Dermal thickness and echogenicity using DermaScan C high frequency ultrasound: methodology and reliability testing in people with and without primary lymphoedema

Short running title: High frequency ultrasound in primary lymphoedema

J. Jane Phillips[1, 2], Karen J. Reynolds[3], Susan J. Gordon[1]

1 Caring Futures Institute, College of Nursing & Health Sciences, Flinders University, South Australia

2 Murdoch Children's Research Institute, Parkville, Victoria Australia

3 Medical Device Research Institute, College of Science & Engineering, Flinders University

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Abstract

Background: DermaScan C high frequency ultrasound was investigated for image capture and analysis of dermal measures in people with and without primary lymphoedema.

Method: Three repeated images were taken at six sites in people without lymphoedema (NLO). Intra-rater reliability was assessed by taking three sets of measures on images from ten people and inter-session reliability by capturing three images, lifting the probe from the skin in between. Methods were adjusted, repeated images from four sites were taken in people with primary lymphoedema (PLO) and reliability re-assessed.

Results: Intra-rater reliability in NLO and PLO for echogenicity measures were excellent (NLO $ICC_{(3,1)}$: 0.989; PLO 0.997) across all sites and specific to each site (calf: $ICC_{(3,1)}$: 0.989; and foot: $ICC_{(3,1)}$: 0.999 respectively). Inter-session reliability was moderate for NLO ($ICC_{(3,1)}$: 0.727), improving after method modifications for PLO ($ICC_{(3,1)}$: 0.916). When investigated by site, inter-session reliability was good in the foot ($ICC_{(3,1)}$: 0.811) and moderate in the calf ($ICC_{(3,1)}$: 0.616). Mean thickness analysed by site resulted in good inter-session reliability only in the foot ($ICC_{(3,1)}$ 0. 838).

Conclusion: Intra-rater reliability was excellent using the DermaScan C for dermal measures in people with primary lymphoedema. Inter-session reliability required particular attention to method and gain settings.

1 Introduction

Ultrasound has been used to measure in vivo skin thickness and fluid content since the 1980s [1-4]. In particular, the superficial focus of high frequency ultrasound (HFU) (15-22MHz) results in an image of 1-2mm depth where the dermis and epidermis are clearly demarcated and accessible for measurement. The distance from the entrance echo (on the surface of the epidermis) to the dermal-subcutaneous tissue interface measures total skin thickness. This has been used for optimal site selection of dermal injections in diabetes [5] and vaccines in children and adults [6], and enabled skin and subcutaneous tissue assessment following prednisolone treatment[7]. The validity of HFU to measure skin thickness was demonstrated in early investigations using a 15MHz ultrasound and A-mode images by charting the image against an x-ray at the same magnification as the ultrasound [8,9]. As well, HFU (22MHz) measurement of epidermal thickness has been validated in healthy people (25-40 years) compared with confocal microscopy[10].

Skin changes are a feature of lymphoedema. The backlog of lymph that characterises lymphoedema accumulates predominantly in the subcutaneous tissues, but is also evident in the dermis[11]. Skin in lymphoedema progresses from soft skin which easily indents or 'pits' when pressed in early stages, to hard inflexible non-pitting skin, which may have wart-like papillomatosis or keratosis and skin folds in later stage lymphoedema[12-15]. HFU, along with tissue histology, magnetic resonance imaging and spectroscopy have been used to compare and contrast tissue changes of lymphoedema in the dermis [16,17] as well as the subcutaneous tissue[18]. Skin thickness measured by HFU increases with advancing stages or severity of lymphoedema [19]. Furthermore, skin is the interface for treatment of lymphoedema, whether by manual lymph drainage (a form of massage) or compression applied by elasticised garments or a pneumatic sleeve around the limb. Indeed, five days of intensive lymphoedema treatment using manual lymph drainage, pneumatic compression and bandaging has resulted in measurable differences in dermal thickness detected with HFU [20].

High echogenicity, seen on an HFU image as greater brightness, occurs when tissues reflect more HFU waves. Echogenicity varies with tissue density and content. Tissues with greater water content are hypoechoic [21-23]. HFU studies have described a relatively hypoechoic dermis on the affected side in lymphoedema [24-26]. HFU has been used to document and

describe dermal oedema in a range of conditions (noting all chronic oedema may now be regarded as a lymphatic issue [27]). Significantly less echogenicity was found in the dermis of people with chronic oedema (regardless of whether oedema was due to lymphoedema, lipodermatosclerosis or cardiac insufficiency) compared with healthy skin [28]. HFU has also been used to differentiate between lipoedema and lymphoedema with a blinded assessor correctly diagnosing 100% of lymphoedema images (which were clearly hypoechoic) with no false positives [29].

