Improving the Diagnosis and Measurement of Tropical Lymphoedema in Ethiopia

A thesis submitted to Brighton and Sussex Medical School in partial fulfilment of the requirement for the degree of Doctor of Philosophy

Abdi Samuel Temesgen

Brighton and Sussex Medical School

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This work was supervised by

Prof Gail Davey,

Professor of Global Health Epidemiology, Brighton & Sussex Medical School, UK,

Prof Chris Chatwin

Professor in Engineering and Design, University of Sussex, School of Engineering and Informatics, UK

J. Brandon Dixon, Ph.D.

Professor and Woodruff Faculty Fellow, George W. Woodruff School of Mechanical Engineering, USA

Professor Abebaw Fekadu

Head, CDT-Africa (Centre for Innovative Drug Development and Therapeutic Trials for Africa), Addis Ababa University

Dr Kebede Deribe

Wellcome Trust Intermediate Fellow, Brighton & Sussex Medical School, UK,

The project was conducted in collaboration with

University of Sussex, School of Engineering and Informatics, UK

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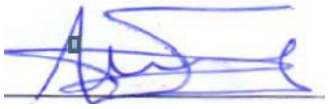
Global good, INTELLECTUAL VENTURES, GH Labs, Seattle, USA

CDT-Africa, Addis Ababa University, Addis Ababa, Ethiopia

Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other University for a degree and does not incorporate any material already submitted for a degree.

Signed 

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Abstract

Background: In the last decade, though significant progress had been made on tropical lymphoedema (mainly podoconiosis research), little has been achieved on the diagnosis and volume measurement aspect, so podoconiosis remains a diagnosis of clinical exclusion. Therefore, the objectives of this PhD thesis were to improve the diagnosis and measurement of podoconiosis in clinical and research settings. The project validated a novel portable three-dimensional infrared imaging device for lymphoedema volume measurement, and a Digital Light Processing technology based on near infrared spectroscopy Analyser (DLP-based NIR spectrometry) and ZI MFIA digital impedence analyser devices for diagnostic purposes.

Methods: To validate the novel portable three-dimensional infrared imaging device, 106 participants were recruited into the study. All the study participants were assessed first using the novel portable three-dimensional infrared imaging system and then by the gold standard water displacement method by two independent raters, with each rater performing duplicate measurements in quick succession.

To test the diagnostic capacity of the DLP based NIR Spectrometry, we recruited patients with lymphoedema due to podoconiosis, lymphoedema due to lymphatic filariasis, lymphoedema due to other causes (other than podoconiosis and lymphatic filariasis) and healthy controls. The ZI MFIA digital impedence analyser study aimed to test whether it can acquire readings from the lower limbs of patients with podoconiosis and those who do not have podoconiosis. Furthermore, the project conducted a community based cross-sectional study to estimate the burden of lymphatic filariasis on chronic lymphoedema patients.

Results: The findings of the novel portable three-dimensional infrared imaging device indicated a strong positive correlation ($r=0.96$; $p<0.001$) and good agreement between the methods. For inter-rater reliability, the ICC was 0.817 (95% CI: 0.738, 0.874) for the water displacement method and 0.9 (95% CI: 0.894, 0.951) for the novel portable three-dimensional infrared imaging device. Intra-rater reliability gave an ICC of 0.969 (95% CI:

0.954, 0.979) for the water displacement method and 0.702 (95% CI: 0.585 to 0.791) for the novel portable three-dimensional infrared imaging device.

The results of the DLP based NIR spectrometry showed the best sensitivity was 0.96, specificity was 0.93 and the area under the curve (AUC) was 0.945. The burden study showed an overall lymphatic filariasis prevalence of 13.3%. Sex and age were independent predictors. The results of the ZI MFIA digital impedence analyser showed that it could collect scans and the scans could be reconstructed in the form of graphs.

Conclusion: The novel portable three-dimensional imaging device can substitute the water displacement technique. The DLP-based NIR spectrometry and the ZI MFIA digital impedance analyser appear to be promising for the diagnosis of tropical lymphoedema, however there are remaining further works.

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Abbreviations/Acronyms

3D	Three-Dimensional
AFS	Acid Fast Stain
BSMS	Brighton and Sussex Medical School
CDC	Centers for Disease Control and Prevention
CDT	Center for Innovative Drug Development & Therapeutic Trials
CI	Confidence intervals
DEC	Diethylcarbamazine
DEC	Diethylcarbamazine Citrate
DLP	Digital Light Processing
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FMOH	Federal Ministry of Health
FTS	Filariasis Test Strip
HCUR	High current Terminal
HLA	Human Leukocyte Antigen
HPOT	High potential terminal
Hx	History
ICC	Interclass correlation coefficient
ICT	Filariasis Immunochromatographic Test
ICT	Immunochromatographic Test

IDA	Ivermectin, Diethylcarbamazine & Albendazole
IEC	International Electro-Technical Commission
LF	Lymphatic Filariasis
LCUR	Low current terminal
LOA	Limits of agreement
LPOT	Low potential terminal
M1	Measurement-1
M2	Measurement -2
M3	Measurement-3
M4	Measurement-4
MDA	Mass Drug Administration
MFIA	Multi Frequency Impedance Analyzer
MFITF	Multi Frequency Impedance Test Fixture
NIR	Near-Infrared
NTD	Neglected Tropical Diseases
OR	Odds Ratios
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
R1	Rater one
R2	Rater two
RGEC	Research Governance & Ethics Committee

ROC	Receiver Operating Characteristic
S1	Scan-1
S2	Scan-2
S3	Scan -3
S4	Scan-4
SG	Savitzky-Golay
SD	Standard deviation
SNV	Standard Normal Variate
SPSS	Statistical Package for Social Sciences
STH	Soil Transmitted Helminthes
TAS	Transmission Assessment Surveys
TMLC	Tape Measures of Limb Circumference
USA	United States of America
WHO	World Health Organization
ZI	Zurich Instruments

Acknowledgements

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Thank you, Jesus,

Chapter one: Introduction

1.1. Overview of lymphoedema

Lymphoedema is the abnormal accumulation of interstitial fluid and fibroadipose tissues resulting from injury, infection, or congenital abnormalities of the lymphatic system (Figure 1.1). Lymphoedema most commonly affects the extremities but can also occur in other areas of the body such as abdomen, genital region, face, and neck. The swelling may range from mild to severe and disfiguring (1-3).

Fig 1.1 Lower exteremity lymphoedema (Picture by Abdi Samuel)

It can be primary or secondary. Primary lymphoedema is present at birth and it is usually due to an inherited condition or birth defect, whereas secondary lymphoedema develops because of damage or dysfunction of the lymphatic system, frequently due to cancer treatment, systemic problems, leprosy, onchocerciasis, podoconiosis or lymphatic filariasis (1, 4-8).

1.2 General Introduction of Podoconiosis and Lymphatic filariasis

Professor Ernest Price first coined the term ‘podoconiosis’ from the Greek words Podos meaning “foot” and Konion meaning “dust”. Due to the mossy appearance of the skin on the feet it is also known as “mossy foot” (9).

Podoconiosis is a form of non-infectious elephantiasis caused by longstanding exposure to red clay soil of volcanic origin (10, 11) (Figure 1.2). In Afaan Oromoo Podoconiosis is called as Tonnoo.

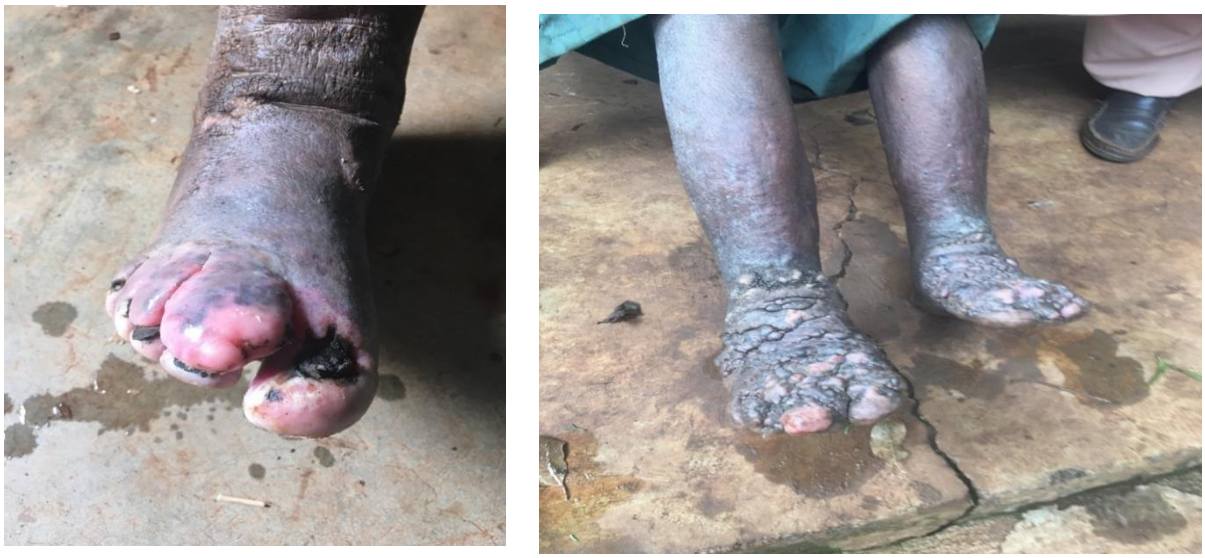


Figure 1.2 Patients with podoconiosis in Western Ethiopia, Oromia Regional State, East Wollega Zone, Wayu Tuka District (Pictures by Abdi Samuel)

Lymphatic filariasis is a major public health problem in tropical regions. It is a disease caused by filarial worms living in the human lymphatic system. The common causative agents are *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* (12-15). *W. bancrofti* is the most well-documented and widespread cause of lymphatic filariasis and makes up 90% of the disease burden (16). These parasites lodge in the lymphatic system and live for four to six years, producing millions of minute larvae that circulate in the blood. Large numbers are present in the lymphatics of the lower extremities (inguinal & obturator groups), upper extremities (axillary lymph nodes), and male genitalia (epididymis, spermatic cord, testicle) (17-21).

1.3 Pathogenesis and Genetics of Podoconiosis

The exact pathogenesis of podoconiosis is still not well understood, however it is generally thought to involve the absorption of mineral particles through the skin of the foot which are taken up into macrophages in the lower limb lymphatics and are thought to induce an inflammatory response in the lymphatic vessels, leading to lymphatic obstruction and the clinical consequences of gross lower leg lymphoedema, which progresses into elephantiasis (8, 12-14) (Figure 1.3).

In a study conducted on patients and non-patients living barefoot on red clay soil, colloid-sized particles of elements common in irritant clays (aluminium, silicon, magnesium, and iron) have been demonstrated in the lower limb lymph node macrophages of both groups. Some soil types such as clay and silt, fine textured and sticky soils are reported to be associated with a higher prevalence of podoconiosis (22-24).

Figure 1.3 A farmer working barefoot in a podoconiosis endemic area in East Gojam zone of north Ethiopia, (Picture taken by Yordanos B. Molla)

Another study conducted by Price showed a rapid fall of podoconiosis prevalence outside red soil areas. The prevalence of podoconiosis decreased from 6.92% to 2.96% at the edge of the red soil, and further decreased to 0.79% and 0.98%, 25 kilometres away from the edge in two different directions (25).

As to the genetics, a strong genetic element has been identified, with the disease found to cluster in families (10, 26). Different expert observations, epidemiological studies, and pedigree analyses reported familial aggregation of podoconiosis. Observations in Ethiopia and Rwanda showed that several households have more than one affected member (27, 28). Other studies in north and west Ethiopia showed one-third to half of patients have other affected close relatives (29).

Among many families, though they have similar exposure to irritant soil, not all family members will develop podoconiosis during their lifetime. Price performed segregation analyses on 80 families with more than one affected child, having adjusted appropriately for increased likelihood of a family with more than one affected individual being included. He calculated the proportion of siblings affected as approximately 0.2, with 95% confidence limits including 0.25, suggesting an autosomal recessive trait (27).

Another 59 multi-generational families were studied, and sibling recurrence risk calculated to be 5.07, and heritability 0.629. Segregation analysis showed the most parsimonious model to be that of an autosomal co-dominant major gene [18].

A case control study by Tekola *et al*. showed that genetic variants in the human leukocyte antigen (HLA) locus of chromosome 6 confer susceptibility to podoconiosis. Specifically, single nucleotide polymorphisms in or near the class II HLA genes namely *HLA-DQA1*, *HLA-DRB1*, and *HLA-DQB1* were found to be at significantly higher frequency among podoconiosis cases than controls. The study also suggested that podoconiosis is a T-cell mediated inflammatory condition (30).

Lymphatic filariasis major mode of transmission is by the bite of female mosquitoes of the genera Culex, Aedes, Anopheles and Mansonia (31) (Figure 1.4).

Figure 1.4 Anopheles mosquito that transmits Lymphatic filariasis. credit: CDC

The parasite requires a human being as a definitive host and mosquitoes as an intermediate host. Infective larvae deposited onto human skin during the mosquito's blood meal enter through the mosquito bite puncture wound or local abrasions. In the human being, the parasites pass to the lymphatic system and undergo further moults and become adult male and female worms (Figure 1.5). Adult female worms produce thousands of sheathed microfilariae per day which are normally found in the peripheral circulation in the evening (nocturnal periodicity) (Figure 1.6). The adults are long thread-like worms which measure 2 cm – 120 cm (4 – 10 µm wide) (31).

