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Hand Cutaneous Perfusion Dynamics in Plastic Surgery:

A Feasibility Study of Hand Cutaneous Microcirculation During Standard Practices in Plastic Surgery Using Laser Doppler Flowmetry

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A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the degree of Master of Science by Research (Applied Health Sciences)

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CONTENTS

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Chad Chang

DECLARATION

I hereby declare that this piece of work has been composed entirely by myself. All of the experimental work was wholly carried out by myself on participants who have all agreed to enter in the study following informed consent.

Name: Chad Chang MBChB, MRCS (Eng.)

Signature: 

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ABBREVIATIONS

AU	Arbitrary Units
AAA	Abdominal Aortic Aneurysm
ANOVA	Analysis of Variance (Statistical Analysis)
AVA	Arteriovenous Anastomoses
BMI	Body Mass Index
CTS	Carpal Tunnel Syndrome
CVA	Cerebrovascular Accident
DGH	District General Hospital
DH	Drug History
DM	Diabetes Mellitus
ETOH	Alcohol
F	Female
FM	Family History
HRA	Health Research Authority
HTN	Hypertension
IF	Index Finger
IHD	Ischaemic Heart Disease
IP	Ischaemic Preconditioning
LDF	Laser Doppler Flowmetry
LDI	Laser Doppler Imaging
LF	Little Finger
LHD	Left Hand Dominant
LSCI	Laser Speckle Contrast Imaging

M	Male
MF	Middle Finger
NICE	National Institute for Health and Care Excellence
O2C	Oxygen to See
OA	Osteoarthritis
OCP	Oral Contraceptive Pill
PDE5I	Phosphodiesterases Type 5 Inhibitor
PDF	Portable Document Format (Computer File)
PMH	Past Medical History
PORH	Post-Occlusive Reactive Hyperaemia
PPI	Proton Pump Inhibitor
RF	Ring Finger
RHD	Right Hand Dominant
RIPC	Remote Ischaemic Preconditioning
RP	Raynaud's Phenomenon
SD	Standard Deviation
TH	Thumb

ABSTRACT

Introduction

Upper limb injuries are common, accounting for a significant proportion of unplanned visits to hospital services in the UK. The severity of such injuries varies significantly. In its most severe form, substantial injuries require immediate specialist input and subsequent long-term rehabilitation. Poor management can result in loss of function and disability, leading to loss of productivities and independence.

Elevation of the limb is a routine practice following an injury to the limbs, as it is thought to be able to reduce oedema secondary to inflammation and venous pooling through gravity. However, it has been suggested that elevation may reduce local blood flow in selected situations due to increased hydrostatic pressure created by limb elevation. There appears to be no census to the ideal duration and position of limb elevation. The evidence of ideal duration and position of elevation remains sparse.

Laser Doppler Flowmetry (LDF) is a non-invasive, probe-based perfusion monitoring technique which has been used in many clinical and research settings unrelated to hand surgery. This feasibility study aims to determine whether LDF can be used for research of hand cutaneous perfusion dynamics in common hand surgery practices such as limb elevation.

Methods

The PeriFlux 5000 LDF system was used to investigate how cutaneous perfusion alters according to the different hand locations in healthy participants. From this initial hand mapping experiment, an optimal point of measurement was found. This point was used for the subsequent elevation experiment where the cutaneous perfusion level was measured as the participants place their arm and forearm into five different positions of varying degrees of elevation.

Results

Laterality and hand dominance status does not influence the level of cutaneous circulation on the hand. There are significant differences in the level of circulation between the volar and dorsal aspect of the hand, as well as between digital and hand measurements. Cutaneous perfusion levels do not alter significantly between different digits provided the same locations are measured. Elevation of the limb leads to a reduction of cutaneous perfusion in a degree dependent manner.

Discussion

This study appears to be the first mapping study of cutaneous perfusion of the hand with LDF to date. Further insight into upper limb perfusion dynamics is gained from the elevation study. This feasibility study has found that LDF is well tolerated by participants and provides objective measurements of cutaneous perfusion. Further research would be helpful to further our understanding and further optimise outcomes in patients with upper limb conditions.

CHAPTER ONE

INTRODUCTION

1.1 Laser Doppler Flowmetry

1.1.1 Introduction of Laser Doppler Flowmetry

Laser Doppler Flowmetry (LDF) is a non-invasive, probe-based perfusion monitoring technique used to measure perfusion of a single unit of tissue. The nomenclatures of LDF vary between different pieces of literature, other terms used include fluximetry[1], anemometry[2], and velocimetry[3]. All of these terms are considered interchangeable.

Perfusion can be measured through LDF through the use of a probe containing afferent and efferent optical fibres. A probe is positioned in direct contact to the tissue of interest of testing subjects. The velocity of moving objects (i.e. red blood cells) within the tested area is measured through emitting low-level laser light and receiving backscattered signals from the tissue.[4] The principle utilised by LDF to measure perfusion is called the Doppler effect.

Through the use of LDF, one can obtain valuable information about the microcirculation and endothelial function of a given unit of tissue, which may be too subtle to be reliably measured by other vascular radiological techniques such as ultrasound Doppler or contrast angiogram. For this thesis, microcirculation is defined as the circulation of terminal arterioles, capillaries, and venules.

1.1.2 Background Principles of Laser Doppler Flowmetry

<u>The Doppler Effect</u>

As suggested by the nomenclature, Laser Doppler Flowmetry employs the Doppler effect to quantify tissue blood flow. The Doppler effect was first described by the Austrian physicist Christian Doppler in 1842. It describes the phenomenon of observed changes

of frequency and wavelength of waveform energy by the relative motion between the source and the observer. A common application of such phenomenon in medicine is ultrasonography, where soundwaves exceeding the upper audible range of human hearing is used to measure the movement of tissues and fluids in the body.

In the case of LDF, laser light of known frequency and wavelength is emitted through the afferent optical fibre of the probe into the tissue of interest. As the laser light reaches the moving red blood cells, it is reflected back into the probe with an altered wavelength due to the Doppler effect. Through machine analysis by the LDF machine, a measurement of blood flow is calculated in the form of 'Flux'.

Measurement Parameters, 'Flux'

Laser Doppler provides information in the form of 'Flux' (F). Its definition is varied according to different scientific disciplines. In this thesis, flux is defined as a measurement of the *rate of flow per unit area*, measured in 'perfusion units'. It is the function of mean speed (s) of the measured flow and concentration (N) of blood cells, with an arbitrary constant (k).[5]

As flux is arbitrarily expressed in *perfusion units*, this measurement is therefore individual specific and does not offer absolute value of blood flow to allow for inter-individual comparison. However, this problem can be resolved through the measurement of dynamic response to physiological or therapeutic stimuli. By measuring the individual fluctuations of flux throughout stimuli, comparison of the dynamic response can be made between individuals. It should be noted that flux is otherwise referred to as arbitrary unit (AU).

There are several synonymous terms which are used interchangeably in existing literature to describe the microcirculation of the skin detected by Laser Doppler. These are cutaneous microcirculation, cutaneous blood flow, cutaneous perfusion, flux and cutaneous flow measurements. In this thesis, cutaneous perfusion is used as the term of choice.

Electrical Zero and Biological Zero

Electrical Zero describes the residual signal detected in LDF modules secondary to the electrical components of the system, resulting in a small level of background 'shot' or 'dark' noise even when measuring inert materials.[6] This level of background signal is theoretically constant for the same Laser Doppler module. Therefore, provided all measurements are performed on the same module with the same settings, inaccuracies secondary to Electrical Zero should not be of concern. The effect of electrical zero is commonly eliminated through the standardised calibration process in setting up the LDF device.

Biological Zero represents the signal obtained from tissues in the state of complete vessel occlusion. This can be considered as the 'baseline noise' of a unit of tissue which remains as detectable flux signal. The biological zero is a constant which is independent of regional blood flow. This phenomenon is secondary to a multitude of factors, inclusive of cell motion within deeper structures and Brownian motion (random traffic of particles suspended in a fluid secondary to collisions with atoms and molecules) of cells within the interstitial fluid.[1]

While it is theoretically considered as a constant background signal, biological zero is problematic due to the nature that it is variable between different individuals.[7] This may potentially increase the risk of inaccuracy when comparing inter-individual cutaneous perfusion, whether in absolute values or dynamic responses. It is the opinion of some authors that biological zero should be routinely obtained and deducted from all measurements to acquire an accurate representation of flow.[6]

Motion Artefact

Motion artefacts in LDF describe inaccuracies in measurement due to unexpected movement of either the measurement system (i.e. probe and lead) or the study subject.

This unexpected movement can lead to alteration in the alignment of the optical fibres against the measured tissue. As the optical fibres alter orientation, the emitted laser photons will reach and interact a different part of the tissue. This will result in a different reflected laser and therefore produce variations in cutaneous perfusion measurements which is otherwise unrelated to the physiological change observed.

Motion artefacts can occur in a spectrum of severity. In minor cases of subtle movements, insignificant temporary variations in cutaneous perfusion measurements may be observed, which can be readily identified and removed before data analysis. Severe motion artefacts due to either excessive magnitude or frequency of movement can render the measurement tracing impossible to extrapolate data for analysis.

There are several strategies which can be adopted to minimise the effect of motion artefacts. One can limit the effect of subject movement by proving clear briefing,

instructing to the test subjects to remain still during measurement. One should be mindful of subject comfort and minimise the measurement period where possible. A balance should be made to limit the measurement period to a narrow time window which allows for the collection of sufficient data for accurate reading. Utilising adhesive tapes to attach the probe and lead against the subject can further limit unintentional dislodging or movement of the measurement system.

Probe Attachment Pressure

Variations in pressures applied to the LDF probe can lead to significant differences in perfusion measurements.[4,8-10] Several studies have demonstrated reductions (and obliteration) in perfusion measurements with increased pressures applied to the measurement probe. Therefore, tension-free fixation of the measurement probe is advised to ensure reliable and accurate reading.

1.1.3 Applications of Laser Doppler Flowmetry

Laser Doppler Flowmetry has many potential uses in clinical and research settings. The ability to measure tissue perfusion in real-time allows one to investigate and determine the quality of target tissue microcirculation and endothelial function. Examples include perfusion assessment and monitoring following surgical procedures (i.e. organ transplant surgery, free flap surgery), and following administration vasodilatory therapeutics in Raynaud's Phenomenon. The documented applications of LDF are outlined as followed:

Applications outside of Plastic Surgery

Diabetology: Diabetes mellitus is a metabolic disease associated with prolonged hyperglycaemia, leading microvascular and macrovascular complications. Diabetic microvascular complications occur in the retina, kidneys and nerves, leading to diabetic retinopathy, nephropathy and neuropathy respectively.

LDF has been used to expand pre-existing knowledge in the pathophysiology of diabetes. The pilot study by Lal and Unni measured microcirculatory perfusion from the feet of diabetic patients and healthy volunteers. They have found a difference between diabetic patients in LDF waveform response to central and local regulatory mechanisms of blood perfusion between the two groups.[11] It is thought that the finding could be used to differentiate diabetic patients with healthy controls, with potentials as a staging tool to assess treatment effectiveness. There are other researches comparing the microcirculation of diabetic subjects against healthy individuals. These studies recorded and analysed autonomic response under dynamic testing conditions such as thermal challenges[12] and post-occlusive reactive hyperaemia.[13,14] It was found that diabetic individuals generally have a lower response from heat provocation compared to nondiabetic individuals. Similar comparative research has been performed for patients with ischaemic heart diseases. Ovadia-Blechman et al. measured both microcirculatory and central circulatory parameters during exercise in individuals with known myocardial ischaemia against healthy individuals. It was noted that microcirculatory measurements differ between the two groups while no differences were found in the central variables (heart rate and blood pressure) between the two groups.[15] Rother et al. used LDF as a monitoring tool to assess skin perfusion in critical limb ischaemia before and after leg revascularisation procedures.[16]

In diabetic retinopathy, several studies were published on their experiences assessing the retinal microcirculation in diabetic retinopathy against hypertensive individuals, as well as the effect of smoking on retinal circulation. It was found that retinal circulation is impaired by smoking status.[17] Jumar et al. found hypertrophic remodelling of retinal arterioles in individuals with diabetes with prolonged duration of disease.[18] The effect of exercise on endothelial function in diabetic patients has been assessed using LDF, which demonstrated a positive effect of regular low-intensity aerobic exercises on peripheral microcirculation.[19]

Rheumatology: Laser Doppler flowmetry is widely used in recent years in researches related to Raynaud's phenomenon to observe and quantify cutaneous microvascular function. Raynaud's phenomenon (RP) is a condition characterised by reversible vasospasm of digital arteries and arterioles, which manifests clinically as paleness and pain secondary to digital ischaemia.

Laser Doppler has been compared against other pre-existing investigative and research instruments to determine the following: its feasibility as a diagnostic tool in RP[20-24], whether it was possible to differentiate subtypes of RP[25,26], and to evaluate physiological and microvascular structural difference between healthy individuals and patients with RP[27-30].

Furthermore, LDF has been utilised as an assessment tool to measure the effect of therapeutic interventions such as iloprost[31,32], calcium channel blockers[33,34], Phosphodiesterases type 5 (PDE5) inhibitors[35-39], and prostaglandin E_1 infusion[40].

Wound Healing: LDF has been used to evaluate the microvascular state of healing tissues, and therefore and predict the time required for healing. This has been studied in patients with diabetic foot ulcers by Becket et al, who concluded that LDF could be used as a reliable noninvasive measurement of microcirculation.[41]

Arverud et al. used LDF to measure the microcirculation in the paratenon of Achilles tendons in healthy individuals against those who sustained Achilles tendon ruptures. It was found that LDF is a feasible tool to assess the microcirculatory status of Achilles tendons.[42]

LDF has also been used in the assessment of cutaneous perfusion in those with venous ulcerations, which found an elevated basal LDF measurement when compared to healthy controls regardless of the ulceration healing status.[43]

Dermatology: In dermatology, LDF has been used to assess segmental-type vitiligo lesions against the normal skin, it was found that vitiligo skin has increased cutaneous perfusion with associated sympathetic dysfunction.[44]

Transplant Surgery: There have been several studies which utilise LDF to measure organ microcirculation in perioperative settings. Ladurner et al. have conducted a pilot study measuring hepatic microcirculation intraoperatively, which has demonstrated that LDF is a reproducible method for evaluating the perfusion of the liver.[45] Similar study has been performed in renal[46] and small bowel[47] transplantation surgery with similar conclusions stating its effectiveness in post-operative monitoring.

Application of LDF in Other Medical Specialties: A study has been performed to measure tumour vasculature and perfusion following isolated limb infusion of chemotherapy agents utilising LDF and white light spectroscopy. It was found that there was no evidence of tumour vasculature destruction following the therapy.[48] Lenasi has demonstrated subtle changes in endothelial function and sympathetic reactivity in hypothyroidism from LDF measurements cutaneous perfusion. LDF measured oesophageal mucosal blood flow in patients with nutcracker oesophagus has been found to be significantly lower, suggesting hypoxia as a possible underlying mechanism of oesophageal pain in this patient group.[49]

The effect of cigarette smoking on cutaneous perfusion and the microcirculatory response has been researched extensively using LDF in various settings. Examples include acute consumption of cigarettes[50], assessment of endothelial function following transient ischaemia in chronic smokers[51-53], and following smoking cessation[54]. Interestingly, Reuther et al. have demonstrated a transient rise in buccal blood flow following the usage of electronic cigarettes in healthy volunteers despite having consumed nicotine. The finding may be secondary to the negative pressure elicited while the subjects are using the electronic cigarettes.[55]

Application in Plastic Surgery

Free Flap Surgery/Microvascular Surgery: Free autologous tissue transfer, otherwise known as free flap, is a reconstructive technique used widely in plastic surgery. Free flap surgery is performed to reconstruct tissue defects when local or regional tissue reconstruction was not possible or unsuitable. Common indications may include

coverage of defects following trauma, tumour or scar excision, or to provide healthy and well-vascularised tissue coverage of poorly healing wounds.

The first human free autologous tissue transfer was performed in 1957, following resection of a recurrent squamous cell carcinoma of the cervical oesophagus. The cervical oesophagus was reconstructed with a section of jejunum taken from the abdomen.[56] In 1973, Taylor and Daniel published their experience performing the first cutaneous free flap for reconstruction of a compound lower limb injury.[57] The free flap used was known as the groin flap, based on the superficial branch of the circumflex iliac artery. Since, many other free flaps have been described and utilised for defects of different locations and reconstructive needs. The nomenclature of free flaps is dependent on the arterial supply, location of donor site and primary tissue composition.

The process involves dissecting and raising a unit of tissue with a pre-existing vascular architecture from its original location, otherwise known as the 'donor site'. The donor site should ideally be amenable to direct closure or small skin graft to minimise donor site morbidity. Following dissection, the arterial and venous supply of the flap is detached before the flap is transferred to a distant location, the 'recipient site'. The blood supply of the transferred free flap is re-established through surgical anastomosis to local blood vessels. After blood supply to the free flap is restored, flap in-setting is performed. This involves using sutures or staples to secure the flap to the surrounding tissues of the recipient site.

Following free flap surgery, there is a period where close monitoring of flap tissue perfusion is essential. As the flap is kept alive by the vascular anastomosis initially, the

success of the procedure (i.e. survival of the transplanted free flap) is dependent on the patency of the anastomosis. Early detection of issues associated with anastomotic patency would therefore be of interest, in order to trigger a prompt return to operating theatre for exploration and flap salvage procedures. It is known that early recognition of flap compromise increases the chances of successful surgical salvage.[58,59]

Another situation where close monitoring of tissue viability is replantation and revascularisation procedures following devascularisation injuries. These procedures involve reattachment of divided blood vessels at the site of injury, which is not dissimilar to that of free flaps. Likewise, close monitoring and early detection would enable early salvage procedures to be performed. This period of close monitoring typically involves clinical examination of skin colour, temperature and capillary refill time, along with adjunct monitoring such as the hand-held Doppler. Laser Doppler flowmetry offers another alternative as an adjunct monitoring device.

Perioperative Monitoring of Microvascular Surgery: In 2011, Kraemer et al. shared their experience monitoring 54 free flaps using LDF combined with surface temperature measurements. It was found that skin temperature has a close correlation with flap microcirculation. The technique was able to detect microcirculatory compromise secondary to anastomoses failure promptly to allow early flap salvage to be performed successfully.[60] This finding is consistent with a study performed by Rothenberger et al, who assessed the intra- and post-operative perfusion dynamics of 34 elective breast reconstruction free flap patients using LDF. It was concluded that LDF measurement is a reliable, objective, and non-invasive device for the monitoring of free flaps. Thus, it may improve flap survival rates by detecting vascular compromise at an early stage.[61] Similar

finding has been found in non-elective settings with patients presented with traumatic injuries requiring replantation and revascularisation in an earlier work performed by Hedén et al. in 1985 in a series of 40 free flaps and replantations. In the study, LDF has been used successfully for post-operative monitoring when used in conjunction with routine clinical observations. It has effectively diagnosed two arterial thromboses with a characteristic flow pattern; it was noted that the technique could effectively distinguish venous and arterial occlusion through perfusion patterns measured on LDF. It was found that LDF is particularly useful when measuring perfusion in the flaps otherwise difficult to assess clinically such as buried flaps.[62] Hovius et al. monitored the post-operative perfusions of 79 microvascular operations (i.e. a combination of free flap, replantation and revascularisation procedures) over a 3 year period, has found that an adjustable critical alarm value could be reliably defined with a sensitivity of 93% and a specificity of 94% in patients for replants and revascularizations.[63]

Perfusion Dynamic Studies of Microvascular Surgery: Laser Doppler flowmetry has been used to assess perfusion dynamics following free-flap surgery in standard conditions[64] as well as under various physiological stressors to determine the optimal post-operative recovery condition. In 1992, van Adrichem LN et al. investigated the acute effect of cigarette smoking on replanted digits, which has found the adverse effect of smoking on the microcirculation in replanted digits. As a result, they have recommended that smoking should be prohibited to optimise circulation post replantation.[65] Dornseifer et al. assessed the effect of thermoregulation on the microcirculation of free flaps. It was shown that active warming of free flaps leads to best microvascular blood flow as opposed to passive warming or cooling of free flaps.[66]

Assessment of Ischaemic Preconditioning of Free Flap Surgery: Ischaemic preconditioning (IP) is a method of introducing ischæmic tolerance to target tissues using cycles of brief periods of mild ischaemia with subsequent reperfusion. The aim of ischaemic precondition is to increases the target tissue's resistance to ischaemic injury. Application of IP would theoretically reduce the risk of free flap necrosis following surgery. Remote ischaemic preconditioning (RIPC) is a further development of the technique to reduce the extent of trauma to the target tissue and vessels from the application of direct stressors to the area. The mechanism is thought to be due to a systemic release of biochemical messengers following ischaemic reperfusion of one organ, which promotes a protective effect to remote organs.[67] A study has used LDF to investigate the effect of RIPC, which has demonstrated an increment of anterolateral thigh blood circulation by a third following cyclical intermittent ischaemia of the contralateral upper limb.[67] Similar studies of RIPC has been successfully conducted using LDF as an investigative method to monitor cutaneous perfusion in healthy individuals[68], as well as those undergone flap reconstructive surgeries[69].

Assessment of Burns: Burns is a form of tissue injury as a result of many causes. Examples of such causes include extremes of temperature, electricity, chemicals, friction or radiation. It typically causes cutaneous injury of various depth with deeper burns possibly involving underlying structures. The assessment of the depth of burn wound is of vital importance to its subsequent management, as it provides prognostic information to the healing potential to the burn wound and therefore determines whether surgical debridement and subsequent reconstructions are required.

Clinical assessment of burn depth can be subjective and dependent on clinician experience. Burn wounds are considered as a dynamic wound which evolves with time. Furthermore, variations in skin tone (or birthmarks and tattoos), extremes of age and intra- and inter-individual variation skin thickness may further exacerbate this diagnostic challenge. Research has suggested that clinical assessment of burn depth has an accuracy ranging between 60-80%.[70]

In recent years, there has been a considerable amount of literature validating the use of Laser Doppler Imaging (LDI) as a tool for burns depth assessment.[71-74] In 2011, National Institute for Health and Care Excellence (NICE) published a medical technology guidance recommending the use of laser Doppler imager to guide treatment decisions for patients with indeterminate or uncertain burn depths.[75]

However, LDF has also been used as an assessment tool for burns. Merz et al. assessed 173 areas of burns of variable depths within 24 hours of injury, excluding catecholamine-dependent patients.[76] All burns were labelled into three categories: 'superficial dermal', 'deep dermal' and 'full thickness' by a single burns surgeon at the time of study enrolment. Digital photography and LDF measurements were taken at the time of assessment and serially at day 3, 6 and 14. LDF measurements to the contralateral site were measured as the control group. The authors reported a statistically significant difference between the average perfusion value measured in all three groups, as well as control group vs burn group. A cut off point at the time of initial assessment was identified. This was found to be a reasonable predictor of spontaneous healing within three weeks, with a positive predictive value of 93.1% and negative predictive value of 88.2%.[76] Other studies have also supported the finding by Merz et al, proving the benefit of more accurate and prompt

identification of burns requiring surgical management, thus reducing the duration of overall hospital stay.[77]

It should be noted that while LDF can aid in the assessment of burns depth, there are several challenges related to its use in the acute burns setting. These include the cost and size of the equipment; measurement area is limited to the probe location, and therefore assessment of more substantial burns of mixed depth can be laborious. The need for probe contact to the area of interest means potential inaccuracies related to pressure artefacts can occur along with a potential risk of burn wound contamination.

Potential for Greater Utilisation in Plastic Surgery

It is evident from the examples mentioned above that LDF has many potential uses in both clinical and research settings. The capability to non-invasively assess tissue microcirculation in real-time has been proven to be useful in helping clinicians monitor the perfusion state of free-flap reconstructions and revascularised or replanted tissues.

Studies of perfusion dynamics of the skin and soft tissues have helped clinicians decide the optimal pre-operative conditioning[67-69] and post-operative regimes[64-66] to minimise morbidity and improve clinical outcomes following microvascular surgeries.

Hand surgery is a branch of plastic surgery which encompasses the management of a wide scope of acute and chronic conditions related to the upper limb. Hand surgery focuses on the restoration and reconstruction of form and function of the hand, as they are essential in performing activities of daily living, providing tactile feedback to the body

and communication through body language. Poor outcomes can cause significant morbidity and impairment of day to day function.

There are several procedures in hand surgery which require careful dissection and surgery of the upper limb vasculature. Examples include Duputryen's surgery, trauma replantation surgery, toe to hand transfer and free tissue transfer for reconstruction of upper limb defects. All the above procedures require robust perfusion of the tissues perioperatively to ensure the survival of the tissues around the surgical site and proper post-operative healing of the tissues, akin to free flap surgery.

There are several practices that are performed perioperatively on a routine basis to optimise post-operative recovery. Examples of these practices include limb elevation, immobilisation using splints and application of surgical tourniquets intraoperatively. Studying the perfusion dynamics of the upper limb in such practices may further the current understanding and knowledge of peri-operative perfusion of the hand. This may provide a theoretical foundation for one to optimise pre-existing peri-operative practices in hand surgery further. It would be of interest to study upper limb tissue perfusion with LDF to assess how cutaneous perfusion alters under such conditions.

1.1.4 Other Laser Doppler Based Techniques

Other Laser Doppler based techniques include Laser Speckle Contrast Imaging (LSCI) and Laser Doppler Imaging (LDI). Despite the similarity in terminology, these techniques are vastly different from LDF in terms of measurement parameters, equipment and theoretical backgrounds. Instead of delivering laser through optical fibres, these techniques utilise different delivery devices to project lasers across a larger area of tissue,

thus simultaneously measures perfusion over an area instead of a single point of tissue. This can be advantageous as it negates the potential problems related to the variability of perfusion between different points of the tissue of interest. Such an assessment method is particularly useful in pathologies where the microvascular circulation varies significantly between different areas of the skin, such as systemic sclerosis, LDI may be more advantageous.[78] This is because a simultaneous assessment of multiple areas can be performed, therefore reducing the risk of sampling error as well as reducing the time required for measurement.

These methods are non-contact and rely on the laser travelling through the air between the device and tissue. The absence of a measuring probe in LDI means the measurement does not require contact to testing subjects – this offers the advantage of reduced risk of measurement artefacts secondary to probe movement or pressure. The non-touch measurement also reduces the risk of cross-contamination of micro-organisms through repeated use of the same measurement probe. This is particularly important when measuring open wounds such as burns or diabetic ulcers. Conversely, the fact that lasers are required to travel an extended distance of air may lead to inaccuracies and unwanted interference artefacts through the environment.

1.2 The Circulatory System of the Upper Limb

To understand the relevance of this study, understanding of relevant basic knowledge in anatomy and physiology of the upper limb circulatory system is necessary. A brief overview of these concepts is discussed in the following paragraphs in this section. For this thesis, the upper limb is defined as the region extending from the deltoid region to the hand.

1.2.1 Arterial Blood Supply of the Upper Limb

The arterial supply of the upper limb is responsible for carrying nutrient-rich, oxygenated blood from the heart to the living tissues of the upper limb. This begins with a pair of arteries called the subclavian arteries. There is one subclavian artery per side, which runs from within the thoracic cavity to the axilla. Within the axilla, this artery is called the axillary artery. As it exits the axilla and enters the arm, it becomes the brachial artery before bifurcating into the ulnar and radial arteries in the cubital fossa, an anatomical area located around the inner aspect of the elbow. These two arteries are responsible for the blood supply of the forearm and the hand. It should be noted that as the vessels travel along the upper limb, various smaller arterial branches are given off to supply different structures of the upper limb such as bone, muscle, subcutaneous adipose tissue and skin.

1.2.2 Venous Drainage of the Upper Limb

The upper limb venous system returns the deoxygenated blood from the upper limb back to the heart. Anatomically, these veins can be divided into two groups: superficial veins and deep veins. These two systems are connected by perforating veins.

The superficial veins lie within the subcutaneous tissue of the upper limb and can be readily visualised in most individuals. There are two major superficial veins: cephalic and basilic veins. The cephalic vein ascends on the lateral aspect of the upper limb before joining the axillary vein in the axilla. The basilic vein travels along the medial aspect of the upper limb and combines with the brachial veins in the arm to form the axillary vein.

The deep veins are otherwise known as vena comitantes, which are paired veins travelling alongside an artery in the same distribution as the arterial tree.

1.2.3 Cutaneous Blood Supply of the Upper Limb

Anatomy of the Skin and Subcutaneous Tissues

The skin is the largest organ of the body with numerous essential physiological functions. It acts as a sensory organ, allow one to detect and differentiate various modalities such as pressure, pain, temperature and vibration. It provides a protective barrier against trauma from mechanical pressures, thermal injuries, micro-organisms, ultraviolet radiations and injuries from other hazardous substances. The skin is one of the organs that enables thermoregulation to occur through various mechanisms of piloerection, perspiration, vasoconstriction and vasodilatation. It is also responsible for the production of vitamin D. Aside from the aforementioned physiological functions, the skin has significant implications for the psychosocial functioning of individuals which is outside the scope of this thesis.

The skin has two layers. The outer layer is called epidermis, which has four histologically discrete layers, with an additional layer in the glabrous skin (i.e. the palms of the hands and soles of the feet). This is a layer of the skin that does not contain blood vessels and is nourished through diffusion from the upper layers of the dermis. It has a variable thickness dependent on anatomical areas.[79] The dermis is the deeper layer of the skin. The dermis has two further divisions, the superficial papillary dermis and deeper reticular dermis. The reticular dermis contains various structures such as sweat glands, hair follicles and sensory receptors of the skin. Deeper to the dermis lies the subcutaneous tissue. This is a layer of tissue which is primarily used for fat storage, it

contains mostly connective tissues and adipocytes. Subcutaneous tissue is not considered part of the skin.

Vascular Plexuses of the Skin

The blood supply of the skin originates from deep vessels throughout the body. For the upper limb, these are the named arteries as discussed in the previous section, *'Arterial Blood Supply of the Upper Limb'*. As these deep vessels travel along its course, interconnecting vessels emerge from the deep vessels or their associated branches. These interconnecting vessels perforate through overlying structures such as muscles and deep fascia to reach the subcutaneous tissue and the skin.

Dependent on the structure traversed, these perforating vessels are classified as fasciocutaneous or musculocutaneous perforators. On the upper limb, the skin is more commonly supplied by fasciocutaneous perforators. As these perforators emerge from the deep fascia, they travel between the lobules of the subcutaneous fat and reach the dermis. A vascular network called 'subdermal plexus' is formed. The subdermal plexus is considered to be the predominant plexus for cutaneous circulation. As the subdermal arterioles traverse more superficially into the dermis, it becomes the smaller dermal arterioles. These terminal arterioles are found in the reticular dermis and form capillary networks, which are responsible for the transportation of oxygen, nutrients, and wastes to and from the local tissue. Precapillary sphincters form the gateway between arterioles and capillaries. Arteriovenous anastomoses (AVAs) are alternative connections between afferent arterioles and efferent venules. Blood flowing through these anastomoses bypasses the capillary networks and predominantly serve thermoregulatory functions.

