisition. This room is defined by the author as a semi-controlled environment.

5.1.2. Pre-imaging protocol

Once a semi-controlled environment had been established, it was crucial to make sure that the equipment was measuring the intended variables. In other words, before every imaging session, the lights needed to warm up to reach the optimal temperature for a stable intensity and spectral output. Once the lights had heated up \( t > 30 \text{ minutes} \), it was critical to record a calibration reference image of where the patient ROI will be. This was done by covering the field of view of the imager at the imaging distance of the ROI with a known diffuse reflectance surface (99% reflectance over the range of 250-2450 nm) (SphereOptics, 2015). The lights and the exposure settings were adjusted so that the intensity measurement from the reflectance target would cover 90% of the maximum dynamic range of the instrument. The 90% threshold was made to not risk overexposing the image and losing valuable information.

After the image was acquired, a dark reference image was acquired by covering the lens with the lens cap to give a measure of the noise in the measurement. As long as the illumination conditions, the field of view, camera settings and camera performance did not change, a relative reflectance measurement could be made by modifying Equation 5 to that of Equation 19.

\[
R(\lambda) = \frac{I(\lambda) - D(\lambda)}{W(\lambda) - D(\lambda)}
\]  

(19)

where \( R(\lambda) \) is the relative reflectance, \( I(\lambda) \) is the intensity of the measurement made, \( D(\lambda) \) is the noise (dark) measurement and \( W(\lambda) \) is the white diffuse reflectance surface.
5.1.3. **Patient imaging protocol (renal and liver)**

Once the patient had signed the consent form for participating in the study, the patient was given a questionnaire to fill in as part of the case report form. The questionnaire outcome provides the researcher with a measure of the patient’s skin type, following the Fitzpatrick’s skin typing (Fitzpatrick, 1988; Australian Radiation Protection and Nuclear Safety Agency, 2014).

The patient was then asked to dress into a gown, leaving the back unexposed, as an image would be acquired of the patient’s back and face. The rationale for including the back of the patient in the protocol was to have a reference measurement of the same patient and see if a less patient identifiable region could be used for obtaining a clinically significant measurement.

After the patient redressed into a gown, a thermal image was acquired of the patient’s back, face and one of a blackbody reference target. The curtain was opened and the patient was then seated on a chair, if the patient was well enough, or on a height adjustable trolley. The trolley/seat height was always adjusted to make the ROI fall within the straight line of sight of the hyperspectral imager. The distance between the ROI and the hyperspectral scanner was measured up, using a tape measure to ensure that the image was in focus, as no focusing of the imager at this point is possible and was checked in the waterfall setting. Once the correct position was established, the patient was told to sit as still as possible, look straight into the window of the mirror scanner, try not to blink until the acquisition was over. The patient was given an indication of when half the time had passed of the acquisition, to encourage them sitting still, as the total acquisition time was longer than one minute.

A series of context images (Nikon D70s and Nikon D5000) and multispectral images were also acquired of the ROI.

Once the hyperspectral acquisition of the face was complete, the same procedure was completed for the patient’s back surface.

At the end of the session, the curtain was drawn and the patient could change back to their clothes, was thanked for participating in the study and was free to leave. In the meantime,
another white and dark calibration was performed to account for any potential changes in the environment (between sessions).

5.1.4. Patient imaging protocol (dialysis)
The dialysis imaging protocol was very similar to the one of the renal and liver, described above. The only difference was that the patient would return after 4 hours of dialysis and the same procedure would be performed again. In most cases however, the patient was too unwell to follow through with the precise instructions, thus reducing the integrity of the protocol.

5.1.5. Patient imaging protocol (tan)
The idea behind the tanning study is to establish the effects of fake tan and natural tanning on subjects to see if these affect any parts of the spectrum of interest in any of the other studies. It was therefore crucial that the individuals had an absence of illness, would not have had any prolonged sun exposure before the initial image acquisition.

The image acquisition protocol is identical to the liver and renal, except that no back images were acquired. The individuals were then followed up a few days later after the application of fake tan for another imaging session, using the same method as in the first. Then finally, after a prolonged sun-exposure, the individuals were followed up again, imaged, to see what the spectral changes were like.