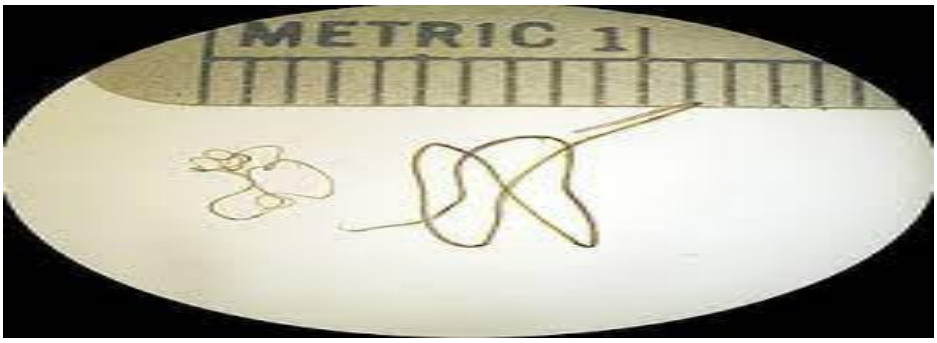


Figure 1.5 Adult worm of *W. bancrofti*. Credit: CDC

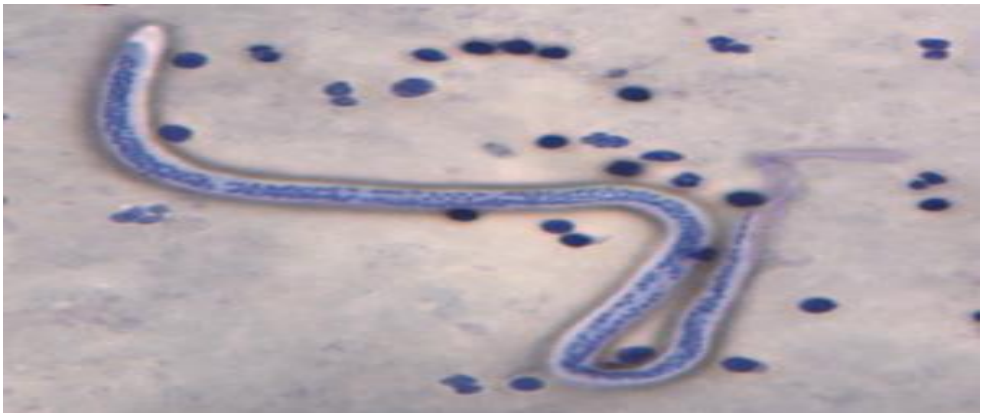


Figure 1.6 Microfilariae of *W. bancrofti*. Credit: CDC

The cycle in the mosquito starts when microfilariae are ingested during a blood meal from an infected person. These then penetrate the mosquito stomach wall, enter the body cavity (hemocoel), migrate to the insect's flight muscles for growth and after 2 moults, the L_3 (the final developmental stage of *W. bancrofti*, which is infective to the human being) migrate through the head, to reach the proboscis of the mosquito (31) (Figure 1.7).

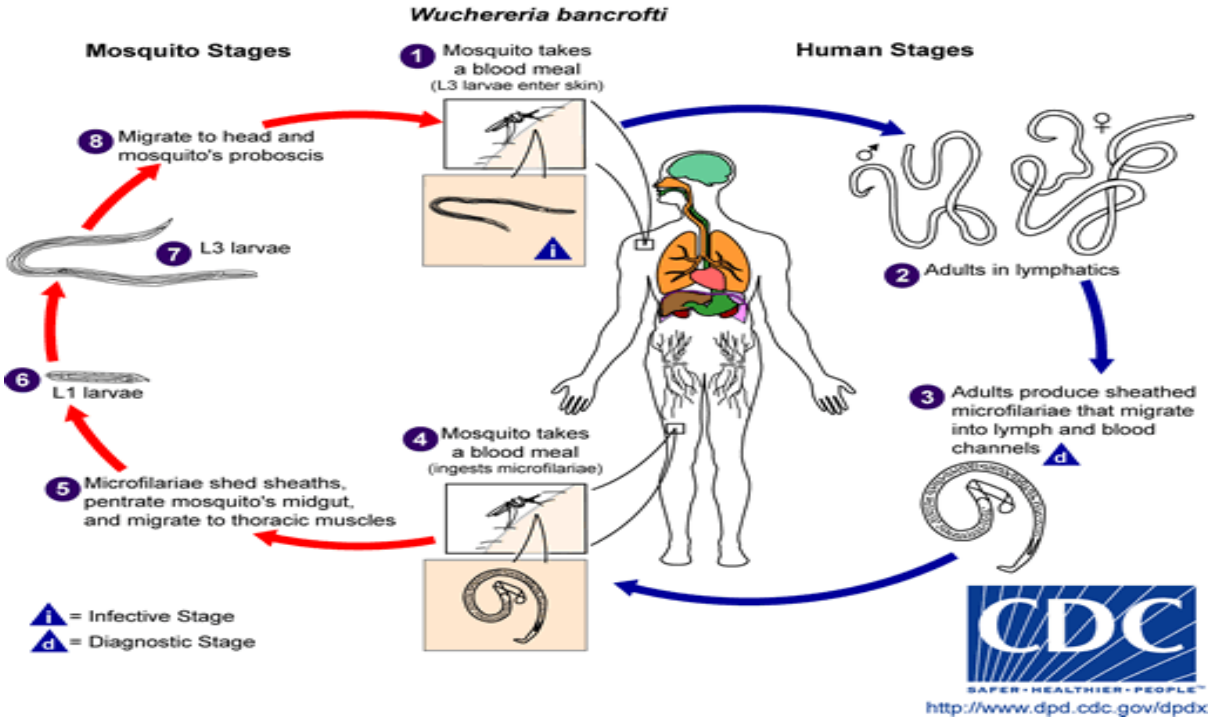


Figure 1. 7 Life cycle of W. bancrofti

1.4 Epidemiology of podoconiosis and lymphatic filariasis

Globally, podoconiosis has been reported in 25 countries in tropical Africa, Southeast Asia, and Latin America (32), with an estimated 4 million cases (11, 33). It is found in several countries in tropical Africa (in Ethiopia, Rwanda, Burundi, Cape Verde, Guinea, Cameron, Sudan, Tanzania, and Uganda), where red clay soils coexist with high altitude, high seasonal rainfall, and low income (28, 34-41). It typically affects agrarian barefoot workers in the tropics in areas located over 1000 m above sea level with an annual rainfall of more than 1000 mm (22).

Cases have also been reported from the Central American highlands in Mexico and Guatemala south to Ecuador and Brazil in South America. It has also been reported from northwest India, Sri Lanka and Indonesia. Podoconiosis is no longer found currently in North

Africa (Algeria, Tunisia, Morocco, and the Canary Islands) or Europe after footwear has become standard, although it was common in the past (9, 32, 42) (Figure 1.8).

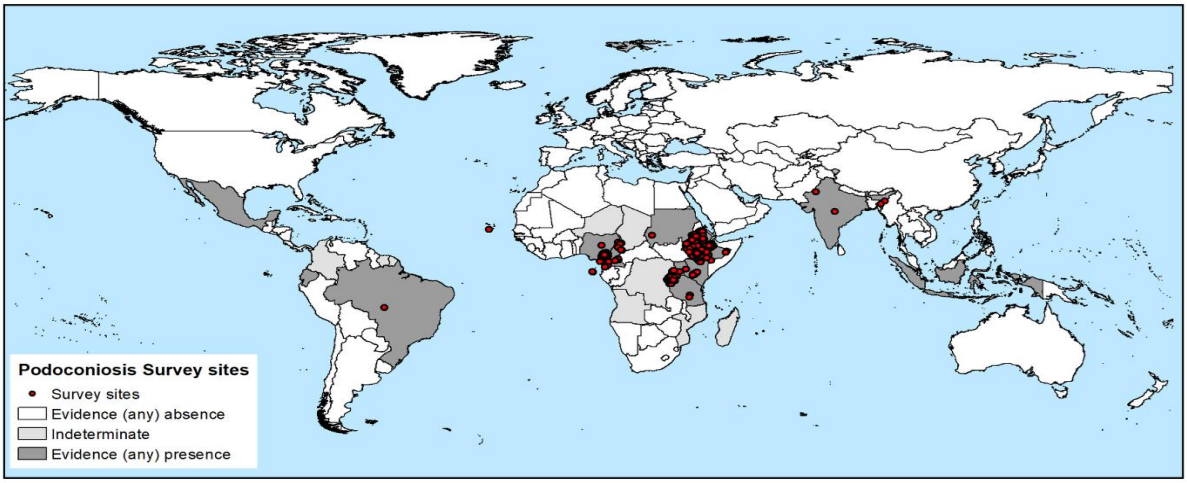


Figure 1.8 Global Distribution of podoconiosis (Deribe K, et.al., 2019)

W. bancrofti was once widespread in tropical regions globally but control measures have reduced its geographic range. It is currently endemic throughout Sub-Saharan Africa, Madagascar, several Western Pacific Island nations and territories and parts of the Caribbean. Bancroftian filariasis also occurs sporadically in South America, India, and Southeast Asia (43).

Brugia spp. associated with LF are more geographically limited and occur only in Southeast Asia. Like *W. bancrofti*, control measures have reduced the occurrence and endemic range considerably. *Brugia timori* is restricted to the Lesser Sunda Islands of Indonesia (43).

An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; of these, almost 25 million men have genital disease (most commonly hydrocoele) and almost 15 million, mostly women, have lymhoedema or elephantiasis of the leg. A recent estimation of the impact of MDA during the past 13 years suggests >96.71 million cases were prevented or cured, yet as many as 36 million cases of hydrocoele and lymphoedema remain. Of the total population requiring preventative

chemotherapy, 57% live in the South-East Asia Region (9 countries) and 37% live in the African Region (35 countries) (44).

1.5 Podoconiosis and Lymphatic filariasis in Ethiopia

Ethiopia is located in East Africa. Administratively, the country is divided into 11 regions, Afar, Amhara, Benishangul-Gumuz, Gambella, Harari, Oromia, Sidama, Somali, South West Ethiopia Peoples', Southern Nations, Nationalities and Peoples (SNNP) and Tigray, and two municipal administrations, Addis Ababa and Dire Dawa.

The current population is about 112.1 million with growth rate of 2.6% per year. The male to female ratio is 100.12:100. The age distribution data show the population < 5 years makes up 16% of the whole, school-age children (5 – 14 years) 31.2%, those of working age (15–65 years) 49%, and those over 65, 4%. Women of reproductive age (15–49 years) make up 23% of the population, the total fertility rate is 4.6 per woman, and the crude birth rate 32 per 1000 (45).

Nearly 80% of the population of Ethiopia lives in rural areas, mainly depending on subsistence agriculture (45). Agriculture accounts for 39% of the gross domestic product, and farmers depend on rainfed agriculture for their livelihood. Ethiopia reported two-digit (10.8%) annual economic growth in recent years; however, it is a low-income country (gross national income, ≤ US$ 1045 in 2020), with 23.5% of its population living below the poverty line (US$ 1.9 per day)(46).

Ethiopia is the country that bears the highest burden of podoconiosis globally, with an estimated 35 million people at risk and 1.5 million cases across 345 districts. The national average prevalence was 4% with the highest prevalence in SNNPR (8.3%) followed by Oromia (4%) and Amhara (3.9%) regional states (47-49) (Figure 1.9).

The disease has been recognised in Ethiopia since 1936 and is endemic in many parts of the country where red soil is found (50-53). In Ethiopia, the soil responsible for the disease is estimated to cover 24% of the surface area on which an estimated 43.8% of the population lives (47).

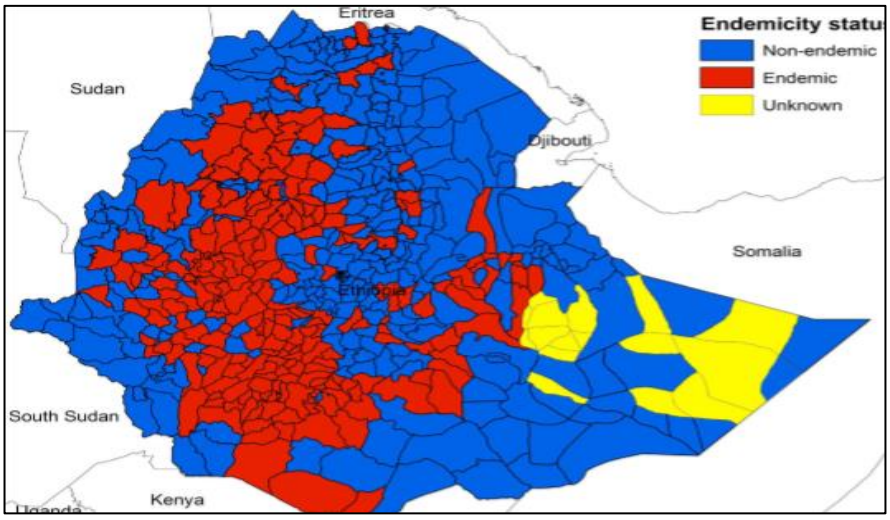


Figure 1.9 Distribution of podoconiosis in Ethiopia.(Deribe K., et.al,2015)

In Ethiopia, LF is endemic in 34 districts. The overall prevalence rate in these districts was 3.7%, but high geographical clustering and variation in prevalence (ranging from 0% to more than 50%) was found (44). The estimated total population at risk of LF in the 70 endemic districts in Ethiopia is 5.9 million. Among the 70 LF-endemic districts, 29 are co-endemic with podoconiosis, 45 are co-endemic with onchocerciasis and 69 are co-endemic with soil transmitted helminths (STH) (54) (Figure1.10).