These AVAs are commonly found on the glabrous skin, namely the palmar surface of the hands and plantar surfaces of the feet.

The Angiosome Concept

The concept of vascular territories was first studied by Manchot in 1889, who charted vascular territories of the body through identification of cutaneous perforators and underlying source vessels.[80] Salmon subsequently expanded this in 1936, who utilised contrast radiographs in his cadaveric study, mapping out both cutaneous and muscular circulation of the body.[81] In 1987, Taylor and Palmer published their cadaveric study using a combination of ink injection, dissection, perforator mapping and radiographic techniques.[82] This study introduced the term 'Angiosome', which is defined as a three-dimensional block of composite tissue supplied by a single source artery.

Furthermore, it was found that adjacent angiosomes have interconnections in different tissue layers. These can either be true anastomoses where no change in vessel calibre is observed or reduced-calibre 'choke vessels'. A large proportion of these anastomoses are of the latter variety. The concept explains the phenomenon where flap necrosis tends to occur in adjacent angiosomes, as there is insufficient blood flow across the choke vessels to keep the tissues viable. These choke vessels are important in 'delay phenomenon', where a strategic partial division of flap blood supply is implemented prior to definitive flap elevation. This manoeuvre is performed to improve flap survivability by encouraging a permanent dilatation of choke vessels by vessel wall hypertrophy and hyperplasia.[83]

1.2.4 Physiological Factors Affecting Cutaneous Blood Supply

Cutaneous perfusion can demonstrate significant variability due to various physiological and pathological factors. Most of the factors discussed in this section involve changes in blood flow through the alteration of the luminal diameter of arterial vessels. Therefore, a proper understanding of Poiseuille's law is vital to appreciate the relationship between vessel diameter and blood flow.

According to Poiseuille's Law, a small change in luminal radius (r) results in a substantial change in blood flow (Q).[84] The blood flow is also dependent on other factors such as pressure difference (ΔP), vessel length (l) and blood viscosity (η). The formula is shown below:

$$Q = {}^{\Delta P \pi r^4}/_{8\eta l}$$

Myogenic Response

The myogenic response was described by Bayliss in 1902 as an autoregulatory mechanism to maintain consistent blood flow in arteries and arterioles.[85] This is found to be independent of the nervous mechanism. As vascular smooth muscles stretch due to increased intraluminal pressure (via increased flow), a stretch-activated ion channel opens and causes the muscle to contract, resulting in a reduction of luminal size and subsequent reduction of blood flow ensues. On the other hand, as intraluminal pressure decreases, the vascular smooth muscles relax and cause vasodilation to increase blood flow. The result of such mechanism ensures a consistent blood flow to the target tissue and capillaries.

Temperature

As previously discussed, AVAs are small direct connections between arterioles and venules which bypasses capillary networks in the skin. These anastomoses are abundant in the glabrous skin and have an important role in thermoregulation of the body. The AVAs are densely innervated by adrenergic axons.[86] These AVAs receive impulses originating from the thermoregulatory centre in the hypothalamus with variable frequency and amplitude dependent on the ambient temperature.[87] Consequently, the AVAs of the skin open and close in variable proportions in a temperature-dependent fashion. As the ambient temperature approaches the lower end of the thermoneutral zone, the majority of the AVAs closes and reduces the overall cutaneous blood flow in the area. Conversely, towards the higher end of the thermoneutral zone, most AVAs open up to increase skin surface perfusion, leading to loss of excess heat.[87] It is known that superficial veins in the skin also react in a similar manner. The superficial veins constrict to divert venous return through deeper veins in colder temperatures to preserve heat and dilate to perfuse the skin surface to aid loss of excess heat. It is estimated that blood flow fluctuations between the two extremes of the thermoneutral zone in an adult subject can be up to around 20% of the resting cardiac output.[88] In LDF studies, it was found that temperature variations may lead to up to 30% variation in cutaneous perfusion measurements.[4] It is suggested by Barwick et al. that use of LDF with heated probe produces the most reliable method of measurement of skin vasodilatory capacity.[89]

Autonomic Nervous System

In the autonomic nervous system, various neurotransmitters such as adrenaline, noradrenaline, dopamine and neuropeptide-Y are released to act on different receptors of vascular smooth muscles to alter vascular calibre and blood flow. Sympathetic nervous

system stimulation results in the release of noradrenaline from post-ganglionic sympathetic neurons. Noradrenaline activates alpha-1 adrenergic receptors won vasculatures, causing vasoconstriction.

Research by Thomsen et al., who induced regional sympathetic block with guanethidine to the arms of healthy volunteers, has found the removal of sympathetic stimulation has resulted in an increment of 50% to cutaneous perfusion by LDF measurement when compared to control.[90] In the clinical setting, digital sympathectomy is used as a treatment for chronic digital ischaemia refractory to medical treatments.[91] When Yang et al. measured palmar skin perfusion with LDI of patients treated with thoracic sympathectomy for palmar hyperhidrosis, it was found that palmar skin perfusion has improved following the procedure.[92]

Local Control

Various vasoactive chemicals are secreted by endothelial cells and perivascular tissues to encourage vasodilation (histamine, bradykinin, prostaglandins, nitric oxide) and vasoconstriction (endothelins) dependent on physiological states of the local tissue. These vasoactive chemicals act to regulate blood flow at a local level.

In hypoxic tissues, production and accumulation of metabolites such as CO_2, H+, lactic acid, and adenosine occur. These metabolites can stimulate and promote vasodilation to increase local tissue perfusion to cope with the associated metabolic demand. As these metabolites are removed from the local tissue, the vessels constrict. This phenomenon can be observed clinically following the release of tourniquets or blood pressure cuffs. The previously occluded limb or digit develops post-occlusive reactive hyperaemia

(PORH) as a compensatory mechanism to remove hypoxic metabolites. Various endothelial diseases have been shown to demonstrate abnormal PORH patterns on LDF measurements when compared to normal controls.[3,93-97]

Diurnal Variation:

Smolander et al. performed a physiological study measuring core temperature and cutaneous perfusion in various sites (fingers, forehead and forearm). It was found that peripheral skin experiences significant circadian variation when compared against central circulation, which suggests that peripheral cutaneous circulation may be a possible regulatory mechanism for core temperature.[98] This finding is further supported by experiments performed by Aoki et al., who found that the cutaneous vasoconstriction and vasodilatory system respond to ambient temperature changes differently according to the timing of the day.[99] This variation in the cutaneous response to ambient temperature changes according to the timing of the day therefore indicates the need to keep cutaneous perfusion measurements to the same time of the day to avoid experimental bias.

Sex

Researches into gender difference in cutaneous perfusion in healthy individuals have found that female subjects generally exhibit a greater response to skin cooling, through both local vasoconstriction and systemic vasoconstriction.[100] The study has tested female subjects in different phases of the menstrual cycle and has noted intra-menstrual cycle variability in cutaneous responses to skin cooling.[100] This finding is consistent with an earlier study by Bartelink et al., who noted a significant intra-menstrual variation in skin temperature and blood flow to digits, as well as forearm blood flow.[101] It was

suggested in the same paper that compared to men, women of fertile age demonstrate lower peripheral blood flow by up to 50%. This difference of blood flow by gender is not observed when compared to women before menarche and after menopause. Therefore, gender variation in cutaneous vascular response to local temperature changes may be, in part, secondary to a difference in the level of circulating sex hormones. The phase of the menstrual cycle should hence be considered when measuring the state of the peripheral circulation of women.

Body Mass Index (BMI)

Recent studies have found that BMI had an inverse correlation with capillary density at rest, suggesting a reduced baseline cutaneous flow compared to individuals with lower BMI.[102,103] In addition, it is found that obesity is associated with impaired basal vasodilatory response to acetylcholine in women, indicating a level of impaired microvascular function associated with increased BMI.[103]

Challenges in Comparing Study Results

It should be noted that due to the numerous variables mentioned above which influence cutaneous perfusion, comparisons between different studies can be difficult. Therefore, one should be mindful of the specifics in methodology and settings in which measurements were obtained when comparing pre-existing LDF studies. Comparisons should only be attempted where the study settings are similar between the studies.

The above also highlighted the importance to ensure all the aforementioned variables are controlled where possible to ensure the validity of the results is not compromised.

1.3 Laser Doppler Flowmetry Measurements of the Hand

Laser Doppler flowmetry has been used to study the upper limb cutaneous circulations in several specialities in various settings. There appears to be no consensus with regards to the optimal location of measurement. From the previous section 'Applications of Laser Doppler Flowmetry', several studies have measured cutaneous circulation on different locations of the upper limb, such as: index finger[36,104], thenar eminence[13], dorsum proximal phalanx of right thumb[105], forearm[14,51,52], dorsum surface of fingers of the non-dominant hand[19,38], dorsum of distal phalanx of left ring finger[30], unspecified finger pulps[2,24,28,32,33], middle finger pulp[34,39,52,53], and variable 'most clinically affected' site or by random selection[2,26,54]. While some of these studies have a specific site of interest due to the clinical condition being studied, there appears to be a lack of pre-existing literature on the optimal location for LDF measurements of the upper limb. It would be therefore of interest to observe how cutaneous measurements of the upper limb vary according to hand dominance, glabrousness of skin, and distance from the central circulation. This may provide valuable information as a basis for future researchers when designing LDF measurement protocols to the upper limb.

1.4 Trauma of the Upper Limb

1.4.1 Introduction

Traumatic Injuries to the upper limb are common, accounting for a significant proportion of unplanned visits to hospital services in the United Kingdom.[106] These injuries can be the result of many mechanisms of varying severity. Typical examples include lacerations from sharp objects, crush injuries from doors or machinery, assault-related injuries, and animal bites.

The severity upper limb hand trauma can range widely depending on the location and mechanism of injury. Injuries may range from a simple laceration of the skin without underlying structure injuries, to the highly complex 'mangled hand' with substantial injury or loss of multiple anatomical structures of the upper limbs requiring immediate specialist hand surgery and subsequent prolonged physiotherapy and rehabilitation. The diagnostic process of upper limb injuries requires examination from trained clinicians from specialist hand services with a good appreciation of anatomy to ensure accurate diagnosis and prompt management.[106]

Poor management of such injuries can result in chronic stiffness and pain, leading to persistent disability and loss of function. The anatomy of the upper limb is complex, beneath the skin envelope and subcutaneous tissues lie multiple anatomical structures such as muscles, tendons, nerves, vessels, ligaments and bones that contribute to the form and vital function of the upper limb. Injuries to these structures may lead to permanent functional deficits leading to long-term disabilities, affecting one's abilities to work or perform day-to-day activities, loss of work and/or income.[107,108]

The management of these injuries is dependent on clinical finding and mechanism of injury. Regardless of the final management route (i.e. operative or conservative management), there are some routine practices that one may implement to optimise recovery from the injury. Examples of these practices include limb elevation and immobilisation using external splints.

1.4.2 Epidemiology

In the United Kingdom, there is no national database of upper limb injuries. Therefore, it is difficult to estimate the incidence of such injuries accurately. A prospective study performed in an emergency department in Glasgow recorded 1,074 upper limb injuries within an eight-week period in 1985. It was found that attendances related to hand problems accounted for 20% of all attendances. A large proportion of these patients were young males between the age of 12 to 29 years old.[109] A prospective survey of a single hand unit in the UK has found its local incidence of hand injuries to be around 475 per 100,000 inhabitants per year.[110]

Similar studies have been performed in other countries, estimating emergency services attendances due to upper limb injuries to be between 12% to 28.6% of all attendances.[111,112] A retrospective analysis of referral data in a Swedish hospital over eight years has calculated their local incidence to be 700 per 100,000 inhabitants per year.[112] A Danish study surveying multiple emergency departments over two year period has found that upper limb injuries affect 3.7 per 100,000 inhabitants per year.[111] While the variation can be attributed to the sample size, the population profile and the scope of local industries, the fact remains that upper limb injuries are the most common presentation to emergency departments.

The costs associated with the treatment of hand injuries can be extensive. These costs include initial management, surgical treatment, inpatient stay and outpatient attendances for rehabilitation and review. An estimate based on single unit data in 1999 has placed the direct cost of the treatment of acute upper limb injuries to approach 90 million per annum nationally.[113] The figure does not consider the indirect costs of upper

limb injuries, such as loss of productivity, loss of income for the patient and family, loss of productivity of the employers and industry. An estimation based in Europe has placed the indirect costs of upper limb injuries to be up to six times of the direct treatment.[114]

1.4.3 Elevation in Upper Limb Trauma

Limb elevation is a routine practice in medicine following an injury to the limbs. It is thought that elevation of the injured part can reduce oedema secondary to inflammation and venous pooling through gravity. There have been several early pieces of research advocating the use of elevation to avoid complications and undesirable sequelae following injuries to both upper and lower limbs[115,116]

The reason that post-injury limb elevation is practised is due to its effect on the reduction of tissue oedema. The reduced oedema is thought to reduce compression of perforating vessels and capillary beds, thereby improving overall tissue perfusion.[105]

Elevation of the upper limb has been shown to reduce forearm and hand volume in healthy individuals. Using a water displacement device, Boland and Adams measured 45 healthy individuals before and after periods of recumbency and overnight sleep with arm elevation. It was found that arm elevation reduces forearm and hand volume significantly, with an average hand volume reduction of 51mls.[117] However, there have been some studies that have suggested that routine limb elevation does not reduce swelling following elective hand procedures. Fagan et al. compared the effect of high arm elevation with a home elevation device against conventional crepe sling in reducing post-operative swelling following carpal tunnel decompression.[118] The experiment found no significant difference in post-operative swelling between the two group at day five post-

operation. It was concluded that high arm elevation in day case elective procedure does not provide significant benefit over standard crepe sling elevation. Baker et al. prospectively randomised 113 patients undergoing fasciectomy or trapeziectomy.[119] Using volumetric measurements, the study found that while hand elevation reduces the extent of post-operative swelling when compared to the non-elevated group, this difference was not statistically significant. Baker et al. therefore concluded that routine elevation following the above procedures was not necessary. There have been no studies that investigated the effect of elevation on tissue oedema following the upper limb following acute trauma.

However, arterial blood flow to the elevated body parts may be reduced due to the increased hydrostatic pressure created by the column of blood between the elevated parts and the heart. There have been studies which suggest that routine use of elevation may reduce local blood flow in selected situations for both upper limbs and lower limbs using a number of medical devices such as transcutaneous oxygen pressures[120-122], and digital blood pressure[123,124]. Khan et al. used digital plethysmograph to measure digital blood pressures in 30 hospital staff performing four hand positions with increasing level of elevation.[123] A sustained drop in digital blood pressure is associated with hand elevation, in an elevation-degree dependent fashion. Sansosti et al. measured lower limb digital blood pressure in 20 healthy volunteers in various lower limb positions.[124] It was found that digital blood pressure is lower when the lower limb is in a higher position. Decreased perfusion pressure from elevation would reduce local tissue oxygenation. Consequently, patients may complain of pain and paraesthesia following prolonged elevation.[125] There appears to be a delicate balance between the reduction of unwanted

swelling following injury and decreased perfusion pressure secondary to excessive elevation.

1.5 Upper Limb Cutaneous Perfusion Dynamics

While there have been some investigations of perfusion dynamics of upper limbs under elevation using LDF[105], evidence of ideal duration and position of elevation remains sparse. There appears to be no census to the ideal duration and position of limb elevation. In addition, the lack of pre-existing literature on the optimal location for LDF measurements of the upper limb indicates the need for further investigation.

1.5.1 Aim and Purpose of Study

The purpose of this feasibility study is to determine whether if LDF can be used as a research tool in hand surgery, through the following experiments:

1) Hand Mapping: measurement of hand cutaneous perfusion by anatomical location, to determine the effect of distance from central circulation, hand dominance and glabrousness of the skin.
2) Elevation: measurement of the effect of upper limb elevation on cutaneous perfusion.

The above experiments would hopefully provide an improved understanding of cutaneous perfusion and tissue perfusion of healthy individuals. It is hoped that the above experiments would provide a basis for future experiments based on patients with upper limb trauma. Studying the perfusion dynamics of the upper limb in such condition may provide valuable scientific evidence to compliment pre-existing knowledge to

further optimise current hand surgery practices in the management of those with upper limb trauma.

1.5.2 Hypotheses

- Cutaneous perfusion of the hand varies according to the anatomical location due to variable distribution of AVA networks.
- Cutaneous perfusion of the hand varies according to hand dominance, due to the effect of vascularisation through preferential use.
- Upper limb elevation of healthy individuals, in the absence of trauma, will reduce cutaneous circulation in an elevation height dependent fashion.
- As a research tool, LDF provides reliable measurements of the upper limb cutaneous perfusion.

CHAPTER TWO

MATERIAL AND METHODS

2.1 Study Design

A prospective feasibility study was conducted at a district general hospital (DGH) in the North of England. The study was designed to assess the cutaneous perfusion of upper limbs in healthy volunteers using the PF 5010 LDPM unit of the PeriFlux System 5000. Data collection period was between April and June 2017.

2.1.1 Participant Recruitment

Healthy individuals working in the DGH with no acute episodes of illness or significant chronic illness were identified and approached for the study. All participants approached were healthy volunteers known to the author (C Chang) through his social network.

Following a verbal explanation of the project, interested participants were invited to a windowless, temperature and light controlled room based in the non-clinical site of the hospital. In this room, a comprehensive information leaflet (see Appendix 1) was provided to the participant. After adequate time to read and understand the information sheet, a standard informed consent form (see Appendix 2) was signed. Ample opportunities and time were given for the participants to ask questions to clarify the purpose of the study further.

Following consent, a health questionnaire was given for the participants to complete (see Appendix 2), with the relevant information immediately recorded into the participants' individual records for this study. It is thought that this provided sufficient time for the participant to become acclimatised to the environment of the room.

2.1.2 Exclusion Criteria

Individuals were excluded from the study if:

- **Lack capacity to consent**: if the potential participant is unable to understand and retain the study information to make a reasonable judgement and express their wish to enter the study, then they are deemed lacking in capacity to consent.
- **Age Restrictions:** those below 18 years of age were excluded.
- **Personal History of Circulatory Disease:** potential participants with conditions such as peripheral vascular disease, systemic sclerosis, Raynaud's disease, congenital vascular conditions requiring medical or surgical interventions were excluded from the study.
- **Suffering from acute illness/injury**
- **Pregnancy**
- **Allergy to transparent adhesive dressing (Tegaderm™, 3M, St Paul, Minnesota):** as the transparent adhesive dressing is used to adhere the measurement probe against the skin. Therefore, any reported allergy to such dressing is a contraindication for the study.

2.1.3 Ethics

Universal application of medical ethics principles such as autonomy, beneficence, non-maleficence and respect for human rights were applied throughout the research. In addition, participants have rights to informed consent, privacy for research participants, the right to withdraw from the study as well as the right to be informed of research outcomes. As the research project did not involve treatment or therapies, shared decision making as an ethical code of practice was not applicable. The research project did not

involve obtaining specimens from individuals; therefore the return of results was not applicable. All individual data are kept confidential as standard practice.

No obvious ethical issues arose in this research. Potential ethical issues such as confidentiality of individual information and informed consent prior to the study were followed throughout the study without issues.

At the time of commencing the study approvals, the Health Research Authority (HRA) guidance was that HRA approval was not required for projects taking place in a non-NHS setting with healthy volunteers. This study was completed on a single non-clinical site. All identified potential participants are known to the author personally and were recruited as such through his own social network.

Application through alternative research ethics committee was made as per HRA recommendation. All required forms were submitted through the School of Human and Health Sciences Research Ethics Panel prior to the commencement of the study. These forms included an application form, supervisor report and university risk analysis and management form. Ethics approval was granted by the panel on the 1st March 2017 (SREP/2017/240217), as shown in the letter included in Appendix 3. Senior clinicians and trust research management department were informed of the feasibility study before the start of the study.

2.2 Equipment

2.2.1 PeriFlux 5000 System and PF5010 Module

The PeriFlux 5000 System (Perimed, Stockholm, Sweden) is a multifunctional system designed by Perimed, which incorporates a modular design which can incorporate different functional units to accommodate specific clinical and research needs. [Figure 1] shows the Periflux 5000 System with a single LDPM unit installed. For this study, the PeriFlux 5001 Main Unit (Perimed, Stockholm, Sweden) was used, which utilises a solid-state diode laser with 780nm wavelength. According to the manufacturer information, with a probe with standard fibre separation (0.25 mm), the LDF system has a measuring depth of 0.5 to 1mm in normal skin. An additional PF5010 LDPM unit was installed to allow for real-time microvascular perfusion measurements required in this study. The probe used with the system is Probe 404-1 Suturable Angled Probe. This is a single cable probe with a low profile and fenestrations, which enables fixation of the probe on the skin surface either through adhesives or sutures.

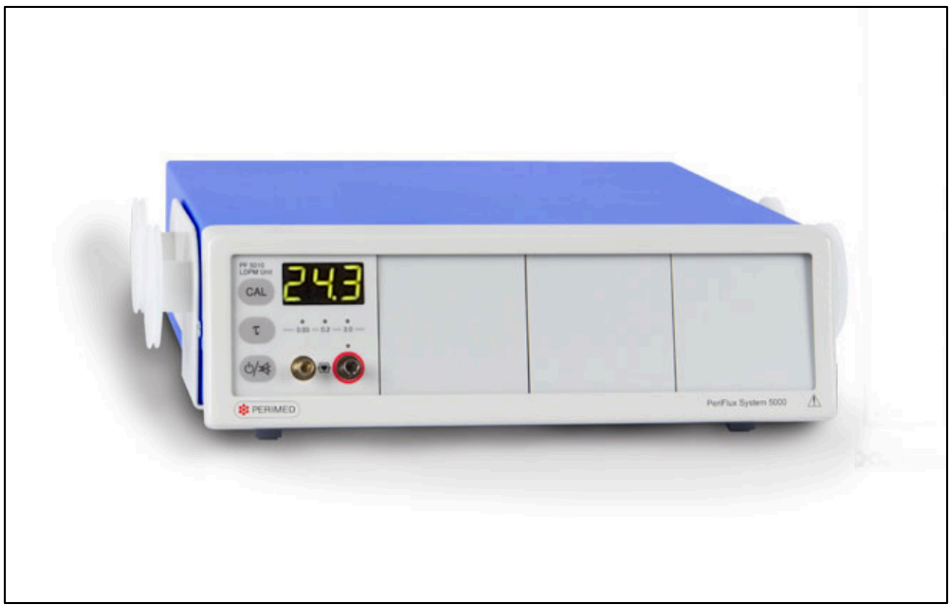

Figure 1: PeriFLux 5000 System

The Periflux 5000 LDF system is attached to a laptop for data collection purposes, and both devices can be easily transported with a trolley. The dimension of the Periflux 5000 machine is akin to that of an electrocardiogram machine. It is therefore possible to use the system in clinical settings by the bedside by healthcare staff. The machine could provide objective data of the level of perfusion of different tissues dependent on the probe placement.

2.2.2 PeriSoft for Windows

PeriSoft for Windows (Perimed, Stockholm, Sweden) is the software used to measure, record and store data collected from the PeriFlux System. The software is designed by the manufacturers of the machine. Individual participant information is collected and recorded prior to measurement. This creates a dedicated folder for the individual participants within the software for data storage. This design automatically compartmentalises the collected data by individuals, avoiding accidental misfiling of collected data.

The software is designed to collect real-time measurements of perfusion over time. The recording period is flexible dependent on research needs, with additional functions to create data tags or time windows in real-time or post data collection for further analysis. Further analysis of individual data windows can be made following data collection, which allows for the calculation of mean perfusion over various smaller time windows within the study periods. Percentage changes in mean perfusion are automatically calculated in this reported mode.

Further information on recording period used in the experiments is discussed below. Upon completion of data collection, a report is generated, quoting mean perfusion over the data collection period.

2.3 Participant Profile and Health Questionnaire

Upon enrolment, a unique identification number was assigned to the participant for data protection and identification purposes. This was recorded on the consent form, the health questionnaire and in the PeriSoft for Windows software.

A health questionnaire was given to participants following enrolment into the study before data collection. The following information was collected to facilitate data analysis:

- Date
- Participant Identifier
- Age
- Sex
- Hand Dominance
- Smoking Status
- Past Medical History
- Family History
- Drug Allergies
- Medical History
- Alcohol History

The information provided above was recorded in the electronic participant database immediately and suitability to participate in the study was assessed. If deemed suitable to proceed, further explanation of the study was provided, and verbal instruction of the experiment was given to the participants.

2.3.1 Standard Participant Baseline Data Set

Further to the above health questionnaire, additional information was recorded at the time of data collection. This included the presence of decorative items such as pieces of jewellery, watches or electronic fitness devices, the use of nail varnish or makeups, and the area used for measurement.

All decorative items were removed prior to data collection to avoid interference through direct contact with the probe or optic fibre cable lead or constriction or pressure on the skin. While no measurements were performed on the nailplate or the nailbed, all false nails and nail varnishes were removed before measurements.

The area of measurement was coded numerically. The coding system is discussed in the later section, *'Measurement Protocols: Hand Mapping'*.

2.4 Measurement Protocols and Standardising Measures

2.4.1 Machine and Probe

To reduce the risk of equipment errors secondary to different machines or baseline calibration, the same PeriFlux 5001 Unit and Probe 404-1 Suturable Angled Probe (Perimed, Stockholm, Sweden) were used to take all measurements. Prior to every data collection session, the machine was turned on for 30 minutes to ensure it has adequately

warmed up. After this 30 minutes warm-up period, the Perimed PF 1000 calibration device was used to calibrate the baseline of the machine. This ensured the electrical zero measured by this machine was consistent throughout the experiments. The probe was disinfected before and after every participant contact with a disposable alcoholic chlorhexidine 2% wipe.

During the initial trial experiment with the machine, it was found that the probe and the optic fibre cable lead was extremely sensitive to movement, leading to significant movement artefacts.

To eliminate the risk of movement artefacts three points were identified which required immobilisation:

1) Probe
2) Optic Fibre Cable Lead
3) Upper Limb/Measured Area

The probe was attached to the measured skin firmly without any risk of movement for the duration of the experiments. This was achieved by using a 7cm x 8.5cm transparent adhesive dressing (Tegaderm IV™, 3M, St Paul, Minnesota) to hold the probe onto the skin gently. The transparent dressing was cut into 1cm strips to avoid excessive use of dressing material on the skin. Care was taken to not exert excessive pressure on the probe, as this could influence the probe reading. Similarly, insufficient pressure on the probe may lead to loss of reading as the probe falls off the skin. The dressings were lightly placed over the skin and probe. Care was taken to ensure no excessive skin tension was produced by the dressing. The dressing was removed and reapplied if there was clear

evidence of excessive tension being produced such as blanching of the skin or visible striation of the transparent dressing. The skin of the measured area was kept at a 'social clean' state, and hand washing was performed if visibly dirty or contaminated. The cable lead was kept tension free from the probe to the machine and was rested on a flat surface along the entire length of the lead to avoid swinging or dangling of the cable. The measured upper limb was kept in a rested state in various positions as described in the latter sections. No movement of the limb is permitted to limit the risk of movement artefacts.

2.4.2 Interference from Environmental Factors

It is noted that the background lighting level may influence the data measurements from LDF. Previous studies have suggested the use of aluminium foils to eliminate such ambient lights.[105] However, such measures have not been used in this study. This is because the ambient lighting level was controlled through the use of a windowless room with a consistent level of background lighting throughout the experiments. Furthermore, the room was temperature controlled by central air conditioning, with the temperature maintained at 22°C. Maintenance of a consistent temperature would eliminate inaccuracies in the measurement of microcirculation secondary to vasoconstriction or vasodilatation from changes in ambient temperature. All data were collected during two months to minimise the influence of seasonal variation on background temperatures. To account for temporal variation in circulation, all measurements were taken during the same time window between 10:00 to 16:00.

2.4.3 Participant Factors

The patient recruitment process excluded individuals with acute episodes of illness or significant chronic illnesses. This exclusion prevented those with significant physiological deviation to be included in the study. This was designed to ensure proper standardisation and to minimise the influence of abnormal physiology on the result.

In the elevation branch of the study, each patient acted as their internal control, as initial measurements were performed in the baseline state before proceeding to the various angles of limb elevation.

During the data collection period, the participants were instructed to remain stationary, avoid deep breaths and other sudden movements to minimise movement artefacts and sympathetic stimulation.[105] Measurements for participants that have recently eaten (<1 hour) were deferred until one hour after their meal. This is also the case for those recently undergone exercises. These restrictions were in place to avoid significant variations from baseline cutaneous secondary to sympathetic and parasympathetic stimulation.

Furthermore, all audio-visual stimulus (i.e. television programmes, films, and music) were removed from the study room to avoid unnecessary stimulation.

2.4.4 Operator Factors

To ensure standardisation of measurement technique, the same operator (C Chang) has taken all measurements throughout the study. During the three months data collection period only the author (C Chang) has access to the machine and the laptop.

2.4.5 Measurement Protocols: Hand Mapping

Following the aforementioned consenting and briefing process, all participants were instructed to sit in the designated seat, placing both hands in front of a table. For all participants, this position placed their hands at a height just below heart level. The acclimatisation time given for each subject was the time taken for completion of informed consent, health questionnaire and an introduction of the LDF machine. While the duration of this time is not formalised, it typically lasts around 5 minutes per participant.

The participants were instructed to place their palm on the table when the probe is placed on the dorsum of their hand, and vice versa. The unmeasured side was kept in the same position as the measured side throughout the experiment.

The areas measured were divided into 47 zones of interest, with 23 on the palmar surface, and 24 on the dorsal surface. The areas on the digits (1 to 14, 24 to 37) were defined by the natural skin crease present on both the dorsal and the volar surface. [Figures 2 and 3] shows the boundaries and distribution of the hand mapping areas as described in the following paragraphs. Corresponding descriptors are summarised in [Table 1].

The dorsal areas on the non-digital portion of the hand were defined by the natural groove between the metacarpal bones as determined by palpation. This formed five rays, which were further divided by an imaginary midline bisecting these rays into ten areas (38 to 47)

The volar surface was divided into four portions, with further divisions:

1) The area between the digitopalmar crease and the distal palmar crease (15 to 18, subdivision by the natural grooves between metacarpal bones)
2) Thenar eminence (19 to 20, subdivision by an imaginary midline)
3) Hypothenar eminence (22 to 23, subdivision by imagery midline)
4) Midpalm (21, the area between distal palmar crease, hypothenar and thenar eminence).