5.1.6. Patient imaging protocol (skin lesion)
For the skin lesion study, the same calibration procedures were completed as for the other studies. However, during the recruitment procedures, the patient was asked where the skin lesion was present, as the set up would only be suitable for certain configurations, limited by the hyperspectral imager. When the patient was recruited, a pre-cut adhesive aluminium marker was attached next to the lesion for reference and before the curtains were opened, a thermal image was acquired of the lesion. Once the lesion had been thermally imaged a hyperspectral image was acquired of the lesion.
5.2. Results

Over the period of March-August, 2014, 5 dialysis, 6 renal, 8 liver, 13 skin lesion and 2 tan patients were recruited. Out of the 5 dialysis patients, 4 were imaged pre and post dialysis. Due to the small sample sizes, no reliable statistical methods can be employed. Whilst some thermal data was collected for each of the studies, sections 5.1.3-5.1.6, only the skin lesion data was formally considered as thermal infrared data for the other studies did not show any apparent conclusive information for the given ROI. Several papers have been published on thermal imaging on renal dialysis patients, where they investigate peripheral circulation and monitoring of stent insertion sites by imaging the arms and hands, and assessing the need for revision of the arterio-venous fistula (Ring and Ammer, 2012). The arms and hands were however not part of the specific ROI for these studies and have therefore not been considered.

While the protocols were detailed and tried to take as many variables into account as possible - dealing with acutely ill patients does not necessarily allow to remain strict to the protocols as the patient’s health has priority above all investigations. Since the measurements were made in close proximity of the patient, any distance offsets are amplified in accordance with $I \propto 1/r$ of an extended source (filling the FOV), where $I$ is intensity and $r$ is distance. As a result, when comparing spectra, the spectra are first normalised to their own mean value as given by Equation 20.

$$R_{mc}(\lambda) = R(\lambda) - \bar{R}(\lambda)$$  (20)

where $R(\lambda)$ is reflectance, $R_{mc}(\lambda)$ is the mean centered reflectance and $\bar{R}(\lambda)$ is the mean reflectance value.

While intensity values between spectra may be different, due to patient movement, or other effects, this transformation make spectral feature comparison simple.

Several regions of interests were identified on the human face; the eyes (left eye, right eye), the cheeks (left cheek, right cheek), the nasolabial fold (left fold, right fold) and the nose. These spectra were then combined with the spectrum of the back and formed the basis for the inter and intra patient comparisons.
Figure 5.2: A sample hyperspectral image of the author’s face with the different regions of interest identified for analysis. Both the left and right hand sides were considered for the eyes, cheeks and the nasolabial fold.

All of the data processing and visualisation was done in a suite of programs written by the author in IDL and analysis was completed before any clinical diagnostic information was provided to avoid bias in analysis.

5.2.1. Dialysis study results
As difficult as the dialysis patients were to recruit, they proved to be a very challenging task to image. This was mainly because of how acutely ill some of the patients were at the time of image acquisition. The hyperspectral scanner takes of the order one minute to scan the full field of view if no complications arise with the camera control software.

For dialysis patients, the author decided to reduce the number of data points compared due to the vast amount of data presented already and will only consider the eyes, cheeks and back. The patient identifier, skin type and figure number associated with the 4 pre/post dialysis patients are found in Table 12.
While no obvious differences are seen in the four different patients when comparing the effects of pre and post dialysis, other effects are found. One of them is the effect from melanin in the skin in the window ~600-800 nm, with the largest gradient found up to 720 nm. This difference diminishes in the spectrum taken from the sclera (right and left eye images). The effects from subcutaneous fat are also found as a dip just after 900 nm (Jacques, 2013). Furthermore, indicators for elevated bilirubin levels are present in each group, represented by a dip around 450-480 nm. The strongest effect from is seen in the back spectra of the patient. This is either a sign that the back has a larger concentration of bilirubin, or that the back was imaged at a different distance than the face and thus the reflectance reference target. An observation in Figure 5.6 is that the spectral characteristics of the pigments in the skin of this patient with skin type 5 are harder to obtain, and limits the clarity of the spectra. The clearest dataset are those of the sclera, where the shapes are more resolvable.

Table 12: Dialysis patients with pre/post spectra, their skin type and figure numbers.