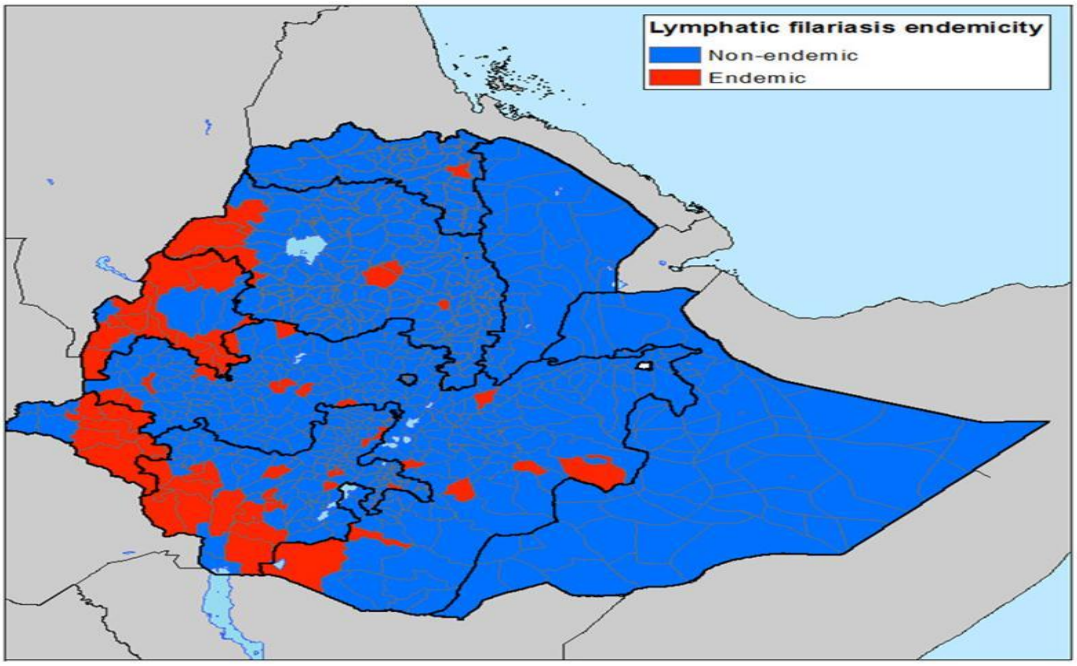


Figure 1.10 Distribution of lymphatic filariasis in Ethiopia. (Mengistu B., et.al., 2017)

1.6 Social and Economic consequences

Due to its symptomatology, podoconiosis has a significant social and economic impact on affected populations. Social stigma towards people with podoconiosis is pronounced, leading to exclusion from school, religious and community gatherings, and a complete bar on marriage into non-affected families. Even health professionals stigmatize patients with podoconiosis. The stigma is not only confined to the patients; it also extends to family members and relatives (49, 55-57).

A study revealed that up to 64% of affected individuals are within the most economically active age groups (29, 58) and it is estimated that podoconiosis patients lose 45% of total working days per year with significant associated loss of income (29). There were an estimated 230.4 million productive days lost per year due to podoconiosis (58, 59).

A study estimated that in 2017 the annual total economic burden due to podoconiosis in Ethiopia was US$213.2 million. The cost related to patients seeking treatment without

access to morbidity-management services were US$17.2 million (8.1%), the cost of patients accessing morbidity-management services was US$1.8 million (0.9%) and the productivity cost associated with podoconiosis morbidity was US$194.1 million (91.1%). The average weighted annual economic burden per podoconiosis case was estimated to be US$136.9, the majority of which resulted from lost productivity (US$126.2). On average, the cost related to patients seeking treatment without access to morbidity-management services was US$11.4 per case annually. They estimated that the average annual economic cost of morbidity management and the associated cost per podoconiosis case treated was US$73.1 (60).

LF is one of the most debilitating and disfiguring diseases. Disfigurement of the limbs and genitals leads to stigma, anxiety, ostracization, psychological trauma, and sexual disability. It impedes mobility, travel, educational opportunities, employment opportunities and marriage prospects (61-64).

1.7 Clinical Features of Podoconiosis and Lymphatic filariasis

Podoconiosis affects the lower limbs, and the swelling is usually limited to below the knees (9, 29, 40, 47, 65). Unilateral leg swelling, thickening of the skin and mossy changes are key early signs (66). Leg swelling is a transient oedema of the lower leg specially the foot which increases following long working days and disappears in the morning after overnight rest. At this stage the oedema can be pitting. The unilateral foot oedema can be associated with pitting on the anterior foot pad and splaying of the forefoot, widening of the forefoot with separation of the toe, particularly between the first and the second toes (67).

Thickening of the skin - The skin over the anterior and dorsum one third of the foot becomes lichenified and thickening can occur which renders the skin, particularly overlying the first toe web space, stiff and unable to be pinched. The increased skin markings, usually longitudinal, may be evident and exaggerated by squeezing together the toes; it is significantly visible between the first and second toes (Figure 1.11).

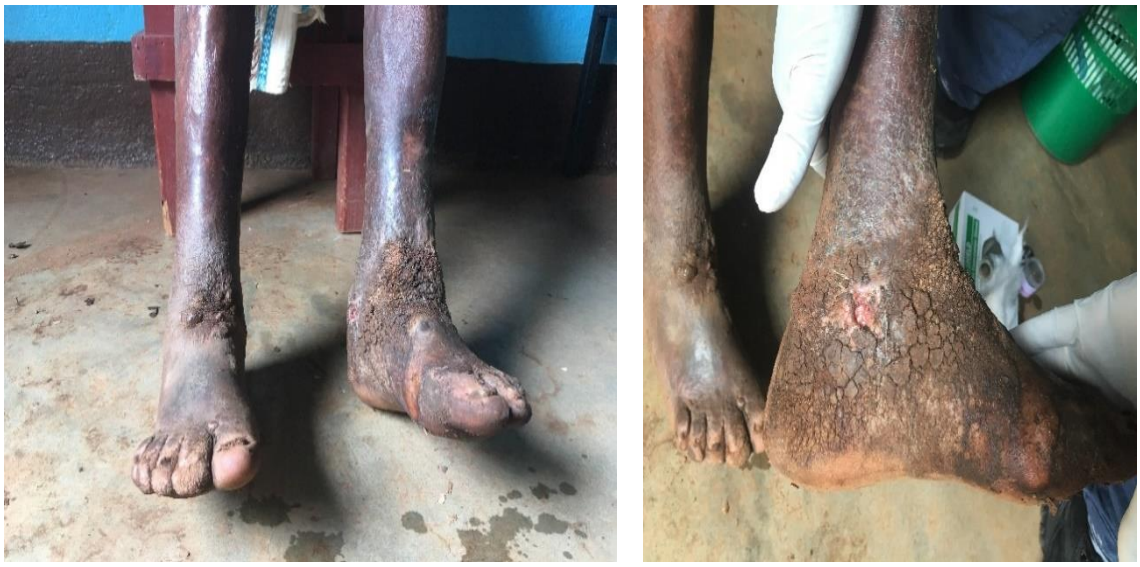


Figure 1.11 Lichenified and thickened skin in patients with podoconiosis (picture by Abdi Samuel).

Mossy changes - warty and papillomatous growths with a rough surface are usually seen on the foot involving the dorsum of foot in the anterior one third and the sole of foot in a ‘slipper’ distribution mainly on lateral side of sole and the heel of foot. With time, the swelling becomes soft and pitting (‘water-bag type’) or nodular and fibrotic (‘leathery type’) (68, 69). Late-stage disease is characterized by fusion of the inter-digital spaces and ankylosis of the inter-phalangeal and ankle joints (49, 66). Researchers developed and validated a clinical staging system for podoconiosis which consists of five stages (70) (Figure 1.12).

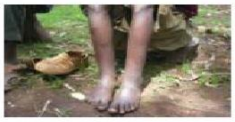
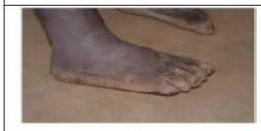
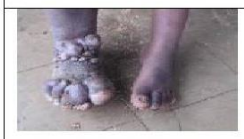
	Stage & Clinical signs		Stage & Clinical signs
	**Stage 1.** Swelling reversible overnight. The swelling is not present when the patient first gets up in the morning.		**Stage 4.** Above-knee swelling that is not completely reversible overnight; knobs / bumps present at any location.
	**Stage 2.** Below-knee swelling that is not completely reversible overnight; if present, knobs / bumps are below the ankle only.		**Stage 5.** Joint fixation; swelling at any place in the foot or leg. The ankle or toe joints become fixed and difficult to flex or dorsiflex. This may be accompanied by apparent shortening of the toes.
	**Stage 3.** Below-knee swelling that is not completely reversible overnight; knobs / bumps present above the ankle.		

Figure 1.12 Clinical staging of podoconiosis, Adapted from

The most prominent clinical feature of lymphatic filariasis is the development of severe lymphoedema of the limbs (“elephantiasis”) and occasionally genitalia (hydrocele) due to dysfunction of lymphatic vessels. Affected limbs become grossly swollen; the skin may become thick and pitted, and secondary infection are frequent due to lymphatic dysfunction. Scrotal hydrocele is also seen in some infected males. Lymphangitis, lymphadenopathy, and eosinophilia may accompany infection in the early stages (71-73) (Figure 1.13).

Figure 1. 13 patients with lymphatic filarisis with lymhoedema (left) and hydocele (right)

1.8 Prevention and Treatment

Podoconiosis is a preventable disease. Primary prevention consists of avoiding or minimizing exposure to irritant soils by wearing shoes or boots and by covering floor surfaces inside traditional huts. Simple lymphoedema management with foot hygiene using soap and antiseptic, bandaging, elevation of swollen legs during the night, and wearing protective shoes consistently make up the strategy to control podoconiosis as a secondary prevention method (74-77) (Figure 1.14).

Figure 1.14 Skin care, washing (foot hygiene) credit; Abdi Samuel

Daily use of socks and shoes, elevation and compression of the affected leg, and in selected cases, removal of prominent nodules are tertiary prevention mechanisms for podoconiosis (Figure 1.15)

Figure 1.15 Use of elastic bandage. Credit: Fasil Tekola

More radical surgery is no longer recommended since patients who are unable to scrupulously avoid contact with soil experience recurrent swelling which is more painful than the original disease because of scarring. Social rehabilitation is vital, and includes training treated patients in skills that enable them to generate income without contact with irritant soil (75).

Another important aspect of the treatment programme is educational and social support, in the form of monthly meetings at which messages on prevention and treatment are given, and social and spiritual support are offered. Generally, the treatment helps eliminate the bad odour, prevent, and heal entry wounds, helps patients become more self-confident, reduce the size of lymphedema, prevent disability, prevent economic loss, and makes the foot fit for a shoe (74-77).

For lymphatic filariasis, WHO's strategy is based on stopping the spread of infection through large-scale annual treatment of all eligible people in an area or region where infection is present; and alleviating the suffering caused by lymphatic filariasis through provision of the recommended basic package of care (61).

Large-scale treatment

The WHO recommended preventive chemotherapy strategy for lymphatic filariasis elimination is mass drug administration (MDA). MDA involves administering an annual dose of medicines to the entire at-risk population. The medicines used have a limited effect on adult parasites but effectively reduce the density of microfilariae in the bloodstream and prevent the spread of parasites to mosquitoes.

WHO recommended MDA regimens are albendazole (400 mg) alone twice per year for areas co-endemic with loiasis, ivermectin (200 mcg/kg) with albendazole (400 mg) in countries with onchocerciasis, diethylcarbamazine citrate (DEC) (6 mg/kg) and albendazole (400 mg) in countries without Onchocerciasis. Single dose of a triple-drug combination comprised of ivermectin, diethylcarbamazine and albendazole (IDA) is dramatically superior to widely used two-drug combinations for clearing larval filarial parasites from the blood of infected persons and it has the potential to accelerate LF elimination in many endemic countries (61).

Morbidity management

Morbidity management and disability prevention are vital for improving public health and are essential services that should be provided by the health care system to ensure sustainability. Surgery can alleviate most cases of hydrocele (78). Clinical severity and progression of the disease, including acute inflammatory episodes, can be reduced, and prevented with simple measures of hygiene, skin care, exercises, and elevation of affected limbs. People with lymphoedema must have access to continuing care throughout their lives, both to manage the disease and to prevent progression to more advanced stages (79-81).

Vector control

Mosquito control is a supplemental strategy supported by WHO. It is used to reduce transmission of lymphatic filariasis and other mosquito-borne infections. Depending on the

parasite-vector species, measures such as insecticide-treated nets, indoor residual spraying or personal protection measures may help protect people from infection (61).

1.9 Ethiopian Ministry of Health Strategy and Current Initiative in the Control of NTDs

The Government of Ethiopia formulated a comprehensive health policy in 1993 to increase access to promotive, preventive, essential, curative and rehabilitative health services for all segments of the population through decentralized, integrated health-care delivery systems. The health services are structured into primary, secondary and tertiary levels of care. The primary care unit comprises five satellite health posts, a health centre and a primary hospital in rural areas and a health centre in urban settings.