The probe was placed directly in the centre of the area measured. This was not possible for the distal portion of digits on the dorsal surface (24, 26, 29, 32 and 35), as it was not possible to measure perfusion through the nailplate. Instead, the probe was placed on the eponychial fold when measuring these areas.

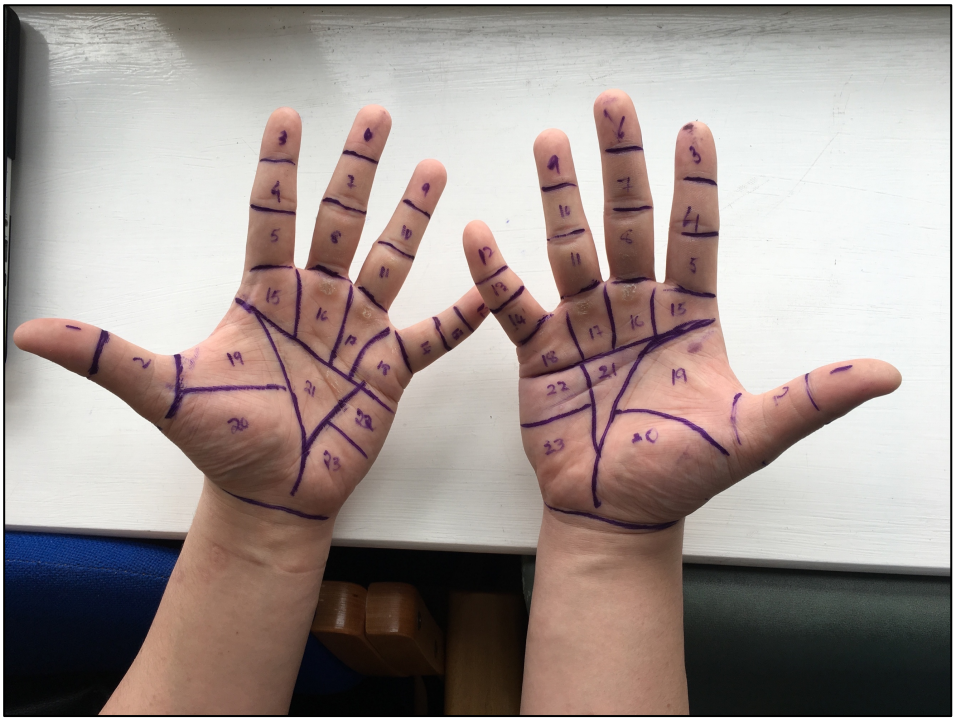

Figure 2: Hand Mapping Areas (Volar)

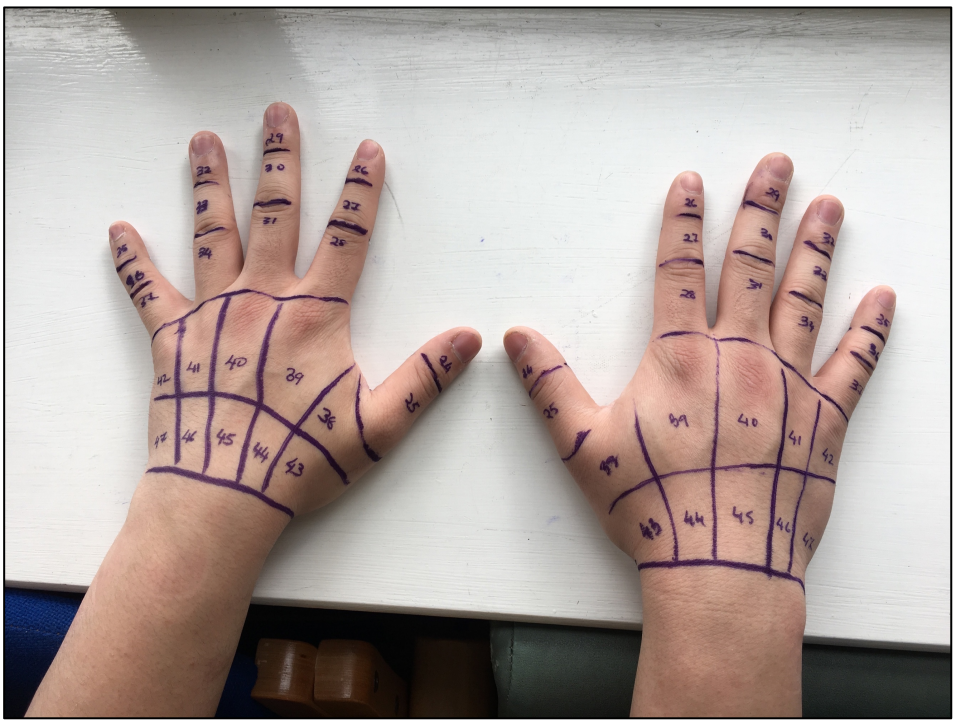

Figure 3: Hand Mapping Areas (Dorsal)

Volar Digit		Volar Hand		Dorsal Digit		Dorsal Hand	
Area	**Description**	**Area**	**Description**	**Area**	**Description**	**Area**	**Description**
1	Thumb Pulp	**15**	Index Finger Distal Palm	**24**	Thumb Eponychium	**38**	Thumb Distal Dorsum
2	Volar Thumb Proximal Phalanx	**16**	Middle Finger Distal Palm	**25**	Dorsal Thumb Proximal Phalanx	**39**	Index Finger Distal Dorsum
3	Index Finger Pulp	**17**	Ring Finger Distal Palm	**26**	Index Finger Eponychium	**40**	Middle Finger Distal Dorsum
4	Volar Index Finger Middle Phalanx	**18**	Little Finger Distal Palm	**27**	Dorsal Index Finger Middle Phalanx	**41**	Ring Finger Distal Dorsum
5	Volar Index Finger Proximal Phalanx	**19**	Distal Thenar Eminence	**28**	Dorsal Index Finger Proximal Phalanx	**42**	Little Finger Distal Dorsum
6	Middle Finger Pulp	**20**	Proximal Thenar Eminence	**29**	Middle Finger Eponychium	**43**	Thumb Proximal Dorsum
7	Volar Middle Finger Middle Phalanx	**21**	Mid-Palm	**30**	Dorsal Middle Finger Middle Phalanx	**44**	Index Finger Proximal Dorsum
8	Volar Middle Finger Proximal Phalanx	**22**	Distal Hypothenar Eminence	**31**	Dorsal Middle Finger Proximal Phalanx	**45**	Middle Finger Proximal Dorsum

9	Ring Finger Pulp	**23**	Proximal Hypothenar Eminence	**32**	Ring Finger Eponychium	**46**	Ring Finger Proximal Dorsum
10	Volar Ring Finger Middle Phalanx			**33**	Dorsal Ring Finger Middle Phalanx	**47**	Little Finger Proximal Dorsum
11	Volar Ring Finger Proximal Phalanx			**34**	Dorsal Ring Finger Proximal Phalanx		
12	Little Finger Pulp			**35**	Little Finger Eponychium		
13	Volar Little Finger Middle Phalanx			**36**	Dorsal Little Finger Middle Phalanx		
14	Volar Little Finger Proximal Phalanx			**37**	Dorsal Little Finger Proximal Phalanx		

Table 1: Description of Hand Mapping Areas

For each area measured, the probe was attached for at least 150 seconds. This period allowed for around 30 seconds for the participants to position their hands back to the default position. Furthermore, this brief period allowed the perfusion measurements to stabilise to a baseline tracing. Once the tracing stabilised, the record mode was switched on for 120 seconds. This measurement window provided adequate data for one to determine an accurate representation of baseline skin perfusion while being sufficiently brief to avoid unnecessary discomfort for the participants from remaining stationary during the data collection.

Measurements were taken for all 47 areas on both hands for the first participant. While this provided a significant amount of data, the time taken for data collection was unacceptably long (3.5 hours in total). To reduce the risk of participant withdrawal from the study, an abbreviation of measurement area was deemed necessary.

From observation during the first participant measurement, it was apparent that certain areas were difficult to measure due to local skin quality and surface anatomy leading to poor probe adherence. These areas were the volar aspects of the middle and proximal phalanx of digits (areas 2, 4, 5, 7, 8, 10, 11, 13, 14) and mid-palm (area 21). Similar areas were removed from the dorsum of the hand to allow for pairing (areas 25, 27, 28, 30, 31, 33, 34, 36, 37). These areas were excluded from the subsequent measurements, reducing the number of areas studied to 18 for each side as shown in [Table 2] below, primarily focused on digital tip measurements and selected hand measurements. This reduced measurement protocol results in a data collection period of around 90 minutes per participant.

Volar Digit		**Volar Hand**		**Dorsal Digit**		**Dorsal Hand**	
Area	**Description**	**Area**	**Description**	**Area**	**Description**	**Area**	**Description**
1	Thumb Pulp	**15**	Index Finger Distal Palm	**24**	Thumb Eponychium	**39**	Index Finger Distal Dorsum
3	Index Finger Pulp	**18**	Little Finger Distal Palm	**26**	Index Finger Eponychium	**42**	Little Finger Distal Dorsum
6	Middle Finger Pulp	**19**	Distal Thenar Eminence	**29**	Middle Finger Eponychium	**43**	Thumb Proximal Dorsum
9	Ring Finger Pulp	**22**	Distal Hypothenar Eminence	**32**	Ring Finger Eponychium	**47**	Little Finger Proximal Dorsum
12	Little Finger Pulp			**35**	Little Finger Eponychium		

Table 2: Abbreviated Hand Mapping Protocol

According to the verbal feedback given by the participants, the time taken was still too long. As a consequence, further exclusion of measurement areas was made to remove measurement of dorsal areas and non-digital areas (area 15, 18, 19, 22, 24, 26,29, 32, 35, 39, 42, 43, 47). These areas were excluded on the basis of inadequate quality and low

average perfusion levels observed. This resulted in a final measurement protocol of five areas: pulps of the thumb, index finger, middle finger, ring finger and little finger (1, 3, 6, 9, 12). These areas were chosen specifically as the distal portion of the digits are generally where perfusion compromises occur in clinical settings (such as inotrope associated digital ischaemia, Raynaud's phenomenon and following digital replantation), and therefore would be of higher clinical relevance. The pulps were preferentially chosen as the eponychium of digits provides poor tracing quality due to limited soft tissue availability for probe adherence. This protocol was better tolerated by the subsequent participants and was used without further alterations.

2.4.6 Measurement Protocols: Elevation

The effect of hand elevation on cutaneous perfusion used measurements from a single optimal point of measurement, as determined by the hand mapping experiment. In this case, the right middle finger pulp was used.

Following the aforementioned consenting and briefing process, all participants were asked to lie on an examination couch set at 45° with right hand resting on a table. Once the participant was in position, the examination couch was adjusted so that the right hand corresponds to the level of the participant's heart level with the elbow straight (180°). This was the baseline position.

The participants right hand was placed in a thermoplastic splint in a position of safe immobilisation (interphalangeal joints straight, metacarpophalangeal joints 90° flexion and wrist joint in neutral position). The immobilisation was used to remove the risk and extent of unintentional movements during periods of elevation, thereby reducing

movement artefacts. The participants were asked to keep their forearm in a neutral position without deliberate supination or pronation.

The following positions were performed in order as listed:

Position	Upper Arm	Elbow
1 (Baseline)	Heart Level	180°
2	Full Elevation	180°
3	Dangling	180°
4	Heart Level	45° Flexion
5	Heart Level	90° Flexion

The participants were asked to raise their right arm fully towards the ceiling in position two, and dangling their right arm fully in position three. In position four and five, a Bradford sling (Capatex, Nottingham, United Kingdom) suspended from an IV drip stand was used to provide support and guide angle of elbow flexion. Examples of the set up can be seen in [Figures 4 to 8] which demonstrates the different positions of the upper limb was placed during this experiment.

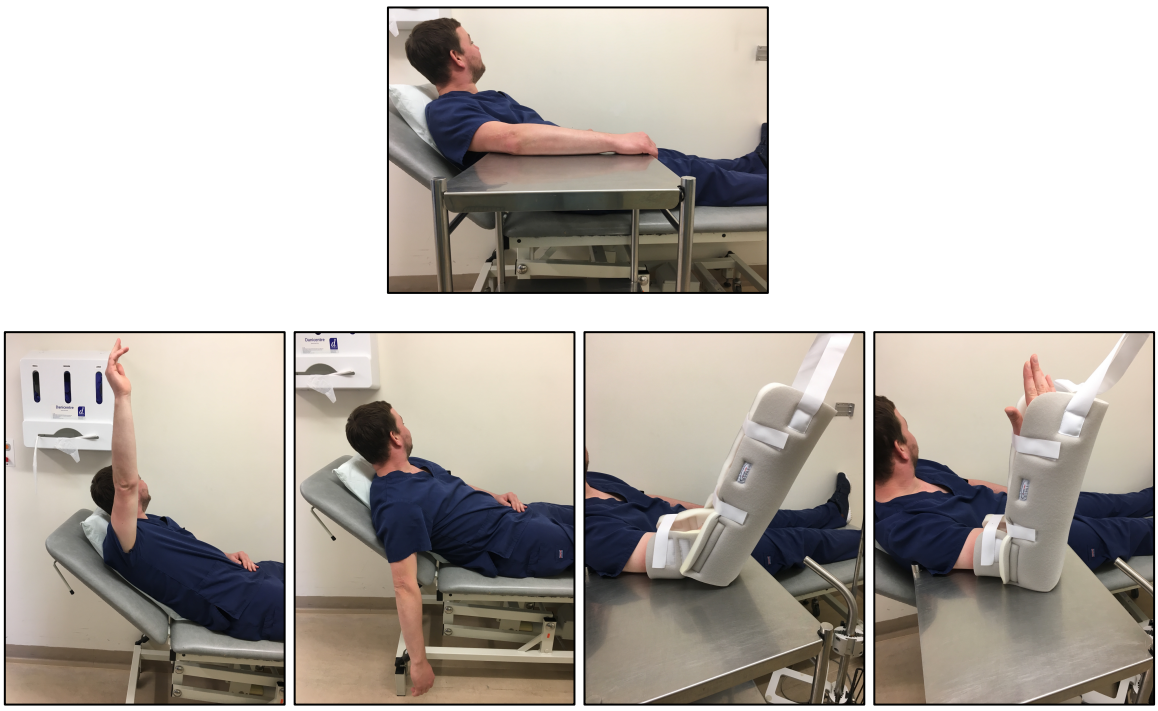

Figure 4-8: Hand Elevation Positions

(Top: position 1, Bottom (left to right): Positions 2 to 5)

Once the probe was attached and the tracing has stabilised with the participant at the baseline position, the record mode was switched on. Each position was recorded for 90 seconds, with 30 seconds interval to allow for the participant to change position and the tracing to stabilise. The data tracing during these time intervals is removed from analysis using the software's selection function. Overall data collection time for individual participants was around ten minutes.

2.5 Unplanned Events

In this study, unplanned events are defined when there is: sudden changes in participant's capacity or availability (acute illness, emergency clinical commitments), the author's ability to collect data (acute illness, equipment failure) or need to terminate study collection due to evacuation needs (fire, flood). In such circumstances, the collected

data would be included where appropriate unless data collection can be performed again on an alternative day. There were no unplanned events that occurred during the course of this study.

2.6 Data Collection, Storage and Analysis

2.6.1 Data Collection and Storage

A password-protected laptop dedicated to the study was installed with the software to facilitate data collection. This laptop was kept secure in a locked room during storage between data collection sessions. The laptop was deliberately kept offline to ensure its data security further. The password was only available to the author (C Chang) and the supervisor (W Gillibrand).

Once the probe was attached to the study area, the perfusion data was generated and displayed on the PeriSoft for Windows software immediately. This data was not automatically recorded unless the record mode is switched on. Once switched on, the displayed data was recorded in real-time. To avoid biases from the observer-expectancy effect, the display was pointed away from the participant during the collection process. To minimise such effect for the researcher, the display was only viewed when inserting annotations (i.e. elevation phase) or starting/stopping record mode.

Once record mode was stopped, the data collected is automatically stored on the hard drive of the password-protected laptop. At the end of each data collection day, the data was exported as a portable document format (PDF) file in various pre-set modes.

In the hand mapping experiments, the general analysis report mode was used. In this mode, the trace is shown in a line graph over time along with basic statistic calculation such as mean value, standard deviation, standard error, maximum and minimum values.

Percent change report was used in the hand elevation experiments. This mode provided the tracing over time, along with the mean value of perfusions within different timeframe area (i.e. different phases of upper limb elevation). The differences in the mean values of these areas are shown by absolute values and percentage differences.

All data was anonymised at the time of participant recruitment, with all data referenced with participant ID only. Participant names and contact details can be traced only through cross-referencing the study consent form and the health questionnaire with the participant ID. These forms are paper-based and stored securely in a locked room.

2.6.2 Data Processing and Analysis

The exported data was recorded in a Microsoft Excel spreadsheet along with individual participant data collected from the health questionnaire. The data was then organised and imported into GraphPad Prism version 7.0d (GraphPad Software, San Diego, California) for further statistical analysis.

Hand Mapping: Each data was assigned into one of eight subgroups to facilitate analysis. These groups were divided by hand dominance, anatomical surface measured, and structure measured, as shown below:

Group	Hand Dominance	Anatomical Surface	Structure Measured
1	Dominant	Volar	Hand
2	Dominant	Volar	Digit
3	Dominant	Dorsum	Hand
4	Dominant	Dorsum	Digit
5	Non-Dominant	Volar	Hand
6	Non-Dominant	Volar	Digit
7	Non-Dominant	Dorsum	Hand
8	Non-Dominant	Dorsum	Digit

It should be clarified that in the results section of the thesis that the 'hand', when compared to digits, describes the area of the non-digit portion of the hand (i.e. the palm and the opisthenar region, denoted by areas 15–23 and 38-47).

The average mean and standard deviation were taken from the general analysis reports and calculated for each group for subsequent comparison. Four main comparisons were made to determine the effect on upper limb cutaneous perfusion from the following factors: hand dominance, anatomical surface, structure measured and digits.

Hand Elevation: The mean perfusion values from each position were extracted from the generated reports of every participant, these values are called *positional perfusion,* with a modifier ranging from one to five depending on the position of the limb when the value was taken. Comparative analyses of the five *positional perfusion* values were performed to determine the relationship of upper limb positional change on cutaneous perfusion.

CHAPTER THREE

RESULTS

3.1 Study Participants

The prospective feasibility study was conducted between April 2017 to June 2017. During the study period, a total of 40 participants were identified to have met the inclusion criteria and recruited for the two experiments. Each experiment had twenty participants. There was no crossover of participants between the two experiments. All identified potential participants consented to take part in the study.

The participant numbers were limited to twenty per group. This arbitrary sample size was decided as a matter of convenience for this time-limited feasibility study. The author is aware of the risk of insufficient statistical power secondary to the small sample size in this study. While the results produced may not necessarily be significant due to this, the results should provide valuable insights into whether future studies would be feasible and worthwhile.

3.1.1 Participant Profile

The breakdown of participant profile details is demonstrated in [Table 3]. Participants 0000 to 0019 (n=20) took part in the hand mapping arm of the study, while participants 0020 to 0039 took part in the limb elevation experiment. The majority of patients were female (n = 29), this is likely reflective of the healthcare workforce distribution. Mean age was 42.6 years (range 22 to 64 years). The participants were predominantly right hand dominant, and non-smokers who consume alcohol. A further breakdown of participant demographics by experiment arms is shown in Appendix 4.

	Hand Mapping	Elevation	Combined
Age (year)	41.2 (22 - 64)	44 (27 - 59)	42.6 (22 - 64)
Gender	7M:13F	4M:16F	11M:29F
Hand Dominance	19RHD 1LHD	17RHD 3LHD	36RHD 4LHD
Smoking Status	2 Smokers 18 Non-Smokers	1 Smoker 19 Non-Smokers	3 Smokers 37 Non-Smokers
Drinking Status	17 Drinkers 3 Non-Drinkers	17 Drinkers 3 Non-Drinkers	34 Drinkers 6 Non-Drinkers

Table 3: Summary Participant Profile

3.1.2 Complications, Side Effects and Unanticipated Events

All participants were able to perform the tasks successfully according to instructions provided at the start of the experiment. There were no unanticipated events which occurred during the study.

For the hand mapping experiment, some participants noted difficulty to remain stationary during the data collection period. However, they were able to persist sufficiently in allowing for the data collection to be completed. Most participants found position two (full elevation) of the elevation study to be the most uncomfortable position. Participants often demonstrated early signs of muscle fatigue and digital paraesthesia towards the end of the 90 seconds period. These symptoms invariably resolve spontaneously shortly following the end of the experiment.

Participant 0013 and 0031 reported a previous allergic reaction to dressings, noting the formation of superficial blisters and rash following previous use of Elastoplast adhesive dressings. Tegaderm™ dressings were used to secure LDF probe for these patients without causing any development of aforementioned allergic reactions.

3.2 Explanation of Results

3.2.1 Normality Testing of Cutaneous Flow Measurements

Normality testing by D'Agostino-Pearson test of combined cutaneous flow measurement (flux) demonstrated a non-Gaussian distribution. It should be noted that the large sample size may cause the test to be too sensitive when detecting differences in the sample group distribution against an ideal Gaussian distribution. This means that a distribution which closely approximates Gaussian distribution may be erroneously considered non-Gaussian if the data set is too large.

This potential influence is considered when selecting relevant statistical tests in this study. Parametric tests such as Student's t-test and analysis of variance (ANOVA) can be sufficiently robust to tolerate some deviation from the Gaussian assumption provided that the sample size is large. However, it was decided that the cutaneous perfusion should be considered non-Gaussian as there is an insufficient number of data for the cutaneous flow of the area on the hand (i.e. areas 15 – 23 and 38 - 47).

Subsequent normality testing of digital cutaneous flow measurement has revealed that the data set is consistent with Gaussian distribution. Therefore, parametric tests were used for comparative analysis for subsections where only digital cutaneous flows are included in the dataset.

3.3 Results: Hand Mapping Study

3.3.1 Hand Mapping Groups

To facilitate analysis of data, the hand mapping perfusion values are grouped by several factors: the hand dominance status, the anatomical surface of the hand, and the structure measured. Accordingly, the hand perfusion values are divided and grouped into eight hand mapping groups. The grouping details along with corresponding areas are shown below:

Group	Hand Dominance	Anatomical Surface	Structure Measured	Corresponding Areas
1	Dominant	Volar	Hand	15-23
2	Dominant	Volar	Digit	1-14
3	Dominant	Dorsum	Hand	38-47
4	Dominant	Dorsum	Digit	24-37
5	Non-Dominant	Volar	Hand	15-23
6	Non-Dominant	Volar	Digit	1-14
7	Non-Dominant	Dorsum	Hand	38-47
8	Non-Dominant	Dorsum	Digit	24-37

3.3.2 Analysis of Participants of Hand Mapping Study

Participant 0000 was the first participant enrolled in the study, who had undergone cutaneous flow measurements of all 94 areas on the hands. This has generated a significant volume of data. A comprehensive breakdown of mean perfusion value of each area is shown in Appendix 5. [Table 4] is a summary of perfusion values of participant 0000 by hand mapping groups.

Group	1	2	3	4	5	6	7	8
Number	9	14	10	14	9	14	10	14
Mean (AU)	92.99	203	12.46	40.19	164.5	245.5	11.91	51.45
SD (AU)	55.68	91.51	4.794	14.93	113.6	92.43	6.215	32.17

Table 4: Summary Mean Perfusion by Group for Participant 0000

As discussed in the previous chapter, due to the practical limitation of time and local skin quality, an abbreviated measurement protocol was trialled for participants 0001 and 0002. The measured area was reduced from 47 to 18 each side. This dramatically reduced the measurement time down from over 210 minutes down to 90 minutes (150 seconds per area).

[Table 5 and 6] are summaries of perfusion values of participants 0001 and 0002, respectively. It should be noted that while the protocol is shortened, all hand mapping groups were sampled. A review was taken to further condense the measurement protocol following verbal feedback from the participants. Several areas were removed due to poor tracing reliability and low mean perfusion value. Examples tracing report along with reasons for exclusion and inclusion is shown in Appendix 6. A final measurement protocol of five areas each side was decided for the subsequent participants for the rest of the study. The finalised protocol only reviews areas from group 2 and 6. Mean perfusion values of participants 0003 to 0019 are shown in [Table 7]

Group	1	2	3	4	5	6	7	8
Number	4	5	4	5	4	5	4	5
Mean (AU)	168.1	244.3	26.57	42.74	104.3	195.5	30.53	51.35
SD (AU)	46.95	69.3	15.28	10.33	63.49	61.71	22.02	21.18

Table 5: Summary Mean Perfusion by Group for Participant 0001

Group	1	2	3	4	5	6	7	8
Number	4	5	4	5	4	5	4	5
Mean (AU)	163.8	198.3	13.57	82.98	67.42	144.7	17.8	48.84
SD (AU)	86.1	86.23	5.022	28.32	28.82	66.14	14.79	51.82

Table 6: Summary Mean Perfusion by Group for Participant 0002

Summary Data Participant 0003 – 0019								
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Number		85				85		
Mean (AU)		247.6				248.3		
SD (AU)		100.5				118.9		

Table 7: Summary Mean Perfusion by Group for Participants 0003 - 0019

3.3.3 Mean Perfusion Comparison by Group

Mean cutaneous perfusion by hand mapping groups were calculated and listed by protocol phases. These values are shown in **[Table 8]** below for further comparison.

	Participant ID			
	0000 Mean (AU)	**0001 Mean (AU)**	**0002 Mean (AU)**	**0003-0019 Mean (AU)**
Group 1	92.99	168.1	163.8	
Group 2	203	244.3	198.3	247.6
Group 3	12.46	26.57	13.57	
Group 4	40.19	42.74	82.98	
Group 5	164.5	104.3	67.42	
Group 6	245.5	195.5	144.7	248.3
Group 7	11.91	30.53	17.8	
Group 8	51.45	51.35	48.84	

Table 8: Combined Summary Mean Perfusion for Hand Mapping Study

[Figure 9] is a chart showing the mean perfusion values by hand mapping group. The groups 2 and 6 (the volar aspect of digits) appear to have the highest mean cutaneous flow across all participants, with the lowest mean cutaneous flow for groups 3 and 7 (dorsal aspect of hands).

The figure demonstrates that the variations between each hand mapping group appear to be comparable between the initial full protocol used for participant 0000 and the abbreviated protocol used by participant 0001 and 0002.

In the most condensed final protocol group, the sampled groups (i.e. groups 2 and 6) seems to have similar mean values compared to the measurements taken from the same groups of the more comprehensive protocols. This perhaps suggests that pulp measurements are representative of volar digital cutaneous perfusion as a whole.

While there appears to be some form of correlation of mean perfusion by hand mapping group, it should be noted that the sample size is too small to determine the significance of correlation. Therefore, no statistical tests were performed to assess for correlation.

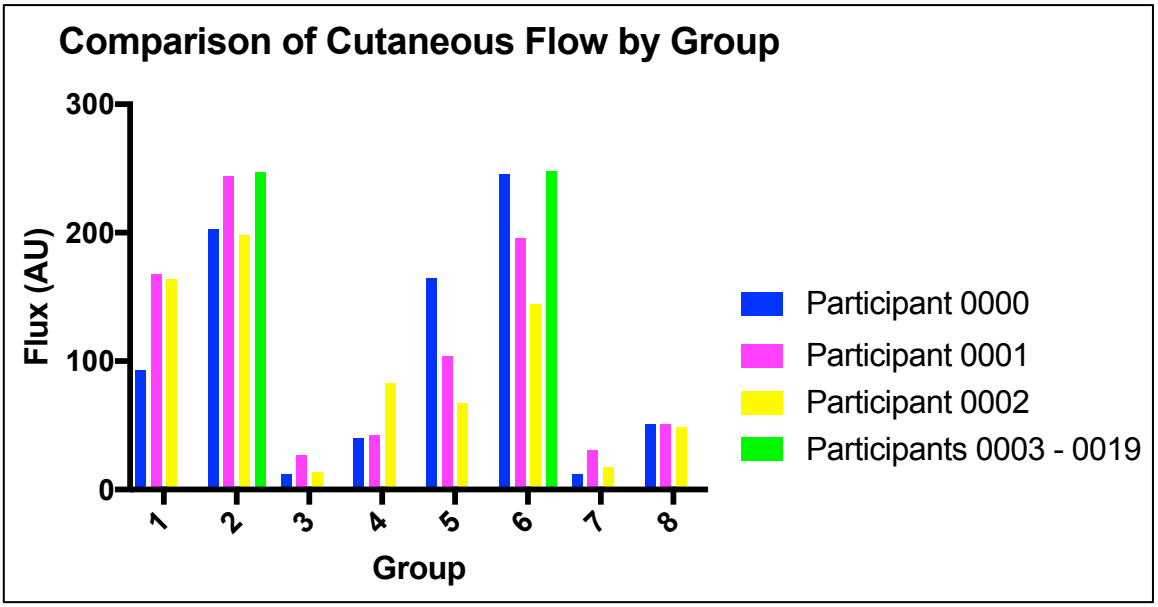

Figure 9: Comparison of cutaneous flow by group, charted for all participants

3.3.4 Effect of Hand Dominance on Cutaneous Flow

The data set is considered non-Gaussian as mentioned in the previous section. As the measurements taken from both groups (i.e. dominant side vs non-dominant side) values taken from corresponding areas of the same set of participants, the data is considered

suitable for paired analysis. Therefore, comparative analysis by Wilcoxon matched-paired signed rank test was performed to determine the effect of hand dominance on cutaneous perfusion.

Three subgroups were analysed: digit, hand and combined. [Table 9] demonstrates the details of descriptive statistics and results from comparative analyses of the subgroups. There is no statistical significance between the cutaneous flow measurements on the dominant and the non-dominant side when comparisons are made between digits (p=0.61), hands (p=0.89) and combined (p=61).

	Digit		Hand		Combined	
	Non Dominant	Dominant	Non Dominant	Dominant	Non Dominant	Dominant
Number	133	133	35	35	168	168
Median (AU)	213.4	208.1	41.61	32.18	172.8	184.8
Mean (AU)	206.5	205.2	70.86	69.98	178.2	177.1
SD (AU)	127	115.5	86.84	74.05	131.7	121.3
p-value	0.61		0.89		0.61	
Significant?	No		No		No	

Table 9: Effect of Hand Dominance on Cutaneous Flow

[Figures 10 to 12] show comparable mean cutaneous flows and standard deviation within individual subgroups.

