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**Lymphatic Research and Biology**

**Title: Investigating Short Term the Effects of Manual Lymphatic Drainage and Compression Garment Therapies on Lymphatic Function using Near-Infrared (NIR) Imaging**

**Running Title: Quantifying the Effects of Lymphatic Drainage**

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**Abstract**

**Background:** Lymphoedema is a chronic peripheral swelling caused by a dysfunction of the lymphatic system, leading to discomfort and loss of upper limb movement. Therapies to treat or manage this swelling have limited evidence, partly due to a paucity in objective lymphatic measures. This study explored the role of near-infrared imaging in evaluating interventions.

**Methods:** Nine healthy volunteers underwent near-infrared fluoroscopy using a micro-dose (50μL, 0.05%w/v) of indocyanine green to quantify lymphatic behaviour before and after a 15 minute period of manual lymph drainage followed by compression garment therapy for a 10 minute period. Images were taken at the forearm and elbow after each intervention. Lymphatic function was defined by the number, size, displacement and speed of lymph packets. The lymph parameters were analysed to assess the effects of the interventions compared to basal values.

**Results:** Baseline parameters of lymph function revealed high variability in the number, size and speed of packets between individuals. Despite this variance, both interventions showed statistically significant improvement (p<0.05) in displacement and speed at the forearm compared to baseline. The velocity of transient lymph packets increased from a median of 6.7mm/sec at baseline to 13.3 mm/sec after manual lymphatic drainage and 10.5 mm/sec after compression garment.

**Conclusion:** Lymphatic activity increased significantly following manual lymphatic drainage, with relative increases being maintained following a short time period of compression garment application. NIR fluoroscopy has the potential to both monitor lymph pathology and provide robust parameters in the assessment of interventions.

**Key words:** Lymphatic System - Lymphoedema- Fluorescence Imaging - Manual Lymphatic Drainage - Compression Garment

1. **Background**

Lymphoedema is a potentially severe and chronic swelling of the limbs triggered by the dysfunction of the lymphatic system. Lymphoedema is caused by the inefficiency of lymphatic vessels to drain fluid and proteins, resulting in an accumulation of lymph, leading to an increase in limb volume 1. This condition leads to a disruption in daily function and adversely affects both gross and fine motor skills, with negative ramifications for work, home, and personal care functions, as well as recreational and social relationships 2. Several causal pathways have been identified in the pathology including primary factors such as genetic abnormalities or secondary external factors such as infection, obesity, 3 injury or [cancer](http://www.nhs.uk/conditions/Cancer/Pages/Introduction.aspx?WT.mc_id=121005) treatment. As an example, a recent study estimates that 21% of women who undergo treatment for breast cancer are diagnosed with lymphoedema 4, with a significant proportion of these developing chronic progressive lymphedema 5. In addition, the presence of lymphoedema in breast cancer survivors leads to both higher medical costs and a higher risk of developing an infection in the limb 6. At present, there are no known curative treatments, either surgical or pharmacological 7. Typically, conservative treatments, such as compression garments and manual lymph drainage are prescribed to manage lymphoedema and promote functional restoration in the limb.

Interventions typically involve one or several components of complex decongestive therapy (CDT) 8, involving a four phase conservative treatment including manual lymphatic drainage (MLD), compression therapy (compression bandages or sleeves), skin care, and lymph-reducing exercises (LREs). However, the evidence to support these interventions remains limited. Indeed, research involving MLD has revealed contradictory findings ranging from no benefit 9 to substantial benefit 10. A recent systematic review found a statistically significant benefit favouring MLD for mild to-moderate lymphoedema patients, with circumference reductions from 12% to 24% (P = 0.05)11. In order to maintain the benefits of MLD physical therapy is typically used in conjunction with compression therapy 12. Compression garments are designed to improve lymphatic uptake and intensify lymphangion pumping13. The efficacy of lymphoedema interventions are typically measured by limb volume using water displacement or circumferential measurements14. This methodology is considered the gold standard as the most accurate for volume, while measurements of arm circumference is used more in clinical practice as it is simpler and less intrusive. However, both methods are prone to error and neither give any information about lymphatic function15.