A health centre provides both preventive and curative services. It serves as a referral centre and provides practical training for health extension workers. It coordinates and supervises all health activities, in the health posts in its catchment area. Primary hospitals, with 25–50 beds, offer inpatient and ambulatory services to about 100 000 people, including emergency surgery. They are referral centres for health centres in their catchment area. General hospitals serve as referral centres for primary hospitals (rural areas) and health centres (in urban settings) and are expected to serve about 1.5 million people. Specialized tertiary referral and teaching hospitals have catchment populations of 3–5 million(82). According to the Health Extension Programme optimization road map(83), health posts are either comprehensive or basic. Comprehensive health posts are staffed by health extension workers, nurses, midwives and other health professionals, while basic health posts are staffed by health extension workers and provide various preventive and health promotion services, in addition to treating conditions such as malaria, pneumonia, scabies, trachoma and mild illnesses. Both types of health post refer clients to health centres for higher-level care. Health extension workers are supported by volunteer community workers, known as the “health development army”, to reach every household. Their interventions comprise 16 health packages, organized into three themes: family health, disease prevention and hygiene and environmental sanitation. Health education and communication is a cross-cutting theme.

Because of the burden of NTDs in Ethiopia, the Ministry of Health developed national NTD strategic plans for the periods 2013–2015 and 2016–2020, prioritizing eight diseases. NTDs were also included in the country's Health Sector Transformation Plans (HSTP I and II).

The Ethiopian Ministry of Health launched its first national NTD Strategic Plan IN 2013 and which defined the burden of NTDs, initiated a national 2013 – 2015 programme and established structures at various levels. The second Strategic Plan (2016-2020) consolidated the NTD programme and scaled up interventions nationally and locally. Operationalization of the Strategic Plan was assessed critically and evaluated at annual review meetings and in disease specific assessments, routine reports and a final evaluation. The reviews showed significant progress in improving people's quality of life and livelihoods through controlling NTDs.

The third National NTD Strategic Plan covers 2021–2025. During this period, the NTD programme will build on the successes and lessons from implementation of the first and second Strategic Plans to consolidate the gains achieved so far in the fight against NTDs. Its aim is to build a sustainable, resilient, high-quality, equitable NTD programme that is fully integrated and mainstreamed into the national health system. The plan is based on in-depth evaluation of the second national Strategic Plan and also the HSTP II, socioeconomic direction, the global NTD road map, ESPEN Strategy Framework 2021-2025 and other global and national commitments to realizing the SDGs.

The third Strategic Plan is intended to govern the prevention, control and elimination of NTDs with new developments in policy and programming and details of the NTD interventions that will be implemented over a 5 year period. It was developed in consultation with the regional health bureaus, sector ministries and agencies, development partners, funding organizations, academia, research institutions and civil society organizations. A core team consisting of experts in the field coordinated the process. The Strategic Plan is intended to be used by the government to guide planning and implementation of NTD programmes, facilitate alignment among stakeholders and accelerate progress towards the prevention, control, elimination and eradication of NTDs. It provides a harmonized tool for all partners working on NTDs to ensure joint support to the country(82).

1.10 Diagnosis and measurement of tropical lymphoedema

Lymphoedema can be successfully managed, and it is possible to improve patient outcomes if it is diagnosed at the earliest stage. Improved diagnosis will help to treat lymphoedema patients in a timely way with effective therapy, so a patient can return to full health and before the disease becomes complicated, disfiguring and stigmatizing. In addition, improved diagnosis is also helpful to study the disease history, drug discovery, improve patient care, clinical trials and enhance the effectiveness of therapy**.**

However, if it is not diagnosed early but is left untreated, lymphoedema leads to chronic inflammation, recurrent infection, reduced mobility, impaired function and hardening of the skin that, in turn, results in further lymph vessel damage and distortion of the shape of affected body parts (84)**.** The lymphoedema of both podoconiosis and lymphatic filariasis is reversible if diagnosed and treated early, but more advanced stages need lifelong treatment.

Regarding lymphoedema limb volume measurement, measuring lower limb volume may be useful in establishing the extent (severity) and staging of lymphoedema. Volume measurement may be used to evaluate treatment and self-management outcomes over time, or to understand whether a true change has occurred during the process of care (85).

Rationale of focusing the study on tropical lymphoedema (Podoconiosis and Lymphatic filariasis)

I grew up in a community suffering from lymphoedema therefore, I know how much the community is suffering and stigmatized from my childhood and had a dream to be part of a solution for this community. I started working on this problem as my career by advising one of my Masters students' theses and finally got a PhD opportunity through a senior, globally renowned professor, Gail Davey, working on this disease. I have got an opportunity to do my entire PhD with her on improving the diagnosis of tropical lymphoedema.

Chapter 2. Concepts of Diagnostic Test Evaluation and Validation Studies

Medical tests are necessary to resolve uncertainty about the health status of an individual. A clinically effective test should reduce ambiguity in clinical decision making, lead to prompt and appropriate intervention, and ultimately improve patient outcomes (86).

Since testing is pivotal to health care, tests should only be recommended for routine clinical use based on evidence of their clinical performance (i.e., diagnostic accuracy) and clinical effectiveness (i.e., benefits and harms) derived from relevant, high-quality studies (87).

The process of test evaluation is multifaceted and challenging, requiring a clear definition of the intended use and role of a test for a specific population within the context of a clinical pathway.

Since test evaluation is multifaceted, evaluation cannot be accomplished in a single study but requires a sequence of studies that address different aspects of test performance. The studies are often undertaken in an order which reflects increasing expense, embedding the tests deeper in clinical pathways, and an appreciation of the resource implications of implementation in clinical practice.

Test evaluation encompasses five phases; analytical performance (technical accuracy), clinical performance (diagnostic accuracy), clinical effectiveness, cost effectiveness and broader impact (societal efficacy). Cost effectiveness is frequently assessed along with clinical effectiveness and is termed societal efficacy when costs are considered from a societal perspective.

The analytical performance phase answers the question; does the test give usable information (reliable and reproducible)?

The diagnostic accuracy phase explores; how well does the test distinguish between diseased and non-diseased individuals?

The diagnostic accuracy phase has different stages. Stage I of diagnostic accuracy answers; do test results in patients with the target condition differ from those in healthy people?

Stage II; are patients with certain test results more likely to have the target condition than patients with other test results?

Stage III; does the test result distinguish between patients with and without the target condition in a clinically relevant population?

Diagnostic thinking**;** Does the test change diagnostic reasoning and decisions? Does the test change patient management?

The clinical effectiveness phase asks; do patients who undergo the test have better clinical outcomes than those who were not tested?

Finally, the societal efficacy phase examines ; is the test resource-efficient and beneficial for the society?

Diagnostic accuracy studies

Diagnostic accuracy is the ability of a test to correctly identify or exclude a target condition. Test accuracy can potentially be linked to the accuracy of clinical decision making through the downstream consequences of true positive, false positive, false negative and true negative test results, but benefits and harms to patients may also be driven by other factors too.

Assessment of diagnostic accuracy is an integral part of test evaluation. Diagnostic accuracy describes the ability of a test to classify individuals, typically simplified into a dichotomy by applying a criterion (referred to as thresholds, cut-offs or cut-points) to define test negatives and test positives.

Several authors have proposed multiple phases in the evaluation of diagnostic accuracy to distinguish between early assessment of test performance (i.e., proof-of-concept or exploratory studies) in a population of known cases and non-cases (case control study), and later assessment in a representative population in an appropriate clinical setting (prospective cross-sectional study of suspected cases)(88-91).

Test accuracy is estimated by comparing results of an index test (a new or existing test of interest) with a reference standard, sometimes known as a ‘gold’ standard. The reference standard is used to verify the presence or absence of the target condition, and may be a single test or a combination of tests and clinical information not routinely available in practice.

Sensitivity, specificity, positive predictive value, and negative predictive value each address diagnostic validity using different and logically opposite clinical perspectives.

Because it is nearly impossible to have a measure that is both perfect at ruling out only

true negative and at ruling in only true positives, the goal instead becomes having the best balance of these tests of validity, in which case all values are as close to perfect as possible. However, in order to accomplish this, there must be variability in both the measure you are testing and the gold standard measure.

Method Comparison Studies

In a method comparison study, the investigator is comparing a less-established method with an established method already in clinical use. The difference in values obtained with the two methods represents the “bias” of the less established method relative to the more established one.

Historically, test evaluations have focused on the accuracy of a single test without making comparisons with alternative tests that can be used at the same point in the diagnostic pathway (92).

Well-designed comparative studies are invaluable for clinical decision making because they can facilitate evaluation of new tests against existing testing pathways and guide test selection. These studies enable unbiased comparisons and increase confidence in the validity of the evidence. Evidence from studies of comparative accuracy can also be used in decision modelling to infer the relative effectiveness of tests when direct evidence from randomized control trials of test effectiveness is unavailable (93).

Generally, there are two comparative study designs—within-subject and between-subject designs.

Robust comparative studies of diagnostic test accuracy use either a within-subject multiple test (sometimes called "paired" or "crossover") design, in which all patients undergo all tests, together with a reference standard, or, more rarely, a between-subject randomized (unpaired or parallel group) design in which all patients undergo the reference standard test but are randomly assigned to have only one of the other tests (94). Such designs ensure validity by comparing like-with-like (either within patients or between randomized groups), thus avoiding confounding by factors such as population characteristics and study methods.

In a within-subject design, all patients undergo all tests and so each patient is their own control. Such designs are potentially resource efficient depending on the extent to which the tests are conditionally dependent. The design minimises between-subject variability and

also allows estimation of the accuracy of combinations of tests. The sequence of testing may be randomized to avoid bias(95).

In a between-subject design, the allocation of tests to patients should ideally be randomized. The randomized design is a valid alternative in situations where a paired design is inappropriate. A disadvantage of this design is that a larger sample is typically required and test combinations cannot be explored unless patients receive all tests in one of the arms of the study.

The core of method validation in general is the investigation of whether their properties are adequate for the intended use. A single laboratory validation is sufficient if the same measuring system is always used when analysing all samples from a population of patients.

Repeatability in a method-comparison study is a necessary, but insufficient, condition for agreement between methods. If one or both methods do not give repeatable results, assessment of agreement between methods is meaningless. Repeatability means how well does one method give the same results when measured over and over again?

Statistics

Measures used to quantify test accuracy are sensitivity and specificity, positive and negative predictive values, and positive and negative likelihood ratios (LR+ and LR–) are typically used to quantify test performance because of the need to distinguish between the presence and absence of the target condition. Sensitivity and specificity are the most commonly reported measures. Sensitivity is the probability that those with the target condition are correctly identified as having the condition, while specificity is the probability that those without the target condition are correctly identified as not having the condition. Sensitivity is also known as the true positive rate (TPR), true positive fraction (TPF) or detection rate, and specificity as the true negative rate (TNR) or true negative fraction (TNF). A receiver operating characteristic (ROC) plot is also a type of measure of accuracy. The ROC plot is a plot of sensitivity against 1-specificity. The position of the ROC curve depends on the discriminatory ability of the test which is illustrated as the degree of overlap of the distributions of test measurements for the diseased and non-diseased groups; the more accurate the test is, the closer the curve to the upper left-hand corner of the ROC plot(96).

A type II error (false-negative) occurs if the investigator fails to reject a null hypothesis that is actually false in the population. Type II errors are more likely to occur when sample sizes are too small, the true difference or effect is small and variability is large in this case.

Relationship between the studies

This PhD project aimed to improve the diagnosis and measurement of tropical lymphoedema . To address this, we validated three different devices, namely the novel three-dimensional infrared imaging device as lymphoedema volume measurement device and the DLP based NIR spectrometer and ZI MFIA digital impedence analyser as diagnostic tools. The volume measurement device was studied on patients with podoconiosis only. The study participants included in the validation of the novel three-dimensional infrared imaging device were also included for both the DLP based NIR spectrometer and ZI MFIA digital impedence analyser studies. The DLP based NIR spectrometer and ZI MFIA digital impedence analyser studies also used the same healthy control group (Fig2.1).