<table>
<thead>
<tr>
<th>Patient ID (IMG4-)</th>
<th>Skin type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>4</td>
<td>Figure 5.3</td>
</tr>
<tr>
<td>006</td>
<td>3</td>
<td>Figure 5.4</td>
</tr>
<tr>
<td>007</td>
<td>4</td>
<td>Figure 5.5</td>
</tr>
<tr>
<td>009</td>
<td>5</td>
<td>Figure 5.6</td>
</tr>
</tbody>
</table>

Figure 5.3: IMG4-001 (skin type 4) pre vs post dialysis mean centered reflectance spectra.
Figure 5.4: IMG4-006 (skin type 3) pre vs post dialysis mean centered reflectance spectra.

Figure 5.5: IMG4-007 (skin type 4) pre vs post dialysis mean centered reflectance spectra.
5.2.2. Renal study results

The renal patient identifiers, skin type and figure numbers are presented in Table 13 below.

<table>
<thead>
<tr>
<th>Patient ID (IMG4-)</th>
<th>Skin type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>3</td>
<td>Figure 5.7</td>
</tr>
<tr>
<td>003</td>
<td>4</td>
<td>Figure 5.8</td>
</tr>
<tr>
<td>005</td>
<td>2</td>
<td>Figure 5.9</td>
</tr>
<tr>
<td>008</td>
<td>2</td>
<td>Figure 5.10</td>
</tr>
<tr>
<td>010</td>
<td>4</td>
<td>Figure 5.12</td>
</tr>
<tr>
<td>011</td>
<td>5</td>
<td>Figure 5.12</td>
</tr>
</tbody>
</table>

Table 13: Renal patients, their skin type and figure numbers.

The renal datasets presented Figure 5.7-Figure 5.12 contain all of the aforementioned ROIs defined. While the spectra are rather variable, both inter- and intra-patients, comparing also with the dialysis patients, the clearest stable pattern is associated with that from the eyes. From Figure 5.8-Figure 5.9 it can also be concluded that the back was at much different positions than the face and caused saturation when the reflectance calibration was made. Similarly, this effect is seen in Figure 5.7 for the nose measurements. Comparing the relative heights of the oxygenated haemoglobin troughs to
those of bilirubin, it can be concluded that there seems to be an indication of higher level of bilirubin concentration in the skin than in the sclera. Furthermore, it can also be concluded that the skin type makes no significant difference to the shape of the spectrum from the sclera (as expected), compared to the shape obtained from the skin. Another effect can be found in Figure 5.7-Figure 5.10 and that is the difference between the right and left hand sides. This is most likely caused by the not sitting with a head on view with respect to the scanning mirror. Another reason for this effect could be the presence of asymmetries in the skin reflectance across the human face. If the effect was due to uneven illumination across the field of view, the reflectance calculations should have accounted for some of that variation. However, since the human face is not a 2 dimensional flat surface, the reflectance conversions are not necessarily always effective and depend on the portion of the face that is under consideration. While the pigment spectral characteristics of the patient’s skin, skin type 5, in Figure 5.12 are harder to resolve than those of lower skin type, the sclera spectra show clear resolvable shapes, suggesting that imaging the sclera would eliminate the problem with skin types.

Figure 5.7: IMG4-002 (skin type 3) renal mean centered reflectance spectra.
Figure 5.8: IMG4-003 (skin type 4) renal mean centered reflectance spectra.

Figure 5.9: IMG4-005 (skin type 2) renal mean centered reflectance spectra.
Figure 5.10: IMG4-008 (skin type 2) renal mean centered reflectance spectra.

Figure 5.11: IMG4-010 (skin type 4) renal mean centered reflectance spectra.
5.2.3. Tan study results

The tan study was performed on two healthy athletic individuals. No control was taken over the type of fake tan that was used, or when obtaining a real natural tan. The data associated with each are found in Table 14 below.

<table>
<thead>
<tr>
<th>Patient ID (IMG3-)</th>
<th>Skin type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>011-01 (no tan)</td>
<td>4</td>
<td>Figure 5.13</td>
</tr>
<tr>
<td>011-02 (fake tan)</td>
<td>4</td>
<td>Figure 5.14</td>
</tr>
<tr>
<td>011-03 (real tan)</td>
<td>4</td>
<td>Figure 5.15</td>
</tr>
<tr>
<td>012-01 (no tan)</td>
<td>4</td>
<td>Figure 5.16</td>
</tr>
<tr>
<td>012-02 (fake tan)</td>
<td>4</td>
<td>Figure 5.17</td>
</tr>
<tr>
<td>012-03 (real tan)</td>
<td>4</td>
<td>Figure 5.18</td>
</tr>
</tbody>
</table>

Table 14: Tan patients in different stages of tan, their skin type and figure numbers.