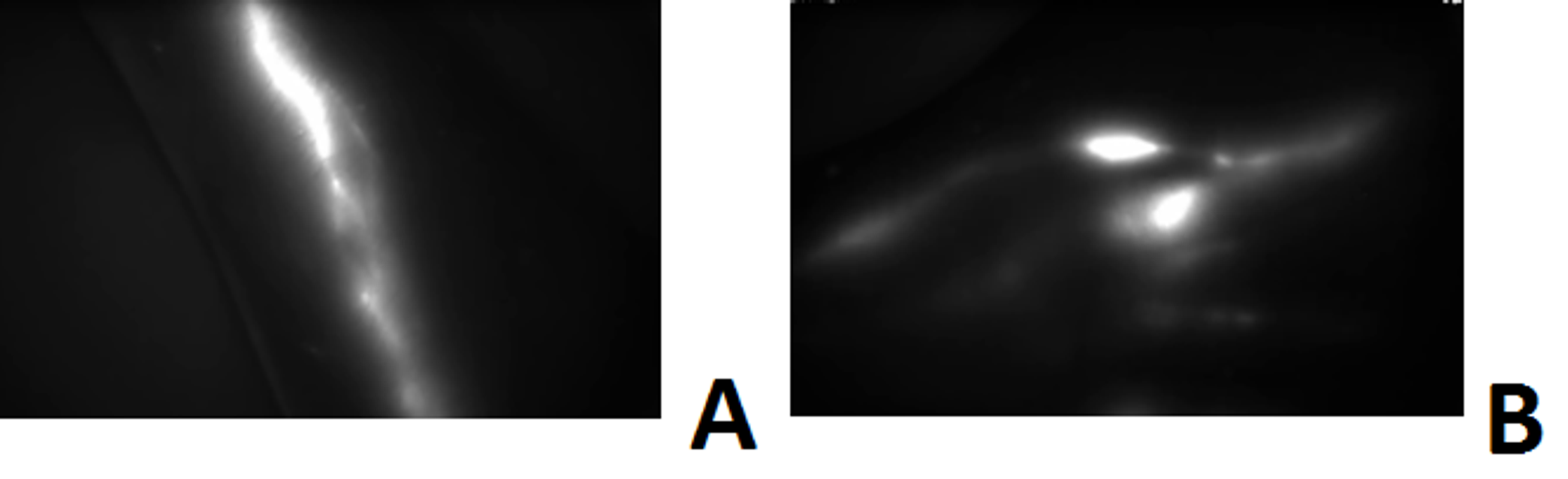
Clinical consensus supports the development of surveillance programmes to provide both early detection and effective management of lymphoedema supported by novel and sensitive diagnostic modalities16. Indeed techniques have been developed to image the lymphatic system and diagnose dysfunction14. One such technique, near-infrared fluorescence lymphatic imaging (NIRFLI) using indocyanine green (ICG), has recently been adopted to visualize the architecture and contractile function of the lymphatic system17. This method has proved both easy and safe to use, and provides relatively high resolution in real time, while remaining relatively economical18. One group has used NIR fluorescence imaging to quantify the effect of MLD on the lymphatic system and found that lymph velocity increased in both healthy participants and lymphoedema patients (p<0.05)14. However, they did not attempt to assess the effects of compression garments (CG) on the lymphatic system, although this is a much more commonly used intervention. The present study was therefore designed to assess the combined effects of MLD and CG and to explore the utility of the NIRFLI technique to assess conservative interventions.

1. **Methods**

The study was a case controlled design using healthy participants. Ethical approval was granted by the University of Southampton ethics committee (REC ID: 19378) prior to data collection. All participants were provided with full details of the study prior to giving their informed consent. Participants with history of thrombosis, liver, kidney or heart disease were excluded, and contraindications for Cardiogreen injections were monitored.