While the presence or absence of pitting adds information regarding the condition of the skin, current objective clinical assessment of lymphoedema severity and change relies on volume measures extrapolated from limb circumference measures and whole limb or segment fluid content using bioimpedance [12]. HFU provides the opportunity for non-invasive, direct, valid and objective measures of dermal thickness and fluid content, yet the equipment is costly and significant training is required. Importantly no standard protocol for HFU measurement is available. Previous studies have used devices with frequencies varying from 10MHz to 20MHz [18,19,25,26,28]. The variation in frequency for image capture has resulted in images of different quality potentially producing non-comparable measures [30]. Other studies have provided little information about the ultrasound settings [19,20,31].

One important setting which has been variously described is time gain compensation (or gain); this operator-dependent control can make small adjustments or amplifications to account for the loss of amplitude that occurs when echoes travel from deeper tissue (attenuation) [32]. These echoes can appear darker than echoes of equal magnitude that are reflected from more superficial structures [33]. The gain compensates for this loss or attenuation of signal and has the effect of increasing both the area and intensity of brightness. Some authors specify keeping the gain setting constant [34,35] whilst more recent studies have adjusted the gain for some images as needed to improve visualisation of the sub-dermal boundary [1,29,36-38]. In particular, this interface of the dermis with the subcutaneous tissue is not as clear as the epidermal-dermal junction [1,29] and adjusting the gain enables the detection of edges. In contrast, for valid dermal fluid content measures, which specifically measure echogenicity, a ‘flat’ or ‘horizontal’ gain, where no compensation in amplification has been made for attenuation is required (P.H. Pedersen, R&D Manager, Cortex Technology, personal communication, May 21, 2019). Hence no one HFU methodology will allow capture of

images to assess both fluid content and skin depth which are both of clinical value to understand the status and change in lymphoedema.

A second requisite for image clarity in ultrasonography is water-based gel, which is used as a coupling medium between the skin and transducer. However gel can alter the distance sound travels depending on its depth, which will alter the echogenicity of images, and then may require gain compensation to produce a clear image. Many specify the standardised application of gel [4,20,29,39], but this is not uniformly followed by all users where gel is generously applied to the skin [36,38].

Once HFU images have been captured, the measurements of dermal thickness and fluid content from the images require standardised methodology. Internal software is available with some equipment and varying description [7,23,40-42] limits method reproduction. Other HFU equipment requires exportation of images to MatLab (a mathematical computing program) [26,38] to perform measures.

High reliability (ICC >0.82) of HFU images using a 20 MHz DermaScan C or DermaLab Combo (both Cortex Technology, Hadsund Denmark) has been reported for dermal thickness measures in post burn scars[43-45] and children[7]. There are few reports about the reliability or reproducibility of dermal thickness measurements in lymphoedema. Dylke et al (2018)[37] reported high inter-image, intra-rater and inter-rater reliability (Cronbach's alpha = 0.995; $ICC_{(3,1)}$ = 0.962 and 0.851; and $ICC_{(2,1)}$ = 0.977) using an 18MHz device to capture images and measure dermal thickness in 38 women with breast lymphoedema secondary to breast cancer. Further, a change in dermal thickness was detected by HFU simultaneously with the development of clinically detected lymphoedema in the arms of women post-surgery for breast cancer, comparing with an unaffected side[25]. However there remains no accepted reliable method for HFU to measure dermal thickness or fluid content in the legs of people with primary lymphoedema.

Measurement error must be minimised to reliably determine the outcome of an intervention and understand the impact of the change[46]. In HFU imaging this requires 1) reliable acquisition of images and 2) reliable analysis of the images. Reliable acquisition of images must occur at the same site at different times with the ultrasound probe being lifted on and off the skin between images (inter-session reliability).

The DermaScan C, a particular type of HFU device (20 MHz; Cortex Technology, Denmark), has been shown to be a valid way of distinguishing changes in water content in the dermis of healthy people (18-65 years) by comparison to MRI[2]. As well it has been shown to be sensitive, detecting significant difference in skin thickness in the healthy between young (2-13yrs) and old (25-40yrs)[47] and between different body sites[34,42] . In a study of healthy skin thickness and echogenicity to assess ageing at different body sites, Gniadecka and Jemec (1998)[23] reported a Spearman correlation coefficient of 0.88 (95% CI: 0.72 – 1.0) between skin thickness and echogenicity.