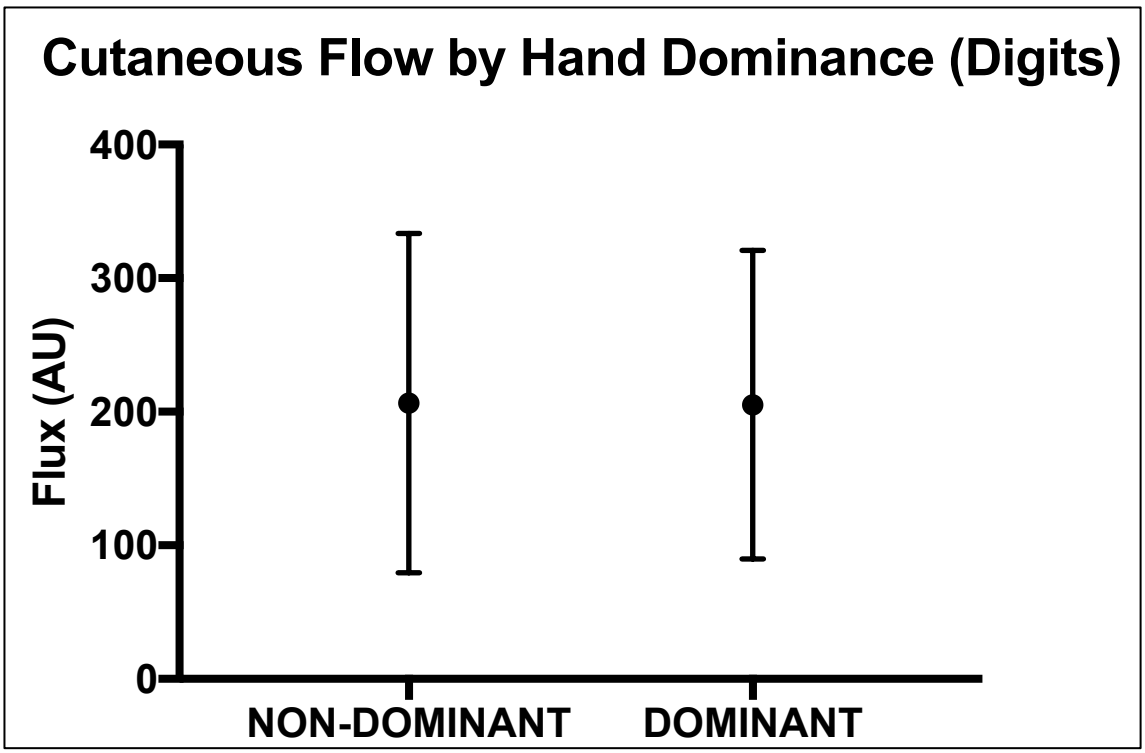

Figure 10: Comparison of Cutaneous Flow by Hand Dominance for Digit Measurements

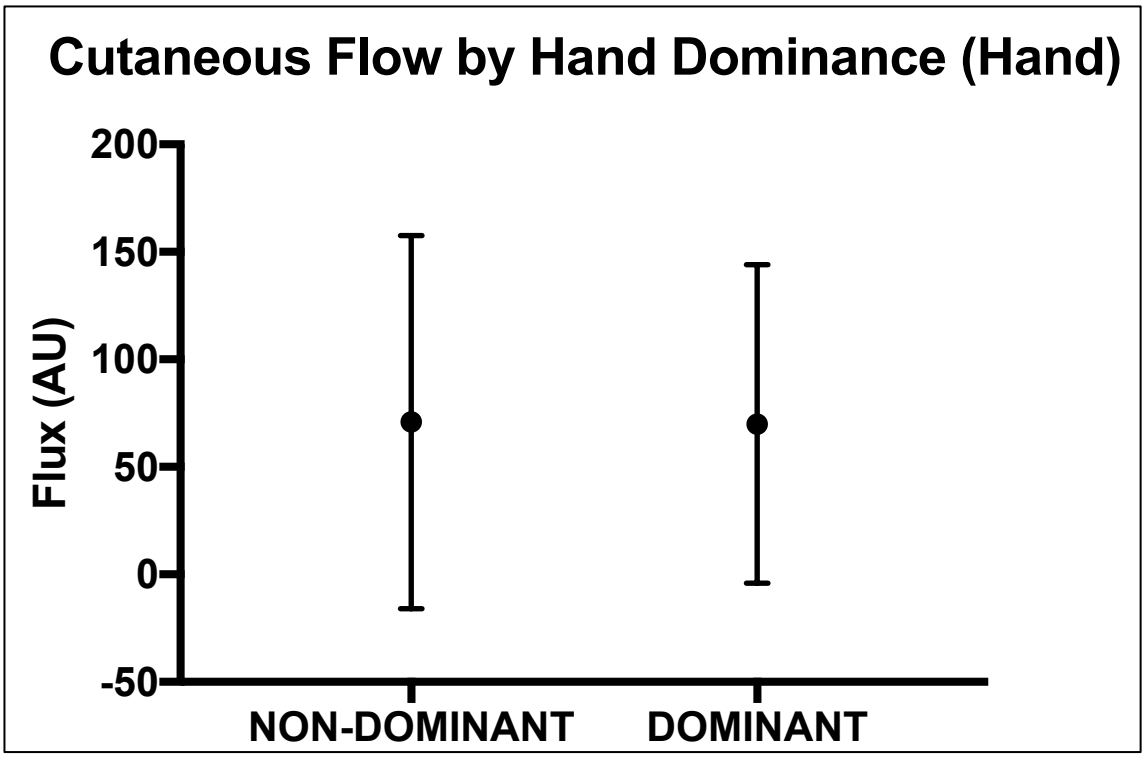

Figure 11: Comparison of Cutaneous Flow by Hand Dominance for Hand Measurements

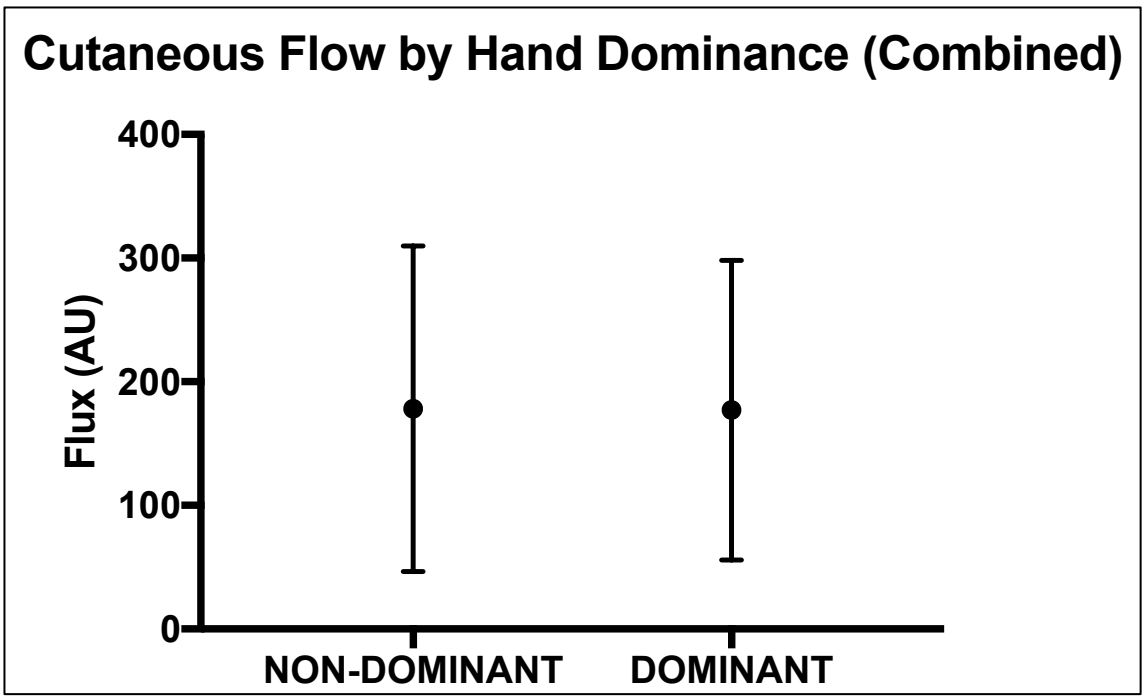

Figure 12: Comparison of Cutaneous Flow by Hand Dominance for Combined Measurements

3.3.5 Effect of Skin Surface (Dorsal vs Volar Skin) on Cutaneous Flow

In this section, comparisons were made from values from dorsal and volar sides of digits and hands. The areas measured dorsal side did not correspond to the volar side and therefore pairing was not deemed suitable. Furthermore, as multiple measurements are collected per study subject, the data is considered to be clustered. Therefore, linear regression is used for analysis.

Three subgroups were analysed: digit, hand, and combined. [Table 10] demonstrates the details of descriptive statistics and results from comparative analyses of the subgroups.

	Digit		Hand		Combined	
	Dorsal	Volar	Dorsal	Volar	Dorsal	Volar
Number	48	218	36	34	84	252
Median (AU)	45.94	239.5	11.77	90.89	28.62	225
Mean (AU)	50.26	240.1	16.6	127.4	35.84	224.9
SD (AU)	29.03	105.9	12.01	82.22	28.62	109.9
p-value	<0.0001		<0.0001		<0.0001	
Significant?	Yes		Yes		Yes	

Table 10: Effect of Skin Surface (Dorsal vs Volar Skin) on Cutaneous Flow

The difference between dorsal and volar cutaneous flows are statistically significant for all three subgroups: digit ($p<0.0001$), hand ($p<0.0001$) and combined ($p<0.0001$). The volar perfusion measurements are generally higher compared their counterparts on the dorsal surface. The differences between dorsal and volar surface mean cutaneous perfusion values for all subgroups can be seen on [Figures 13 to 15].

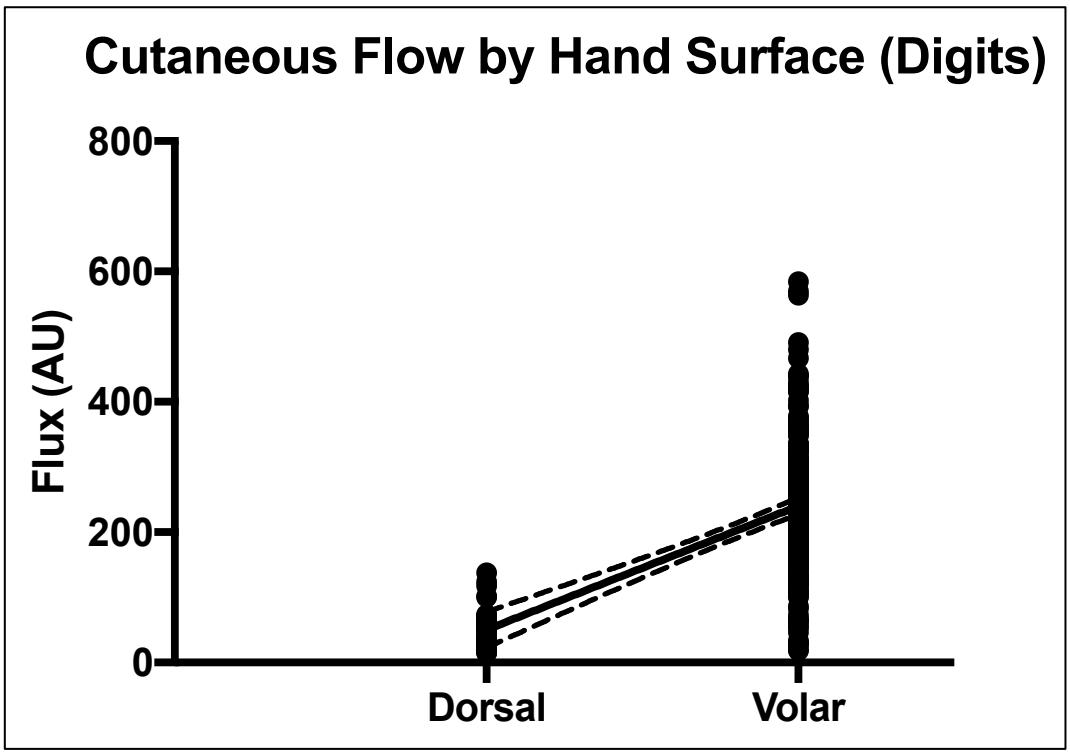

Figure 13: Comparison of Cutaneous Flow by Hand Surface for Digital Measurements

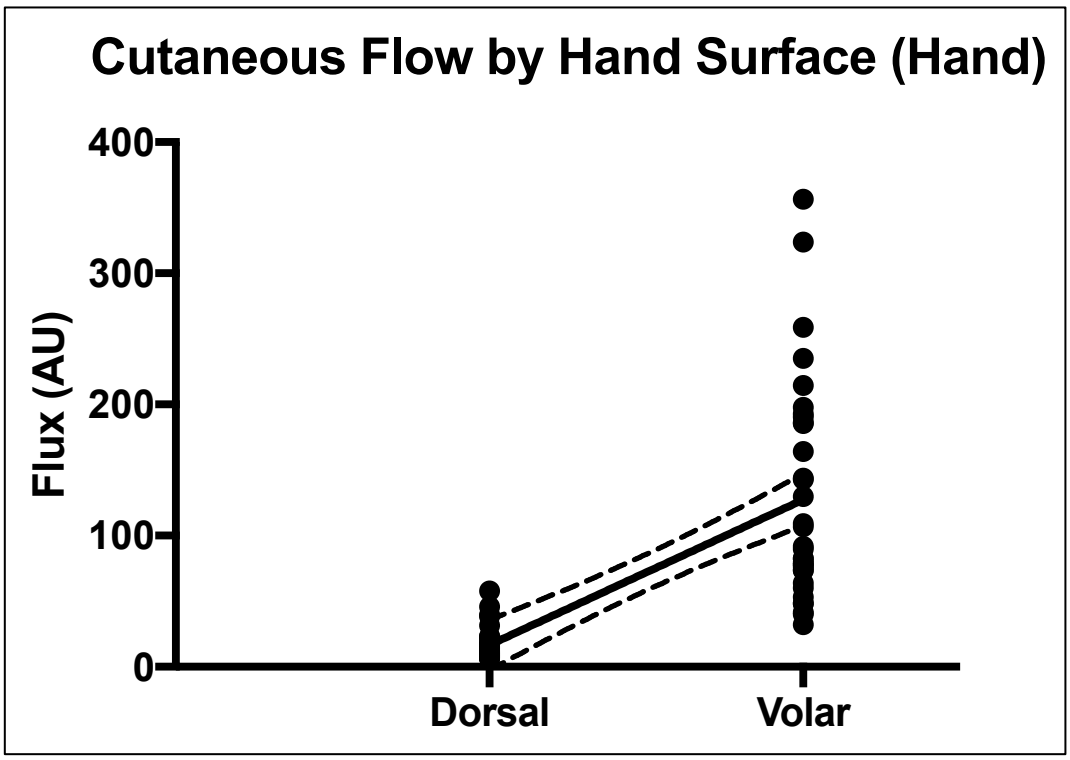

Figure 14: Comparison of Cutaneous Flow by Hand Surface for Hand Measurements

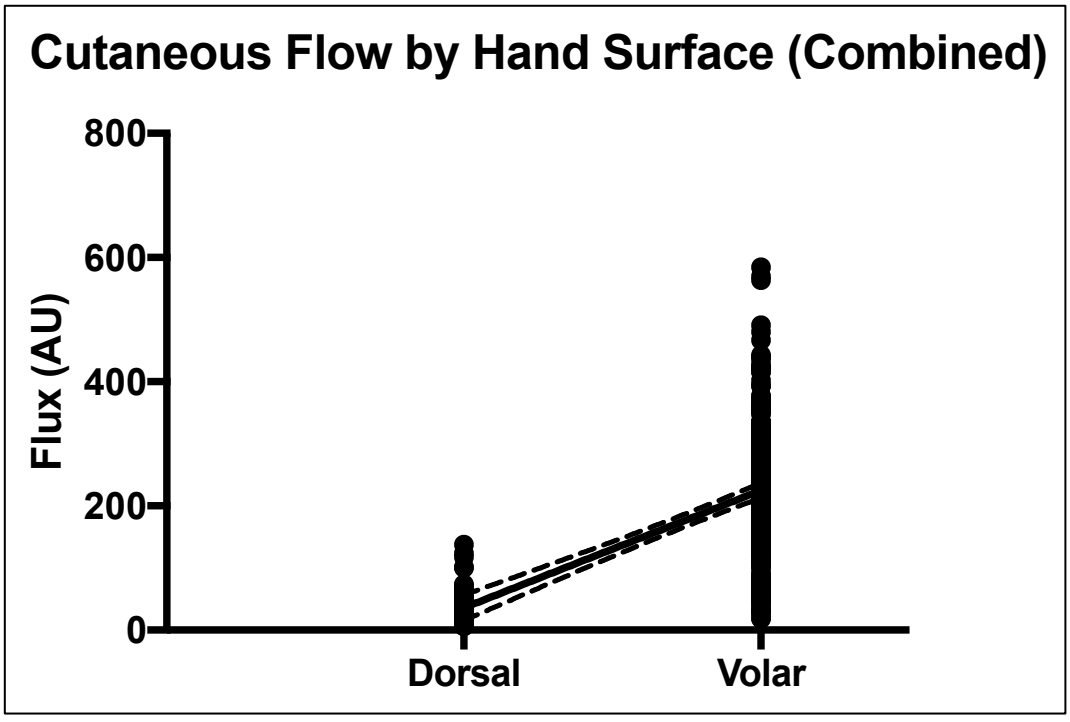

Figure 15: Comparison of Cutaneous Flow by Hand Surface for Combined Measurements

3.3.6 Effect of Structure Measured (Hand vs Digit) on Cutaneous Flow

Further to the above, comparisons were made from cutaneous flow measurements of digits against hands. Again, as the areas measured on the hand does not correspond to the digits in numbers and location, pairing was not appropriate. Linear regression is used in consideration of the data clustering.

Three subgroups were analysed: volar, dorsal, and combined. Details of descriptive statistics and results from comparative analyses of the subgroups are shown in [Table 11].

	Volar		Dorsal		Combined	
	Hand	Digit	Hand	Digit	Hand	Digit
Number	34	135	36	48	70	183
Median (AU)	90.89	246.6	11.77	45.94	39.95	203.5
Mean (AU)	127.4	250.7	16.6	50.26	70.42	198.1
SD (AU)	82.22	113.6	12.01	29.03	80.11	132.4
p-value	<0.0001		<0.0001		<0.0001	
Significant	Yes		Yes		Yes	

Table 11: Effect of Structure Measured (Hand vs Digit) on Cutaneous Flow

The difference between cutaneous flows of the digits and hands are statistically significant for all three subgroups: volar ($p<0.0001$), dorsal ($p<0.0001$) and combined ($p<0.0001$). The digits have consistently higher cutaneous flow compared to the hands. [Figures 16 to 18] demonstrate clear differences in mean cutaneous flows and standard deviations of digits and hands within the above subgroups.

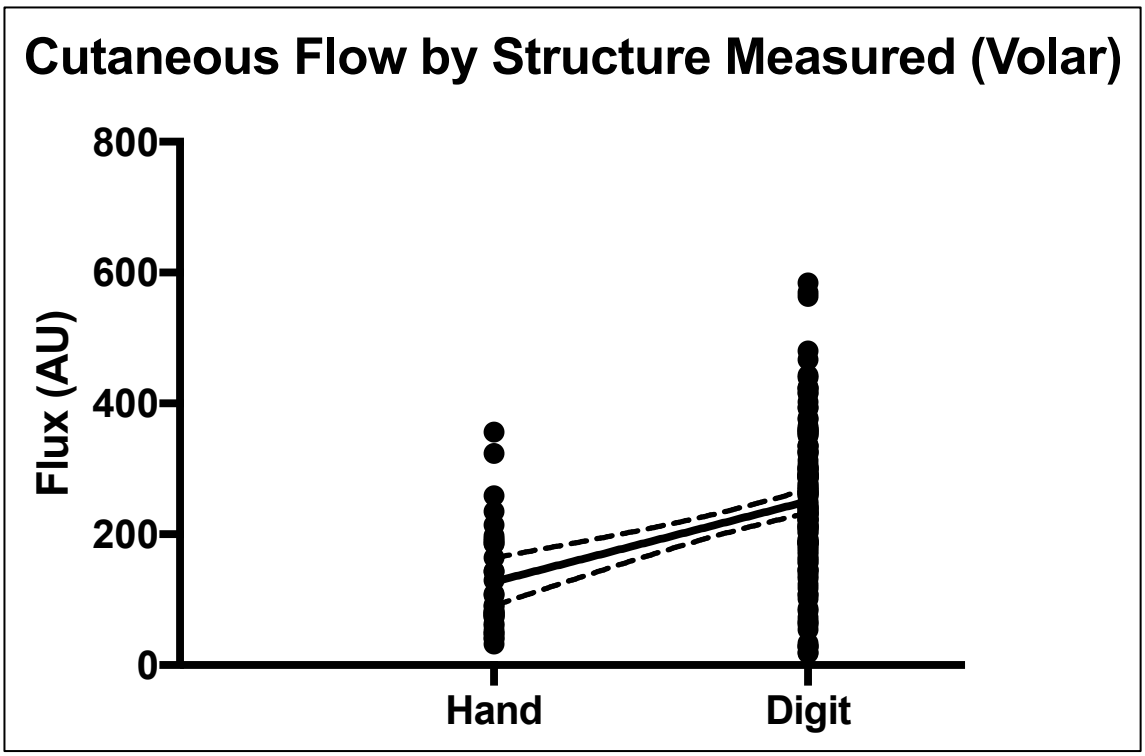

Figure 16: Comparison of Cutaneous Flow by Structure Measured (Hand vs Digit) for Volar Measurements

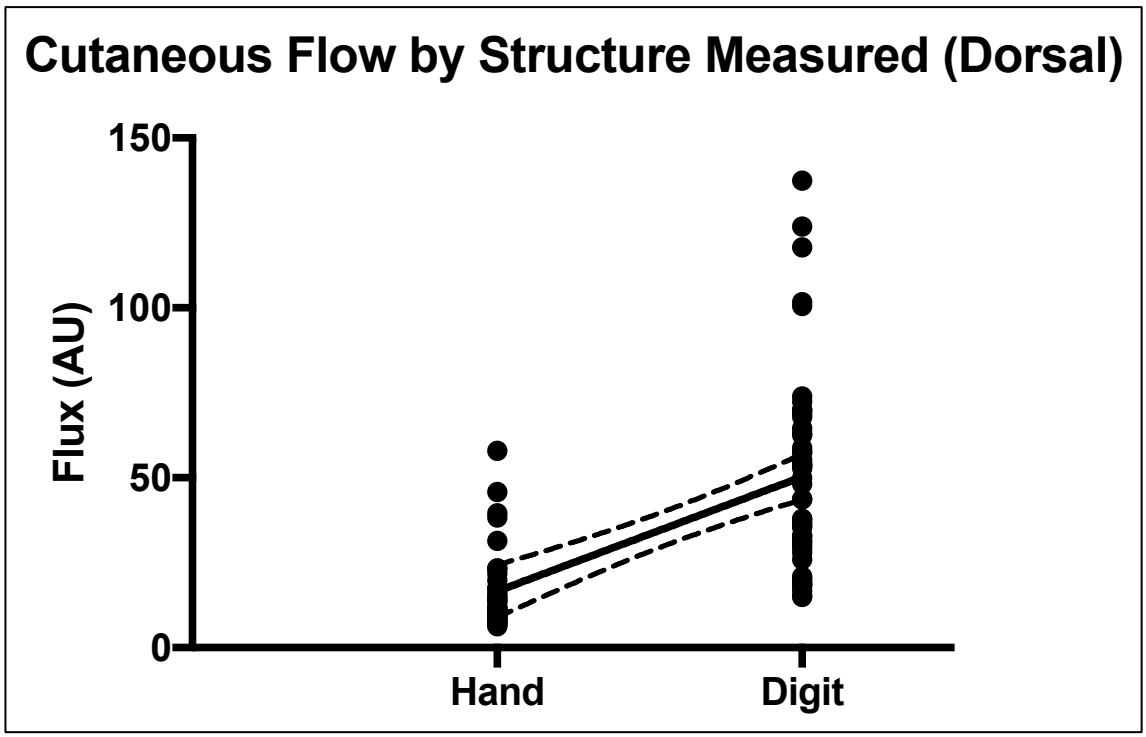

Figure 17: Comparison of Cutaneous Flow by Structure Measured (Hand vs Digit) for Dorsal Measurements

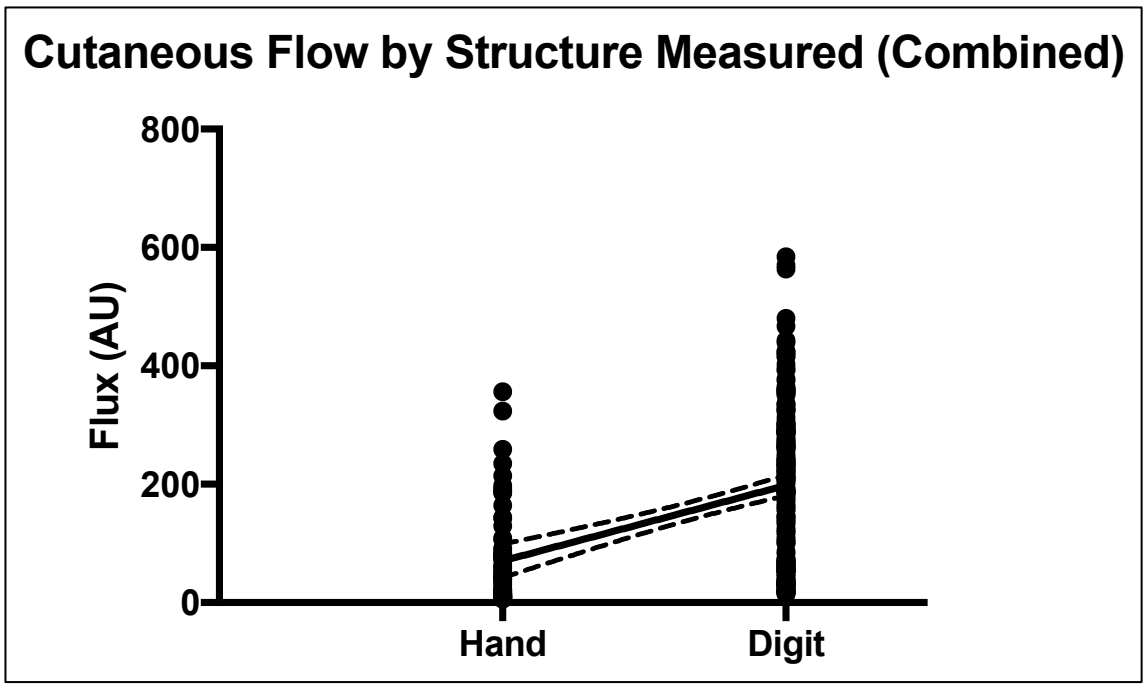

Figure 18: Comparison of Cutaneous Flow by Structure Measured (Hand vs Digit) for Combined Measurements

3.3.7 Effect of Digits on Cutaneous Flow

As mentioned previously, normality testing by D'Agostino-Pearson test of digital cutaneous flow measurement has revealed that the data set is consistent with Gaussian distribution. Since the measurements of different digits were taken from the same set of participants, it was possible to perform paired analysis of the data set. As simultaneous comparisons of several groups were required in this section, therefore repeated measures one-way ANOVA test was used.

Since thumbs are anatomically different the other digits, the number of recorded flow measurement differs. Therefore, the thumb group cannot be included in the ANOVA test. Additionally, participant pairing cannot be assumed because of the difference in the number of data in the thumb and the other digits. Therefore, four separate unpaired

Student's t-tests were used to compare the thumb against index finger, middle finger, ring finger and little finger separately.

Similar to previous sections, three subgroups were analysed: volar, dorsal and combined.

Volar Subgroup

The details of descriptive statistics and results from comparative analyses of the volar subgroup are shown in [Table 12]. There appears to be no statistical significance between the mean cutaneous perfusion between the thumb and the rest of the digits (index p=0.18, middle p=0.10, ring p=0.35, and little p=0.43). Similarly, a comparison between digits excluding the thumb has yielded a p-value of 0.78, indicating no statically difference between the mean perfusion value between index, middle, ring and little fingers. [Figure 19] demonstrate the mean perfusion values of volar aspect of digits.

	Thumb	Index	Middle	Ring	Little
Number	42	44	44	44	44
Median (AU)	213.5	243.7	250	241.7	249.4
Mean (AU)	220.6	245.9	254.5	241.2	237.6
SD (AU)	73.45	97.79	110.7	123.5	117.2
p-value	Th-IF (0.18) Th-MF (0.10) Th-RF (0.35) Th-LF (0.43)	0.78			

Table 12: Effect of Digits on Cutaneous Flow (Volar Subgroup)

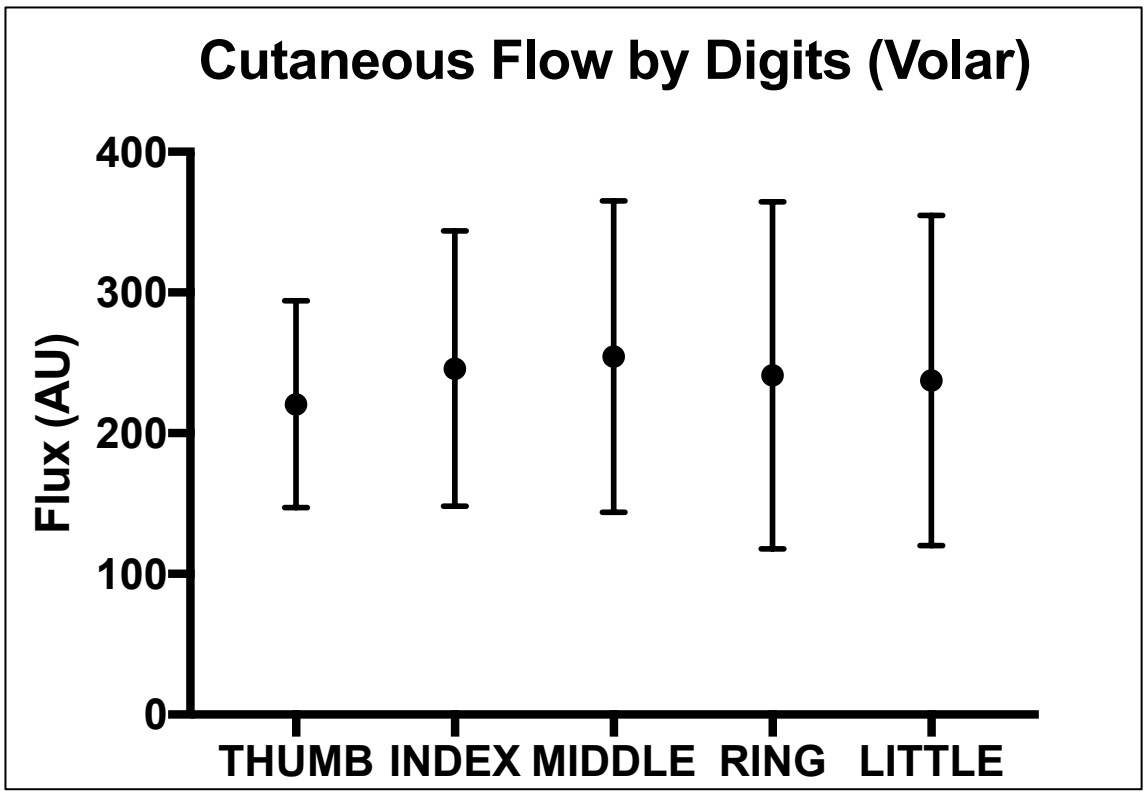

Figure 19: Mean Cutaneous Flow by Digits (Volar Subgroup)

Dorsal Subgroup

Dorsal subgroup analysis demonstrates no statistical difference between the thumb and other digits (index p=0.29, middle p=0.54, ring p=0.37, and little p=0.47). Likewise, comparison with ANOVA showed no statistical significance between the mean cutaneous perfusion between the non-thumb digits (p=0.39). There appears to be some difference between mean perfusion taken from dorsal ring finger when compared to other digits. This is not statistically significant, however. [Figure 20] demonstrate the mean perfusion values of dorsal aspect of digits.