*2.1 NIR fluorescence imaging*

A NIRFLI methodology developed in the host laboratory provided the basis to measure lymphatic function, as detailed in recent papers19, 20. To review briefly, a micro-dose of ICG was tracked by a NIR camera system (Fluobeam® 800, Fluoptics, Grenoble, France), which included a spectrally confined (780nm-centred) laser light source which activates ICG with emitted light at a wavelength of 760nm. The images were recorded by a sensitive charge-coupled device (CCD) camera. Images were collected at both the right forearm and elbow, where active dermal lymphatic vessels were identified for analysis (Figure. 1). A region of interest (ROI) was selected around a well- defined vessel which exhibited contractile activity resulting in transient lymph packets. The camera was located perpendicular to the long axis of the observed vessel in order to obtain high quality images 21. Three videos, each of 5 minutes duration, were captured from the forearm and the elbow at three time phases, namely, baseline (BL) prior to the intervention, after MLD (Post MLD) and after the application of a compression garment (Post CG).



**Figure.1**a) Lymphatic vessels at the forearm. b) Lymphatic vessels at the elbow

* 1. *Manual lymphatic drainage protocol*

MLD was implemented in the right upper limb for 15 minutes, following the Vodder technique22. During therapy participants were positioned supine with the arm elevated. MLD was then administered with nodal massage in the axillary zone using stationary circles. Lymph node massage was performed by a single researcher (CL) with the tips of the fingers, applying slow, deep but gentle pressure. The massage continued from the proximal aspect, working gradually after clearance of each section, to the distal regions23. The pump technique was used in this phase, applying circle-shaped pressure with the entire palm and the proximal phalanges in a transverse direction. Then the scoop technique was performed, in which the palmar surface of the hand moved over the skin, facilitated by movement of the wrist and combined with forearm pronation and supination24. Additional node clearance was made in the anterior aspect at the elbow at the cubital nodes, followed by the pump and scoop technique in the forearm. The final stage of the MLD involved draining the mobilized lymph to the axillary nodes with the pump and scoop technique, performed in a cephalic direction, towards the axilla. Light intensity of MLD was applied throughout the phases to minimise erythema or pain to the participant.