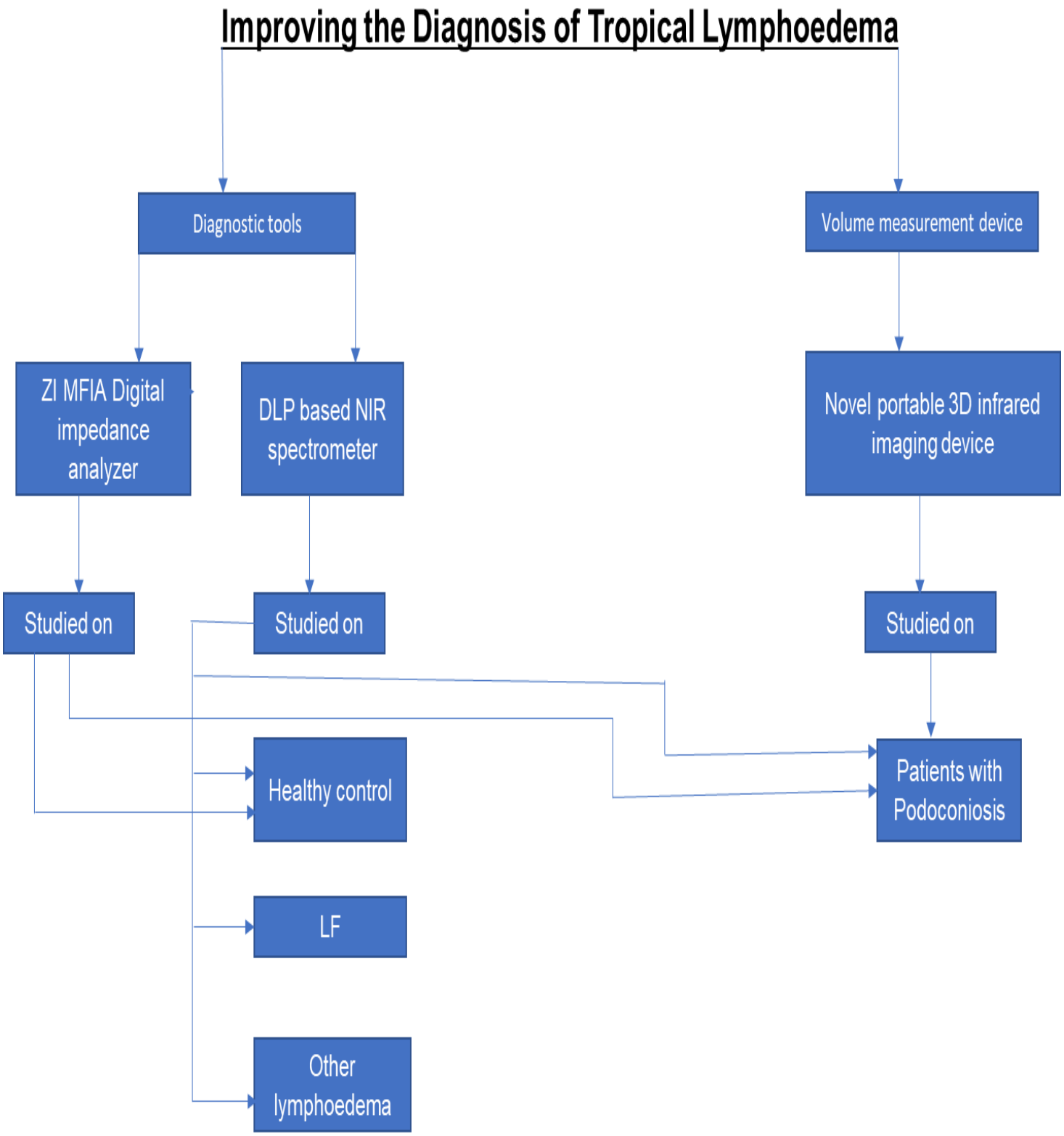


Fig 2.1 The schematic presentation of the relationship between the studies

Overview of the Devices

The water displacement technique is currently the gold standard technique for measuring lymphoedema volume measurement. It's a monitoring tool used to improve assessment of treatment and self-management outcomes.

The water displacement technique we used was a water tank with an overflow tube situated exactly 32cm above the inner base of the tank. This method works based on the principle that, when an object is immersed in a water tank with an overflow, the volume of the object is equal to the volume of the water spilled over through the overflow tube. The water displaced from the water tank was collected until the water stopped dripping and the volume was measured with a calibrated graduated cylinder.

However, this method is cumbersome, not portable, unhygienic, and impractical for use in field studies and is contra-indicated for patients with open skin lesions. In addition, it requires water, and the water needs to be changed for each patient to reduce the risk of contamination, this requires the water to be decanted potentially contaminating the surroundings. Because of these shortcomings, volume measurement by water displacement is not in use in Ethiopian health facilities, or to our knowledge, other podoconiosis-endemic countries. Thus, there is a pressing need to identify and validate a method that is practical to use in the field and in remote settings, where people with podoconiosis are generally to be found. Tape measures of limb circumference (TMLC) are frequently used but can be difficult to standardize and do not predict volume adequately for lower extremity lymphoedema (97, 98).

Therefore, to solve the volume measurement challenges we validated the novel portable three-dimensional imaging device. The novel portable 3D infrared imaging device (LymphaTech, Atlanta, GA, USA) consists of a portable infrared sensor (Structure by Occipital, San Francisco, CA, USA) mounted on a tablet computer. This is a lymphoedema volume measurement device. It has been tested to measure limb volume in patients with lymphoedema secondary to lymphatic filariasis (LF), and has been shown to be feasible to use, producing results that are accurate and reproducible.

To improve the diagnostic challenges , we validated the DLP based NIR spectrometry and the ZI MFIA digital impedance analyser.

The DLP based NIR spectrometry is a device that works based on spectrometric principle. The DLP based NIR spectrometry can be used for product identification, classification and quality control, as well as for the determination of product properties (chemical and physical) and component concentrations in process applications, however not yet used for lymphoedema diagnosis.

The ZI MFIA digital impedance analyser is a new device under development as part of the overall Global Health Research at the University of Sussex, School of Engineering and Informatics. The aim of developing this device is to use this device as a point of care diagnostic technique for tropical lymphoedema diagnosis.

General objective

To improve the diagnosis and measurement of tropical lymphoedema

Specific objectives

1. To validate the 3D portable imaging system for lower limb lymphoedema volume measurement
2. To assess the burden of lymphatic filariasis in chronic lymphoedema patients in Benishangul Gumuz Region, Western Ethiopia
3. To validate the DLP-based NIR spectroscopy device for early and accurate diagnosis and characterization of tropical lymphoedema
4. To assess the novel bioelectrical impedance device for early and accurate diagnosis of tropical lymphoedema

Chapter 3. Validation of a portable three-dimensional imaging Device for measuring lower limb volume of patients with podoconiosis in Ethiopia

3.1 Background

Lymphoedema is a localized lymph retention and tissue swelling resulting from impaired flow of the lymphatic system. The symptoms include swelling in one or more extremities. The swelling may range from mild to severe and disfiguring.

Measuring lower limb volume can be useful for early diagnosis, establishing the disease severity and to measure treatment and self-management outcomes over time. Currently, the water displacement technique is the most used and the reference technique for measuring the limb of lymphoedema cases, however, this method is cumbersome, not portable, unhygienic, and impractical for use in field studies and is contra-indicated for patients with skin lesions. Therefore, the aim of this study was to replace this technique with one or more valid and reliable point of care technique(s) to improve limb volume measurement in remote settings where lymphoedema is prevalent.

3.2 OBJECTIVES

General objective:

The general objective of this study was to validate the novel portable three-dimensional infrared imaging device for measuring lower limb volume of podoconiosis patients against the reference standard water displacement technique.

Specific objectives

- To determine the agreement between the the novel portable three-dimensional infrared imaging device and the reference standard technique for lower limb lymphoedema volume measurement
- To determine the repeatability of the 3D portable imaging system and water displacement volume measurements
- To determine the intra-rater (within rater) reliability of both methods

- To determine the interrater (between rater) reliability of both methods

3.3 Research questions

1. Do the two methods agree to the extent that the index test can replace the reference standard?
2. Using which of the two devices do the two raters produce a more reliable reading?
3. By which device does the rater produce a more reliable reading upon taking repeated measurements?

3.4 Pilot study of the the novel portable three-dimensional infrared imaging device

Before we embarked to validate this device, we conducted a pilot study to help plan a larger study and to check feasibility. This pilot study helped us become familiar with the device, train data collectors, and calculate a sample size for the larger study.

Methodology for the pilot study

A cross sectional study was conducted in Konchi Clinic, Wayu Tuka *Woreda*, Western Ethiopia, between December 1, 2018, and January 15, 2019.

The study was conducted after obtaining ethical clearance from BSMS Research Governance & Ethics Committee (RGEC) ref no 17/023/DAV and Wollega University institutional review board ref no WU/IRB WU-RTTVP/136/09.

After explaining the purpose of the study, patients with lymphoedema were consecutively screened in Konchi Clinic when they came for their regular follow-up visits. Those who were eligible for the study were appointed to come back to stay overnight in the clinic for midnight blood film examination. After they came back to the clinic, they were invited to give signed or finger-print consent.

Since podoconiosis is diagnosed by clinical exclusion, we collected a midnight blood to exclude lymphatic filariasis. To exclude lymphoedema of other causes, the participants were further screened by physical examination, and family history. Then, twenty-four podoconiosis cases were recruited into the study. The study participants were assessed first

using the novel portable three-dimensional infrared imaging device (index test) and then by the gold standard water displacement method by two independent raters, with each rater performing duplicate measurements.

The water displacement technique uses a water tank with an overflow tube, which is situated exactly 32cm above the inner base of the tank. This is based on the principle that, when an object is immersed in a water tank with an overflow, the volume of the object is equal to the volume of the water spilled over through the overflow tube. The water displaced from the water tank was collected until the water stopped dripping and the volume was measured with a calibrated graduated cylinder.

Before the actual data collection, the new water tank used for the water displacement technique was calibrated using eight volunteers, by taking triplicates of measurement from each of them**.**

3.5 Methods for the the novel portable three-dimensional infrared imaging device for the main (larger) study

Study Design: A cross sectional study was conducted on one hundred and six (106) podoconiosis patients in Konchi Clinic, Wayu Tuka *Woreda*, Western Ethiopia, between August 1, 2019, and October 10, 2019.

All the study participants were assessed first using the novel portable three-dimensional infrared imaging device (index test) and then by the gold standard water displacement method by two independent raters, with each rater performing duplicate measurements in quick succession. Then, the two methods were compared using appropriate statistical parameters. The aim of using two independent raters was to assess the inter-rater reliability between the methods, while taking duplicate measurements was to check the intra-rater reliability and repeatability of the methods. For comparing agreement, we used the first measurements of each device taken by the first rater.

Study Area: The study was conducted in Konchi clinic Wayu tuka District, Oromia region, Western Ethiopia. The district is located 316 km from the capital Addis Ababa at an altitude of 1700–2200 m above sea level and has an average annual rainfall of 2400 mm. It is

bounded by Sibu Sire in the north and east, Leka Dulecha in the south, and Guto Gida in the west. Gute is the administrative center. The population of Wayu Tuka 'woreda' is estimated at 75 970, living in 15 930 households, of whom 95% are in rural areas and depend on subsistence farming for their living (Figure 3.1).

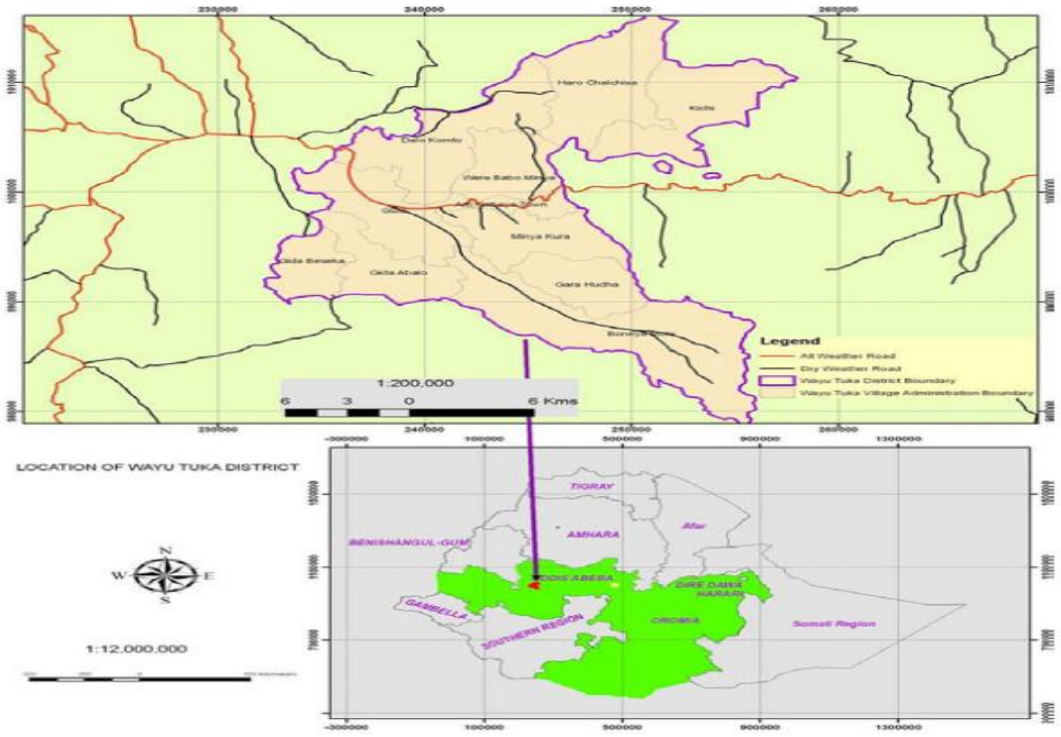


Figure 3.1 Map of Wayu Tuka District

Konchi clinic is a non-governmental organization clinic run by the Catholic Church. It provides a range of medical services to the surrounding community and offers lymphoedema morbidity management for many lymphoedema patients. The 2017 data of the clinic showed that it provided morbidity management for 2,012 patients from twelve (12) villages (Table 3.1).

No	Village	Male	Female	Total
1	Konchi	17	62	79
2	Kura	55	89	144
3	Garjo Fitea	16	62	78
4	Garahudha	56	65	121
5	Gute	48	225	273
6	Bonaya moloo	116	200	316
7	Bata	105	150	255
8	Tulu Gombo	20	54	74
9	Walene	61	121	182
10	M/Kilili	27	72	99
11	Gaba Sanbata	138	161	299
12	Wara Babu	26	66	92
	Total	685	1327	2012

Table 3.1 Morbidity Management services provided by Konchi clinic by village and sex,2017

Since 2017, reliable data have not been collected, but the clinic has extended its services to additional villages and also launched a new site (clinic) in Sibu Sire Catholic Clinic, western Ethiopia, about 280 km away from the capital Addis Ababa.