	Thumb	Index	Middle	Ring	Little
Number	8	10	10	10	10
Median (AU)	49.03	53.11	42.91	36.66	48.61
Mean (AU)	45.18	55.8	54.3	38.71	56.31
SD (AU)	14.87	23.98	39.2	14.9	40.4
p-value	Th-IF (0.29) Th-MF (0.54) Th-RF (0.37) Th-LF (0.47)	0.39			

Table 14: Effect of Digits on Cutaneous Flow (Dorsal Subgroup)

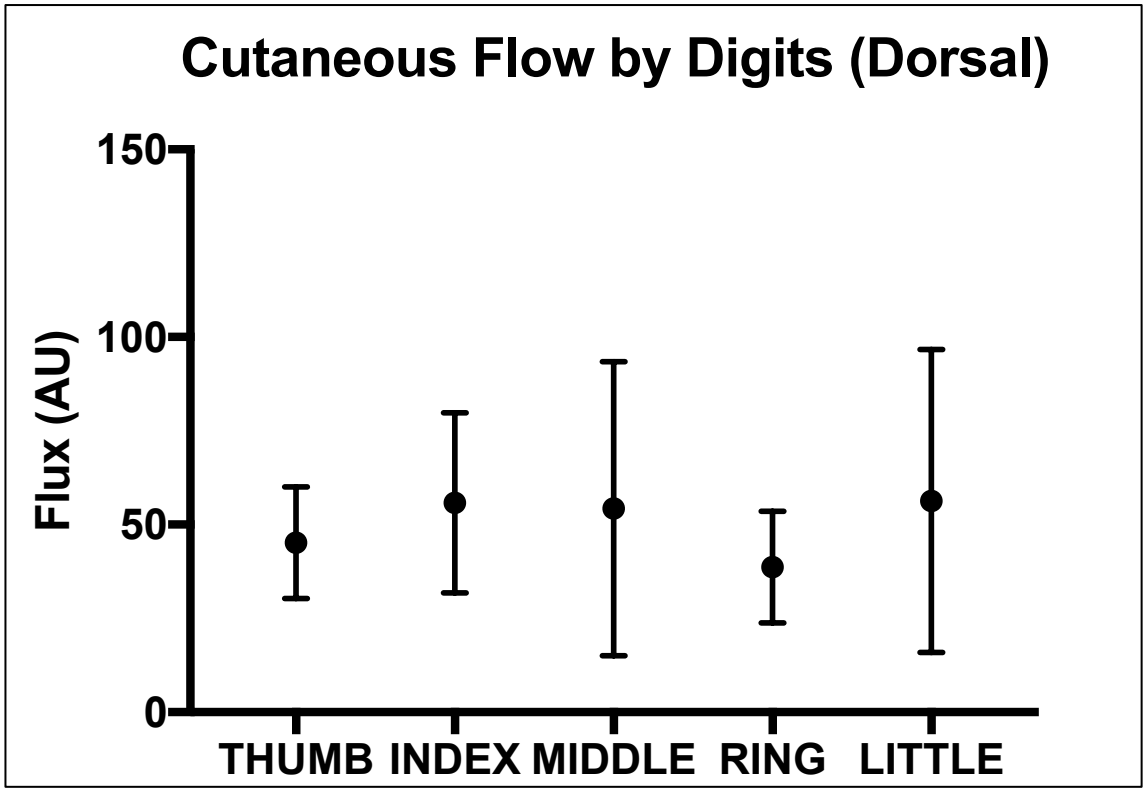

Figure 20: Mean Cutaneous Flow by Digits (Dorsal Subgroup)

Combined Subgroup

In the combined group, no statistical difference was found from the multiple Student's t-tests comparing thumb against other digits (index p=0.38, middle p=0.26, ring p=0.63, and little p=0.61). Comparison of mean cutaneous flow of the non-thumb digits using ANOVA revealed no statistical significance (p=0.74) between the digits. The information

is summarised in [Table 16]. [Figure 21] demonstrate the mean perfusion values of digits when both volar and dorsal aspects are combined.

	Thumb	Index	Middle	Ring	Little
Number	50	54	54	54	54
Median (AU)	190.4	231.8	228.2	192.6	206
Mean (AU)	192.5	210.7	217.4	203.7	204
SD (AU)	93.63	115.8	128	136.8	128.4
p-value	Th-IF (0.38) Th-MF (0.26) Th-RF (0.63) Th-LF (0.61)	0.74			

Table 16: Effect of Digits on Cutaneous Flow (Combined Subgroup)

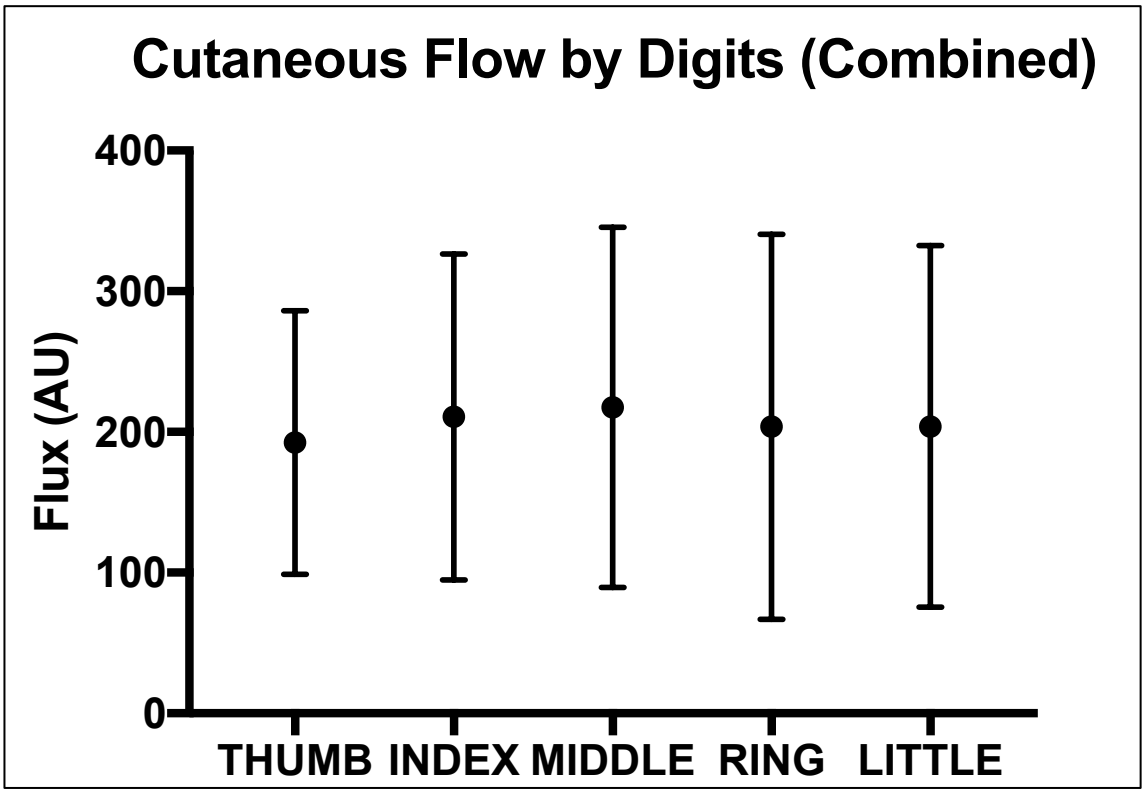

Figure 21: Mean Cutaneous Flow by Digits (Combined Subgroup)

3.3.8 Effect of Digits (Pulp) on Cutaneous Flow

As the pulps are found to have the most consistently high mean cutaneous perfusion, further analysis of the effect of digits on cutaneous flow specific to the pulps was

performed. The number of data per digit is consistent this time; therefore the use of repeated measures one-way ANOVA test to compare all digits was possible. No separate Student's t-test for the thumb was required. [Figure 22] demonstrate the mean perfusion values of the pulps of digits.

	Thumb	Index	Middle	Ring	Little
Number	40	40	40	40	40
Median (AU)	216.7	251.3	254.6	261.4	253
Mean (AU)	221.7	254.7	252.9	253.1	241.7
SD (AU)	75.05	96.77	114.6	122.6	120.8
p-value	0.35				
Significant?	No				

Table 18: Effect of Digits on Cutaneous Flow (Pulp)

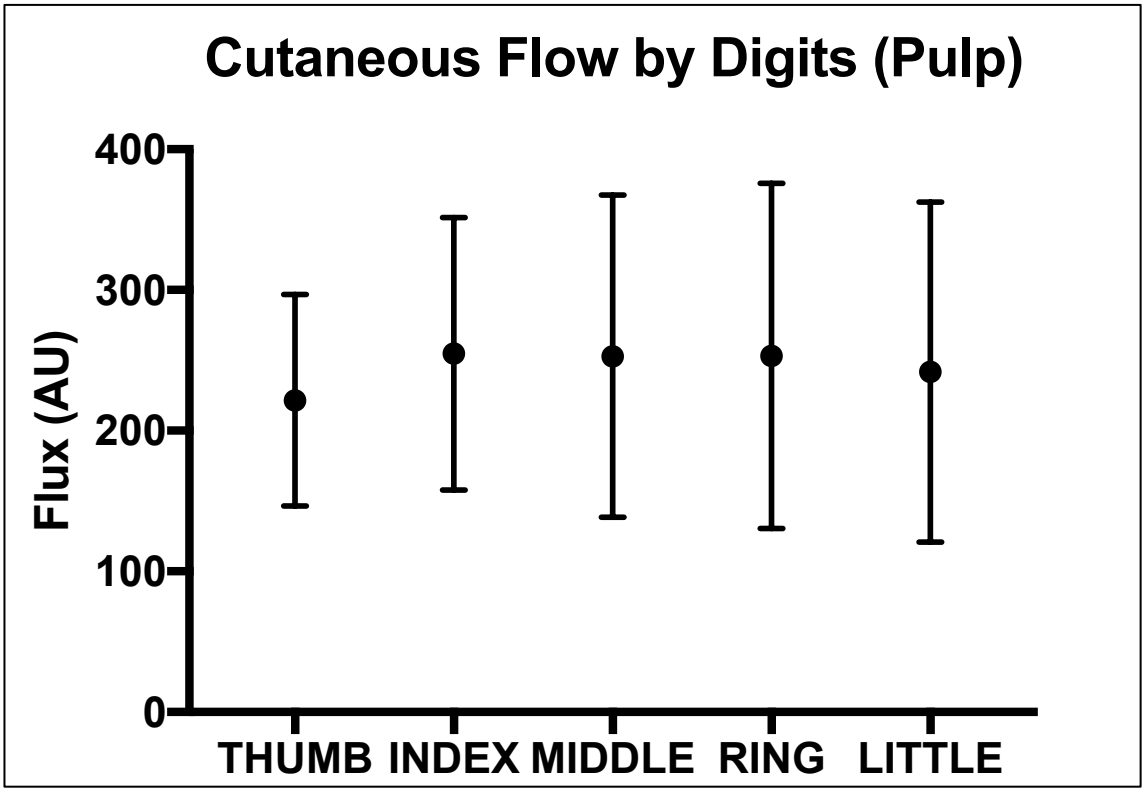

Figure 22: Mean Cutaneous Flow by Digits (Pulp Subgroup)

3.3.9 Comparison of MF Pulp Flow by Laterality and Hand Dominance

The finger pulps were considered to have the highest mean cutaneous perfusion according to the above analyses. There appeared to be no statistical difference between the perfusion measurements of different pulps. The middle finger pulp was arbitrarily chosen to be the most suitable site for further experiments due to its ease of probe application and central location of the digits.

Further analysis was performed to ensure there were no unduly biases by laterality and hand dominance. Normality testing of cutaneous flow measurement of the middle pulps found the data follows a Gaussian distribution. Paired Student's t-test was chosen as the data set was taken from the two sides of the same group of participants. It was found that middle finger pulp cutaneous perfusion was not influenced by laterality (p=0.31) as well as hand dominance (p=0.48) as seen in [Table 20] and [Table 21]. However, it should be noted that the right side has a smaller standard deviation (SD 90.93 vs SD 133.1). A similar finding is found on the dominant side (SD 84.6 vs SD 139.2). [Figures 23 and 24] shows the distribution of cutaneous flow of middle finger pulps by laterality and dominance respectively.

	Left	Right
Number	20	20
Median (AU)	241.6	279.5
Mean (AU)	231.3	274.5
SD (AU)	133.1	90.93
p-value	0.31	
Significance?	No	

Table 20: Comparison of Middle Finger Pulp Flow by Laterality

	Non-Dominant	Dominant
Number	20	20
Median (AU)	241.6	269.9
Mean (AU)	238.7	267.1
SD (AU)	139.2	84.6
p-value	0.48	
Significance?	No	

Table 21: Comparison of Middle Finger Pulp Flow by Hand Dominance

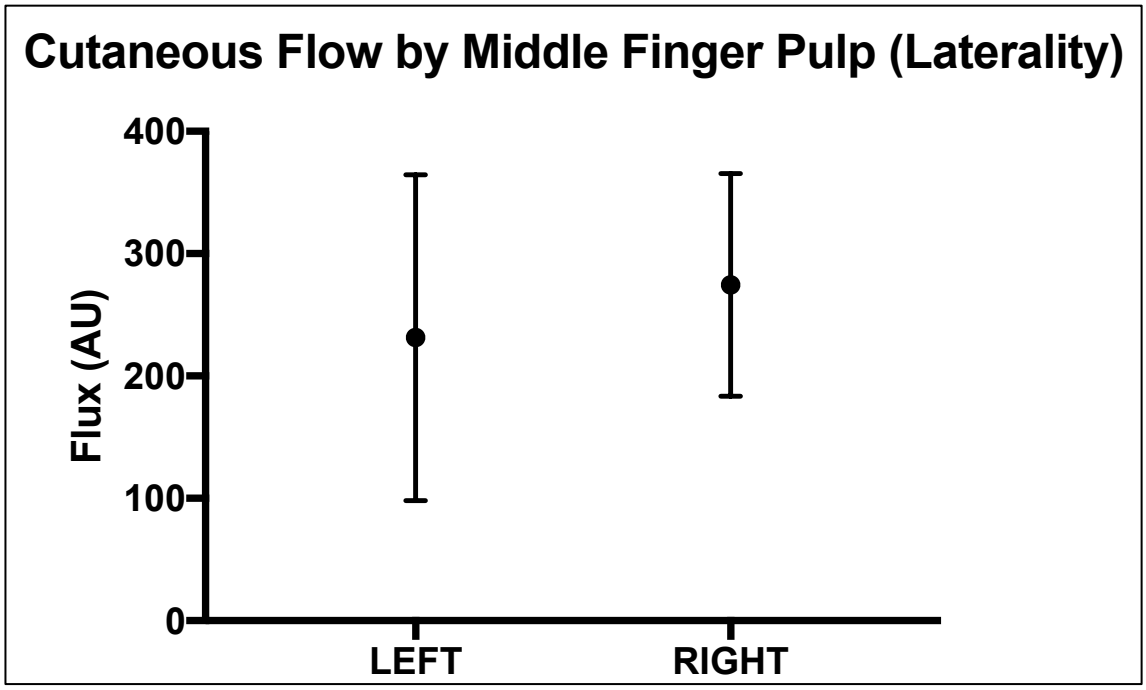

Figure 23: Distribution of Cutaneous Flow of Middle Finger Pulps by Laterality

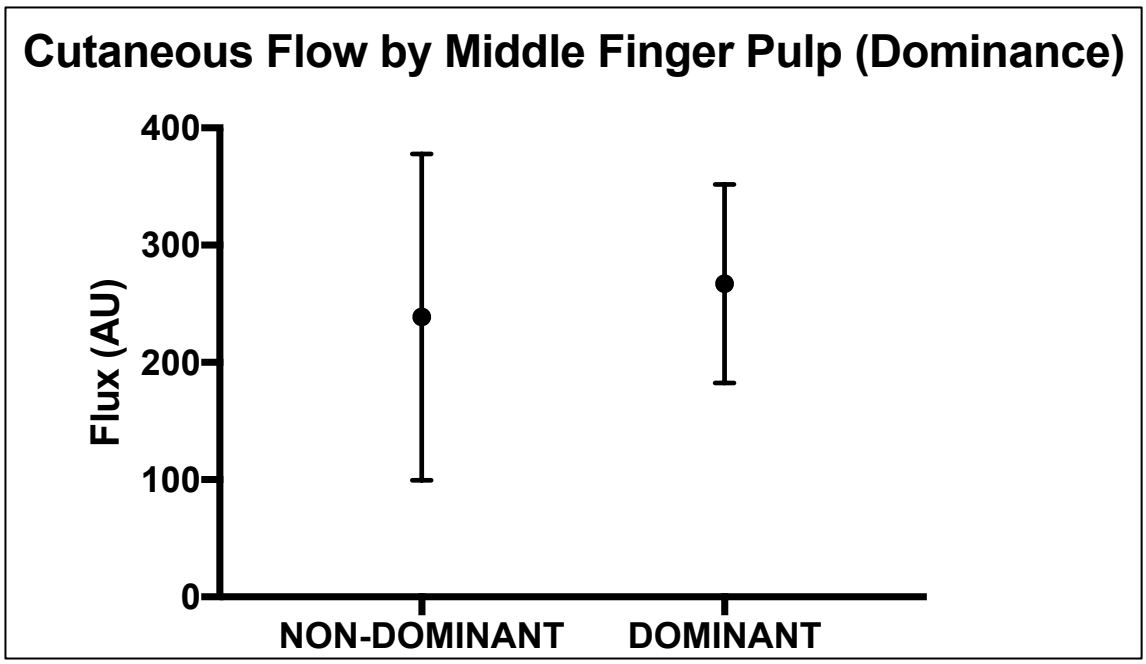

Figure 24: Distribution of Cutaneous Flow of Middle Finger Pulps by Hand Dominance

3.3.10 Comparison of MF Pulp Flow by Gender

The effect of gender on middle finger pulp perfusion was analysed through using unpaired Student's t-test as matching was not possible due to the imbalanced number of data and the difference in participant profile (i.e. gender). As shown in [Table 22], there was no difference of mean cutaneous perfusion between the two genders (p=0.61). [Figure 25] shows the distribution of cutaneous flow of middle finger pulps by gender.

	Female	Male
Number	26	14
Median (AU)	254.6	257.6
Mean (AU)	246.1	265.7
SD (AU)	115.3	116.5
p-value	0.61	
Significance?	No	

Table 22: Comparison of Middle Finger Pulp Flow by Gender

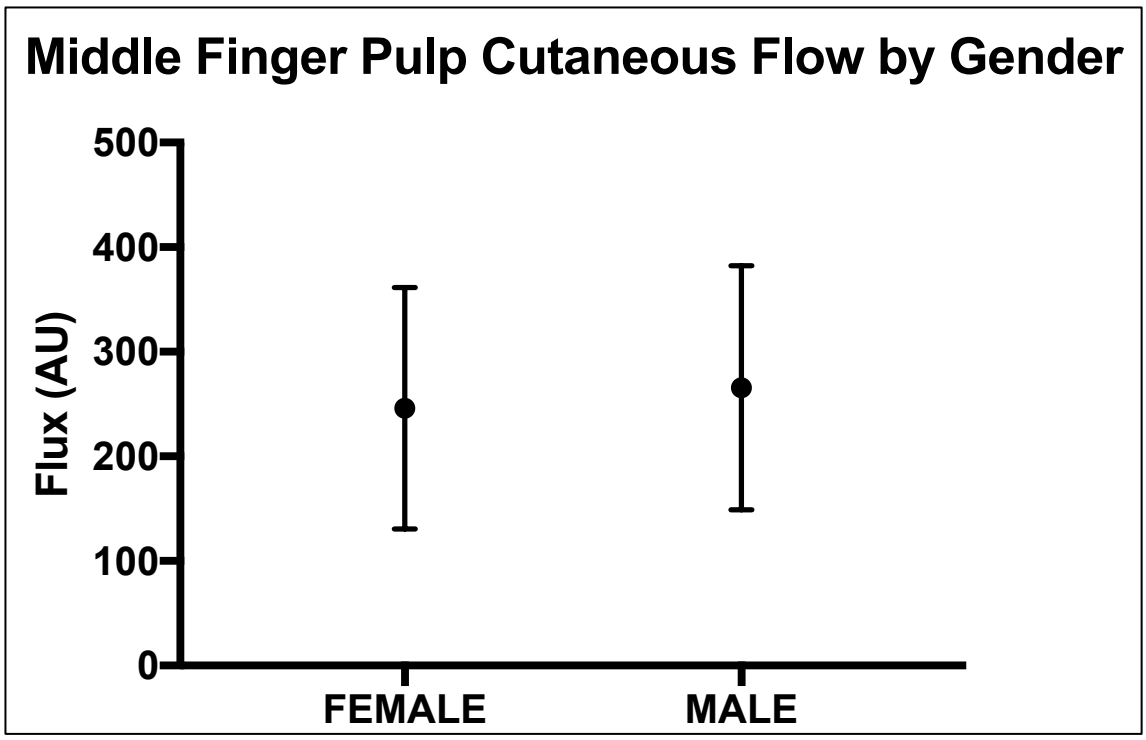

Figure 25: Distribution of Cutaneous Flow of Middle Finger Pulps by Gender

3.3.11 Comparison of RMF Pulp Flow by Age

Following the identification of right middle finger (RMF) pulp as a suitable area for further experimentation, a correlation analysis was made against patient age. Other factors such as smoking status, drug history and past medical history were considered, although the data size was insufficiently powered to calculate meaningful results.

As the values for RMF cutaneous perfusion has been shown to follow a Gaussian distribution through D'Agostino-Pearson normality testing, Pearson's correlation analysis was used. A correlation coefficient of r = 0.1103 is found, indicating that the two analysed factors are unlikely to have a meaningful correlation. R^2 is calculated to be 0.01216, indicating there is around 1% variation of RMF pulp flow is related to age. A scatter plot for this is shown in [Figure 26], which exhibits no apparent correlation of one factor against another.

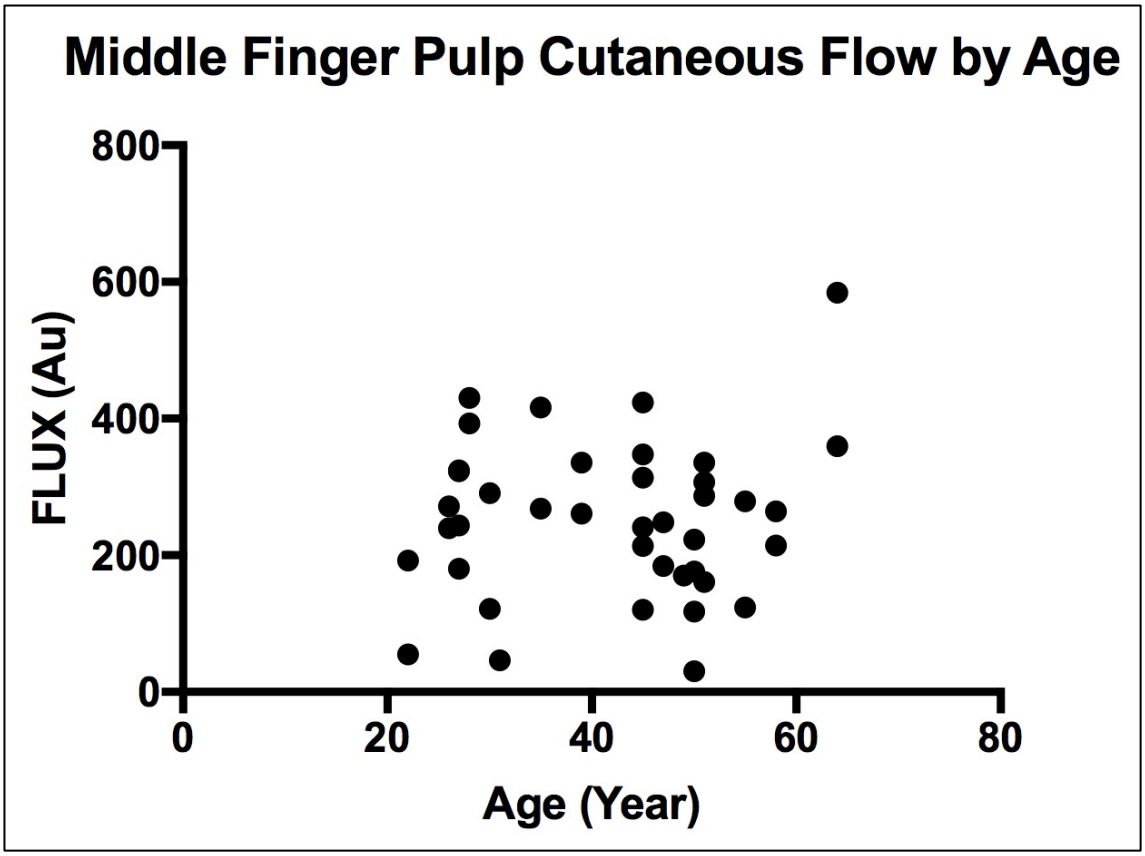

Figure 26: Scatter Plot of Middle Finger Pulp Cutaneous Flow Against Age

3.4 Results: Elevation Study

In this experiment, the effect of hand elevation on cutaneous perfusion is tested from a single optimal point of measurement (RMF). All participants were asked to perform five different elevation positions during the measurement period as shown below. The baseline position is used as the control for comparative statistical tests.

Position	Upper Arm	Elbow
1 (Baseline)	Heart Level	180°
2	Full Elevation	180°

3	Dangling	180°
4	Heart Level	45° Flexion
5	Heart Level	90° Flexion

The experiment generated 20 sets of data, which had five mean RMF positional perfusion values generated from the five positions. Descriptive statistics of the measurements taken from the different positions are shown in **[Table 23].**

Position	1	2	3	4	5
Number	20	20	20	20	20
Median (AU)	261.7	69.08	252.3	211	160.3
Mean (AU)	242.8	111.2	285.1	206.4	177.2
SD (AU)	99.41	103.1	122.3	83.58	106.7
p-value		<0.001	0.23	0.10	0.03

Table 23: Effect of Elevation on Positional Perfusion of the RMF

As exhibited in [Figure 27], upon complete elevation of the upper limb from the baseline, the mean cutaneous flow reduces. As the upper limb is lowered to its full dependence position, the blood flow to the skin is increased. As the forearm is elevated to the final two positions, perfusion measurement gradually reduces.

Repeated measures one-way ANOVA test was used as to compare the mean positional perfusion measurements over different positions. It was found that the experimented positions resulted in statistically significantly different ($p < 0.001$) cutaneous perfusions. Further comparison was made using Dunnett's multiple comparison tests to compare positions 2 to 5 against control position. As shown on [table above] It was found that positions 2 and 5 reduce cutaneous perfusion of the RMF in a statistically significant manner when compared to the control position. However, position 3 and 4 do not appear to alter the cutaneous perfusion in a statistically significant manner.

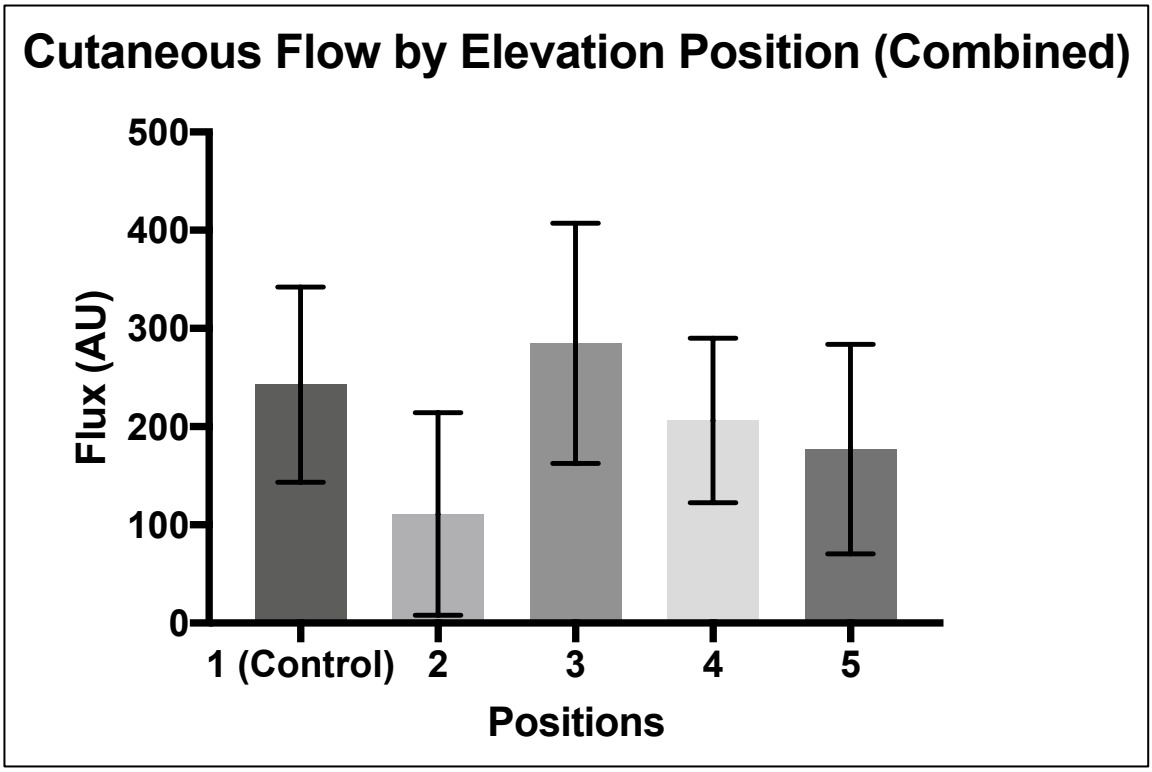

Figure 27: Cutaneous Flow of Middle Finger Pulps by Different Elevation Position

CHAPTER FOUR

DISCUSSION

The results are discussed sequentially according to the order of experiment performed. Initially, the hand mapping experiment results are discussed. Following this, the hand elevation results will be discussed. The final portion of this section will cover the limitations, technical and practical considerations highlighted from this project.