* 1. *Compression garment*

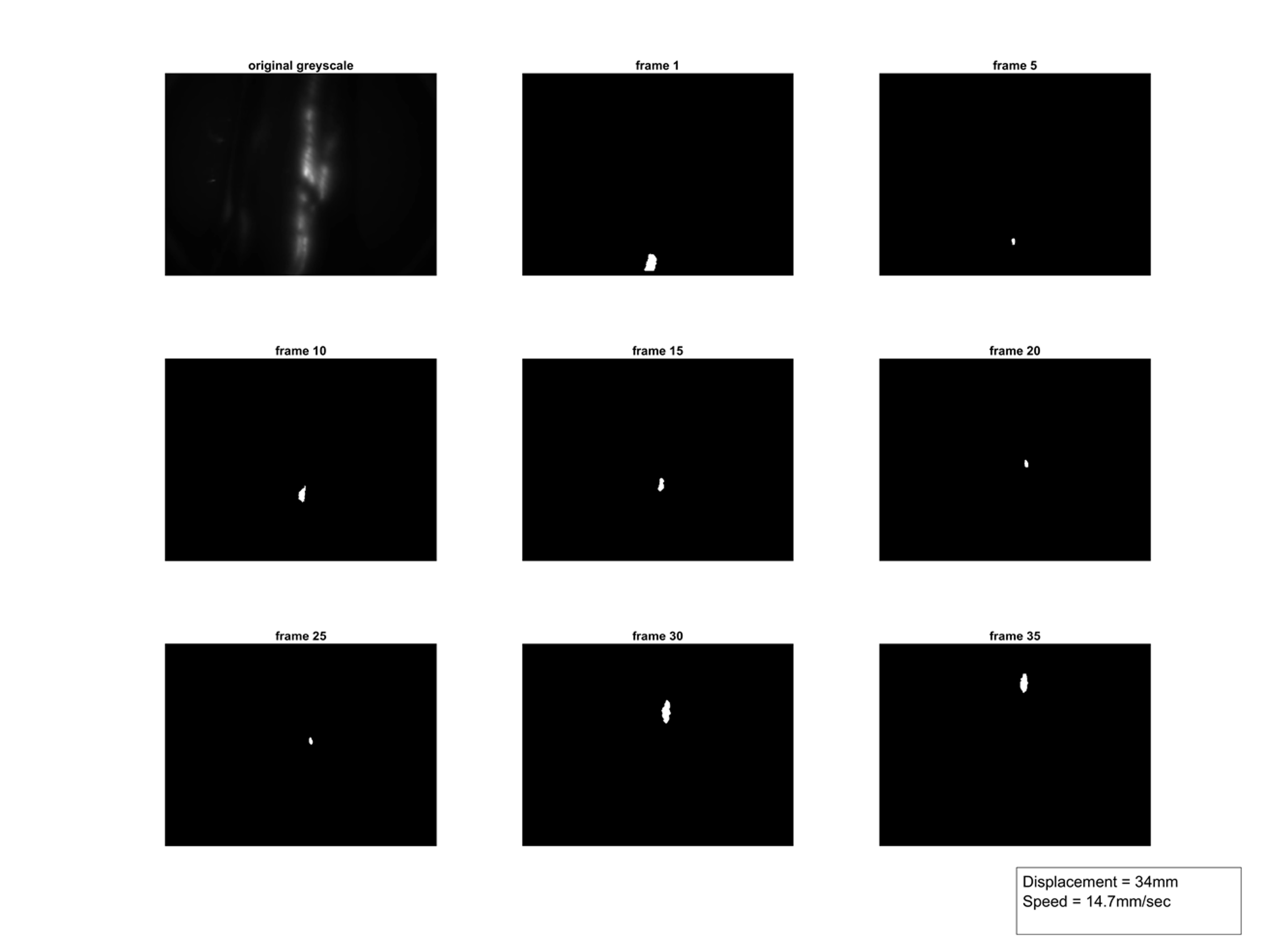
A Juzo class 2 compression arm sleeve was used in the range of 23-32 mmHg, as deemed suitable for moderate levels of lymphoedema. The device was made from a knitted material, which allowed for a component of gradient compression decreasing from distal to proximal locations, whilst avoiding skin damage. The garment was designed to provide a compression effect towards the muscles, promoting lymph drainage during movement. The garment was fitted according to the manufacturer guidelines for sizing using small, medium and large sized sleeve.

* 1. *Test protocols*

During imaging each participant remained in a sitting position with their test limb supported at heart level by a vacuum consolidated pillow. Intradermal injections of 0.1ml of 0.05% w/v ICG were administered by a registered Nurse and delivered equally into the two inter-digital spaces between the thumb and the second finger. A 20 minute period was provided for ICG dissemination, following which an appropriate delineated lymphatic vessel was selected separately at the forearm and elbow to provide reference data for the three data collection periods. Initially, images were acquired for 5 minutes at each site, providing baseline values for lymphatic function at the forearm and elbow. MLD was then performed by a single researcher trained in the technique (CL). Imaging was then repeated for 5 minutes directly after this therapy. Participants were then fitted with a compression garment for a 10 minute period. Upon removing the sleeve, imaging at the wrist and elbow was repeated. The order in which the interventions were delivered was not randomised.

* 1. *Video analysis*

Robust parameters of lymphatic function from the IR imaging sequences were identified using a customised software application (Matlab, Mathworks, USA). The output parameters included the frequency of transient packets, the area of each transient packet, in pixels, the distance travelled or ‘displacement’ of the packets (mm) and the velocity of each packet (mm/sec). To review briefly, the features were established using a droplet morphometry and velocimetry (DMV) tracking approach25. Here image subtraction, binary conversion and centroid tracking provided the basis to identify and measure each transient lymph packet captured within the 5 minute video sequences. Each transient event was analysed to provide x and y coordinates of the packet centroids, whereby, the resultant displacements could be estimated. An Extended Kalman Filter (EKF) was applied to ensure the centroid axes from distinct packets were isolated between the video frames (Figure 2).



**Figure 2.** Track of a single transient lymph packet from participant 5 through time frames at the forearm following MLD intervention.