Sample size: The sample size was determined by considering the sample size suggested by Bland and Altman (99) for method agreement studies, in which they recommend 100 as a good sample size. Considering this, we decided 100 was a fair sample size to provide a valid scientific conclusion about the agreement between the methods. Adding a 6% non-response rate gave us a target of 106 participants.

Source Population

The source population was all lymphoedema patients of varying clinical stages in Wayu tuka district who were 18 years or older attending Konchi clinic for morbidity management during the study period.

Study Population

The study population were selected podoconiosis patients of varying clinical stages who were 18 years or older attending Konchi clinic for morbidity management during the study period and willing to be included in the study.

Inclusion Criteria

Those who fulfilled all these criteria were recruited into the study

- Lymphoedema patients (cases)
- 18 years or older
- Negative for midnight blood film examination

Subject Recruitment strategy

All the study participants were recruited into the study after obtaining ethical clearance from BSMS Research Governance & Ethics Committee (RGEC) ref no 17/023/DAV and Wollega University institutional review board ref no WU/IRB WU-RTTVP/ 136/09.

To recruit podoconiosis patients into the study, we used physical examination, family history, midnight blood film, acid fast stain (to exclude leprosy) and skin slit examination (to exclude onchocerciasis). A podoconiosis case was defined as a person with lymphoedema of the lower limb present for more than 3 months for which other causes (i.e. onchocerciasis, leprosy, systemic disorder) had been excluded, including being negative for *W. bancrofti* microfilariae through blood film examination**.**

The study participants were informed about the study and recruited as they came to the Konchi clinic for their regular follow up schedule during the study period. We used the same approach successfully in the pilot study. Subjects were selected consecutively from eligible lymphoedema patients aged greater or equal to 18 years attending the clinic. After explaining the purpose of the study and what participation would involve in their local

language, eligible participants were invited to give signed consent. For those who were unable to sign their names, we used their fingerprint instead. After obtaining their consent, we gave them an appointment to come to the clinic to stay overnight for midnight blood film examination to diagnose lymphatic filariasis.

During the overnight stay, a small drop (about 50 µl) of blood was collected from the ball of the third or fourth finger between 10:00 PM and 12:00 AM. Thin and thick blood films were prepared and stained using 10% Giemsa solution to examine for *W. bancrofti* microfilaria. Blood taken around midnight enhances the chances of identifying the microfilaria, since the microfilaria are released into the peripheral circulation between 10:00 PM and 12:00 AM to match the biting habits of the mosquito vector. Those found to be positive for microfilaria were regarded as lymphatic filariasis and excluded from the study.

Those negative on blood film examination were further categorized into podoconiosis or lymphoedema due to other causes, based on clinical examination, family history and physical examination. The swelling of podoconiosis starts in the foot and progresses upwards, the lymphoedema is asymmetric, usually confined to below the knees and is unlikely to involve the groin. Patients were asked if they have ever been diagnosed with leprosy, and physical examination was conducted to exclude signs of leprosy including sensory loss. Those with sensory loss were further diagnosed using acid fast staining technique. Though onchocerciasis has clear clinical features which can easily be distinguished from podoconiosis, slit skin examination was done for those who were suspected to have onchocerciasis. Systemic causes of lymphoedema were ruled out by examination of facial, hand and general body swelling. Hereditary causes of lymphoedema were excluded through history since these occur at birth or immediately after birth. A clinical algorithm was used for the recruitment purpose (Figure3.2).

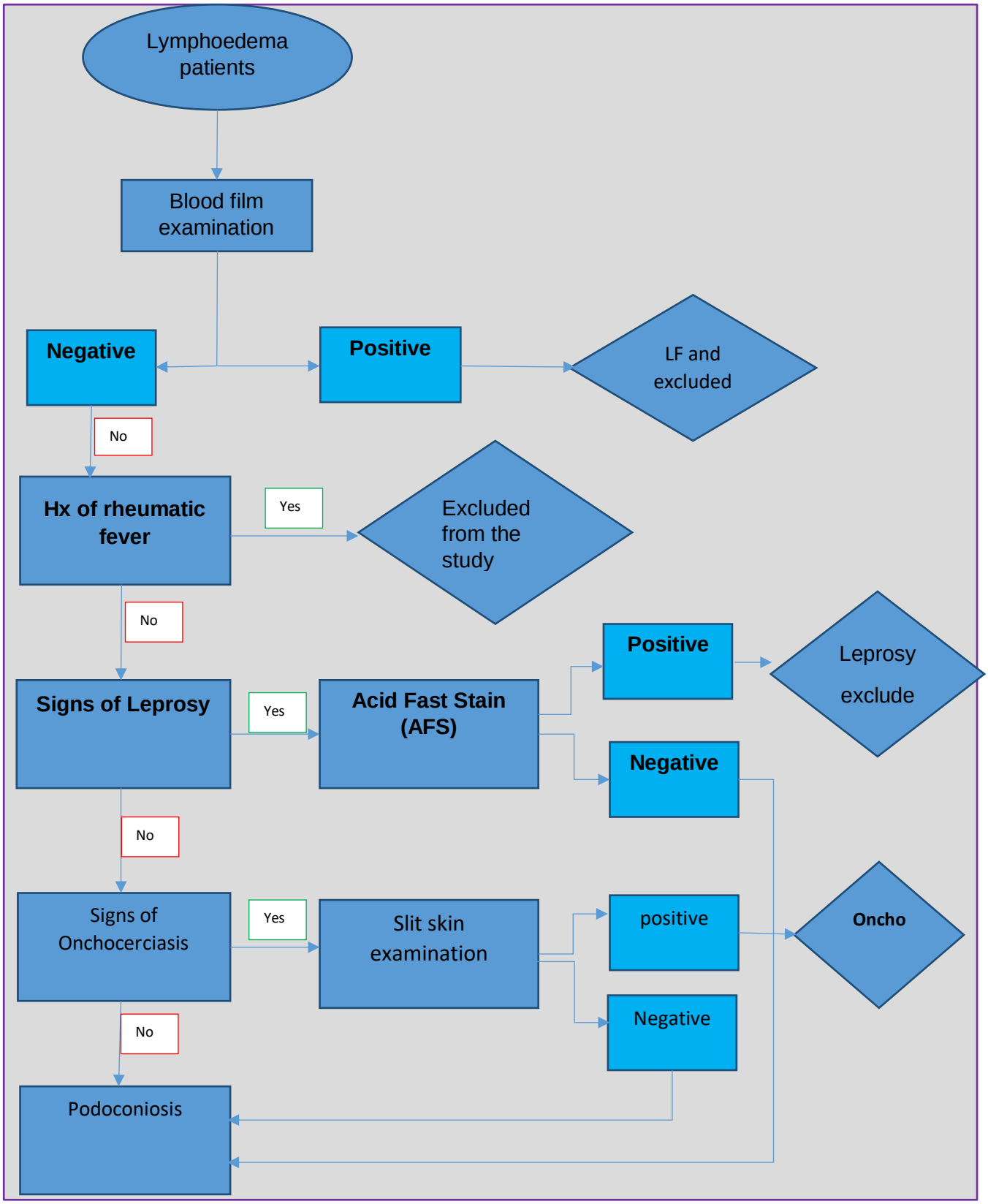


Figure 3.2 Clinical Algorithm for study participant recruitment

Blood film preparation and staining

Before commencing the data collection, we facilitated all the necessary logistics and arranged cars to transport some of the patients who lived a long way from the main road (Figure 3.3).

Figure 3.3 Transport for study participants when they come to the clinic for blood film examination

Accommodation was provided for the study participants and data collectors to stay overnight in the clinic (since the blood must be collected between 10:00PM and 12:00AM). Dinner and breakfast were also prepared for all study participants. We provided dinner for all the study participants before 10:00PM (Figures 3.4 & 3.5).

Figure 3.4 A local common dish (buddeena or Injera) prepared for the study participants

Figure 3.5 Study participants in their accommodation room

Timing of blood sample collection

Microfilariae exhibit a marked periodicity depending on the species involved, therefore the time of specimen collection is critical. The optimal collection time for demonstrating microfilariae of *W. bancrofti* is between 10:00PM and 12:00AM. For the details of blood collection and staininig procedure of blood film see Annex-2.

Staging of podoconiosis

Since the severity of lymphoedema may affect the result of the index test, varying stages of podoconiosis were recruited into the study. For staging, we used a clinical staging system developed and validated by Tekola *et al* (70) as stated under section 1.7 of this thesis. We included stages 2 to 5 and excluded stage 1 since the swelling in this case is reversible. In case of podoconiosis patients with bilateral lymphoedema, the leg with the larger staging was measured. If both had the same staging, the left leg was measured consistently since we evaluated only one of the legs of each study participant.

Data collection using the 3D volume measurement device

This study was conducted on 106 patients with podoconiosis identified using the algorithm described above from among 212 individuals with lymphoedema.

Measurement Technique

Two independent raters were recruited for the study and were trained to perform both the 3D imaging and the water displacement method with volunteers before the actual data collection. During the training common errors and characteristics of good and poor scans were explained, and raters understood the importance of inspecting the scans visually before saving them. The same raters who conducted the pilot study were again recruited for the main study.

After the study participants were recruited into the study, the lower limb volume of all the study participants were first measured using the Novel Portable Three-Dimensional device

(Index test) then, by the reference standard water displacement method by two raters (evaluators) and each of them took duplicate measurements.

Overview of the Novel Portable Three-Dimensional imaging device

The three-dimensional infrared imaging device (LymphaTech, Atlanta, Georgia) consists of a portable infrared sensor (Structure by Occipital, San Francisco, California) mounted on a tablet computer (Figure 3.6). It employs proprietary software to combine depth data from the sensor with accelerometer data from the tablet to create point-cloud reconstructions of the surface of the scanned limbs. The LymphaTech software analyses the 3D model to calculate customizable measurements of limb volume and circumferences. For the detailed procedure of THE Novel Portable Three-Dimensional imaging device see Annex-3.

Figure 3.6 The 3D imaging device attached on an iPad

Overview of Water Displacement Technique

The water displacement technique we used was a water tank with an overflow tube situated exactly 32cm above the inner base of the tank. This method works based on the principle that, when an object is immersed in a water tank with an overflow, the volume of the object is equal to the volume of the water spilled over through the overflow tube. The water displaced from the water tank was collected until the water stopped dripping and the volume was measured with a calibrated graduated cylinder. Before the actual data collection, the water tank was calibrated using eight volunteers, by taking triplicates of measurement from each of them.

Water Displacement Method procedure

The subject was instructed to sit in the waiting room for 30 minutes prior to measurement to stabilize skin temperature with room temperature (20^0 C - 27^0 C). The water tank was first filled to overflow, and the water level was allowed to stabilize. Then, the subject was instructed to slowly place his/her foot into the water tank in a standing position. Patients with trouble balancing could hold a nearby countertop for support. The water displaced from the water tank was collected until the water stopped dripping and the volume was measured with a calibrated graduated cylinder. The volume of the water measured by the graduated cylinder is equal to the volume of the leg of the study participant (Figure 3.7).

Figure 3.7 Taking volume measurement using water displacement method (Picture by Abdi Samuel)

Statistical Analysis Plan

Data description

One hundred and six podoconiosis patients of varying clinical stages were recruited into this study. All the study participants were measured by both reference and index tests by two independent raters. Both raters made duplicate measurements. We labelled the raters as Rater one (R1) and Rater two (R2). Rater one, took duplicates of measurements using the water displacement techniques, and these measurements were labelled M1 and M2. This rater also took two measurements using the 3D imaging system, and these measurements were labelled Scan-1 (S1) and Scan-2 (S2). Similarly, Rater two also took duplicate measurements using both devices. The first measurement by Rater two using the 3D imaging system is labelled Scan-3 (S3), and the second scan Scan-4 (S4), whereas the first water displacement data measurement by Rater two was labelled Measurement-3 (M3) and the second, Measurement-4 (M4).

Statistical analysis methods

The data was entered and analyzed using SPSS version 25. We applied measures of agreement and repeatability. We objectively analyzed the agreement between the two methods, the reliability of each method, between raters (inter-rater) reliability and within raters (intra-rater) reliability.

The agreement between the two methods

Research question: Could the two methods agree to the extent that the index test can replace the reference standard?

Since we are comparing the agreement of the new method, the 3D imaging device, against the reference standard water displacement technique and our outcome measure is continuous, we employed the Bland-Altman limits of agreement (LOA) method.

Before starting the analysis, we checked that our data fulfilled the Bland-Altman assumptions. We assumed the within pair differences were approximately normally distributed and we checked this by plotting a histogram with the normal curve to show the data was appropriate for LOA.

Only the first measurements by each method and by rater one (i.e. M1 and S1) were used to illustrate the comparison of the gold standard and the index test, since the aim of the study was to determine whether the single observations taken by the 3D imaging device could be used to replace the gold standard technique.

After checking that the data were suitable for the Bland-Altman limits of agreement method, we calculated the basic values required for calculating limits of agreement, which are the within pair difference, the mean of the within pair difference and its standard deviation.

Then, we plotted a scatter plot using the mean of the two measurements taken by both methods on the X axis and the difference between them on the Y axis with a horizontal line so that we could visually observe the differences between the pairs of measurements.