4.1 Summary and interpretation of findings

4.1.1 Hand Mapping Experiment

The hand mapping experiment has demonstrated several interesting findings, which is discussed in detail in this section. This appears to be the first mapping study of cutaneous perfusion of the hand with LDF to date, as there are no documented studies in the literature.

Throughout the hand mapping experiment, changes were made to the measurement protocol to reduce measured area due to timing and logistical reasons. While this has reduced the area measured per hand mapping group initially, the selected areas appear to be representative of the hand mapping group as shown in the results section. The author is aware of the fact that the volume of data collected in this study is insufficient for definitive statistical analyses to be performed.

It is clear that, from the data collected in this study, that the hand demonstrates heterogeneity in the level of cutaneous perfusion in different areas of the hand. Further analyses have been performed to understand how the level of cutaneous perfusion varies according to the different areas of the hand, this is discussed in the sections below.

It appears that the first hypothesis has been shown to be at least partly true – the cutaneous perfusion of the hand does vary between different anatomical locations. It is unclear whether if the discrepancy in cutaneous perfusion is solely secondary to variable distribution of AVA networks. The following sections will attempt to explain potential reasons which may have contributed to the difference in cutaneous perfusions between different areas of the hand.

Hand Dominance

The initial hypothesis at the start of the study has been disproved. The cutaneous perfusion of the hand does not vary according to the hand dominance status. The results from the study have suggested that the cutaneous perfusion of the hands in healthy individuals at rest does not alter significantly between the dominant and the non-dominant hand, which is consistent for both the digital measurements and the hand measurements.

The finding from this feasibility study has potentially important research implications, as it validates that hands dominance does not influence on the choice of control or intervention group for studies that investigate hand cutaneous perfusion at resting state. In clinical settings, the results found here can be used to justify the use of contralateral hand cutaneous perfusion as a reference point for comparison in situations such as following replantation surgery or monitoring of skin perfusion following significant tissue injury or following extensive surgery. One should be mindful that this current study is limited by small sample size, and therefore the conclusions deduced from the results should be treated with caution. Further studies with a larger sample size would be beneficial to confirm the validity of finding from this study.

The effect of hand dominance on upper limb circulation has been investigated previously. It has been found that while ophthalmic arterial pressures differ according to laterality and hand dominance status, brachial arterial pressures do not vary according to hand dominance status.[126] A study performed by Chang et al. has demonstrated a similar finding. They used pulsed-wave Doppler ultrasound to visualise palmar arterial branches at rest and with sympathetic stimulation. It was found that hand dominance status had no influence on macrovascular circulation in terms of pulsatility index, arterial diameters and blood flow at rest and under sympathetic stimulation.[127] Currently, there has been no researches that specifically studied the effect of hand dominance on cutaneous microcirculation of the upper limb.

However, upper limb perfusion during exercise appears to differ according to hand dominance status. Kagaya et al. investigated forearm blood flow velocity and vessel diameter using ultrasound Doppler in young female tennis players at rest and during exercise. It was found that vessel diameter measurements in dominant upper limbs appear to be larger both at rest and after exercise when compared to non-dominant upper limb. Forearm blood flow velocity appears to differ only during post-exercise states.[128] It is unclear whether this is an effect which is exclusive to trained athletes as a consequence of training associated physiological changes as no untrained individual were included in the study. This would therefore be of interest for further research following this feasibility study to measure cutaneous perfusion following exercise conditions. While the finding from this study is based on a comparasion of 168 areas, only 20 subjects were measured. It is possible that the sample size is too small to detect subtle differences in perfusion values. Therefore, future studies with larger sample sizes

would be recommended. Furthermore, as this study did not measure any areas proximal to the wrist, and the blood flow measurements of pre-existing research has been focused on named vessels - one should consider including cutaneous perfusion measurements of the forearm to determine whether the current finding applies to the forearms.

Surface (Dorsal vs Volar Skin)

Statistical analyses from the hand mapping experiments have found that there is a significant difference between the mean cutaneous perfusions of the volar and the dorsal surface of the hand. The volar surface appears to have a higher level of cutaneous perfusion compared to the dorsum.

A possible explanation of such result is due to the presence of higher concentration of AVAs on the glabrous skin (i.e. volar surface of the hand). As the participants were studied in a temperature-controlled room within the thermoneutral zone, the AVAs in the participants were likely to be active, leading to increased perfusion detected by LDF which is otherwise not measured on the dorsal surface due to the relative absence of AVAs. While technically it is possible to differentiate the blood flow in the capillaries and AVAs using LDF, this would require the use of probes with different optical fibre separations.[129] The equipment used in this study did not have such probes. Therefore, the measurements taken in this study incorporates both nutritive blood flow of the capillaries and thermoregulatory blood flow of the AVAs. Using probes which allows differential measurements of AVAs and capillaries would allow one to determine whether the significant difference in skin perfusion is due to the variation in AVAs distribution between the two surfaces or related to other factors such as skin thickness or distance of skin to underlying structures such as bone and tendons.

Structure Measured (Hand vs Digit)

It is found that the mean blood flow of the digits is significantly higher than the mean blood flow found on the skin overlying the palm and opisthenar region. Further subgroup analyses have shown the trend to be consistent for both the volar and dorsal aspect. There appears to be no pre-existing literature which investigated the difference between cutaneous perfusion of the digits and the hands.

Using Poiseuille's Law as a theoretical model, one would expect the blood flow to the digits to be lower than the more proximal areas on the palm and opisthenar region due to the longer vessel distance from the heart. In clinical settings, it is commonly observed in those with septic shock or receiving peripheral vasopressors to develop a compromised blood supply of the distal portion of the digits. Therefore, one would expect that the more distal (i.e. digital) cutaneous perfusion measurements to be lower than its proximal counterparts (i.e. hands).

The author has noticed that it was more difficult to secure the LDF probe on the palm when compared to the volar aspect of the digit. This may be a possible explanation of the finding. Poor probe contact to the skin could contribute to a lower reading. However, the trend was consistent for all participants and therefore poor probe adherence is unlikely to be the cause. Furthermore, the lower perfusion values were not isolated to the volar aspect only, but also on the dorsal aspect where probe attachment was not considered problematic.

Reviewing the vascular mapping work by Taylor and Palmer has shown that the digital pulps appear to have more abundant blood supply compared to the rest of the hand.[82] However, no other apparent observable variations in vessel distribution between digital and hand skin are seen. The techniques employed by the study highlights arteries and arterioles to a certain calibre. It is possible that smaller vessels which the LDF used in the current study are aimed to measure is not visualised in the study.

Digital arteries are found on the volar aspect of the digits in a paired fashion, with one on either the radial and the ulnar aspects of the digits. These vessels are situated more superficial to the skin compared to their proximal corresponding vessels in the palm. It is possible that the LDF probe may have detected the flow of digital vessels instead of the flow on the subdermal plexus when measurements on the digital areas are performed. This may have contributed to the higher value as digital arteries would have a considerably higher perfusion value.

Another possible explanation for this result is from an evolutionary biology perspective. As the joints of the hands flex and extend during normal daily functional activities, digital skin appears to be much more compressive stress when compared to the skin covering the palm and opisthenar region. The digital skin blanches as one form a fist due to the pressure exerted by the skeletal system (i.e. phalanges) from the movement. The skin on the palm and dorsum of the hand does not appear to blanch as there is less overall movement associated with that part of the hand. It is possible that the digital cutaneous circulation has evolved to form richer vascular networks to restore perfusion between hand movements quickly.

Digital Choice

The thumb appeared to have a lower level of cutaneous perfusion from the initial comparison of mean cutaneous perfusion levels between different digits of the hand. This was observed for both volar and dorsal measurements. However, statistical tests have determined the lower level of cutaneous perfusion in the thumb was not statistically significant. Further analyses were performed for different surfaces and areas of the digit. The finding was consistent across all subgroups. There was no statistical difference in the perfusion measurements between different digits of the hand at the resting state.

This finding is particularly important in the field of digital cutaneous perfusion research. It was discussed in the previous chapters that there had not been an agreed protocol of digital perfusion measurements with LDF amongst researchers, leading to a significant variation in measurement protocols where different digits are measured between different studies and occasionally within the same study. The finding here can be used to justify that comparisons can be safely made between studies where LDF measurements were performed on different digits, provided they are consistent on the surface of measurement, i.e. volar or dorsal skin.

A possible reason for the observed difference in cutaneous perfusion of the thumb could be related to the order of which areas were measured in the hand mapping experiment. The measurement was consistently performed in a sequential manner from the lowest numbered area to the highest numbered area. Therefore, the thumb pulp was always measured first in every participant. It may be possible that acclimatisation time for the participants was insufficient, and therefore the values obtained from the first few readings were inaccurate as the participants were not adequately acclimatised to the

room temperature. However, if this was the cause for the lower thumb mean cutaneous perfusion measurements, one would expect the lower measurements would not be observed in the dorsal subgroup, as the participant would have had sufficient time to acclimatise during measurements for the volar areas. While the lower mean cutaneous perfusion of the thumb has also been observed in the dorsal subgroup, one should be mindful that the measurement was taken from the initial hand mapping protocols and therefore has a very small sample size. It is therefore difficult to conclude with certainty with the results available. Future studies with a larger sample size would be welcomed to ascertain whether the observation is secondary to the aforementioned factor. In addition, future study design should consider the effect of measurement sequence where multiple areas are measured, as the results for the first few areas measured may be inaccurate if the subject has not fully acclimatised to the ambient temperature.

One may consider investigating this potential source of bias further by repeating the first few measurements to see whether if the cutaneous perfusion level has changed during this time. A simple method to deal with issues associated with temperature acclimatisation would be to increase the acclimatisation time before the measurements are taken. In this study, the acclimatisation time is the time taken for completion of informed consent, health questionnaire and an introduction to the LDF machine. The estimated time for these tasks were around 5 minutes. A study by Roy et al. using digital thermography techniques has found skin surface temperatures begin to acclimatise and stabilise between 8 to 16 minutes following entering a room with new ambient temperature.[130] Similar results have been found by Marins et al., who recommended a minimal acclimatisation time of 10 minutes, provided the newly entered environment was not of extremes of temperature.[131]

Optimal Measurement Area: Middle Finger Pulp

The purpose of the hand mapping experiment was to identify a point of optimal measurement for subsequent experiments. A set of criteria was devised at the start of the hand mapping experiment to help to determine this point. These are as followed:

- Reliable perfusion tracing
- Good level of tissue penetration
- High baseline perfusion (in order to detect subtle changes)
- Easy access to the measurement area, not commonly concealed
- The probe can be easily secured to the measurement area
- The measurement area is of clinical relevance
- The measurement area is not influenced by laterality and hand dominance status
- The measurement area not commonly restricted by pieces of jewellery or watches.

Following the first three participants (0000 – 0002), several measurement areas were removed as they did not satisfy the above criteria. These were due to a number of reasons: unstable tracing (15-18, 21, 36-42), difficult to secure probe onto the skin (21, 24, 27, 29, 32, 35), and low mean perfusion values (24-47). Provisional analysis performed at the time revealed no apparent difference in mean perfusion values of the hand by hand dominance status or laterality. This is later proven in formal statistical tests as discussed in the previous section 'Hand Dominance'.

Subsequent measurements were recorded and analysed as described above, which demonstrated that the volar aspect of digits had the highest mean baseline perfusion.

The pulp was deemed the most suitable area, as they are easily accessed even when the hands are dressed with bandage dressing or placed in cast or thermoplastic splints. Digital circulation is the area of clinical interest in a number of conditions such as systemic sclerosis and Raynaud's Disease, where ulceration commonly occurs at the most distal points of the fingers and thumbs. In digital replantation, clinical monitoring is primarily focused on the distal portion of the reattached digit rather than the area proximal to the injury. Furthermore, inotrope associated tissue necrosis often occurs at the most distal portion of the digits. Finally, monitoring of distal perfusion is commonly performed using capillary refill times of the digits in a number of conditions with the potential of distal circulation compromises, such as circumferential limb burns, tourniquet syndrome to digits and following crush injury or prolonged surgery to limbs. Therefore, one would consider that the measurement of digital perfusions would be of higher clinical relevance and interest.

The middle finger pulp was arbitrarily chosen to be the most suitable site for further experiments due to its ease of probe application and central location of the digits. The ring finger was deemed as an unsuitable option as it is the digit of choice for wedding bands.

Influence of Laterality and Dominance on Middle Finger Pulp Measurement

The middle finger pulp was considered as the optimal measurement site in this study. Further statistical tests were performed to ensure that there were no unduly biases prior to the start of the elevation experiment.

No statistically significant difference was detected between the mean cutaneous perfusion of middle finger pulp measurements between men and women. While there appear to be some variations in the baseline digital skin perfusion in women of different phases of the menstrual cycle[101], this finding is consistent with previously published research. Cankar et al. compared several perfusion metrics inclusive of heart rate, blood pressure, skin temperature and fingertip LDF measurements in women of different menstrual cycle phases and men. It was found that at baseline, there was no statistical difference between LDF measured cutaneous perfusion between men and women regardless of menstrual cycle phases.[100]

Hand dominance status did not affect the level of cutaneous perfusion to the middle finger pulp, consistent with the previous finding in this study. Likewise, laterality appears to have no influence on the level of blood flow to the middle finger pulp. It was noted that the measurements taken from the right side have a smaller standard deviation when compared to the left (SD 90.93 vs SD 133.1). A similar finding is found on the dominant side (SD 84.6 vs SD 139.2). This finding infers that measurements taken from the right middle finger pulps may be more consistent when compared to its left sided counterparts.

As the majority of participants are right hand dominant (n=19), it would be difficult to determine whether if this narrower standard deviation is secondary to the effect of hand dominance or laterality. Selective participant recruitment to match the number of participants of right and left-hand dominance may be used to facilitate comparison. However, such research may have little value in application to real-life settings, as the majority of the population are considered right hand dominant (90%).[132]

The right middle finger pulp was selected to be the area of choice for further experiments in light of the finding above.

Effect of Age on RMF Pulp Measurement

Correlation analysis has found little relationship between cutaneous perfusion of the RMF pulp and age in our study population. The participants studied are generally young and healthy without significant medical issues, which would not necessarily be consistent with that of the general population. It is uncertain whether if the finding can be applied to the general population, where cardiovascular disease and endothelial dysfunction is more prevalent in the elderly.

It would be interesting to review the relationship between age and cutaneous perfusion in future LDF studies with patients against the finding from this study to see whether if the finding holds true across the general population.

4.1.2 Elevation Experiment

The elevation experiment tested the effect of hand elevation on cutaneous perfusion using five different upper limb positions as described in the previous section.

After perfusion value at position 1 was recorded (mean=242.8AU), the participants were asked raised their hand to fully elevated position (position 2). It was found that in this position, the mean cutaneous perfusion was at the lowest point (mean=111.2AU, p=0.001). Once the upper limb is lowered into position 3, where it was hanging fully dependent, the value increased to its highest point, though this was not statistically significantly higher than the neutral position (mean=285.1AU, p=0.23). In position 4 and

5, the arm was placed in the neutral position at heart level similar to position 1, with the forearm elevated in varying degrees. As the forearm is placed at 45 and 90 degrees, the cutaneous perfusion values appear to reduce in an elevation dependent manner. Position 4 has a lower mean perfusion level compared to the baseline position, though this was not statistically significant (mean=206.4AU, p=0.10). In position 5, there is a statistically significant drop in cutaneous perfusion (mean=177.2AU, p=0.03)

From the statistical analysis above, the hypothesis at the start of the study is confirmed. In healthy individuals, elevation of the upper limb without acute trauma will reduce its cutaneous circulation in an elevation height dependent fashion.

In published works, a study performed by Darmanin et al. had the most similar study design and methodology as the elevation experiment performed in this study.[105] Several upper and lower limb positions were tested in their study using an Oxygen to See (O2C) (LEA Medizintechnik, Giessen, Germany) machine. The machine incorporates LDF and tissue photospectroscopy to provide measurements in tissue oxygen saturation, haemoglobin content and blood flow measurements. The volar aspect of proximal phalanx of the thumb (area number 2 of the hand mapping areas) was measured. The O2C probe takes simultaneous measurements at two depths, superficial (2mm) and deep (8mm) measurements. The study presented deeper data only as it was felt that this reflects the perfusion status of the deeper tissues more accurately. The study has noted a significant reduction in LDF detected blood flow in both full elevation and dependence of the upper limb. Forearm position was tested further with the upper arm at heart level. Interestingly, it was found that tissue perfusion increased as the forearm is elevated to 45 and 90 degrees. This increment of perfusion did not appear to have an association

with the height of elevation, as tissue perfusion was found to be at its highest level when the forearm is elevated to 45 degrees.

This finding did not concur with the results of this current study. It was found that elevation of the measured area, regardless of forearm elevation only or with upper arm elevation, leads to a reduction of cutaneous perfusion. One possible reason may be due to the difference in the depth of measurement between the two studies. The deeper measurement performed by Darmanin et al. at 8mm is likely to be measuring the perfusion of deeper structures (i.e. tendon or muscles) rather than the skin. The Periflux 5000 system used in the current study measures tissue perfusion at a depth of 1mm.

A possible explanation of the discrepancies in results between the two studies could be that different structures of the hand respond in a dissimilar fashion from elevation. One may postulate the discrepancy may be secondary to the disparity in pressure gradients between cutaneous capillaries and small arterioles of the deeper structures and their response to elevation. It would be of interest to research this further with LDF systems and probes which allows for simultaneous perfusion measurements at both deeper and superficial levels to determine whether the discrepancies in position associated perfusion changes discussed here is observed. While the O2C has a superficial probe measurement setting (2mm), the tissue penetration is likely still too deep to accurately quantify microcirculation in the skin.

Upper limb elevation is routinely used in clinical settings following surgery or acute trauma because it reduces tissue oedema. The reduction of oedema may reduce compression of blood vessels and capillary beds, therefore improving tissue perfusion.

However, it has been demonstrated that tissue perfusion of limbs may be compromised from limb elevation from a number of studies using different research devices. [121-124] The current study has found that cutaneous perfusion of the hand decreases with elevation in a height dependent manner. Therefore, a fine balance needs to be achieved between adequate reduction of undesirable oedema and decreased tissue perfusion from overzealous limb elevation.

The data from Darmanin et al. have suggested that upper limb elevation with the arm parallel to the heart level and forearm elevated to 45 degrees to be a good compromise between optimising superficial blood flow and reducing venous pooling of the upper limb. It was suggested that in some instances elevation of 90 degrees would be suitable where venous congestion is a potential problem.[105] The conclusion drawn from the results of the current study would be consistent with Darmanin et al. However, the author would suggest that forearm elevation of 45 degrees would be preferable to 90 degrees, as forearm elevation of 90 degrees leads to a statistically significant reduction of cutaneous perfusion. It has been shown that elevation of upper limb reduces oedema in healthy individuals.[117] However, there have been no studies which examine the relationship between degrees of arm elevation and extent of oedema reduction, in either non-traumatised hand or traumatised hand settings. Further understanding of such relationship would allow one to ascertain the optimal position of limb elevation further.

Currently, no studies which examine the effect of elevation on tissue perfusion has been performed in limbs with traumatic injuries. Acute inflammatory response occurs following tissue trauma. The process involves a number of physiological processes which involves cell-derived mediators and several acellular biochemical cascade systems. A

vascular phase occurs, leading to local tissue vasodilatation, increased blood flow and vessel wall permeability. It is possible that tissue perfusion may respond differently upon limb elevation in the acutely injured upper limb. As discussed previously, tissue oedema following trauma could reduce cutaneous perfusion through increased compressive pressures to the blood vessels and capillary beds. While post-traumatic tissue oedema and acute inflammation may have independent roles in altering cutaneous perfusion in the acutely traumatised tissue, it is unclear how they may interact and influence cutaneous perfusion concurrently. The current study provides a useful baseline understanding of how upper limb skin perfusion changes from limb elevation in healthy individuals. It would therefore be of interest to perform similar experiments in those with acute trauma to the upper limb to see whether if similar findings are observed.

4.2 Limitations, Technical and Practical Considerations

4.2.1 Limitations and Learning Points

There are a number of limitations which has been identified in this study. The most significant of which is associated with the small sample size. Although some interesting results have been found from the study, one should be mindful that the study is likely to be underpowered due to the low number of participants included in the study. The conclusions drawn from the results of this study are based on a small sample of healthy individuals and is unlikely to be applicable to the wider population. However, the study and its results offer valuable insights which form the foundation for future studies.

An epidemiological study based on a UK based emergency department has found a significant proportion of patients presented with hand injuries were young males between the age of 12 to 29.[109] This is consistent with the author's experience across

several plastic surgery units across the North of England. The study population was predominantly female, with an average age of 42.6 years. It is possible that the finding may not be directly applicable to the hand trauma patient population. Matching of participant demographics against the patient population would be something that one should consider during the participant recruitment process in the future.

The study population is predominantly Caucasian, except one participant being Asian in ethnic origin. It is therefore difficult to extract useful findings on the influence of ethnicity on cutaneous perfusion. From the author's experience in clinical practice, the patient population of interest is primarily Caucasian. However, it would be of interest to investigate ethnicity variations in cutaneous perfusion of the hand in future studies. This potentially has important implications in clinical research, as comparisons may be made between studies performed in different parts of the world with subjects of different ethnicities.

The study was able to observe the cutaneous perfusion of healthy individuals without acute injuries to the hand. However, the effect of physiological response to hand trauma was something that could not be investigated as such patients were not included in the study. While the findings from the current study can be applied to such patients to a certain extent, further studies with hand trauma patients or post-operative elective hand surgery patients would be useful to confirm whether the finding is applicable in the patient population.

Throughout the study, it was found that the PeriFlux 5000 System was highly sensitive to movements. Motion artefacts readily obscure the perfusion tracing upon minor

movement of the probe lead and/or the participants. In order to reduce movement artefacts, several mechanisms such as Bradford sling and thermoplastic splints have been used in this study to a reasonable level of success. It was found that the area most vulnerable to motion artefacts was the probe lead as it was generally left dangling between the machine and the participant. Several measurement attempts had to be abandoned due to motion artefacts from accidental contact (from the author) to the probe lead. Eventually, this was dealt with by taping the probe lead to the examination couch.

The potential influence of ambient light on measurements has been discussed by a prior study, which suggested that aluminium foil can be used to cover the probe for each reading to eliminate inaccuracies secondary to ambient light interference.[105] This was not adopted in the current study as it was deemed impractical as frequent changes of measurement area were required in the hand mapping study. Therefore, it may be considered as a source of potential error. However, the ambient lighting level was consistent throughout the data collection period, therefore ambient light associated inaccuracies are unlikely to have occurred.

In this study, while acclimatisation time was intended prior to measurement, the duration of this time was not specified. The acclimatisation period is assumed to be the time taken for informed consent, health questionnaires and introduction of the equipment to take place. Further standardisation of acclimatisation time would be required, as inadequate acclimatisation may lead to discrepancies in cutaneous perfusion measurements. It is known that variation in local skin temperature can cause an observable difference in the local skin blood flow. While the room where measurements

were taken was temperature controlled, different clothing style, material and thickness between participants may lead to differences in skin surface temperature. Such a source of error may be eliminated by asking the participants to change into standard clothing such as patient gowns. Temperature controlled LDF probes may reduce the risk of error secondary to skin temperature variation, as the local skin temperature in the area measured can be controlled by the probe. Therefore, future studies would benefit from LDF measurement probe with heating capabilities to enable local skin temperature control.

The PeriFlux 5000 System used in this study had only the laser Doppler perfusion monitoring module installed. While it provided valuable measurements on the level of cutaneous tissue perfusion, it provided no data on tissue oxygenation. Additional modules which support measurements of transcutaneous oxygen/carbon dioxide can be installed, which would allow additional data to be collected to provide a more comprehensive assessment of the perfusion status of the measured tissue. As highlighted previously, the PeriFlux 5000 System offers laser Doppler measurement at a single depth which is relatively superficial (1mm). Therefore, only cutaneous perfusion data were measured, and no information on deeper tissue perfusion was obtained during the study.

4.2.2 Practical Application of Laser Doppler Flowmetry

The project was a feasibility study performed to explore the suitability of laser Doppler flowmetry as a research tool in plastic surgery. The laser Doppler flowmetry machine used in this study was able to provide reliable tracings of real-time cutaneous perfusion measurements. The measurement process was non-invasive, and no participants reported any form of harm secondary to the use of LDF from this study.

Laser Doppler flowmetry has been used extensively as a research tool in various other fields of medicine and has been validated previously. As a research tool, it has a steep initial learning curve. However, it is relatively simple to use once the user is trained. Measurements can be obtained quickly provided the machine is regularly calibrated. Perfusion tracing can be obtained over a prolonged period, as the software does not limit the window of data collection.

Once the probe is attached and software opened, continuous monitoring of real-time perfusion data can be obtained easily over the desired monitoring period. However, the device is highly sensitive to movement artefacts as well as other manoeuvres which causes sympathetic stimulation. Furthermore, the device requires a lead to be attached to the monitored area at all times. Unless they are sedated or intubated it is likely that participants may find this restrictive. Therefore, laser Doppler flowmetry would be more suitable for intermittent monitoring of awake participants or monitoring intervention progress in stages.

Further drawbacks of using LDF in the research setting is that the initial cost of the machine is high, with an on-going need for consumables in the forms of probes, calibration fluids and dressings. Furthermore, the tissue penetration for the LDF system used in this study (PeriFlux 5000 System) is limited to 1 mm. This means that the machine could only provide information on perfusion of superficial tissue only. Therefore, its use in the measurement of perfusion in deeper structures is limited.

4.2.3 Participant Perception of Laser Doppler Flowmetry

In all clinical studies, participant engagement and cooperation are highly important factors to consider. This is particularly relevant in this study. Where new equipment is being trialled, the acceptability of its use should be considered. Throughout the study, all participants were happy to take part in the experiments to have their cutaneous perfusion measured by the laser Doppler flowmetry system. They were not intimidated by the machinery nor felt restricted by the attachment of probes on their skin. Most were interested in observing the real-time feedback on their cutaneous perfusion measurements.

4.3 Summary of Conclusions and Suggestions for Further Work

Laser Doppler flowmetry has been shown to be a useful research tool in this study. The tool allows instantaneous non-invasive cutaneous blood supply measurements. This would help us develop further insight into upper limb perfusion dynamics. The improved understanding of theoretical foundation in cutaneous microcirculation may support pre-existing clinical practices or facilitate changes in these practices to optimise outcomes in patients with upper limb conditions further.

The hand mapping experiment has indicated that there are significant variations in the cutaneous perfusion between different areas of the hand. However, skin perfusion does not seem to vary according to laterality and hand dominance status provided comparison be made in the same area. Similarly, choice of digits does not appear to influence measured perfusion as long as measurement area is matched. An optimal area of measurement was made. This decision was based on practical considerations and statistical analysis of hand mapping experimental results. There appears to be no

standardisation of LDF measurement areas in the current literature. Comparisons of published literature can be difficult as the cutaneous perfusion values are taken from different part of the hand. Our finding suggests that LDF measured cutaneous perfusion between different sites of the hand may be reasonably compared in certain selected situations.

From the second experiment, it was found that upper limb elevation leads to statistically significant changes to cutaneous perfusion. The results demonstrated the perfusion of the skin reduces as one elevate their upper limb from the level of their heart. There appears to be some level of inconsistency with published literature. However, the discrepancy may be secondary to the different depth of tissue penetrations of the laser Doppler flowmetry employed in this study and the published literature.

It should be noted that further repeated studies of a larger cohort of participants over a more extended study period would be required to validate the conclusions drawn from this feasibility study. In addition, the next logical step in further studies in cutaneous perfusion dynamics would include other common clinical practices in the speciality:

1) **Prolonged Elevation:** The elevation time used in this study is relatively short (i.e. minutes of limb elevation) when compared to the typical limb elevation in clinical practice (i.e. over several hours) is quite short in the experiment. Longer elevation period would better simulate the clinical practice. One may postulate that prolonged period of elevation may lead to physiological adaptation and altered perfusion dynamics. Understanding this change of cutaneous perfusion over time may lead to changes to limb elevation recommendations.

2) **Use of Tourniquet:** Tourniquets are frequently used in various settings in hand surgery. It is used to create a bloodless surgical field in both elective and trauma hand surgery. In acute limb trauma where direct compression does not control bleeding, tourniquets may also be used. While the biochemical changes secondary to tourniquet associated tissue, ischaemia has been investigated previously, the effect of tourniquet use on cutaneous perfusion has not been studied. It would therefore be of interest to see whether if there are persistent alterations to cutaneous perfusion following tourniquet use. A proposed method of investigating this would be taking measurements prior to the application of the tourniquet and repeated the measurement following the resolution of the post-occlusive reactive hyperaemia after tourniquet release. Furthermore, as the tourniquet forms a tight, constrictive band microscopic trauma to the soft tissues, it is possible that the skin perfusion may alter as a result of compressive trauma. Therefore, it may be interesting to investigate the perfusion of the area skin which was compressed by the tourniquet to see whether if cutaneous perfusion alters differently compared to non-compressed skin.

3) **Limb Immobilisation:** Splintage immobilisation is a common practice following surgery or acute trauma of the hand where protection of repaired structures from excessive movement is required. Currently, there appears to be no consensus on an agreed immobilisation protocol within the professional body. There exist variations in practices regarding the method of immobilisation (i.e. bulky bandage, plaster of Paris, neighbour strap), the area immobilised (entire hand vs affected digit/joint) and the duration of immobilisation. In the author's experience, at the

start of rehabilitation, the immobilised limbs tend to have an altered skin appearance which gradually returns to normal upon mobilisation and rehabilitation. Prolonged immobilisation of the lower limb can lead to deep vein thrombosis. It is unclear whether if such consequence occurs in the cutaneous perfusion. Further investigation into the cutaneous perfusion following limb immobilisation may help further our understanding of this phenomenon.