* 1. *Statistical analysis*

Statistical analysis was performed using MATLAB (Mathworks, USA). Participant lymph behaviour was analysed during each of the three test phases. All data was presented in non-parametric descriptors (medians) accounting for the relatively small sample size and distribution of the data. The area, displacement and speed parameters from each transient packet were averaged over each test phase. Changes across the Baseline, Post MLD and Post-CG phases were assessed using the Friedman test. Comparisons of lymphatic behaviour between individual time points were conducted using Wilcoxon signed-rank test. Differences were considered to be significant at the 5% level (p<0.05).

1. **Results** 
   1. *Participants*

Nine healthy participants (5 males, 4 females) were recruited with a mean age of 36 years (range 22-58 years), mean height of 1.66±0.11m, mean weight of 65.3±10.8kg and their corresponding BMI was 21.15 ±3.4kg/m2.

* 1. *Lymph Function*

Table 1 provides a summary of the parameters reflecting lymph function from the three phases of data collection. It is evident that there was considerable intra-individual variability across sites and the four parameters. As an example, the number of packets identified at the forearm at baseline ranged between 1 and 10 with a median of 4 packets. Considerable variation was also observed in the size (area) of packets at the elbow with a median of 515 pixels and a range from 214 to 1822 pixels. It should be noted that the baseline values at the elbow were generally higher than the corresponding values measured at the forearm for all four parameters.

Despite the inter-participant variability, there were notable increases in lymphatic activity after the interventions. This trend was consistent across the parameters with enhanced median values following MLD and CG. As an example, MLD induced a statistically significant (p< 0.05) increase for displacement and speed at the forearm (Table 1). In addition, there was a 100% increase in the median area of transient packets following MLD at the forearm.

**Table. 1** Summary of parameters

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Forearm** | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Subject Area (Pixels)** | | | | | | | | **Displacement (mm)** | | | | | | **Speed (mm/Sec)** | | | | | | **No. Packets** | | | | | |
|  | | | BL | | Post MLD | | Post CG | BL | | Post MLD | | Post CG | | BL | | Post MLD | | Post CG | | BL | | Post MLD | | Post CG | |
| 1 | | | *1043* | | *833* | | *291* | *14* | | *17* | | *15* | | *7.0* | | *8.4* | | *7.7* | | 4 | | 8 | | 2 | |
| 2 | | | *254* | | *2510* | | *968* | *3* | | *4* | | *17* | | *1.5* | | *20.2* | | *8.5* | | 10 | | 3 | | 11 | |
| 3 | | | *344* | | *950* | | *2399* | *5* | | *27* | | *22* | | *2.5* | | *13.6* | | *11.1* | | 1 | | 3 | | 4 | |
| 4 | | | *572* | | *1059* | | *1023* | *8* | | *21* | | *17* | | *4.0* | | *10.7* | | *8.3* | | 4 | | 11 | | 9 | |
| 5 | | | *466* | | *1141* | | *2976* | *25* | | *23* | | *25* | | *12.3* | | *11.6* | | *12.6* | | 5 | | 10 | | 6 | |
| 6 | | | *1672* | | *609* | | *497* | *13* | | *24* | | *45* | | *6.7* | | *12.2* | | *22.3* | | 4 | | 3 | | 2 | |
| 7 | | | *1208* | | *1718* | | *834* | *20* | | *29* | | *16* | | *9.8* | | *14.7* | | *8.2* | | 4 | | 5 | | 3 | |
| 8 | | | *471* | | *1462* | | *1740* | *7* | | *34* | | *23* | | *3.5* | | *17.2* | | *11.5* | | 7 | | 5 | | 7 | |
| 9 | | | *3988* | | *1432* | | *3846* | *14* | | *27* | | *21* | | *7.1* | | *13.3* | | *10.5* | | 3 | | 8 | | 6 | |
| Median | | | | |  | |  |  | |  | |  | |  | |  | |  | |  | |  | |  | |
|  | | | 572 | | 1141 | | 1023 | 13 | | 27\* | | 21 # | | 6.7\* | | 13.3 # | | 10.5 | | 4 | | 5 | | 6 | |
| **Elbow** | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Subject Area (Pixels)** | | | | | | | | **Displacement (mm)** | | | | | | **Speed (mm/Sec)** | | | | | | **No. Packets** | | | | | |
|  | BL | | Post MLD | | Post CG | | | BL | | Post MLD | | Post CG | | BL | | Post MLD | | Post CG | | BL | | Post MLD | | Post CG | |
| 1 | *214* | | *297* | | *351* | | | *25* | | *21* | | *18* | | *12.5* | | *10.3* | | *9* | | 15 | | 8 | | 9 | |
| 2 | *375* | | *378* | | *498* | | | *19* | | *24* | | *21* | | *9.7* | | *11.9* | | *10.3* | | 8 | | 7 | | 14 | |
| 3 | *1035* | | *2338* | | *1593* | | | *26* | | *25* | | *28* | | *12.9* | | *12.3* | | *13.8* | | 4 | | 8 | | 8 | |
| 4 | *367* | | *1258* | | *2075* | | | *29* | | *27* | | *23* | | *14.4* | | *13.5* | | *11.4* | | 7 | | 6 | | 9 | |
| 5 | *406* | | *336* | | *1477* | | | *18* | | *04* | | *24* | | *9.1* | | *1.8* | | *12.1* | | 6 | | 4 | | 3 | |
| 6 | *515* | | *305* | | *343* | | | *13* | | *18* | | *22* | | *6.3* | | *9.2* | | *11.2* | | 1 | | 1 | | 4 | |
| 7 | *585* | | *840* | | *2205* | | | *13* | | *32* | | *23* | | *6.4* | | *16.2* | | *11.4* | | 3 | | 4 | | 4 | |
| 8 | *538* | | *1219* | | *1701* | | | *22* | | *21* | | *27* | | *10.8* | | *10.5* | | *13.3* | | 7 | | 7 | | 11 | |
| 9 | *1822* | | *1564* | | *3468* | | | *26* | | *24* | | *34* | | *12.8* | | *12.2* | | *17.0* | | 8 | | 14 | | 10 | |
| Median | | |  | |  | | |  | |  | |  | |  | |  | |  | |  | |  | |  | |
|  | 515 | | 840 | | 1593 # † | | | 22 | | 24 | | 23 | | 10.8 | | 11.9 | | 11.4 | | 7 | | 7 | | 9 | |