By comparing the before tan and after natural tan spectra for both patients, it can be concluded that the skin surface in the face does not have symmetric colour distribution. This statement is supported by the near identical spectra from the sclera, meaning the patient was looking straight into the mirror scanning system. The fake tan spectra suggest
a spectral smoothing of these differences, which is evident in both patients resulting in near identical spectral distributions for the different ROIs.

![Image of mean centered reflectance spectrum for IMG3-011 before tanning.](image)

Figure 5.13: Mean centered reflectance spectrum for IMG3-011 before tanning.

![Image of mean centered reflectance spectrum for IMG3-011 after fake tanning.](image)

Figure 5.14: Mean centered reflectance spectrum for IMG3-011 after fake tanning.
Figure 5.15: Mean centered reflectance spectrum for IMG3-011 after obtaining a natural (real) tan.

Figure 5.16: Mean centered reflectance spectrum for IMG3-012 before tanning.
5.2.4. Liver disease results
The liver patient identifiers, skin type and figure numbers are found in Table 15 below.
Table 15: Liver disease patients, their skin type and figure numbers.

<table>
<thead>
<tr>
<th>Patient ID (IMG4-)</th>
<th>Skin type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>012</td>
<td>5</td>
<td>Figure 5.19</td>
</tr>
<tr>
<td>013</td>
<td>5</td>
<td>Figure 5.20</td>
</tr>
<tr>
<td>014</td>
<td>4</td>
<td>Figure 5.21</td>
</tr>
<tr>
<td>015</td>
<td>3</td>
<td>Figure 5.22</td>
</tr>
<tr>
<td>016</td>
<td>5</td>
<td>Figure 5.23</td>
</tr>
<tr>
<td>017</td>
<td>5</td>
<td>Figure 5.24</td>
</tr>
<tr>
<td>028</td>
<td>2</td>
<td>Figure 5.25</td>
</tr>
<tr>
<td>032</td>
<td>2</td>
<td>Figure 5.26</td>
</tr>
</tbody>
</table>

The liver results, much like the previous study results suggest that there is a spread in the skin surface reflectance as a function of position in the face. For patients IMG4-017, 028, 032 a strong absorption feature seems to be present at around 476 nm. This result was also found by Lamola et al. (2013) when looking into light absorbed by bilirubin as a function of haematocrit values in a laboratory setting. From all the data presented so far, there seem to be a lot of variation in skin reflectance measurements compared to the sclera. The author suggests that rather than looking for potential skin surface variations, the better measure of the blood content would be to image the sclera and thus reduce the effect of skin type, which also seems to contribute to a random variation.
Figure 5.20: IMG4-013 (skin type 5) liver mean centered reflectance spectra.

Figure 5.21: IMG4-014 (skin type 4) liver mean centered reflectance spectra.
Figure 5.22: IMG4-015 (skin type 3) liver mean centered reflectance spectra.

Figure 5.23: IMG4-016 (skin type 5) liver mean centered reflectance spectra.
Figure 5.24: IMG4-017 (skin type 5) liver mean centered reflectance spectra.

Figure 5.25: IMG4-028 (skin type 2) liver mean centered reflectance spectra.
5.2.5. **Intensity-Intensity index: Liver disease**

The author decided to investigate the bilirubin values further. This was done by creating an Intensity-Intensity (II) index based around the relative intensities (from reflectance measurements) of bilirubin at 476 nm to that of oxygenated haemoglobin at 542 nm as shown in Equation 21.

\[
II_{\text{index}} = \frac{I_{476} - I_{542}}{I_{542}} \quad (21)
\]

In Figure 5.27 below is a representation of all of the data presented so far in terms of the II index.
There seems to be a threshold value present around -0.3 that encompasses most values (other than the odd tan and dialysis and liver data points). The data sequence order (sequence number in figures) is the same as in the previous figures on mean centered reflectance, where the first input is the left eye, second input the right eye etc. In order to see the importance of this index, it is useful to consider each of the groups separately only considering the two eyes and cheeks of each patient to reduce the excess of data points.