The aim of this study was to develop and test a standardised HFU image capture method using the DermaScan C. The method was piloted in people without lymphoedema, refined and then tested with people who had primary lower limb lymphoedema. The intra-rater reliability of image measurement for skin thickness and dermal fluid content was investigated, and HFU images captured at different times were investigated for inter-session reliability.

2 Methods

2.1 Ethics

Ethics approval for a study recruiting people with primary lymphoedema across three states was granted by Royal Children's Hospital Melbourne Australia (HREC/16/RCHM/136). Lymphoedema participants gave written informed consent and provided images for assessment in this reliability study.

2.2 Population

Initially people with no lymphoedema (NLO) were recruited from friends and colleagues of the primary researcher to pilot the proposed methodology. After the initial pilot children and adults aged 3- 40 years with primary lymphoedema (PLO) diagnosed by Mercy Health Lymphoedema Services assessment clinic or the Royal Children's Hospital Melbourne were recruited. Exclusion criteria included pregnancy, any skin condition in the assessable area such as dermatitis or eczema; uncontrolled cardiac, embolic or thrombotic conditions; connective tissue conditions such as Marfan's Disease, inflammatory conditions such as rheumatoid arthritis and infective conditions especially history of cellulitis within the past two months.

2.3 Positioning

Participants lay supine on a plinth with one pillow under the head and another under the limb being measured. For posterior limb image capture, they lay prone. .

2.4 High Frequency Ultrasound: equipment, image capture and measurement

The DermaScan C (Cortex Technology Hadsund Denmark) provides 20 MHz B-scanning at 60 x 150-micron resolution, with 13 mm penetration [48].

The head of the transducer (probe) was held perpendicular to the skin [21,41] at a standardised distance from the skin [4,49], producing images where the epidermis is parallel with the membrane within the transducer (Figure 1). Water-based gel (DANE-GEL R1, Rohdé Produits, Gl. Holte, Denmark) was applied within a 'spacer', a slot on the probe head which provides a uniform distance between the HFU transducer and the skin surface. Air bubbles within the gel required removal or re-application of the gel.

An area the size of the transducer head was marked on each image capture site using a body pencil. This ensured repeat placement of the transducer on the same site for multiple image captures. Image capture was performed in a climate-controlled room with the participant always in the same position to avoid discrepancies due to temperature or body position.

One gain setting was consistently used to provide images for fluid content measures (mode one, gain profile 13 in the DermaScan C). To determine the best gain for skin thickness image clarity, three different gain settings were tested: mode one, gain profile 19 and mode two gain profiles 16 and 19, chosen from initial NLO pilot testing and with reference to the manufacturer's manual on image capture [49]. For repeat image capture, the head of the probe was removed from the skin, gel re-applied and the head of the probe replaced on the same site (dorsum of the foot or calf) for three successive sets of four images. This provided images for the inter-session reliability analysis.

Ten images were chosen randomly across sites and people. Measures were taken on all images before being repeated twice more, separated in time by approximately two hours, ensuring no recall of individual images between measurement sessions.

2.4.1 Intra-rater reliability: dermal thickness measures Images with the same gain setting were used for repeated dermal thickness measures at the same site. Lines were established to include both the epidermis and the dermis, along the entrance echo on the surface of the

epidermis and the underside of the dermis (along the interface with the subcutaneous tissue), using automated edge detection software from the DermaScan C (DScan version 3 application software for Windows, advanced configuration), with the threshold set at 20 [49]. The line produced by the edge detection function, determined 'automatically' by default in the DermaScan software, may also be manipulated manually. In some images, a small gap in echogenicity allowed the measurement line to follow the threshold (of echogenicity it was following) within the dermis, which created a loop that deviated in and out of the dermis at the same point and affected the minimum measure (Figure 2). The small gap in echogenicity was 'bridged' manually, to avoid an artificial minimum.