BL (Baseline). Post MLD (manual lymphatic drainage). Post CG (compression garment).

BL vs. Post MLD. #; p< 0.05 BL vs. Post CG †; p<0.05 Post MLD vs. Post CG

The application of CG generally maintained the trend of increased values following MLD when compared to basal values. In some cases, the post-CG values were significantly higher than baseline, for example, in displacement values from the forearm. In addition, there was an increase in median value of frequency and area of transient packets at the elbow, with the latter being statistically significant (p=0.038).

1. **Discussion**

The present study was designed to provide a quantitative assessment of lymphatic function before and after MLD and compression garment therapy using NIR fluoroscopy. Robust parameters indicative of dermal lymphatic function were extracted using a standardised image processing technique recently developed by the authors20. The results indicated that MLD had a significant effect on lymph activity, with increases in both speed and displacement of transient packets at the forearm. These behavioural changes in lymph function were maintained with the application of a compression garment over a relatively short time period. The methodology was successfully adopted to define suitable dermal lymphatic vessels, which were used to define parameters related to the flow of transient packets. These parameters were sensitive to detect changes pre- and post- physical interventions in a healthy population.

Similar changes in lymph behaviour following MLD have been reported by Tan et al. (2011), with 5 of 6 healthy subjects demonstrating increased lymph velocity after MLD, with 3 individuals revealing statistically significant differences in 1 or both arms. The magnitude of velocity changes reported by Tan and colleagues (range = 7.4-52.7%) was lower than the changes reported in the present study (range = 10-98%). These differences could be explained by the differences in analytical approaches to quantify velocity, with Tan and colleagues assessing between 28-1095 transient packets and the present study choosing an average of 4 packets. Nonetheless the order of magnitude in velocity was very similar between studies, with the present study ranging from 2.5-22.3mm/sec and Tan et al. (2011) ranging from 6.2-12.7mm/sec. In a separate study, velocities of normal lymph flow were reported to range between 5-12 mm/sec in 24 healthy control arms26. This highlights that despite the differences in settings and protocols, NIRFLI is producing parameters which are consistent in distinct populations.