When considering the II index for the liver disease patients alone, Figure 5.28 (plot of eyes and cheek index only), the last three patients suggest significantly elevated levels of bilirubin, especially patient 032, where the second data point (right eye) that seems to have a low intensity was only low as a result of the patient blinking during image acquisition.

After the analysis was completed, bilirubin and albumin values were made available to the author, and the clinical results are found in Table 16.
Table 16: The bilirubin and albumin blood results for the liver disease patients. Note that no blood results were made available to the author of patient IMG4-014.

While there is a good correlation between the II index results displayed in Figure 5.28 and the bilirubin values obtained from blood tests, the relation is not obvious. This is because the measurements are based on oxygenation levels, and different tissues have different oxygenation levels, limiting the effectiveness of this index. Therefore, for future work, another point of reference for normalisation would be useful, or potentially the use of Monte-Carlo simulations with different chromophore concentration levels that could give a better result. Nevertheless, the technique works as a first order indication of raised bilirubin values, especially when comparing the eyes.
5.2.5.1. **Intensity-Intensity index: Dialysis**

When comparing the II index for the dialysis patients, Figure 5.29 to the overall index found in Figure 5.27, the only measurement that is anomalous is IMG4-009-01, left eye. The eyes post dialysis in IMG4-009-02 (same patient) suggests that this is not a significant result. No bilirubin measurements were available for the dialysis and renal patients.

![II index Dialysis (eyes and cheeks)](image)

Figure 5.29: The II index for the dialysis patients. The dashed orange line represents the threshold at -0.3.

5.2.5.2. **Intensity-Intensity index: Renal**

Similarly, the renal patients, Figure 5.30 seem to be free from elevated bilirubin levels at 476 nm, especially if considering the response from the eyes (the first two data points in each dataset) that suggest the blood bilirubin concentration is not significantly larger in amplitude than the oxygenated haemoglobin level.
5.2.5.3. Intensity-Intensity index: Tan

In the tanning patients, Figure 5.31 the scleral bilirubin content is suggesting a different story than the apparent skin spectrum, where the skin seems to indicate false positive bilirubin levels as expected from the tanning process, along with larger variations associated with skin measurements. As expected the eye measurements show no large variations and are ‘normal’ as expected.

Figure 5.30: The II index for the renal patients.
5.2.6. Skin lesions results

Each of the skin lesions were grouped up based on reflectance characteristics and their mean centered reflectance spectra are found in Figure 5.32 below. The mole reflectance spectra could be divided into 4 different groups (A-D) by eye and spectral shape. Group A seemed to have very similar spectra; group B were spectra that did not seem to belong in any of the other groups; group C seemed to form a group of their own, and group D lacked distinct features. Group A was assumed to be part of a ‘normal’ population and its mean was plotted into each group for reference.

Figure 5.31: II index for the tanning patients.
Figure 5.32: The visual grouping of skin lesions, where group A was found to have similar characteristics and was included in the other groups as a reference spectrum.

Healthy skin measurements were made from a nearby location of the lesions and are displayed in Figure 5.33.

Figure 5.33: The healthy nearby skin associated with the skin lesions.

The author decided to make use of the method provided by Diebele et al. (2012), where they present a parameter $p$, to differentiate between melanoma and nevus as seen in Equation 22.

$$p = OD_{650} + OD_{950} - OD_{540}$$ (22)
The optical density at 650 nm represents the minimal haemoglobin absorption while melanin absorption is more pronounced. 950 nm represents the maximum light penetration in their set up and 540 is the peak absorption of haemoglobin.

The author decided to adopt this parameter for the lesion, but call it $q$, as the values plotted would correspond to reflectance rather than optical densities. The same calculation was made for the skin, calling it $q_0$. By subtracting $q-q_0$, the difference between the two can easier be interpreted and is displayed in Figure 5.34.

![Figure 5.34: $q-q_0$ plot, skin lesion - skin.](image)

Similarly, the temperature distribution of each of the lesions and surrounding skin can also be considered and incorporated in the $q-q_0$ plot. This is shown in Figure 5.35.
Figure 5.35: $q-q_0$ vs $T-T_0$, where $q$ is the parameter for skin lesion, $q_0$ for skin, $T$ is the temperature for skin lesion and $T_0$ is that of skin.

This analysis was performed before the clinical outcome was known, unfortunately, the two suggested outliers, IMG4-029 and IMG4-026 were not clinically significant.