2.4.2 Intra-rater reliability: dermal fluid content measures were determined by using the 'region of interest' (ROI) function in the DermaScan software. This is a standardised shape and size, which may be placed within the dermis to establish the area for assessment. Shape 1, with a standard rectangular area of 6.894712 mm^2, was set with the long boundary along the underside of the epidermis, completely within the dermis extending the length of the field of view (12.1mm). The threshold for detection was set to 30 [2,4,22,47] and on requesting 'segmentation', the area (mm^2) and intensity (pixels and percentage) is produced of both the total ROI and the proportion (segmented area) of the ROI which was represented by 0-30, or fluid.

Measures were exported in a .csv (comma separated values) file and the image showing the ROI, segmented area and dermal thickness measurements was saved as an image in the DermaScan software.

2.4.3 Inter-session reliability Images captured successively at approximately 5 minute intervals were analysed for dermal thickness and fluid content as described above.

2.5 NLO pilot reliability study

Ten people with no lymphoedema (NLO), eight females and two males, aged 17 – 54 years, provided sites on one upper and one lower limb each for ultrasound image capture. Six sites were imaged: dorsum of the foot, posterior mid-calf, posterior mid-thigh and dorsum of the hand, medial anterior forearm, a quarter of the way from wrist to medial epicondyle, and anterior upper arm, a quarter of the way from the medial epicondyle to the anterior edge of the acromion in the anatomical position. Ten images from both upper and lower limbs were randomly chosen for image analysis and consisted of ten images for fluid analysis and ten for thickness measure analysis.

2.6 Statistical Analysis

The intra-class correlation co-efficient (ICC), used to assess intra-rater or test-retest reliability, combines both correlation and agreement [50,51]. SPSS version 25 was used to calculate ICC, denoted $ICC_{(3, 1),}$ using a two-way model (mixed effects), single score and absolute agreement [52].

Both the ICC and the confidence intervals were considered in interpretation of reliability scores. ICC values over 0.90 indicate excellent reliability (repeatability), while 0.75 to 0.90 indicate good reliability and 0.50 to 0.75 moderate reliability [50,51]. Where the confidence interval extended below 0.75, even with a higher value ICC, the reliability was rated as a range indicating the lower level (for example, an ICC of 0.92 with a lower level CI of 0.70 would be rated as good to excellent, not excellent).

Intraclass correlation coefficients (ICC) calculated for intra-rater reliability were good to excellent (CI: 0.836 to 0.998) (Table 1A) while inter-session reliability was lower, being generally moderate to good (Table 2).

2.7 Pilot methodology modifications for PLO measurements

2.7.1 Image capture. To enable more reproducible image capture, vertical lines were drawn on the screen of the monitor to assist visual vertical alignment of the epidermis for each image (checking that the probe is held perpendicular to the skin). A visual check of the screen marks against the image also highlighted discrepancies in the thickness of the gel [1,4]. Site position reproduction accuracy is important as is standardisation of coupling gel thickness [2,22,41,53,54]. Attention was paid to scraping excess gel from the probe surface as small variations in gel were seen to increase the gap between probe membrane and epidermal surface. Secondly, to ensure that the placement of the probe was consistent on the same site, instead of using pen markings on the skin, a small adhesive template was used, just big enough for the head of the probe.

Based on the NLO pilot, mode two gain profile level 16 (2/16) produced most images enabling edge detection of the sub-dermal boundary. However, as it was unclear if the same would be true in participants with PLO, three gains were captured to enable the clearest image to be used for skin thickness measures. Images using the same gains were used for reliability analysis.

A set of four images using four different gain settings (comprising one to enable fluid measures (mode one, gain profile 13) and three for thickness measures: mode one, gain profile 19 and mode two gain profiles 16 and 19,) were taken three times at the same site, lifting the probe and re-applying gel between image capture; this provided data for the inter-session reliability analysis.

2.7.2 Intra-rater reliability: skin thickness measures. Repeated measures of skin thickness (comprising both epidermis and dermis) were taken on ten randomised images from ten lymphoedema (PLO) participants, (as per NLO participants), but with an amended measurement procedure. The central six millimetres of the full image length (12.1mm) was used for edge detection (whereas the full length of the image had been used for those with NLO); this enabled a more consistent edge detection process than when including the top and bottom of the image. Edge detection lines for both the outer surface of the epidermis and the sub-dermal boundary began on the scale line mark at 3.5cm and finished at 9.5cm; the minimum, maximum and mean distance between the two boundary lines were used for analysis.