After checking these, we calculated limits of agreement, which is the mean of the within-pair difference plus/minus 1.96(SD). We then calculated confidence intervals for the mean and the LOA. To construct confidence intervals for both, we need to calculate the standard error of the mean and the LOA. For the LOA we calculated the CI for both the lower and upper limits of agreement. Finally, we plotted the result on a Bland-Altman graph.

Inter-rater Reliability (between the two raters)

Research question: on which of the two devices do the two raters produce a more reliable reading?

To address this question, we did a separate and independent repeatability analysis and compared the repeatability parameters between the two methods.

To calculate the inter-rater difference, both raters took duplicates of measurements using both devices, however, the inter-rater reliability was analyzed using only the first measurements taken by both raters of both devices, i.e., M1 and S1 for Rater-1 and M3 and S3 for Rater-2.

To calculate the inter-rater reliability of the water displacement method, we analyzed the first measurements taken by both raters (M1 and M3).

First, we plotted a graph, then we calculated the mean and standard deviation (SD) of the two raters and displayed this in a table. Then, we calculated an interclass correlation coefficient (ICC). In this case, we used ICC equation (3,1) which was calculated from a two-way ANOVA. The ICC was estimated using the single measure option, absolute agreement definition and two-way mixed effects ANOVA, and the ICC was provided with the 95% confidence interval.

Interpretation: An ICC is measured on a scale of 0 to 1. 1 represents perfect reliability with no measurement error, whereas 0 indicates no reliability.

We also used the Bland-Altman method since it would help us in providing estimates of agreement between the two raters and support our earlier parameters. To do this, we calculated the mean, SD and difference of the two sets of readings (those first readings by both raters). Then, we plotted a scatter plot using the paired differences against the paired means. Then, we calculated the 95% LOA (which is paired mean differences ± 2 SD of the differences)

Interpretation: in this case, a value closer to zero indicates better agreement in measurements between the raters.

After that, we exactly repeated the same approach to analyse the reliability between the raters for the 3D imaging devices and then compared with the finding.

Intra-rater reliability

Research question: by which device does the rater produce a more reliable reading upon taking repeated measurements?

We did the analysis using the repeated measurements taken by Rater one only. First, we plotted the duplicate measurements taken by Rater-1 on a separate plot for both methods for visual inspection of intra-rater reliability between the two methods.

Then, we assessed the repeatability of the water displacement technique (M1 and M2) using the ICC (1,1). The data were analyzed using a single-measurement type, absolute-agreement definition and 2-way mixed-effects model and reported the estimated ICC with its 95% Confidence Interval. After that, we repeated the same approach for the 3D imaging devices (S1 and S2) and then compared the finding, again employing the Bland-Altman method.

3.6 Results of the pilot study

Twenty-five (25) study participants were initially included in the study, but one had learning difficulties and was unable to insert his leg into the tank and stand until the dripping stopped, so he was excluded from the study. Of the remaining twenty-four, twelve (33%) were 50 years or older and 14 (58%) were females. With respect to their clinical staging, five were stage II, eight stage III, four stage IV and six stage V. Two raters each took two scans from each patient, so in total 96 scans were taken for the study.

Volumes measured by the gold standard method ranged from 1595ml to 9275ml, while those measured by the index test ranged between 1664ml and 9443 ml. The mean volume differences indicated a bias of 269 ml, in which the scanner underestimated the limb volume compared to water displacement, but with a high degree of consistency thus resulting in a strong correlation coefficient. The correlation coefficient between the scanner and water displacement measurements was 0.98.

3.7 Results of the main study

Correlation between the novel portable three-dimensional infrared imaging device and water displacement method

To assess the correlation between the methods, we have used the first measurements of both devices taken by rater one (S1and M1). The Pearson correlation coefficient r = 0.96 and p<0.001, therefore we have enough evidence to reject the null hypothesis and so, we have concluded that there is a nearly perfect positive relationship (association) between the the novel portable three-dimensional infrared imaging device and the water displacement technique (Figure 3.8).

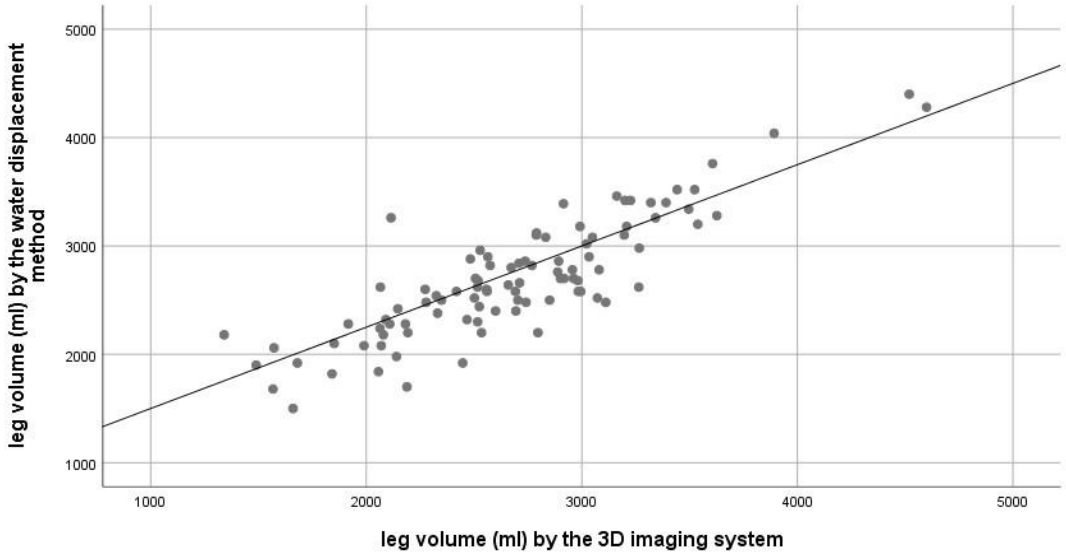


Figure 3.8 Scatter plot showing leg volumes in patients with podoconiosis assessed using a portable the novel portable three-dimensional infrared imaging device and the water displacement method

Assessing the agreement between the methods

We first ran a one sample t-test for the paired difference variable (M1-S1) to assess whether the two devices agree at least on average using target (test value) of 0 by assuming there is no difference between the two measurements, that is our null hypothesis. The results of the one sample t-test for the paired difference showed a mean value of 12.61 and standard deviation of 302.83 and p=0.684, so we did not reject the null hypothesis. This finding showed, there was no statistically significant difference between the water displacement and

the 3D imaging devices, which means there was a certain level of agreement on average between them. Having demonstrated a certain level of agreement between the methods, we further assessed the data using the Bland and Altman test to estimate the level of that agreement. Had the P-value been <0.05 we would have rejected the null hypothesis, meaning a statistically significant difference between the two devices and no need to use a Bland Altman graph.

Before we ran the Bland and Altman test, we checked the normality of the paired difference variable using a histogram with a normal curve as depicted below. The graph did not show any strong skew (approximately normally distributed), so we proceeded to the test since it fulfilled the assumption (Figure 3.9).

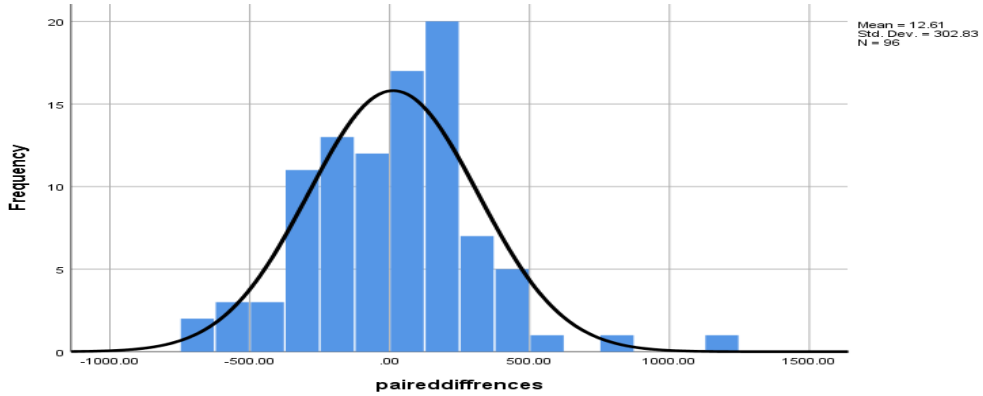


Figure 3.9 Histogram with normal curve for the paired difference variable

For the Bland and Altman test, we calculated the mean of the difference, standard deviation, the lower and the upper 95% CI. As above, the mean and standard deviation were 12.61 and 302.83 respectively. The upper 95% Limits of agreement (LOA) was calculated using mean of the difference + (SD*1.96) and the lower LOA as mean of the difference - (SD*1.96). Using this formula, the upper 95% LOA was 593.55 and the lower was -580.94. Then, we plotted the Bland and Altman plot using these parameters (Figure 3.10).

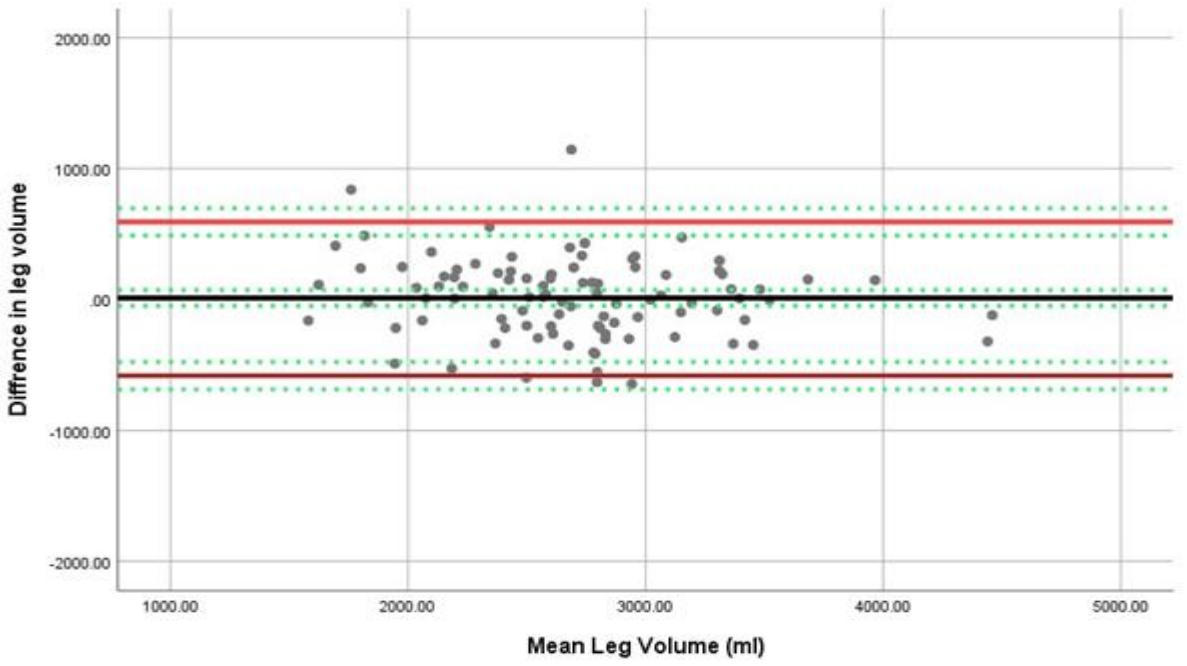


Figure 3.10 Bland-Altman plot showing agreement between the novel portable three-dimensional infrared imaging device and water displacement method for assessment of lower limb volume in patients with podoconiosis.

The y-axis shows the difference in leg volume (ml) measured using the water displacement method minus the volume measured using the novel portable three-dimensional infrared imaging device as assessed by rater one. The x-axis shows the mean of leg volume (ml) measured using the water displacement method and 3D imaging system. Data are shown for individual patients (grey dots), the mean of the difference between the two methods (black line) with upper and lower limits of agreement (red lines) with 95% confidence intervals (dashed lines).

After plotting the Bland and Altman graph, we ran a linear regression analysis using paired difference as a dependent variable and the mean as independent to determine the proportional bias between the methods (for the presence of a linear trend above or below the line of our mean difference). The result showed a beta coefficient of -0.09 which is close to zero and p=0.105, so we accept the null hypothesis showing we have enough evidence to talk about the lack of proportional bias.

The plot showed that, except for four measurements, two above the upper 95% LOA and two below the lower LOA, 95.8% of the data fall within the 95% limits of agreement (in less than 2SD). Even those two points below the lower limit of agreement are very close to the line.

From the graph, the mean of the difference was 12.61ml with 95% CI (-48.75, 73.96) and standard deviation of 302.83ml. The mean difference was 12.1ml, meaning the novel portable three-dimensional infrared imaging device slightly underestimated the volume of the lower limb compared with the reference standard. The confidence interval shows that the water displacement method could measure 48.75ml lower or 73.96ml higher than the novel portable three-dimensional infrared imaging device.

Since the paired mean difference confidence interval crosses one, we do not have enough evidence to conclude that the average 12.61ml difference is statistically significant therefore, we do not need to adjust for this difference to be closer to the reference standard.

The upper 95% LOA was 593.55ml and the lower was -580.94, i.e., the the novel portable three-dimensional infrared imaging device may measure 593.55 ml below or 580.94ml above the water displacement technique making it difficult to conclude the two methods agree sufficiently.