4) **Cutaneous Perfusion in the Traumatic Limb:** Currently, no cutaneous perfusion studies have been performed in limbs with acute traumatic injuries. The role of acute inflammation and oedema on cutaneous perfusion is not well understood and would benefit from further study. It is uncertain how upper limb cutaneous perfusion may acutely change following traumatic injuries. Furthermore, it is unknown that whether such changes will occur throughout the entire injured limb or limited to the injured region. An obvious challenge to the study would be patient standardisation. Due to the unpredictable nature of acute trauma, the injury pattern, severity and extent of injury may differ significantly between each patient. It may be difficult to group patients and injuries to allow for reasonable comparison to take place.

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APPENDICES & SUPPLEMENTARY MATERIALS

Appendix 1: Health Qustionnaire

Skin Perfusion from Plastic Surgery Practices
Laser Doppler Flowmetry Study

HEALTH QUESTIONNAIRE FORM FOR STUDY PARTICIPANTS

Name of Principle Investigator: Chad Chang
Name of Orgnaisation: University of Huddersfield

This health questionnaire form is for study participants who have agreed to participant in research on the blood supply of the skin. The title of our research project is *Skin Perfusion from Plastic Surgery Practices - Laser Doppler Flowmetry Study.*

Date: _____ **Participant No:** _____

What is your age?

_____ years

What is your sex?

Male ☐ Female ☐

Which is your dominant hand?

Right ☐ Left ☐

What is your smoking status?

Never ☐ Smoker ☐ _____/day
Ex-Smoker ☐ E-Cigarette ☐

Do you have the following medical conditions?

High blood pressure	☐	Sickle Cell Disease	☐
Ischaemic Heart Disease	☐	Carpal Tunnel Syndrome	☐
Diabetes (include diet controlled)	☐	Other Hand Conditions	☐
Peripheral Vascular Diease	☐	Previous Hand Trauma	☐
Vascullitis	☐	Previous Hand Surery	☐
Raynaud's Disease	☐	Vibration White Fingers	☐
Other Conditions, as below	☐		

_____
_____

Any significant medical condition affecting your family?

Yes ☐ No ☐
If yes, please give further details: _____

Do you have any allergies?

Yes ☐ No ☐
If yes, please give further details: _____

Do you take any medications, prescribed or otherwise?

Yes ☐ No ☐
If yes, please give further details: _____

Do you drink alcohol?

Yes ☐ _____unit/week No ☐

If any queries, please contact Mr Chad Chang on Chad.Chang@hud.ac.uk

Appendix 2: Information Sheet and Informed Consent Form

Skin Perfusion from Plastic Surgery Practices
Laser Doppler Flowmetry Study

INFORMED CONSENT FORM FOR STUDY PARTICIPANTS

Name of Principle Investigator: Chad Chang
Name of Orgnaisation: University of Huddersfield

This Infomed Consent From is for healthy volunteers and individuals who attended the trauma services in the department of Plastic, Reconstructive and Burns surgery in Pinderfields General Hospital. We invite you to participant in research on the blood supply of the skin. The title of our research project is *Skin Perfusion from Plastic Surgery Practices - Laser Doppler Flowmetry Study.*

This Informed Consent Form has two parts:

1. Information Sheet (to share information about the research with you)
2. Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form.

PART I: Information Sheet

Introduction

My name is Chad Chang, and I work as a research registrar in Plastic, Reconstructive and Burns surgery. I am also a full time Masters of Research student attending the University of Huddersfield. We are doing research on the blood supply of the skin and how some common practices in Plastic Surgery may influence it, and we would like to invite you to be part of this research. You do not have to decide immediately about your participation. Before you decide, you can talk to anyone you feel comfortable with about this research.

There may be some words you do not understand or used in unconventional ways. Please ask me to stop and explain as we go through this. If you have any questions, please do not hesitate to ask me at any point.

Purpose of Research

Uppler limb injuries are common, accounting for a large proportion of unplanned visits to hospital services in the United Kingdom. These injuries require careful examination from trained clinicians to ensure accurate diagnosis and prompt management. Whether the injury is managed with or without an operation, there are some common practices that would be adviced. These includes the use of immobilisation (plasters/splints) and elevation of the injured limb. If surgery is required, a finger or an arm tourniquet may be used to stop bleeding to allow a view of the injury for surgery. We would like to investigate how these practices may alter the blood supply of the upper limb skin.

Type and Proces of Intervention

This research will involve using a Laser Doppler Flowmetry (LDF) to measure the blood supply of your skin. This is done by attaching a lead that is taped to your skin. This is a non-invasive procedure. You will be asked to perform a series of practices whilst measuring your skin blood supply, which may include 1) moving your hands in various elevated positions, 2) putting you in and out of splints (only if you already need splints for your hand injury) and 3) placing tourniquet on your arm/fingers for various durations and pressure and releasing them. You may be asked to be measured on more than one occasion.

If you agree to participate, you will be asked to complete a health questionnaire. We will then measure your blood pressure and heart rate whilst I explain the LDF machine and the probe. I will then direct you though the process. We anticipate that (except for the splintage part of the study) only one visit is required and the study should last for no longer than one hour in total.

Participant Selection/Voluntary Participation

You are invited because you are of similar demographic to our common patient population, with medical backgrounds which may influence the LDF reading. Your participation in this research is entirely voluntary. Whether you choose to participate or not, all the services and treatment you receive will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

Potential Risk/Side Effects

There are no known side effects of skin measurement with the LDF except potential skin reaction from the adhesive dressing, in which case we do have alternatives if you are prone to skin irritation. The interventions discussed above are performed routinely daily and contribute to very little risk, but we will discuss this further when we apply this.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be kept secure and no-one but the researchers will be able to see it. Any information about you will be kept annoymous by having a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up in a password protected computer.

Sharing the Results

The knowledge that we get from doing this research will be shared with you through community meetings before it is published and made available to the public and other researchers. Confidential information will not be shared.

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

If any queries, please contact Mr Chad Chang on Chad.Chang@hud.ac.uk

PART II: Certificate of Consent

- I have read the information sheet about this study
- I have had an opportunity to ask questions and discuss this study
- I have received satisfactory answers to all my questions
- I have received enough information about this study
- I understand that I am free to withdraw from this study:
    - At any time
    - Without giving a reason for withdrawing
- I agree to take part in this study

Signature (participant): _____
Date: _____
Print Name: _____

Statement by the researcher/person taking consent

- I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the information as stated.
- I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability.
- I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Signature (researcher): _____
Date: _____
Print Name: _____

Appendix 3: Ethics Approval from University of Huddersfield School of Human and Health Sciences Research Ethics Panel

From: **Warren Gillibrand** W.P.Gillibrand@hud.ac.uk
Subject: FW: SREP Application - Chad Chang (MSc by Res) - The Influence of Plastic and Hand Surgery Practices on Upper Limb Cutaneous Blood Flow - SREP/2017/240217
Date: 20 September 2018 at 14:17
To: Chad Chang (changchad@doctors.org.uk) changchad@doctors.org.uk,
CHANG, Chad (SOUTH TEES HOSPITALS NHS FOUNDATION TRUST) chad.chang@nhs.net

WG

Best wishes,

Warren

Dr. Warren Gillibrand RN, M.Sc. PGCE, PhD, FHEA, PGCEP
Acting Deputy Director of Graduate Education (Health)
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University of Huddersfield
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Continuing your Professional Development (CPD) booklet 2018/19
: https://hhs.hud.ac.uk/ssprd/DVL/reader.html
Twitter feed: @HuduniSSPRD_HSC Facebook
page: https://www.facebook.com/HuddersfielduniversitySSPRD

From: SHUM Research Ethics
Sent: 20 September 2018 14:12
To: Warren Gillibrand <W.P.Gillibrand@hud.ac.uk>
Subject: SREP Application - Chad Chang (MSc by Res) - The Influence of Plastic and Hand Surgery Practices on Upper Limb Cutaneous Blood Flow - SREP/2017/240217

Dear Warren,

I confirm that Chad Chang's SREP Application as detailed above was approved outright on 01-Mar-2017.

Regards,

Kirsty
(SREP Administrator)

Kirsty Thomson
Research Administrator

: 01484 471156
: hhs_srep@hud.ac.uk
: www.hud.ac.uk

Appendix 4: Participant Profile

ID	Age (yr)	Gender	Hand Dom	Smoking	PMH	FH	Allergy	DH	ETOH
0000	28	M	RHD	Nil	Nil	Nil	NKDA	Nil	<10 units
0001	27	F	RHD	Nil	Nil	Nil	NKDA	Nil	15-20 units
0002	29	M	RHD	Nil	Nil	Nil	NKDA	Nil	15-20 units
0003	45	M	RHD	Nil	Seasonal Rhinitis	IHD	NKDA	Gabapentin	20 units
0004	30	F	RHD	Nil	Left thumb laceration	DM	NKDA	OCP	20 units
0005	50	F	RHD	Nil	DM, asthma	Stroke	NKDA	Inhalers	<10 units
0006	45	F	RHD	Nil	Nil	IHD	Penicillin	Nil	<10 units
0007	58	F	RHD	Nil	Hypothyroid, OA	IHD, DM, AAA	NKDA	Thyroxine	<10 units
0008	51	M	RHD	Nil	HTN	IHD, HTN	NKDA	Lisinopril	20 units
0009	45	M	RHD	Nil	Nil	Nil	NKDA	Nil	Nil
0010	39	F	RHD	Nil	Nil	Nil	NSAID	Venlafaxine	<10 units
0011	51	F	RHD	Nil	Nil	Nil	Morphine	Thyroxine	<10 units
0012	22	F	RHD	Nil	Nil	Nil	NKDA	Nil	Nil
0013	64	F	RHD	5-10/day	OA	Nil	Elastoplast NSAIDs	Co-codamol Tramadol	<10 units
0014	47	F	RHD	Nil	Hypothyroid Reflux, CTS	HTN	NKDA	Thyroxine, lansoprazole	<10 units
0015	50	F	RHD	Nil	Nil	Diabetes	NKDA	Nil	10-15 units
0016	27	M	RHD	<5/day	Nil	IHD, DM CVA, bowel ca	NKDA	Nil	<10 units
0017	26	M	RHD	Nil	Nil	Diabetes	NKDA	Nil	Nil
0018	35	F	LHD	Nil	Nil	Diabetes, IHD, Leukaemia	NKDA	Morphine Patch	<10 units
0019	55	F	RHD	Nil	Nil	IHD	NKDA	Nil	<10 units

0020	37	M	RHD	Ex	Nil	Nil	Nil	Nil	<10 units
0021	36	F	RHD	Nil	Cold intolerance	Nil	Hay fever	Nil	10-15 Units
0022	27	F	RHD	Ex	Nil	Nil	Nil	Nil	<10 units
0023	54	F	RHD	Nil	Nil	Nil	Nuts	Nil	<5 units
0024	56	F	RHD	Ex	Nil	Nil	Nil	Thyroxine, Iron, Folic Acid, PPI	Nil
0025	38	F	RHD	Nil	Nil	Nil	Nil	Thyroxine, Iron, Folic Acid, Lansoprazole	<5 units
0026	43	M	RHD	Nil	HTN	Nil	Nil	Betablocker	<10 units
0027	28	F	RHD	Ex	Nil	Nil	Nil	Nil	<5 units
0028	57	F	RHD	Nil	HTN OA	HTN IHD	Nil	Analgesia, lecardepine	<10 units
0029	37	F	RHD	Ex	Psoriatic arthritis	Nil	Nil	Methotrexate, Humira, thyroxine	<5 units
0030	43	F	RHD	Nil	Cold intolerance	Nil	Nil	Sertraline	<5 units
0031	53	F	RHD	Nil	Nil	DM, IHD	Elastoplast	Nil	Ni
0032	54	F	RHD	Nil	Nil	Nil	Trimethoprim	Nil	<5 units
0033	34	M	LHD	Nil	Nil	Nil	Nil	Nil	<10 units
0034	45	M	RHD	Nil	Nil	IHD	Hay fever	Nil	20 units
0035	49	F	RHD	Ex	Nil	N	N	Ramipril	20 units
0036	57	F	RHD	10/day	Nil	Nil	Nil	Paroxetine	20 units
0037	59	F	RHD	Ex	HTN	Nil	Penicillin	Bisoprolol,Amlodipine, thiazide, ramipril, statin, amitriptyline	<10 units
0038	38	F	LHD	Ex	Nil	DM, IHD	Penicillin	OCP	<5 units
0039	35	F	LHD	Nil	Nil	Nil	Nil	Nil	Nil

F = Female, M = Male, RHD = Right hand dominant, LHD = Left hand dominant, OCP = Oral contraceptive pill, DM = Diabetes Mellitus, IHD = Ischaemic heart disease, HTN = Hypertension, OA = Osteoarthritis, AAA = Abdominal aortic aneurysm, PPI = Proton pump inhibitor, CVA = Cerebrovascular Accident, CTS = Carpal Tunnel Syndrome

Appendix 5: Hand Map Data (Participant 0000 to 0002)

Participant 0000						
Area	Side	Mean (AU)	SD (AU)	Side	Mean (AU)	SD (AU)
1	L	300.52	35.80	R	157.29	22.22
2	L	215.86	13.30	R	183.03	24.17
3	L	333.55	28.77	R	293.5	23.56
4	L	128	8.91	R	100.51	21.26
5	L	247.03	12.53	R	157.24	26.94
6	L	430.43	21.39	R	392.87	47.27
7	L	371.79	22.23	R	230.6	31.98
8	L	251.57	10.67	R	225.79	55.33
9	L	260.18	21.55	R	307.54	30.46
10	L	168.61	23.77	R	106.46	19.32
11	L	148.18	15.28	R	64.48	8.54
12	L	266.27	24.64	R	154.75	31.55
13	L	200.64	12.40	R	281.75	38.29
14	L	113.71	33.40	R	186.24	62.3
15	L	356.42	44.33	R	185.55	57.88
16	L	53.03	7.35	R	82.49	21.79
17	L	73.7	11.63	R	73.6	15.04
18	L	143.89	20.18	R	78.25	17.24
19	L	323.74	58.67	R	82.4	14.42
20	L	79.4	20.09	R	32.18	16.64
21	L	60.14	27.03	R	76.09	27.23
22	L	197.68	20.35	R	186.03	47.32
23	L	192.9	63.34	R	40.31	22.96
24	L	58.87	6.11	R	32.99	11.67
25	L	48.13	9.16	R	35.92	9.11
26	L	73.95	19.01	R	53.46	19.05
27	L	72.41	8.90	R	37.66	8.74
28	L	16.86	5.20	R	36.72	10.01
29	L	117.82	22.96	R	64.62	11.19
30	L	30.22	8.05	R	31.58	7.72
31	L	18.14	4.64	R	19.5	4.71
32	L	20.99	2.67	R	57.81	9.19
33	L	43.69	5.94	R	35.44	9.13
34	L	31	5.39	R	32.81	4.09

35	L	101.69	37.87	R	68.92	8.69
36	L	67.92	15.77	R	29.29	11.49
37	L	18.64	5.01	R	25.97	7.96
38	L	8.11	2.22	R	11.86	5.36
39	L	23.25	7.02	R	9.59	2.89
40	L	13.7	5.09	R	17.5	4.67
41	L	6.6	2.84	R	23.29	5.89
42	L	21.83	9.72	R	9.23	2.47
43	L	6.31	0.93	R	14.63	3.54
44	L	13.33	4.36	R	11.43	7.35
45	L	7.59	2.58	R	9.09	2.21
46	L	11.23	3.05	R	7.6	2.81
47	L	7.19	2.51	R	10.37	4.53

Participant 0001						
Area	**Side**	**Mean (AU)**	**SD (AU)**	**Side**	**Mean (AU)**	**SD (AU)**
1	L	128.19	18.62	R	160.84	20.04
3	L	150.33	43.84	R	232.64	33.7
6	L	180.07	30.34	R	324.5	43.79
9	L	270.24	29.83	R	199.1	31.51
12	L	248.78	60.04	R	304.59	35.33
15	L	41.61	14.61	R	235.21	25.2
18	L	77.79	31.49	R	164.19	25.53
19	L	106.98	29.44	R	142.89	19.86
22	L	190.64	29.33	R	129.91	41.41
24	L	53.71	6.33	R	49.92	7.54
26	L	69.91	8.85	R	43.74	14.41
29	L	62.61	7.39	R	54.23	6.54
32	L	55.27	18.45	R	37.88	11.51
35	L	15.27	1.55	R	27.94	10.03
39	L	38.37	9.38	R	45.8	2.77
42	L	57.96	11.55	R	31.45	11.87
43	L	16.01	4.97	R	17.34	2.38
47	L	128.19	18.62	R	11.68	1.51

Participant 0002						
Area	Side	Mean (AU)	SD (AU)	Side	Mean (AU)	SD (AU)
1	L	185.67	15.65	R	324.91	66.49
3	L	147.96	24.68	R	120	23.31
6	L	46.08	10.8	R	170.11	88.91
9	L	123.93	16.28	R	245.58	40.99
12	L	219.89	49.53	R	130.85	39.18
15	L	63.68	20.73	R	214.57	37.1
18	L	109.26	26.01	R	89.82	22.96
19	L	48.56	21.75	R	258.81	41.56
22	L	48.16	27.37	R	91.95	15.17
24	L	18.75	5.91	R	63.14	10.33
26	L	52.75	19.96	R	100.56	15.46
29	L	20.34	9.55	R	123.93	16.29
32	L	14.89	5.83	R	57.33	12.58
35	L	137.48	30.61	R	69.93	13.74
39	L	14.36	8.6	R	19.77	6.05
42	L	39.58	10.34	R	15.27	2.9
43	L	7.82	2.45	R	8.37	1.73
47	L	9.43	2.91	R	10.87	2.29

Appendix 6: LDF Tracing General Analysis Report

Examples: Poor Tracing Consistency

General Analysis Report

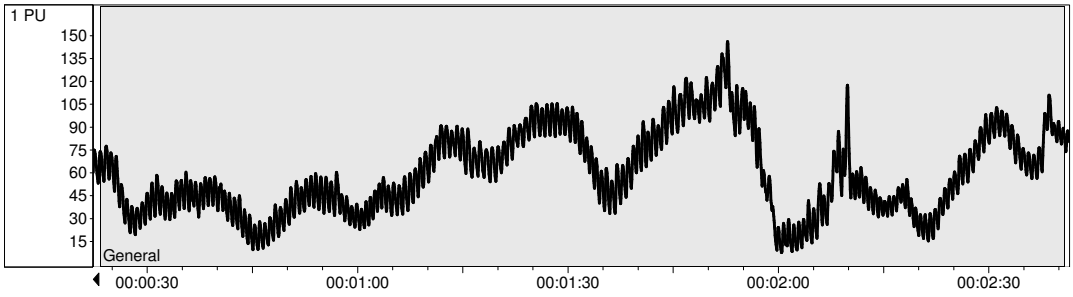
Patient Information

Comment: 28M
Nil PMH
Non Smoker

Recording Information

Comment: l21

Trace



Calculations

Item		1 PU
Mean value	(Unit)	60.14
Standard Deviation	(Unit)	27.03
Standard Error	(Unit)	0.41
Maximum value	(Unit)	146.18
Minimum value	(Unit)	7.69

Item		1 PU
First value	(Unit)	73.94
Last value	(Unit)	80.23
Percent change first to last value	(%)	8.50
Area under curve	(Unit*sec.)	8269.09
Slope	(Unit/sec.)	0.20

Item		1 PU
Duration	(sec.)	137.55
Relative start time	(hh:mm:ss.fr)	00:00:23.31
Relative stop time	(hh:mm:ss.fr)	00:02:40.86

General Analysis Report

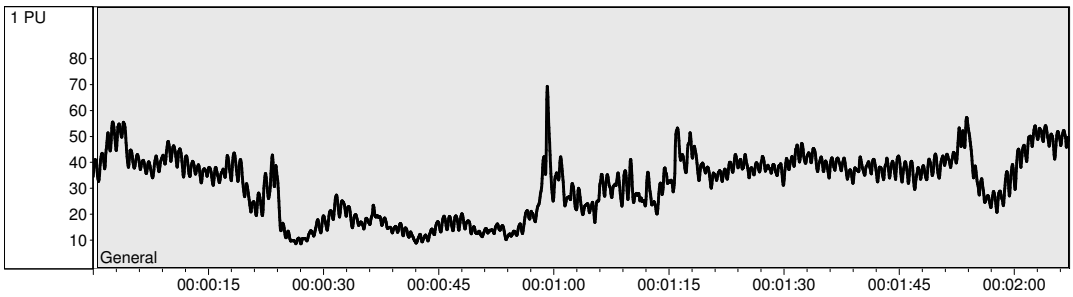
Patient Information

Comment: 27F
Nil PMH
Non Smoker

Recording Information

Comment: R42

Trace



Calculations

Item		1 PU
Mean value	(Unit)	31.45
Standard Deviation	(Unit)	11.87
Standard Error	(Unit)	0.19
Maximum value	(Unit)	69.27
Minimum value	(Unit)	8.58

Item		1 PU
First value	(Unit)	34.45
Last value	(Unit)	49.47
Percent change first to last value	(%)	43.58
Area under curve	(Unit*sec.)	3980.65
Slope	(Unit/sec.)	0.11

Item		1 PU
Duration	(sec.)	126.54
Relative start time	(hh:mm:ss.fr)	00:00:00.53
Relative stop time	(hh:mm:ss.fr)	00:02:07.12

Examples: Low Tracing Value

General Analysis Report

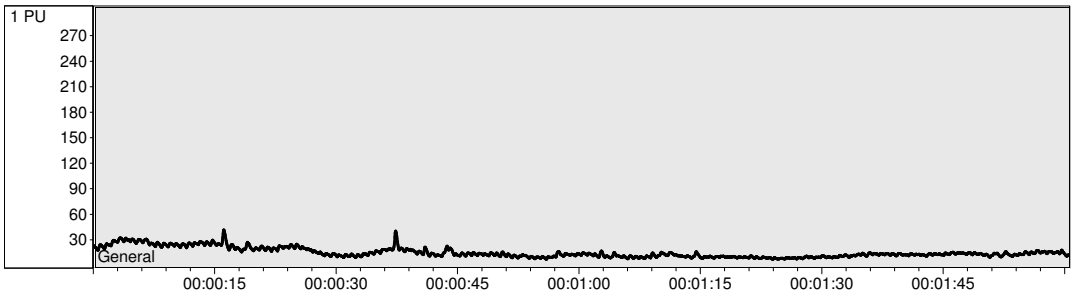
Patient Information

Comment: 29M
Nil PMH Non Smoker

Recording Information

Comment: L32
Hand Mapping

Trace



Calculations

Item		1 PU
Mean value	(Unit)	14.89
Standard Deviation	(Unit)	5.83
Standard Error	(Unit)	0.09
Maximum value	(Unit)	41.78
Minimum value	(Unit)	7.17

Item		1 PU
First value	(Unit)	21.27
Last value	(Unit)	11.66
Percent change first to last value	(%)	-45.19
Area under curve	(Unit*sec.)	1791.11
Slope	(Unit/sec.)	-0.11

Item		1 PU
Duration	(sec.)	120.31
Relative start time	(hh:mm:ss.fr)	00:00:00.20
Relative stop time	(hh:mm:ss.fr)	00:02:00.53

General Analysis Report

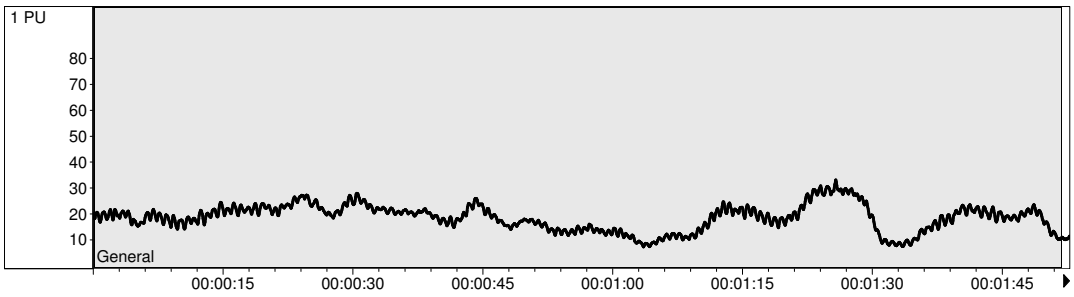
Patient Information

Comment: 28M
Nil PMH
Non Smoker

Recording Information

Comment: l37

Trace



Calculations

Item		1 PU
Mean value	(Unit)	18.64
Standard Deviation	(Unit)	5.01
Standard Error	(Unit)	0.08
Maximum value	(Unit)	33.08
Minimum value	(Unit)	7.29

Item		1 PU
First value	(Unit)	18.37
Last value	(Unit)	10.38
Percent change first to last value	(%)	-43.52
Area under curve	(Unit*sec.)	2082.07
Slope	(Unit/sec.)	-0.03

Item		1 PU
Duration	(sec.)	111.66
Relative start time	(hh:mm:ss.fr)	00:00:00.10
Relative stop time	(hh:mm:ss.fr)	00:01:51.80

Examples: High Value Stable Tracing

General Analysis Report

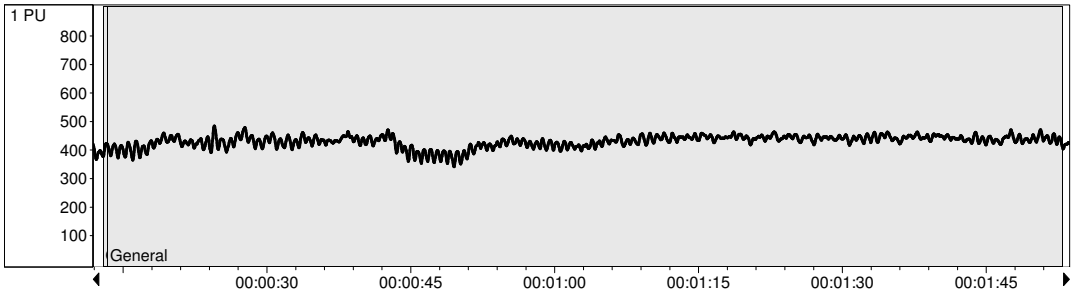
Patient Information

Comment: 28M
Nil PMH
Non Smoker

Recording Information

Comment: l6

General Area 1 Trace



General Area 1 Calculations

Item		1 PU
Mean value	(Unit)	430.43
Standard Deviation	(Unit)	21.39
Standard Error	(Unit)	0.38
Maximum value	(Unit)	484.80
Minimum value	(Unit)	341.49

Item		1 PU
First value	(Unit)	377.66
Last value	(Unit)	441.35
Percent change first to last value	(%)	16.86
Area under curve	(Unit*sec.)	42944.64
Slope	(Unit/sec.)	0.29

Item		1 PU
Duration	(sec.)	99.79
Relative start time	(hh:mm:ss.fr)	00:00:12.89
Relative stop time	(hh:mm:ss.fr)	00:01:52.70

General Analysis Report

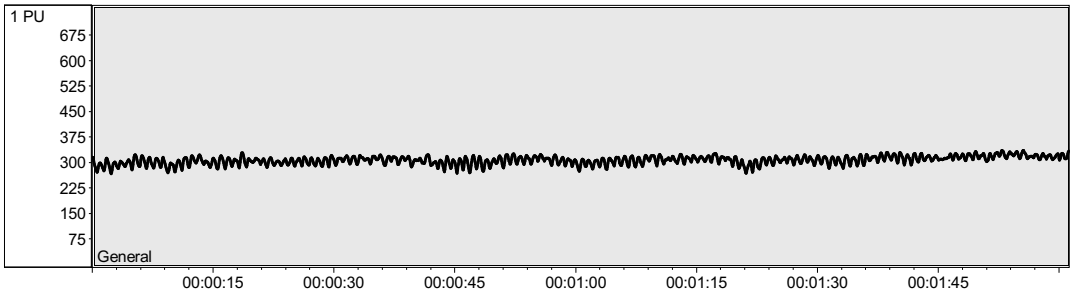
Patient Information

Comment: 51F
RHD
Non smoker
No PMH
No FH
Allergy Morphine
DH Thyroxine
ETOH 10 unit/week

Recording Information

Comment: l6

Trace



Calculations

Item		1 PU
Mean value	(Unit)	306.76
Standard Deviation	(Unit)	12.17
Standard Error	(Unit)	0.19
Maximum value	(Unit)	336.30
Minimum value	(Unit)	267.64

Item		1 PU
First value	(Unit)	289.49
Last value	(Unit)	331.57
Percent change first to last value	(%)	14.54
Area under curve	(Unit*sec.)	37080.42
Slope	(Unit/sec.)	0.15

Item		1 PU
Duration	(sec.)	120.87
Relative start time	(hh:mm:ss.fr)	00:00:00.30
Relative stop time	(hh:mm:ss.fr)	00:02:01.22