2.7.3 Intra-rater reliability: dermal fluid content measures. Consistent with skin thickness analysis, the centre of the image was used for LO images to assess fluid content: a small standardised rectangle set by the DermaScan C software (Shape 3: 2.287931mm^2; previously Shape 1 with 6.894712 mm^2 was used in NLO) was chosen as the Region of Interest (ROI). This ROI was set around the centre of the vertical scale (at 6.5), and was aligned with the underside of the epidermis, with the edge of the ROI just brushing the line of brighter intensity of the epidermis, rather than a rectangle that stretched the whole length of the image (as used for those with NLO).

2.7.4 Inter-session reliability

The reliability of image capture at intervals of approximately five minutes with the probe removed in between image capture was assessed as for NLO. The procedure for dermal thickness and fluid content measurement from images was the same used in intra-rater reliability.

2.8 Data Cleaning

To check data entry, a random 15% of all data entered for NLO were double checked with no errors found. For the PLO data, having twice the amount of data entered (measurement of both legs), double data entry into Microsoft Excel (Microsoft, Washington, US) was used to check for errors.

3 Results

Ten people with primary lymphoedema of the lower limb provided ultrasound images. Images using mode one gain profile 13 and mode two gain profile 16 were used for fluid content and thickness measure analysis respectively.

3.1 Intra-rater reliability: Images from five people (two females aged three and thirteen; three males aged eight, eleven and thirty-four) provided measures using images from the foot and calf in affected and unaffected lower limbs. Intra-class correlation coefficients ($ICC_{(3, 1)}$) calculated for intra-rater reliability were good to excellent (95% confidence interval (95% CI): 0.991 to 1.000) (Table 1B).

3.2 Inter-session reliability: Images were provided by four participants (a male aged 16 and three females aged 31 and two aged 40) from the foot and calf in affected lower limbs. Inter-session reliability improved compared to NLO results, with good to excellent ICCs for mean thickness measures (95% CI: 0.809 to 0.980) and fluid measures (total intensity within range) (95% CI: 0.783 to 0.976) (Table 3).

Further analysis was undertaken with the data divided by site. Whilst the analysis of intra-rater reliability in the PLO population shows reliability increased with technique improvements compared with the NLO population (Table 1A), the separation of PLO data into specific sites for fluid analysis (Table 4) resulted in slightly lower reliability, although all higher than ICCs than in the NLO reliability study, and still all excellent.

However, inter-session reliability was not as high. In the NLO population, both fluid and thickness measures generally had good reliability (Table 2), although minimum thickness was moderate (ICC 0.667; CI 95% 0.543 - 0.772), as was the measure of the range representing fluid within a specified area of the image. Technique improvements implemented in the PLO population increased the ICC generally from good to excellent (Table 3). However, when refining the data by site (Table 5), reliability became variable at specific sites in those with

lymphoedema. Measures for fluid were generally good (ICC > 0.765) although again, the measure representing fluid within a specified area ('total intensity within range' represented by 0-30 from the intensity range 0-255) was lower, particularly in the calf (ICC: 0.616, CI: 0.332 to 0.828). Measures from the foot however were good (ICC 0.811; CI: 0.623 to 0.922). The foot also had higher reliability for minimum and mean thickness measures than the calf.

4 Discussion

Future research and clinical use of high frequency ultrasound (HFU) would benefit from using a standardised method to allow comparison of outcomes from intervention studies and consistent description of the tissues of people with and without lymphoedema. The method described here was developed and piloted to reliably measure dermal thickness and fluid content in people with (PLO) and without primary lymphoedema (NLO).

4.1 Device Those using the DermaScan C are advised to develop their own skill by practice, there being "no formal training or education in dermatological echography" [4, p.478]. A training pathway with supporting manuals would assist in the reliable clinical and research use of this device. Key factors for good imaging are the situation and angle of the probe, the gain setting and the gel layer [4]. Attention to these factors, particularly the use of a fixed gain setting, the addition of marks on the screen to monitor the verticality of the probe and the thickness of the gel, improved the reliability of the capture and analysis of HFU images in this study, in assessing and comparing different body sites and different populations.