5.3. Conclusions

Protocols have been developed for making use of pushbroom type hyperspectral imaging systems, and step-stare multispectral imaging in a real clinical environment. An attempt has been made to standardise the image acquisition when looking for chromophore changes as a response to a presence of disease. However, there are a number of improvements that need to be taken into consideration for future work and will be discussed further in chapter 6.

Face and back images of patients with chronic kidney disease were taken and analysed to see if there were any visible indicators of disease, more specifically presence of urea. No specific trend could be detected for either the renal group or the dialysis group. What was established however was that the back images had a slight different signal compared to the spectra from the face. One reason for this would be the distance between the imager and the patient was hard to control, especially for the hyperspectral imager and the reflectance conversion was made at the set focusing distance, unless the researcher noticed a large discrepancy and tried to control for it by moving the calibration target.
Another problem was found associated with distances and calibration. Since the human face is not a 2D surface, and at a distance of 29 cm, the spatial variation in a face has significant impact on the light distribution across the face and the reflectance correction, the values obtained from the nose, eyes, cheeks and the nasolabial fold would give rather different concentration of chromophores present. This could be due to the tissue itself, as tissues have different level of chromophore concentration depending on the health level of the tissue, but also due to the experimental set up and calibration process.

It was however found that the sclera is a good region of interest for imaging in order to compare the concentration of different chromophores present in the skin between different patients. The reason for this is that the melanin concentration in skin is not as strong in the sclera and would enable more direct measurement of haemoglobin and bilirubin for comparison. On the basis of this, an intensity-intensity index was developed and the bilirubin concentration was compared for different regions of interest and between different patients. It was found that fake tan reduces the spectral variation of the skin and tanning changes the skin colour in the similar region as the bilirubin signature. However, by looking at the spectrum obtained from the eye measurement, these false positive results can be found. It was also found that the presence of high bilirubin values, associated with liver disease, was found via the II index and correlates well with clinical results.

When applying the same protocol for face imaging to imaging skin lesions, it was found that the limiting factor of using the hyperspectral imager is the spatial resolution. The lesion would only be covering an area of ~100 pixels per waveband and the camera would not be able to resolve the lesions to the extent so that the ABCDE rule could be applied effectively. While two skin lesions were found to be marginally different from a sample of 10, none of the lesions were clinically significant and the protocol and analysis was not sensitive enough to detect the presence of cancerous lesions.

A further improvement would be to move away from a steady state type of imaging of skin lesions, where local circulatory and metabolic effects are dominating, and investigate them under a thermal stimulus (cooled or heated). By applying a thermal stimulus to the region of interest, the recovery can be monitored over time and thus the local circulatory and metabolic effects can be evaluated as a function of time, especially because cancerous lesions recover at a different rate and with a different pattern compared to a thermally
stimulated healthy tissue (Herman, 2013; Bonmarin and Le Gal, 2014; Gurjarpadhye et al., 2015). This was however not possible to investigate under this project specific ethics and research protocol.
Chapter 6  Conclusions and future work

6.1. Thermal infrared imaging

Thermal infrared imaging study protocols were successfully devised for a lab and a clinical setting. The lab environment data consisted of healthy volunteers with absence of illness whilst the clinical environment consisted of controls and patients with fever, infection and sepsis.

Differences between fever and control patients have been found using different methods. By drawing a line along the midsection of the arm, the effects of peripheral cooling can be seen. In fever, there is an elevation of temperature along the arm and in some cases a mottling type of patterns can be found, especially for patients with the presence of an infection, which could potentially be an indicator of poor perfusion. In Sepsis, the effects of systemic vascular resistance can be seen in terms of cooling away from the midline of the arm, while the upper to lower arm gradient is higher than for that of fever. The largest statistical significance has been found to be attributed to the upper arm temperature variation and was investigated further by using the whole upper arm region rather than a line. Both the upper arm and the upper arm segmentation temperature distributions were successfully able to differentiate between fever and control patients. While they are not sensitive enough to detect sepsis, it could be due to the low sample sizes and the stage of sepsis the patients may be in.

Therefore, future work includes recruiting more patients for the fever and sepsis study, especially patients with sepsis to try to determine if a marginally different pattern can be found that is associated with sepsis compared to fever. More in depth analysis should be performed on the other regions of the arms or other limbs, not just the upper arm to detect if any patterns are present. It is understood that such a study of patients in the High Dependency Unit is being planned.