Appendix 7: Summary Hand Map Data

ID	Side	Area	Age	Sex	Dom Side	Mean (AU)	SD (AU)	VOLAR DORSUM	HAND/ DIGIT	DOM HAND
0000	L	1	28	M	RHD	300.52	35.80	VOLAR	DIGIT	NO
0000	R	1	28	M	RHD	157.29	22.22	VOLAR	DIGIT	YES
0000	L	2	28	M	RHD	215.86	13.30	VOLAR	DIGIT	NO
0000	R	2	28	M	RHD	183.03	24.17	VOLAR	DIGIT	YES
0000	L	3	28	M	RHD	333.55	28.77	VOLAR	DIGIT	NO
0000	L	7	28	M	RHD	371.79	22.23	VOLAR	DIGIT	NO
0000	L	8	28	M	RHD	251.57	10.67	VOLAR	DIGIT	NO
0000	L	9	28	M	RHD	260.18	21.55	VOLAR	DIGIT	NO
0000	L	10	28	M	RHD	168.61	23.77	VOLAR	DIGIT	NO
0000	L	11	28	M	RHD	148.18	15.28	VOLAR	DIGIT	NO
0000	L	12	28	M	RHD	266.27	24.64	VOLAR	DIGIT	NO
0000	L	13	28	M	RHD	200.64	12.40	VOLAR	DIGIT	NO
0000	L	14	28	M	RHD	113.71	33.40	VOLAR	DIGIT	NO
0000	L	15	28	M	RHD	356.42	44.33	VOLAR	HAND	NO
0000	L	16	28	M	RHD	53.03	7.35	VOLAR	HAND	NO
0000	L	17	28	M	RHD	73.7	11.63	VOLAR	HAND	NO
0000	L	18	28	M	RHD	143.89	20.18	VOLAR	HAND	NO
0000	L	19	28	M	RHD	323.74	58.67	VOLAR	HAND	NO
0000	L	20	28	M	RHD	79.4	20.09	VOLAR	HAND	NO
0000	L	21	28	M	RHD	60.14	27.03	VOLAR	HAND	NO
0000	L	22	28	M	RHD	197.68	20.35	VOLAR	HAND	NO
0000	L	23	28	M	RHD	192.9	63.34	VOLAR	HAND	NO
0000	L	24	28	M	RHD	58.87	6.11	DORSUM	DIGIT	NO
0000	L	25	28	M	RHD	48.13	9.16	DORSUM	DIGIT	NO
0000	L	26	28	M	RHD	73.95	19.01	DORSUM	DIGIT	NO
0000	L	27	28	M	RHD	72.41	8.90	DORSUM	DIGIT	NO
0000	L	28	28	M	RHD	16.86	5.20	DORSUM	DIGIT	NO
0000	L	29	28	M	RHD	117.82	22.96	DORSUM	DIGIT	NO
0000	L	30	28	M	RHD	30.22	8.05	DORSUM	DIGIT	NO
0000	L	31	28	M	RHD	18.14	4.64	DORSUM	DIGIT	NO
0000	L	32	28	M	RHD	20.99	2.67	DORSUM	DIGIT	NO
0000	L	33	28	M	RHD	43.69	5.94	DORSUM	DIGIT	NO
0000	L	34	28	M	RHD	31	5.39	DORSUM	DIGIT	NO
0000	L	35	28	M	RHD	101.69	37.87	DORSUM	DIGIT	NO
0000	L	36	28	M	RHD	67.92	15.77	DORSUM	DIGIT	NO
0000	L	37	28	M	RHD	18.64	5.01	DORSUM	DIGIT	NO
0000	L	38	28	M	RHD	8.11	2.22	DORSUM	HAND	NO
0000	L	39	28	M	RHD	23.25	7.02	DORSUM	HAND	NO

0000	L	40	28	M	RHD	13.7	5.09	DORSUM	HAND	NO
0000	L	41	28	M	RHD	6.6	2.84	DORSUM	HAND	NO
0000	L	42	28	M	RHD	21.83	9.72	DORSUM	HAND	NO
0000	L	43	28	M	RHD	6.31	0.93	DORSUM	HAND	NO
0000	L	44	28	M	RHD	13.33	4.36	DORSUM	HAND	NO
0000	L	45	28	M	RHD	7.59	2.58	DORSUM	HAND	NO
0000	L	46	28	M	RHD	11.23	3.05	DORSUM	HAND	NO
0000	L	47	28	M	RHD	7.19	2.51	DORSUM	HAND	NO
0000	R	3	28	M	RHD	293.5	23.56	VOLAR	DIGIT	YES
0000	L	4	28	M	RHD	128	8.91	VOLAR	DIGIT	NO
0000	R	4	28	M	RHD	100.51	21.26	VOLAR	DIGIT	YES
0000	L	5	28	M	RHD	247.03	12.53	VOLAR	DIGIT	NO
0000	R	5	28	M	RHD	157.24	26.94	VOLAR	DIGIT	YES
0000	R	7	28	M	RHD	230.6	31.98	VOLAR	DIGIT	YES
0000	R	8	28	M	RHD	225.79	55.33	VOLAR	DIGIT	YES
0000	R	9	28	M	RHD	307.54	30.46	VOLAR	DIGIT	YES
0000	R	10	28	M	RHD	106.46	19.32	VOLAR	DIGIT	YES
0000	R	11	28	M	RHD	64.48	8.54	VOLAR	DIGIT	YES
0000	R	12	28	M	RHD	154.75	31.55	VOLAR	DIGIT	YES
0000	R	13	28	M	RHD	281.75	38.29	VOLAR	DIGIT	YES
0000	R	14	28	M	RHD	186.24	62.3	VOLAR	DIGIT	YES
0000	R	15	28	M	RHD	185.55	57.88	VOLAR	HAND	YES
0000	R	16	28	M	RHD	82.49	21.79	VOLAR	HAND	YES
0000	R	17	28	M	RHD	73.6	15.04	VOLAR	HAND	YES
0000	R	18	28	M	RHD	78.25	17.24	VOLAR	HAND	YES
0000	R	19	28	M	RHD	82.4	14.42	VOLAR	HAND	YES
0000	R	20	28	M	RHD	32.18	16.64	VOLAR	HAND	YES
0000	R	21	28	M	RHD	76.09	27.23	VOLAR	HAND	YES
0000	R	22	28	M	RHD	186.03	47.32	VOLAR	HAND	YES
0000	R	23	28	M	RHD	40.31	22.96	VOLAR	HAND	YES
0000	R	24	28	M	RHD	32.99	11.67	DORSUM	DIGIT	YES
0000	R	25	28	M	RHD	35.92	9.11	DORSUM	DIGIT	YES
0000	R	26	28	M	RHD	53.46	19.05	DORSUM	DIGIT	YES
0000	R	27	28	M	RHD	37.66	8.74	DORSUM	DIGIT	YES
0000	R	28	28	M	RHD	36.72	10.01	DORSUM	DIGIT	YES
0000	R	29	28	M	RHD	64.62	11.19	DORSUM	DIGIT	YES
0000	R	30	28	M	RHD	31.58	7.72	DORSUM	DIGIT	YES
0000	R	31	28	M	RHD	19.5	4.71	DORSUM	DIGIT	YES
0000	R	32	28	M	RHD	57.81	9.19	DORSUM	DIGIT	YES
0000	R	33	28	M	RHD	35.44	9.13	DORSUM	DIGIT	YES
0000	R	34	28	M	RHD	32.81	4.09	DORSUM	DIGIT	YES
0000	R	35	28	M	RHD	68.92	8.69	DORSUM	DIGIT	YES

0000	R	36	28	M	RHD	29.29	11.49	DORSUM	DIGIT	YES
0000	R	37	28	M	RHD	25.97	7.96	DORSUM	DIGIT	YES
0000	R	38	28	M	RHD	11.86	5.36	DORSUM	HAND	YES
0000	R	39	28	M	RHD	9.59	2.89	DORSUM	HAND	YES
0000	R	40	28	M	RHD	17.5	4.67	DORSUM	HAND	YES
0000	R	41	28	M	RHD	23.29	5.89	DORSUM	HAND	YES
0000	R	42	28	M	RHD	9.23	2.47	DORSUM	HAND	YES
0000	R	43	28	M	RHD	14.63	3.54	DORSUM	HAND	YES
0000	R	44	28	M	RHD	11.43	7.35	DORSUM	HAND	YES
0000	R	45	28	M	RHD	9.09	2.21	DORSUM	HAND	YES
0000	R	46	28	M	RHD	7.6	2.81	DORSUM	HAND	YES
0000	R	47	28	M	RHD	10.37	4.53	DORSUM	HAND	YES
0000	L	6	28	M	RHD	430.43	21.39	VOLAR	DIGIT	NO
0000	R	6	28	M	RHD	392.87	47.27	VOLAR	DIGIT	YES
0001	L	1	27	F	RHD	128.19	18.62	VOLAR	DIGIT	NO
0001	R	1	27	F	RHD	160.84	20.04	VOLAR	DIGIT	YES
0001	L	9	27	F	RHD	270.24	29.83	VOLAR	DIGIT	NO
0001	L	12	27	F	RHD	248.78	60.04	VOLAR	DIGIT	NO
0001	L	15	27	F	RHD	41.61	14.61	VOLAR	HAND	NO
0001	L	18	27	F	RHD	77.79	31.49	VOLAR	HAND	NO
0001	L	19	27	F	RHD	106.98	29.44	VOLAR	HAND	NO
0001	L	22	27	F	RHD	190.64	29.33	VOLAR	HAND	NO
0001	L	24	27	F	RHD	53.71	6.33	DORSUM	DIGIT	NO
0001	L	26	27	F	RHD	69.91	8.85	DORSUM	DIGIT	NO
0001	L	29	27	F	RHD	62.61	7.39	DORSUM	DIGIT	NO
0001	L	32	27	F	RHD	55.27	18.45	DORSUM	DIGIT	NO
0001	L	35	27	F	RHD	15.27	1.55	DORSUM	DIGIT	NO
0001	L	39	27	F	RHD	38.37	9.38	DORSUM	HAND	NO
0001	L	42	27	F	RHD	57.96	11.55	DORSUM	HAND	NO
0001	L	43	27	F	RHD	16.01	4.97	DORSUM	HAND	NO
0001	L	47	27	F	RHD	9.78	2.62	DORSUM	HAND	NO
0001	L	3	27	F	RHD	150.33	43.84	VOLAR	DIGIT	NO
0001	R	3	27	F	RHD	232.64	33.7	VOLAR	DIGIT	YES
0001	L	6	27	F	RHD	180.07	30.34	VOLAR	DIGIT	NO
0001	R	6	27	F	RHD	324.5	43.79	VOLAR	DIGIT	YES
0001	R	9	27	F	RHD	199.1	31.51	VOLAR	DIGIT	YES
0001	R	12	27	F	RHD	304.59	35.33	VOLAR	DIGIT	YES
0001	R	15	27	F	RHD	235.21	25.2	VOLAR	HAND	YES
0001	R	18	27	F	RHD	164.19	25.53	VOLAR	HAND	YES
0001	R	19	27	F	RHD	142.89	19.86	VOLAR	HAND	YES
0001	R	22	27	F	RHD	129.91	41.41	VOLAR	HAND	YES
0001	R	24	27	F	RHD	49.92	7.54	DORSUM	DIGIT	YES

0001	R	26	27	F	RHD	43.74	14.41	DORSUM	DIGIT	YES
0001	R	29	27	F	RHD	54.23	6.54	DORSUM	DIGIT	YES
0001	R	32	27	F	RHD	37.88	11.51	DORSUM	DIGIT	YES
0001	R	35	27	F	RHD	27.94	10.03	DORSUM	DIGIT	YES
0001	R	39	27	F	RHD	45.8	2.77	DORSUM	HAND	YES
0001	R	42	27	F	RHD	31.45	11.87	DORSUM	HAND	YES
0001	R	43	27	F	RHD	17.34	2.38	DORSUM	HAND	YES
0001	R	47	27	F	RHD	11.68	1.51	DORSUM	HAND	YES
0002	L	9	32	M	RHD	123.93	16.28	VOLAR	DIGIT	NO
0002	L	12	33	M	RHD	219.89	49.53	VOLAR	DIGIT	NO
0002	L	15	34	M	RHD	63.68	20.73	VOLAR	HAND	NO
0002	L	18	35	M	RHD	109.26	26.01	VOLAR	HAND	NO
0002	L	19	36	M	RHD	48.56	21.75	VOLAR	HAND	NO
0002	L	22	37	M	RHD	48.16	27.37	VOLAR	HAND	NO
0002	L	24	38	M	RHD	18.75	5.91	DORSUM	DIGIT	NO
0002	L	26	39	M	RHD	52.75	19.96	DORSUM	DIGIT	NO
0002	L	29	40	M	RHD	20.34	9.55	DORSUM	DIGIT	NO
0002	L	32	41	M	RHD	14.89	5.83	DORSUM	DIGIT	NO
0002	L	35	42	M	RHD	137.48	30.61	DORSUM	DIGIT	NO
0002	L	39	43	M	RHD	14.36	8.6	DORSUM	HAND	NO
0002	L	42	44	M	RHD	39.58	10.34	DORSUM	HAND	NO
0002	L	43	45	M	RHD	7.82	2.45	DORSUM	HAND	NO
0002	L	47	46	M	RHD	9.43	2.91	DORSUM	HAND	NO
0002	L	1	29	M	RHD	185.67	15.65	VOLAR	DIGIT	NO
0002	R	1	47	M	RHD	324.91	66.49	VOLAR	DIGIT	YES
0002	L	3	30	M	RHD	147.96	24.68	VOLAR	DIGIT	NO
0002	R	3	48	M	RHD	120	23.31	VOLAR	DIGIT	YES
0002	R	9	50	M	RHD	245.58	40.99	VOLAR	DIGIT	YES
0002	R	12	51	M	RHD	130.85	39.18	VOLAR	DIGIT	YES
0002	R	15	52	M	RHD	214.57	37.1	VOLAR	HAND	YES
0002	R	18	53	M	RHD	89.82	22.96	VOLAR	HAND	YES
0002	R	19	54	M	RHD	258.81	41.56	VOLAR	HAND	YES
0002	R	22	55	M	RHD	91.95	15.17	VOLAR	HAND	YES
0002	R	24	56	M	RHD	63.14	10.33	DORSUM	DIGIT	YES
0002	R	26	57	M	RHD	100.56	15.46	DORSUM	DIGIT	YES
0002	R	29	58	M	RHD	123.93	16.29	DORSUM	DIGIT	YES
0002	R	32	59	M	RHD	57.33	12.58	DORSUM	DIGIT	YES
0002	R	35	60	M	RHD	69.93	13.74	DORSUM	DIGIT	YES
0002	R	39	61	M	RHD	19.77	6.05	DORSUM	HAND	YES
0002	R	42	62	M	RHD	15.27	2.9	DORSUM	HAND	YES
0002	L	6	31	M	RHD	46.08	10.8	VOLAR	DIGIT	NO
0002	R	43	63	M	RHD	8.37	1.73	DORSUM	HAND	YES

0002	R	6	49	M	RHD	170.11	88.91	VOLAR	DIGIT	YES
0002	R	47	64	M	RHD	10.87	2.29	DORSUM	HAND	YES
0003	L	1	45	M	RHD	234.69	48.83	VOLAR	DIGIT	NO
0003	R	1	45	M	RHD	208.05	11.49	VOLAR	DIGIT	YES
0003	L	9	45	M	RHD	368.37	80.01	VOLAR	DIGIT	NO
0003	L	12	45	M	RHD	260.91	25.28	VOLAR	DIGIT	NO
0003	L	3	45	M	RHD	190.16	18.24	VOLAR	DIGIT	NO
0003	R	3	45	M	RHD	239.02	45.98	VOLAR	DIGIT	YES
0003	L	6	45	M	RHD	120.06	16.55	VOLAR	DIGIT	NO
0003	R	6	45	M	RHD	213.54	8.08	VOLAR	DIGIT	YES
0003	R	9	45	M	RHD	237.85	30.29	VOLAR	DIGIT	YES
0003	R	12	45	M	RHD	415.67	17.99	VOLAR	DIGIT	YES
0004	L	6	30	F	RHD	121.85	22.45	VOLAR	DIGIT	NO
0004	L	9	30	F	RHD	312.15	47.81	VOLAR	DIGIT	NO
0004	L	12	30	F	RHD	255.88	28.65	VOLAR	DIGIT	NO
0004	R	6	30	F	RHD	290.97	56.63	VOLAR	DIGIT	YES
0004	L	1	30	F	RHD	263.14	30.05	VOLAR	DIGIT	NO
0004	R	1	30	F	RHD	187.36	12.19	VOLAR	DIGIT	YES
0004	L	3	30	F	RHD	289.44	31.3	VOLAR	DIGIT	NO
0004	R	3	30	F	RHD	235.03	61.93	VOLAR	DIGIT	YES
0004	R	9	30	F	RHD	186.31	42.57	VOLAR	DIGIT	YES
0004	R	12	30	F	RHD	284.94	64.27	VOLAR	DIGIT	YES
0005	L	9	50	F	RHD	196.74	64.82	VOLAR	DIGIT	NO
0005	L	12	50	F	RHD	256.86	52.92	VOLAR	DIGIT	NO
0005	L	1	50	F	RHD	232.05	39	VOLAR	DIGIT	NO
0005	R	1	50	F	RHD	230.82	42.13	VOLAR	DIGIT	YES
0005	L	3	50	F	RHD	132.82	23.73	VOLAR	DIGIT	NO
0005	R	3	50	F	RHD	306.62	45.7	VOLAR	DIGIT	YES
0005	R	9	50	F	RHD	163.19	24.06	VOLAR	DIGIT	YES
0005	R	12	50	F	RHD	250.04	46.17	VOLAR	DIGIT	YES
0005	L	6	50	F	RHD	176.05	22.08	VOLAR	DIGIT	NO
0005	R	6	50	F	RHD	222.69	33.31	VOLAR	DIGIT	YES
0006	L	9	45	F	RHD	491.13	31.03	VOLAR	DIGIT	NO
0006	L	12	45	F	RHD	213.43	68.27	VOLAR	DIGIT	NO
0006	L	6	45	F	RHD	313.53	29.67	VOLAR	DIGIT	NO
0006	R	6	45	F	RHD	241.32	36.36	VOLAR	DIGIT	YES
0006	L	1	45	F	RHD	178.94	18.41	VOLAR	DIGIT	NO
0006	R	1	45	F	RHD	222.15	9.73	VOLAR	DIGIT	YES
0006	L	3	45	F	RHD	316.08	79.21	VOLAR	DIGIT	NO
0006	R	3	45	F	RHD	224.29	36.36	VOLAR	DIGIT	YES
0006	R	9	45	F	RHD	289.3	63.49	VOLAR	DIGIT	YES
0006	R	12	45	F	RHD	157.62	38.74	VOLAR	DIGIT	YES

0007	L	12	58	F	RHD	135.24	32.75	VOLAR	DIGIT	NO
0007	L	9	58	F	RHD	438.96	51.15	VOLAR	DIGIT	NO
0007	L	1	58	F	RHD	297.03	17.97	VOLAR	DIGIT	NO
0007	R	1	58	F	RHD	298.05	17.74	VOLAR	DIGIT	YES
0007	L	3	58	F	RHD	256.03	48.92	VOLAR	DIGIT	NO
0007	R	3	58	F	RHD	214.18	24.54	VOLAR	DIGIT	YES
0007	R	9	58	F	RHD	136.55	26.97	VOLAR	DIGIT	YES
0007	R	12	58	F	RHD	563.89	50.32	VOLAR	DIGIT	YES
0007	L	6	58	F	RHD	214.3	52.66	VOLAR	DIGIT	NO
0007	R	6	58	F	RHD	264.05	39.13	VOLAR	DIGIT	YES
0008	L	9	51	M	RHD	326.69	92.05	VOLAR	DIGIT	NO
0008	L	12	51	M	RHD	402.53	67.73	VOLAR	DIGIT	NO
0008	L	1	51	M	RHD	150.67	19.69	VOLAR	DIGIT	NO
0008	R	1	51	M	RHD	187.29	28.95	VOLAR	DIGIT	YES
0008	L	3	51	M	RHD	332.78	31.17	VOLAR	DIGIT	NO
0008	R	3	51	M	RHD	377.35	20.07	VOLAR	DIGIT	YES
0008	R	9	51	M	RHD	220.7	24.11	VOLAR	DIGIT	YES
0008	R	12	51	M	RHD	268.78	40.91	VOLAR	DIGIT	YES
0008	L	6	51	M	RHD	161.27	41.94	VOLAR	DIGIT	NO
0008	R	6	51	M	RHD	335.89	34.09	VOLAR	DIGIT	YES
0009	L	6	45	M	RHD	347.34	17.95	VOLAR	DIGIT	NO
0009	L	9	45	M	RHD	262.69	57.85	VOLAR	DIGIT	NO
0009	L	12	45	M	RHD	288.5	78.81	VOLAR	DIGIT	NO
0009	L	1	45	M	RHD	192.99	10.39	VOLAR	DIGIT	NO
0009	R	1	45	M	RHD	146.31	13.9	VOLAR	DIGIT	YES
0009	L	3	45	M	RHD	262.38	17.02	VOLAR	DIGIT	NO
0009	R	3	45	M	RHD	326.68	36.29	VOLAR	DIGIT	YES
0009	R	9	45	M	RHD	439.84	36.51	VOLAR	DIGIT	YES
0009	R	12	45	M	RHD	231.66	45.83	VOLAR	DIGIT	YES
0009	R	6	45	M	RHD	423.5	63.63	VOLAR	DIGIT	YES
0010	L	6	39	F	RHD	260.82	38.89	VOLAR	DIGIT	NO
0010	R	6	39	F	RHD	335.63	39.17	VOLAR	DIGIT	YES
0010	L	9	39	F	RHD	141.61	20.8	VOLAR	DIGIT	NO
0010	L	12	39	F	RHD	264.92	14.14	VOLAR	DIGIT	NO
0010	L	1	39	F	RHD	277.44	34.52	VOLAR	DIGIT	NO
0010	R	1	39	F	RHD	187.87	24.85	VOLAR	DIGIT	YES
0010	L	3	39	F	RHD	287.43	28.99	VOLAR	DIGIT	NO
0010	R	3	39	F	RHD	233.8	73.74	VOLAR	DIGIT	YES
0010	R	9	39	F	RHD	480.21	67.71	VOLAR	DIGIT	YES
0010	R	12	39	F	RHD	259.25	113.71	VOLAR	DIGIT	YES
0011	L	9	51	F	RHD	422.66	120.64	VOLAR	DIGIT	NO
0011	L	12	51	F	RHD	442.98	46.71	VOLAR	DIGIT	NO

0011	L	1	51	F	RHD	313.23	31.31	VOLAR	DIGIT	NO
0011	R	1	51	F	RHD	301.05	10.45	VOLAR	DIGIT	YES
0011	L	3	51	F	RHD	569.68	14.47	VOLAR	DIGIT	NO
0011	R	3	51	F	RHD	352.02	74.11	VOLAR	DIGIT	YES
0011	R	9	51	F	RHD	334.02	23.12	VOLAR	DIGIT	YES
0011	R	12	51	F	RHD	467.3	19.97	VOLAR	DIGIT	YES
0011	L	6	51	F	RHD	306.76	12.17	VOLAR	DIGIT	NO
0011	R	6	51	F	RHD	287.28	79.93	VOLAR	DIGIT	YES
0012	L	6	22	F	RHD	55.07	29.96	VOLAR	DIGIT	NO
0012	R	6	22	F	RHD	192.2	88.74	VOLAR	DIGIT	YES
0012	L	9	22	F	RHD	28.66	25.94	VOLAR	DIGIT	NO
0012	L	12	22	F	RHD	27.78	19.29	VOLAR	DIGIT	NO
0012	L	1	22	F	RHD	146.03	50.64	VOLAR	DIGIT	NO
0012	R	1	22	F	RHD	82.77	20.25	VOLAR	DIGIT	YES
0012	L	3	22	F	RHD	64.96	31.51	VOLAR	DIGIT	NO
0012	R	3	22	F	RHD	187.98	51.89	VOLAR	DIGIT	YES
0012	R	9	22	F	RHD	54.09	24.87	VOLAR	DIGIT	YES
0012	R	12	22	F	RHD	29.98	16.85	VOLAR	DIGIT	YES
0013	L	9	64	F	RHD	290.02	12.12	VOLAR	DIGIT	NO
0013	L	12	64	F	RHD	107.62	27.55	VOLAR	DIGIT	NO
0013	L	1	64	F	RHD	211.17	10.2	VOLAR	DIGIT	NO
0013	R	1	64	F	RHD	258.85	8.93	VOLAR	DIGIT	YES
0013	L	3	64	F	RHD	355.6	60.87	VOLAR	DIGIT	NO
0013	R	3	64	F	RHD	351.33	13.58	VOLAR	DIGIT	YES
0013	R	9	64	F	RHD	300.7	39.79	VOLAR	DIGIT	YES
0013	R	12	64	F	RHD	361.65	47.85	VOLAR	DIGIT	YES
0013	L	6	64	F	RHD	584.44	16.77	VOLAR	DIGIT	NO
0013	R	6	64	F	RHD	359.65	52.22	VOLAR	DIGIT	YES
0014	L	9	47	F	RHD	222.32	66.89	VOLAR	DIGIT	NO
0014	L	12	47	F	RHD	209.98	57.9	VOLAR	DIGIT	NO
0014	L	1	47	F	RHD	209.39	37.83	VOLAR	DIGIT	NO
0014	R	1	47	F	RHD	230.73	23.4	VOLAR	DIGIT	YES
0014	L	3	47	F	RHD	325.21	21.34	VOLAR	DIGIT	NO
0014	R	3	47	F	RHD	272.2	60.84	VOLAR	DIGIT	YES
0014	L	6	47	F	RHD	248.42	44.38	VOLAR	DIGIT	NO
0014	R	6	47	F	RHD	184.07	17.42	VOLAR	DIGIT	YES
0014	R	9	47	F	RHD	188.18	72.83	VOLAR	DIGIT	YES
0014	R	12	47	F	RHD	202.09	92.45	VOLAR	DIGIT	YES
0015	L	9	50	F	RHD	19.85	20.16	VOLAR	DIGIT	NO
0015	L	12	50	F	RHD	18.34	26.35	VOLAR	DIGIT	NO
0015	L	1	50	F	RHD	86.54	11.61	VOLAR	DIGIT	NO
0015	R	1	50	F	RHD	101.92	13.72	VOLAR	DIGIT	YES

0015	L	3	50	F	RHD	61.53	15.24	VOLAR	DIGIT	NO
0015	R	3	50	F	RHD	108.63	28.8	VOLAR	DIGIT	YES
0015	L	6	50	F	RHD	30.09	14.68	VOLAR	DIGIT	NO
0015	R	6	50	F	RHD	117.8	40.82	VOLAR	DIGIT	YES
0015	R	9	50	F	RHD	66.97	24.19	VOLAR	DIGIT	YES
0015	R	12	50	F	RHD	33.34	50.53	VOLAR	DIGIT	YES
0016	L	6	27	M	RHD	243.35	67.62	VOLAR	DIGIT	NO
0016	R	6	27	M	RHD	323.21	67.59	VOLAR	DIGIT	YES
0016	L	9	27	M	RHD	288.91	66.38	VOLAR	DIGIT	NO
0016	L	12	27	M	RHD	291.44	54.17	VOLAR	DIGIT	NO
0016	L	1	27	M	RHD	359.36	55.76	VOLAR	DIGIT	NO
0016	R	1	27	M	RHD	286.24	75.85	VOLAR	DIGIT	YES
0016	L	3	27	M	RHD	230.89	70.92	VOLAR	DIGIT	NO
0016	R	3	27	M	RHD	302.39	40.77	VOLAR	DIGIT	YES
0016	R	9	27	M	RHD	394.41	40.31	VOLAR	DIGIT	YES
0016	R	12	27	M	RHD	300.14	62.3	VOLAR	DIGIT	YES
0017	L	6	26	M	RHD	239.92	20.51	VOLAR	DIGIT	NO
0017	R	6	26	M	RHD	271.78	86.6	VOLAR	DIGIT	YES
0017	L	9	26	M	RHD	169.5	28.81	VOLAR	DIGIT	NO
0017	L	12	26	M	RHD	375.43	54.33	VOLAR	DIGIT	NO
0017	L	1	26	M	RHD	156.11	31.34	VOLAR	DIGIT	NO
0017	R	1	26	M	RHD	276.22	50.39	VOLAR	DIGIT	YES
0017	L	3	26	M	RHD	292.75	31.61	VOLAR	DIGIT	NO
0017	R	3	26	M	RHD	203.51	18.69	VOLAR	DIGIT	YES
0017	R	9	26	M	RHD	188.42	41.6	VOLAR	DIGIT	YES
0017	R	12	26	M	RHD	173.81	51.85	VOLAR	DIGIT	YES
0018	R	6	35	F	LHD	416.14	148.09	VOLAR	DIGIT	NO
0018	L	6	35	F	LHD	268.09	49.21	VOLAR	DIGIT	YES
0018	R	9	35	F	LHD	424.45	123.71	VOLAR	DIGIT	NO
0018	R	12	35	F	LHD	180.9	53.81	VOLAR	DIGIT	NO
0018	R	1	35	F	LHD	364.7	47.36	VOLAR	DIGIT	NO
0018	L	1	35	F	LHD	355.44	52.44	VOLAR	DIGIT	YES
0018	R	3	35	F	LHD	357.55	73.37	VOLAR	DIGIT	NO
0018	L	3	35	F	LHD	240.9	40.21	VOLAR	DIGIT	YES
0018	L	9	35	F	LHD	263.52	9.12	VOLAR	DIGIT	YES
0018	L	12	35	F	LHD	177.42	22.87	VOLAR	DIGIT	YES
0019	L	9	55	F	RHD	73.2	32.83	VOLAR	DIGIT	NO
0019	L	12	55	F	RHD	290.34	81.9	VOLAR	DIGIT	NO
0019	L	6	55	F	RHD	278.92	50.49	VOLAR	DIGIT	NO
0019	R	6	55	F	RHD	123.7	44.23	VOLAR	DIGIT	YES
0019	L	1	55	F	RHD	242.15	87.68	VOLAR	DIGIT	NO
0019	R	1	55	F	RHD	132.33	25.8	VOLAR	DIGIT	YES

0019	L	3	55	F	RHD	246.56	76.08	VOLAR	DIGIT	NO
0019	R	3	55	F	RHD	162.71	57.39	VOLAR	DIGIT	YES
0019	R	9	55	F	RHD	295.68	70.13	VOLAR	DIGIT	YES
0019	R	12	55	F	RHD	144.09	90.7	VOLAR	DIGIT	YES

Appendix 8: Summary Elevation Data

ID	Area	Age	Sex	Dom Side	Mean 1 (AU)	Mean 2 (AU)	Mean 3 (AU)	Mean 4 (AU)	Mean 5 (AU)
0020	6	37	M	R	288.52	166.77	299.83	255.92	232.91
0021	6	36	F	R	361.18	16.96	480.41	236.97	80.61
0022	6	27	F	R	300.27	52.01	402.41	142.98	87.55
0023	6	54	F	R	384.01	239.94	397.21	418.07	380.21
0024	6	56	F	R	382.10	114.54	452.65	242.67	271.44
0025	6	38	F	R	134.54	78.26	208.42	265.10	231.38
0026	6	43	M	R	129.71	39.08	168.58	133.80	92.49
0027	6	28	F	R	217.83	21.65	304.63	124.86	42.90
0028	6	57	F	R	246.20	97.34	276.70	191.95	167.86
0029	6	37	F	R	283.09	233.81	227.86	270.63	223.66
0030	6	43	F	R	380.40	242.28	384.80	322.83	309.54
0031	6	53	F	R	277.21	416.07	146.10	251.68	420.68
0032	6	54	F	R	119.40	59.90	455.75	145.65	177.20
0033	6	34	M	L	196.93	29.88	199.60	200.68	141.54
0034	6	45	M	R	312.78	88.92	188.28	221.35	185.21
0035	6	49	F	R	77.54	56.60	72.47	90.18	59.98
0036	6	57	F	R	281.81	58.08	442.35	244.00	138.85
0037	6	59	F	R	181.42	150.98	186.60	175.67	152.69
0038	6	38	F	L	79.02	19.91	196.47	89.64	84.54
0039	6	35	F	L	221.05	41.74	210.08	104.25	63.51