Images vary in brightness and in clarity of image at depth due to tissue properties (eg. density), device properties (both fixed properties eg. frequency, and variable settings eg. time gain compensation) and procedural differences (eg. thickness of gel, angle of probe on the skin) [4]. The time gain compensation may be adjusted to allow for signals from those deep tissues to be intensified, making up for the attenuation of echoes originating from deeper tissue that occurs with high frequency. Attenuation can make echoes of the same echogenicity appear darker, if originating from deeper tissue [39,55]. Given the time gain compensation may be altered to make images brighter, it is important to standardise this setting when making comparisons across anatomical sites, participants and repeated measures[39]. Images used for assessment of dermal thickness require enough clarity for an edge to be seen, which software can detect, so that measurements can be made. When using HFU diagnostically, the ability to adjust settings (gain) in real-time has allowed for

visualisation of structures not otherwise clearly identifiable. A relatively high gain (mode four gain profile 13, using the DermaScan C) was used for image capture to measure thickness in post-burn scars, which produce hypoechoic images [56]. However, when assessing change in tissue over time, or differences between populations, many factors may affect repeated scanning, with accurate repositioning and the gain settings being key factors. Previous studies utilising HFU vary, with the gain setting being adjusted if thickness measures are being assessed [42,43,53], or standardised if echogenicity is assessed [41]. The importance of using the same specific gain settings for each site in each person in follow up images was stressed by Schou et al [7] in an investigation of changes in skin thickness in children over weeks of prednisolone use. Gel depth can also alter echogenicity of images by altering the distance sound travels; the DermaScan C has addressed this by adding a spacer to the head of the probe. However, where gel is applied non-uniformly and the gain requires adjustment for image clarity as a result, there is room for variation and technical error. Thickness measures rely on echogenicity thresholds for edge detection in the DermaScan C software, therefore any device property or method that affects echogenicity and attenuation including gain and gel depth, would ideally be kept constant to reduce any potential source of error. Images used for fluid assessment rely on the echogenic properties of the tissue so the gain setting of the HFU needs to be constant if tissue properties are to be compared with others [4]. Determining what differences are due to tissue change or true difference between populations, is central to a study of this nature.

4.2 Population differences Standardised settings become problematic where there is marked lower echogenicity due to tissue type, as in lymphoedematous skin [28]. Ideally, the same settings should be used for comparison of dermal thickness between lymphoedematous skin and normal skin, but the low echogenicity of the dermis in lymphoedema means that the gain setting that produces acceptable images in normal dermis are too low to produce images in lymphoedematous dermis that provide clear depiction of the lower boundary of the dermis (and allow thickness measurements to be made). On the other hand, if settings are used that produce acceptable images in lymphoedema, (time gain compensation ‘turned up’), the resultant dermal image in healthy normal skin may be too bright (hyperechoic). Consequently, the lowest gain setting that produced acceptable images in both was sought.

4.3 Body site differences Further, when comparing sites around the body, higher echogenicity has been observed in limb skin than truncal skin in previous studies, prompting the gain to be altered to obtain images depicting clear boundaries [23]. Different echogenicity

was evident according to site during preparations for this current study, with proximal limb segments (upper arm and thigh) generally higher in echogenicity than distal segments. Investigation according to site however, exposed the variability of HFU images in some areas of the body. The reliability of measures from the posterior calf specifically rated far lower when analysed individually than when it was included with measures from all sites. A reason for this may be the variable under-side of the dermis (deep boundary), where "shadows" frequently appear in the calf image possibly representing veins (Figure 3). Generally, foot images were higher in intensity, except in lymphoedematous feet (Figure 4) where images were found to require a higher gain for accurate thickness measures to be taken; the presence of extra fluid in the dermis has been noted to disrupt collagen fibres [41], resulting in less density, lower resolution and clarity in the image [57].

In this study, reliability investigation of the capture and analysis of HFU images, undertaken in people with no lymphoedema, led to improvements in technique which resulted in greater reliability in a dermal study in people with lymphoedema. **Intra-rater reliability** outcomes in the non-lymphoedema population were excellent, but thickness measures had lower confidence limits below 0.9. Ideally excellent scores above 0.90 were sought for clinical measures. Methods amended for both image capture and image analysis following the NLO pilot outcomes, resulted in subsequent images taken from ten PLO participants showing improved intra-rater reliability, achieving 'excellent' for all measures.

However **inter-session reliability** investigated by site showed relatively low ICC for images taken over the calf in the leg, raising questions as to the variability of the tissue itself, or if technical error occurred. Inter-session reliability analysis by site in the NLO population may reveal whether this low reliability extends to both populations or if it was specific to the PLO calf for repeated measures. Thickness measures had lower reliability than fluid measures; possible causes of this lower reliability may include the tissue responding differently to prolonged ultrasound transmission [32] or the lower uniformity of the underside of the calf dermis, resulting in greater variability in echogenicity and the measurement line detecting that border curving inwards frequently.