6.2. Spectral imaging

While no spectral differences were found in the VIS/NIR bands for patients with chronic kidney disease, a method was devised to find abnormal levels of bilirubin present in patients. The region of interest recommendation is the sclera, based on the inter- and intra-patient skin variability. The problem with skin imaging is that the tissue chromophore
concentrations vary depending on ROI and the signal measured is affected by skin thickness, melanin content, potential skin treatment and tanning.

Two skin lesions were identified as marginally different from the sample of 10 lesions examined; however the clinical evaluation showed these not to be clinically significant (i.e. malignant).

While data was recorded for each of the patients in the spectral studies, utilising the multispectral imager (SpectroCAM), there was insufficient amount of time to analyse the data, thus the data is stored for future analysis.

Hyperspectral imaging in a real clinical environment has been found to be at best difficult and at worst impractical. This is mainly because it is difficult to control for the lighting conditions that is so crucial for image acquisition and data integrity. The fluorescent light present in most hospitals are detrimental to image acquisition and so are windows that let external light in; as these are usually of varying intensity and hue. Some equipment in hospitals might also make use of LEDs for lighting, and these would also contaminate the spatial and spectral ROI and could be difficult to account for in image processing stages unless specific measures have been taken in the image acquisition protocol.

Another problem during acquisition is imaging acutely ill patients. They could have movement restrictions and would have difficulties displaying the ROI in question for a longer period of time. Depending on what type of spectral and spatial ROI is of interest, the patient would have to remain completely still for a longer period of time in order to maintain data integrity, which has proven to be difficult even for healthy patients.

There is still however a place for spectral imaging in a clinical environment. However, it would have to remain in a semi-controlled or fully controlled room if possible, where all the environmental variables can be controlled, except for the patient. The room should be kept well ventilated and light-tight, restricting the light contamination from other areas. The ventilation is important to maintain a constant and pleasant temperature and humidity. The illumination needs to be restricted to either halogen lights or specific LEDs that are customised for the type of imaging that is being done. Ideally the room needs to be fitted with diffusing material to allow evenly diffused light across the region of interest.
so that shadowing effects are kept to a minimal. Similarly, the patient should be covered with a white reflective cloth to reduce contamination caused by light scatter from their clothing. Depending on the type of imaging that is being performed, a more complex set up would be needed to reduce patient discomfort and to aid them to remain still by the use of different types of supporting materials.

A more flexible imaging set up is needed. For better accuracy, a higher imager specification needs to be used as the imager employed here has many limitations in both spectral and spatial resolution. It also suffers from many operational faults that would limit the data integrity. Regardless of imaging type however, it is imperative that the reflectance corrections are done and that the appropriate optical settings are used to obtain in focus images. If areas are imaged with a large spatial variation at a short distance, a 2D correction might not be sufficient and it would be of importance to know the variation of illumination with distance.

Another way to control for many of the issues mentioned above would be to construct an MRI scanner type of bed, where the patient lies down on a “bed” and is placed inside a larger tube which illuminates to body evenly. This tube would have a rotating part which would be composed of either a scanning system or a wide angle optical zoom that can focus on the region of interest. This would potentially be a good alternative to reduce time in full body imaging projects.

Alternatively, smaller devices can be constructed that would be able to do operate in most environments, but would be restricted to looking at a certain type of disease or spectral ROI. The author’s recommendation is to focus on imaging the eye when trying to determine chromophore concentrations activated in the blood. The sclera which is not affected by skin type or thickness would be a good spatial target to look for a clear bilirubin or oxy-, deoxygenated haemoglobin concentration. Care has to be taken to not damage the eye with the illumination when performing these measurements, especially if UV light is being used. The other advantage of looking at the white of the eye is that it is not contaminated by skin care products like moisturisers, fake tan or sunscreen.

In terms of skin lesions, there are many different techniques and imagers that have been recently well established (since starting the work described here) in detecting disease and
are customised for the specific disease under investigation. A way of improving the ABCDE rule would be to implement VIS/NIR spectral scan of the lesion in high resolution and use the images in conjunction with the established computerised analysis (Ogorzalek et al., 2011). This has been done to an extent by Elbaum et al. (2001), but the author is not aware of the exact algorithms that are employed, due to intellectual property restrictions, to reach the final diagnosis or what spectral and spatial resolution the images have.
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