The changes in transient lymph velocity can be related to the principles of MLD, namely that it enhances movement of lymph fluid by stimulating the natural peristaltic contractions of the lymphangions, reducing hydrostatic resistance to lymph flow and increasing velocity11. According to the theory the distance and speed of an individual packet in the lymphatic vessels will be influenced by the force of the lymphangion contraction. Therefore, stronger contractions would propel fluid further with higher ejection fractions27. However, the effects of MLD may not be as pronouced in lymphoedema patients, with their tortuous network of capillaries and reduced number of functional lymph vessels28, increasing dermal back flow and extravascular lymphatic fluid leakage29. The backflow events have also been recently described in lymphatic vessels which have been exposed to a period of uniaxial mechanical compression 20.

The present study also revealed that compared to baseline values the increases in lymphatic activity following MLD were maintained with the application of a compression garment over a 10 minute period. The specific protocol adopted did not permit the evaluation of whether compression garments used in isolation could result in an improvement of lymphatic activity. Indeed, for most parameters there was a small and insignificant reduction in speed and displacement, with an increase in area. However, the present findings do suggest that hosiery can maintain improvement in lymph function following MLD over short time period. Further studies are required employing a randomised cross over design with longitudinal measurements to assess the efficacy of MLD and CG therapies in isolation and when they are combined. It is of note that there is limited evidence that active pneumatic compression devices can increase lymph activity in both healthy control and breast cancer-related lymphedema (BCRL) subjects, when assessed with NIR fluorescence lymphatic imaging30.

The number of participants in the present cohort study limits the generalisability of the results. Indeed, all the participants included were healthy volunteers and thus any extrapolation of the findings to individuals with lymphoedema must proceed with caution. However, it has been shown that similar changes in lymph function have been identified in both healthy and lymphoedema patients14. In addition, the delivery of the interventions was not randomised, with the MLD intervention being applied intially with its inevitable influence on the subsequent compression garment phase. Further research incorporating longitudinal analyses could derive wether MLD used in isolation has similar effects to its use in combination with compression garments. The CG was also applied for a relatively short period of 10 minutes whereas, in practice, individuals may wear CGs continuously, or at least during waking hours. Evaluation of the temporal effects of CG application warrants further examination. It must also be recognised that the infrared imaging system is limited in terms of depth resolution 31 and thus is sensitive to only superficial dermal lymphatic vessels to a depth of a few centimetres. However, it has been suggested that for both healthy individuals or lymphoedema patients with incipient symptoms, the superﬁcial lymphatics may play a greater role in lymph drainage than deep lymphatic vessels18.

The valuable information provided using NIR fluoroscopy could enhance the diagnosis and treatment of lymphatic disorders through the visualization and quantification of changes in lymphatic transport either before or at the early stages of symptoms. It also offers the opportunity to evaluate MLD methods with a real-time feedback, permitting a spatial resolution of the lymphatic network, indicating the affected areas, and the lymphatic vessels that are still functional, allowing for targeted physical therapy32. A further benefit might be to use NIR imaging to stratify which subjects could respond to therapy, decreasing unnecessary financial cost for the patient and the health care system. Targeted elements of CDT could be applied to specific sub-populations at varying stages of lymphoedema.

1. **Conclusion**

The studyquantitatively evaluated lymphatic functional behaviour following a short period of MLD and compression garment interventions using NIR fluoroscopy. There was an increase in lymphatic activity in the majority of the participants, with a statistically significant improvement in transient lymph velocity and displacement. NIR fluoroscopy has the potential to provide an insight for investigating who will respond to lymphoedema treatment or measure whether contractile function is enhanced by the techniques used to manage this complication. However, further randomised studies are required on symptomatic patients over prolonged periods of intervention.

**Ethics approval and consent to participate**

This project has been approved by the University of Southampton ethics committee (REC ID: 19378).

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**Author Disclosure Statement**

There were no conflicts of interest in this study.

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