4.4 Limitations of this study The reliability outcomes of this study are specific to the populations assessed, to one operator and to the DermaScan C; outcomes may not apply to other operators and other high frequency ultrasound devices. The number of images captured using different gains varied between participants, in order to investigate optimal gain settings.

Repeated images for each type of reliability (inter-session in particular) were restricted to a subset of participants (as described in the methods).

5 Conclusion

Known procedural factors in high frequency ultrasound image capture such as gain setting, and operator technique such as gel thickness and probe angle affect dermal measures produced using the DermaScan C. Based on the pilot study in people with no lymphoedema, amended methods improved reliability in a subsequent study in the primary lymphoedema population. Reliability outcomes determining the repeatability of HFU measures in both these populations suggest that the time gain compensation and measurement method for thickness and echogenicity be specified by anatomical site in the method of ultrasound studies and particularly in the use of the DermaScan C. Further analysis of lymphoedematous images showed good to excellent intra-rater reliability in measurement of images and good inter-session reliability for fluid measures.

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Address: J J Phillips
Caring Futures Institute
College of Nursing & Health Sciences
Flinders University
Sturt Road, Bedford Park South Australia 5042
Email: jane.phillips@flinders.edu.au

Table 1 Intra-rater Reliability (Image analysis) [ICC = Intraclass Correlation Coefficient]										
A. NLO Reliability Pilot						**B. PLO Reliability Pilot**				
Measure Single measures	**N**	**ICC**	**Confidence Interval**		**Result**	**N**	**ICC**	**Confidence Interval**		**Result**
			Lower	**Upper**				**Lower**	**Upper**	
Minimum Distance	**10**	**.951**	**.868**	**.986**	**Good - excellent**	10	.997	.992	.999	Excellent
Maximum Distance	**10**	**.940**	**.836**	**.983**	**Good - excellent**	10	.999	.998	1.000	Excellent
Average Distance	**10**	**.962**	**.898**	**.990**	**Good - excellent**	10	1.000	.999	1.000	Excellent
Segmented Area (mm)	**10**	**.991**	**.943**	**.998**	**Excellent**	10	.999	.998	1.000	Excellent
Segmented Area (pixels)	**10**	**.991**	**.943**	**.998**	**Excellent**	10	.999	.998	1.000	Excellent
Total Intensity	**10**	**.993**	**.966**	**.998**	**Excellent**	10	1.000	.999	1.000	Excellent
Total Intensity within range %	**10**	**.989**	**.925**	**.998**	**Excellent**	10	.997	.991	.999	Excellent

Table 1 Lymphoedema Reliability Study: Intra-rater reliability comparing outcomes from amended methodology of primary lymphoedema (PLO) with initial methodology in non-lymphedema (NLO) population

(Reliability rating based on ICC <0.5= Poor; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90)

Table 2 Non-lymphoedema Pilot: Inter-session Reliability [ICC = Intraclass Correlation Coefficient]									
Measure Single measures	**N**	**ICC**	**Confidence Interval**		**Result**	**F Test with True Value 0**			
			Lower	**Upper**		**Value**	***df*1**	***df*2**	**Sig**
Minimum Distance	**59**	**.667**	**.543**	**.772**	**Moderate**	**7.034**	**58**	**116**	**.000**
Maximum Distance	**59**	**.784**	**.692**	**.857**	**Good**	**11.9**	**58**	**116**	**.000**
Average Distance	**59**	**.813**	**.731**	**.877**	**Good**	**13.897**	**58**	**116**	**.000**
Segmented Area (mm)	**57**	**.867**	**.804**	**.914**	**Good**	**20.631**	**57**	**112**	**.000**
Segmented Area (pixels)	**57**	**.867**	**.804**	**.914**	**Good**	**20.631**	**57**	**112**	**.000**
Total Intensity	**57**	**.890**	**.836**	**.930**	**Good**	**25.47**	**57**	**112**	**.000**
Total Intensity within range %	**57**	**.727**	**.614**	**.817**	**Moderate**	**8.889**	**57**	**112**	**.000**

Table 1 Non-lymphoedema Pilot: Inter-session Reliability

Reliability rating based on ICC <0.5= Poor; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90

Table 3. PLO Inter-session reliability [ICC = Intraclass Correlation Coefficient]											
Measure Single measures	**N**	**ICC**	**Confidence Interval**		**Result**	**F Test with True Value 0**				**Mean of 3 means**	**Mean of 3 variances**
			Lower	**Upper**		**Value**	***df*1**	***df*2**	**Sig**		
Minimum Distance	10	.881	.705	.966	Moderate to good	22.915	9	18	.000	.894	.209
Maximum Distance	10	.908	.765	.974	Good - excellent	29.866	9	18	.000	1.469	.210
Average Distance	10	.929	.809	.980	Good - excellent	36.816	9	18	.000	1.177	.214
Segmented Area (mm)	10	.917	.786	.977	Good - excellent	35.953	9	18	.000	1.701	.168
Segmented Area (pixels)	10	.917	.786	.977	Good - excellent	35.953	9	18	.000	2255.633	294843
Total Intensity	10	.892	.693	.970	Moderate to good	35.564	9	18	.000	9.302	11.266
Total Intensity within range %	10	.916	.783	.976	Good - excellent	36.164	9	18	.000	6.466	1.818

Table 3. Primary Lymphoedema (PLO) Reliability Study: Inter-session reliability

Images from two lymphoedema participants utilising two sites (dorsum foot and calf).

(Reliability rating based on ICC <0.5= Poor; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90)

Table 4 PLO Reliability Study: Intra-rater Reliability Image Analysis by site [ICC = Intraclass Correlation Coefficient]						
	Measure Single measures	N	ICC	Confidence Interval		Result
				Lower	Upper	
Segmented Area (mm)	**Calf**	**10**	.992	.977	.998	Excellent
	Foot	**10**	.999	.998	1.000	Excellent
Segmented Area (pixels)	**Calf**	**10**	.992	.977	.998	Excellent
	Foot	**10**	.999	.998	1.000	Excellent
Total Intensity	**Calf**	**10**	.997	.992	.999	Excellent
	Foot	**10**	1.000	1.000	1.000	Excellent
Total Intensity within range %	**Calf**	**10**	.989	.968	.997	Excellent
	Foot	**10**	.999	.996	1.000	Excellent

Table 4 Primary Lymphoedema (PLO) Reliability Study: Intra-rater Reliability (Specific to site)

(Reliability rating based on ICC <0.5= Poor; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90)

Table 5 PLO Reliability Study : Inter-session reliability by site											
Measure Single measures	**Site**	**N**	**ICC**	**Confidence Interval**		**ICC rating**	**F Test with True Value 0**				**Cronbach's Alpha**
				Lower	**Upper**		**Value**	***df*1**	***df*2**	**Sig**	
Minimum Distance	**Calf**	16	.302	.014	.622	Poor	2.380	15	30	.021	.580
	Foot	16	.834	.667	.932	Good	15.606	15	30	.000	.936
Maximum Distance	**Calf**	16	.864	.722	.945	Good	19.814	15	30	.000	.950
	Foot	16	.705	.460	.872	Moderate	7.940	15	30	.000	.874
Average Distance	**Calf**	16	.321	.032	.637	Poor	2.522	15	30	.015	.603
	Foot	16	.838	.673	.934	Good	15.748	15	30	.000	.936
Segmented Area (mm)	**Calf**	16	.767	.551	.903	Good	12.289	15	30	.000	.919
	Foot	16	.887	.765	.955	Good	24.175	15	30	.000	.959
Segmented Area (pixels)	**Calf**	16	.767	.551	.903	Good	12.289	15	30	.000	.919
	Foot	16	.887	.765	.955	Good	24.175	15	30	.000	.959
Total Intensity	**Calf**	16	.765	.540	.902	Good	12.709	15	30	.000	.921
	Foot	16	.872	.737	.949	Good	21.375	15	30	.000	.953
Total Intensity within range %	**Calf**	16	.616	.332	.828	Moderate	5.544	15	30	.000	.820
	Foot	16	.811	.623	.922	Good	13.071	15	30	.000	.923

Table 5 Primary lymphoedema (PLO): Inter-session reliability by site

(Reliability rating based on ICC <0.5= Poor; Moderate: 0.5-0.75; Good 0.75-0.90 and Excellent >0.90)

List of figure captions

Figure 1 DermaScan C image of dermis & epidermis

Figure 2 DermaScan C image showing edge detection line following area of low echogenicity and resultant ‘false’ minimum skin thickness.

Figure 3 DermaScan C image from the leg over the calf muscle, showing dermis with variable sub-dermal border

Figure 4 DermaScan C image from the foot showing oedematous dermis with low echogenicity