Finally, we calculated the 95%CI for each estimate. The 95% CI for LOA was calculated using the formula: estimate $\pm$ (1.96*standard error). Standard error of the mean was calculated using the formula $\sqrt{\sigma 2}/n$ and the standard error for the LOA was calculated as $\sqrt{3 * \sigma 2}/n$. Using this formula, the SE for the mean was 30.9 and for the LOA was 53.53(table 3.2)

Parameter	Estimate	95% CI	SE
Mean difference	12.61	-48.75, 73.96	30.9
Upper 95 % LOA	593.55	488.63, 698.47	53.53
Lower 95% LOA	-580.94	-476.02, -685.86	53.53

Table 3. 2 Bland-Altman estimate of the mean of the difference (with 95% upper and lower LOA and 95% CI) between the novel portable three-dimensional infrared imaging device and water displacement method for assessment of lower limb volume in patients with podoconiosis

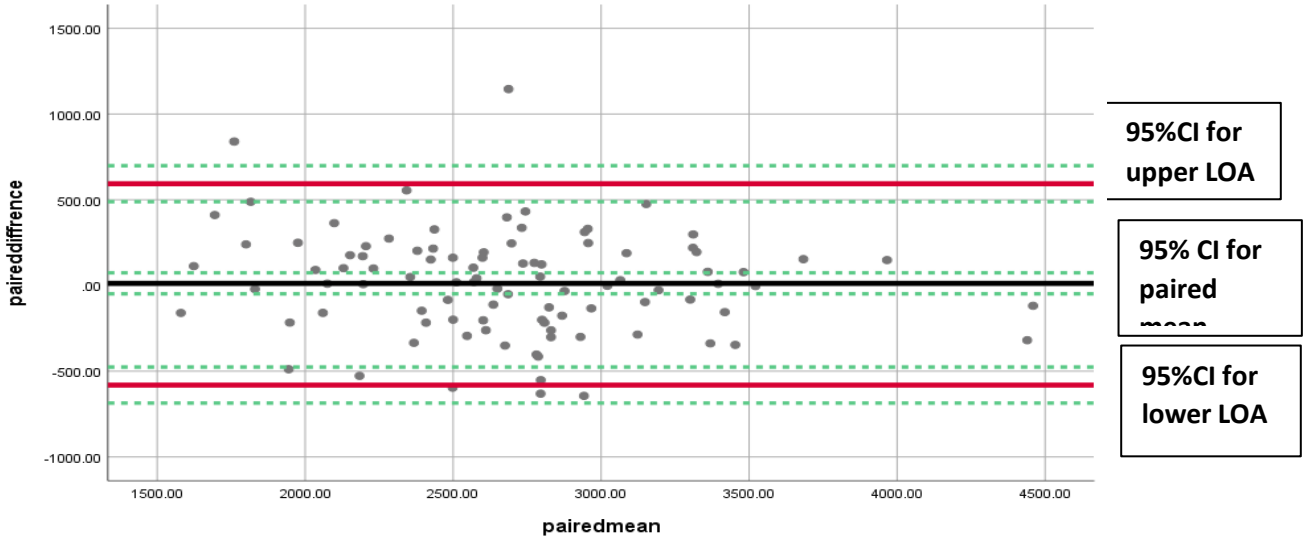


Figure 3.11 Bland and Altman plot for the method agreement with each estimate indicated with their 95% CI

In Figure 3.11 above, all the green broken lines showed the 95% CI for their estimates.

After the 95% CI was calculated for both the upper and the lower limits of agreement, only two measurements fell out of the range. So, we are 95% confident that the difference between the measurements taken by both devices were between 488.63ml and 698.4688ml above zero and between -476.02 ml and -685.86ml below zero (Fig 3.11).

Calculating the 95% CI for each estimate helped us to figure out the most and least optimistic conclusions about the agreement between the two methods. So, based on the finding, the most optimistic conclusion is that the water displacement method may measure 488.63ml above or 476.02ml below the novel portable three-dimensional infrared imaging device, and the least optimistic that it may measure between 698.47ml above and 685.86ml below the 3D device.

3.9 Assessing reliability

Inter-rater reliability

The mean ± SD leg volume measured using the water displacement technique was 2649.7 ± 546.4 ml when assessed by rater one and 2748.3 ± 551.3 ml when assessed by rater two. For the 3D device, the mean ± SD leg volume was 2682.1 ± 595.1 ml when assessed by rater one and 2698.0 ± 606.9 ml when assessed by rater two.

Measurements by both raters showed slightly greater variability (higher SD) when they used the water displacement method but, overall, the variabilities of each rater/method were similar.

Both raters produced more reliable readings (better agreement between raters) when using the portable three-dimensional infrared imaging device than the water displacement method The ICC for the water displacement method was 0.82 (95% CI: 0.74 to 0.87) (Figure 3.12 & 3.13).

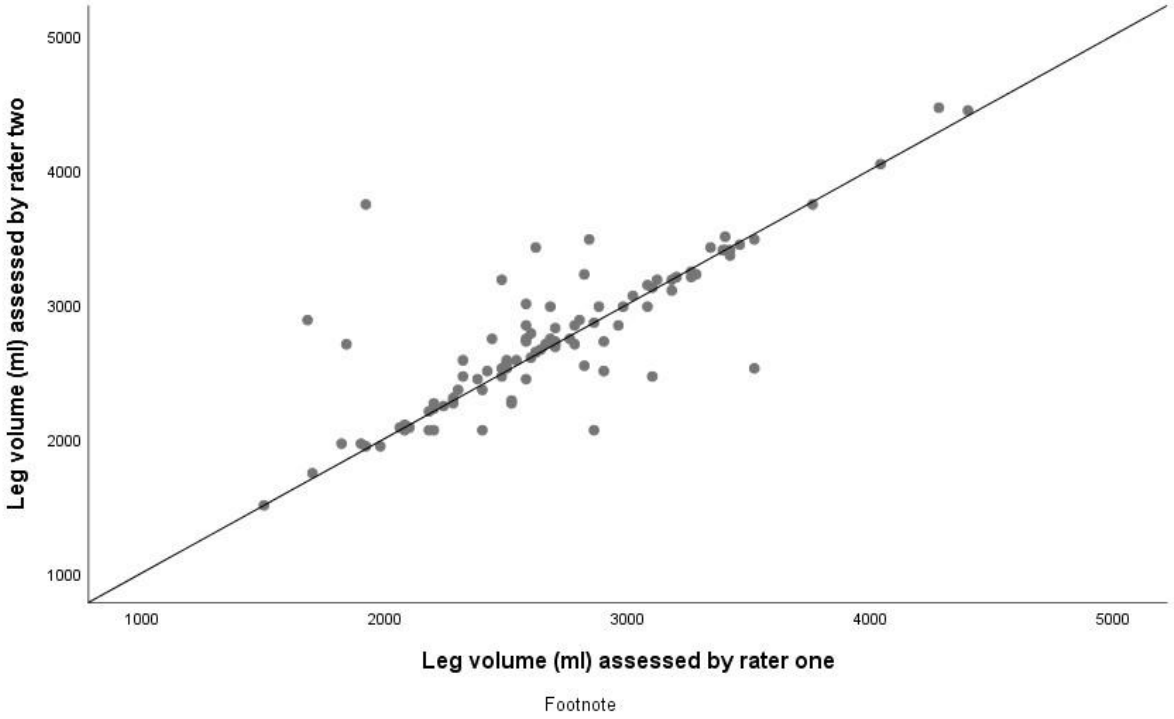


Figure 3.12 Scatter plot showing the correlation between lower limb volume assessed by rater one and rater two using the portable the novel portable three-dimensional infrared imaging device

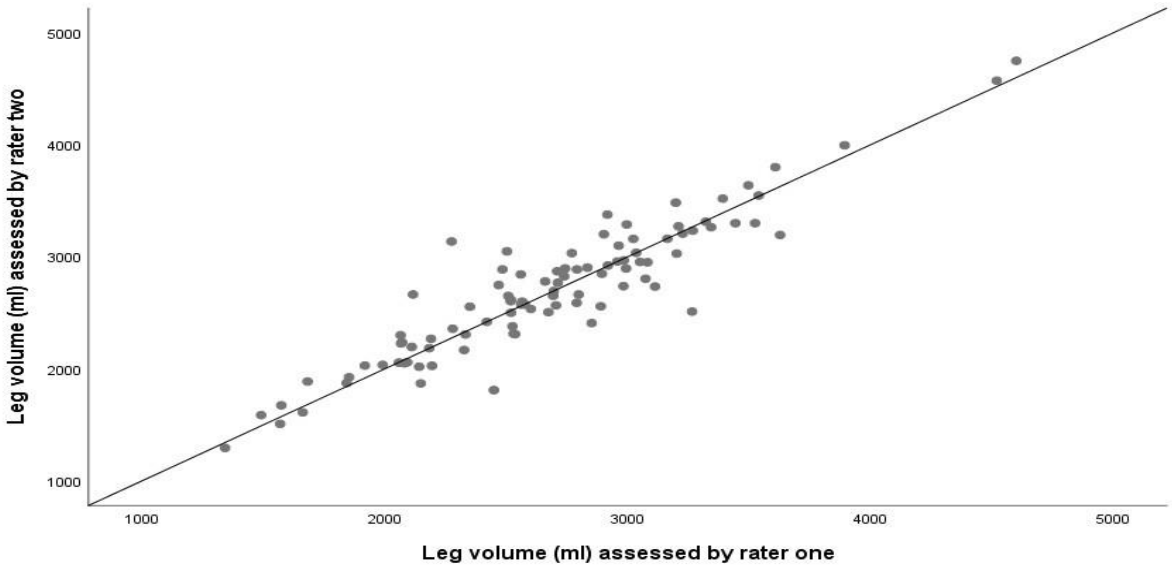


Figure 3.13 Scatter plot showing the correlation between lower limb volume assessed by rater one and rater two using the water displacement

The mean ± SD of the inter-rater difference for the water displacement technique was − 53.6 ± 329.8 ml (95% LOA: − 700.1, to 592.8 ml (Figure 3.14 and Table 3.3).

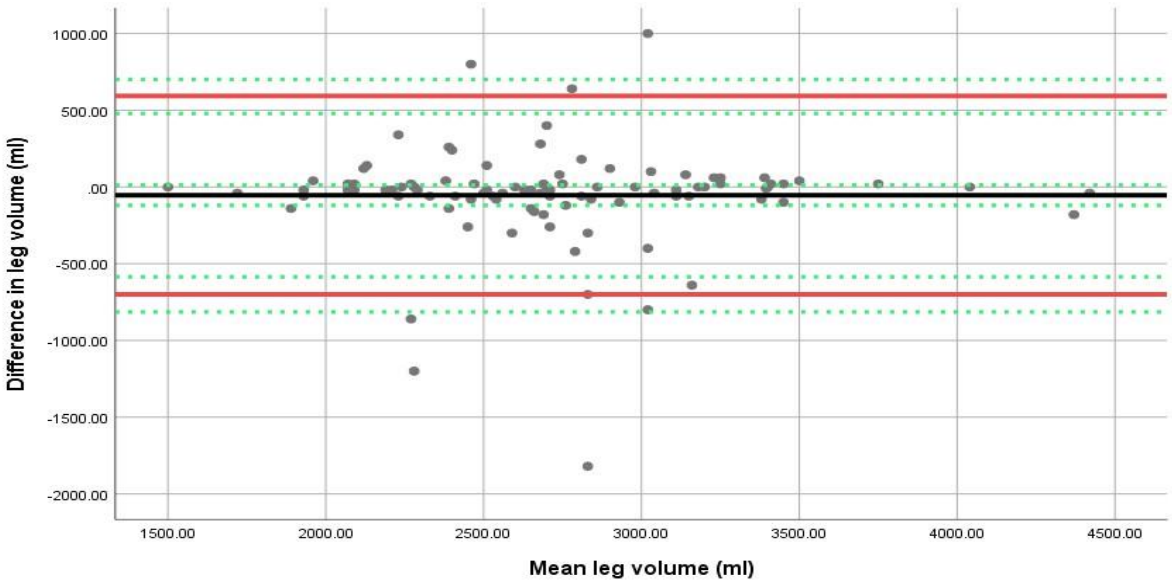


Figure 3.14 Bland-Altman plot showing inter-rater reliability for assessment of lower limb volume using the water displacement method

The y-axis shows the difference in leg volume (ml) measured using the water displacement method by rater one minus the volume measured using the same method by rater two. The x-axis shows the mean leg volume (ml) measured using the water displacement method by rater one and rater two. Data are shown for individual patients (grey dots), the mean of the difference between the two raters (black line), upper and lower limits of agreement (red lines), and 95% confidence intervals (dashed green lines).

Parameter	Mean difference(ml)	95%CI
Mean difference	-53.6 ml	-119.9, 12.4
Upper 95% LOA	592.8 ml	478.5, 701.1
Lower 95% LOA	-700.1 ml	-814.4, -585.8

CI: confidence interval; LOA: limits of agreement

Table 3.3 Bland-Altman estimate (with 95% upper and lower LOA and 95% CI) of inter-rater reliability when using the water displacement method to assess lower limb volume in patients with podoconiosis.

The ICC for the novel portable three-dimensional infrared imaging device was 0.93 (95% CI: 0.89, 0.95) (Figure 6B) and the mean ± SD of the inter-rater difference was 15.9 ± 229.1 ml (95% LOA: 465.0, 433.2 ml) (Figure 3.15 and Table 3.4).

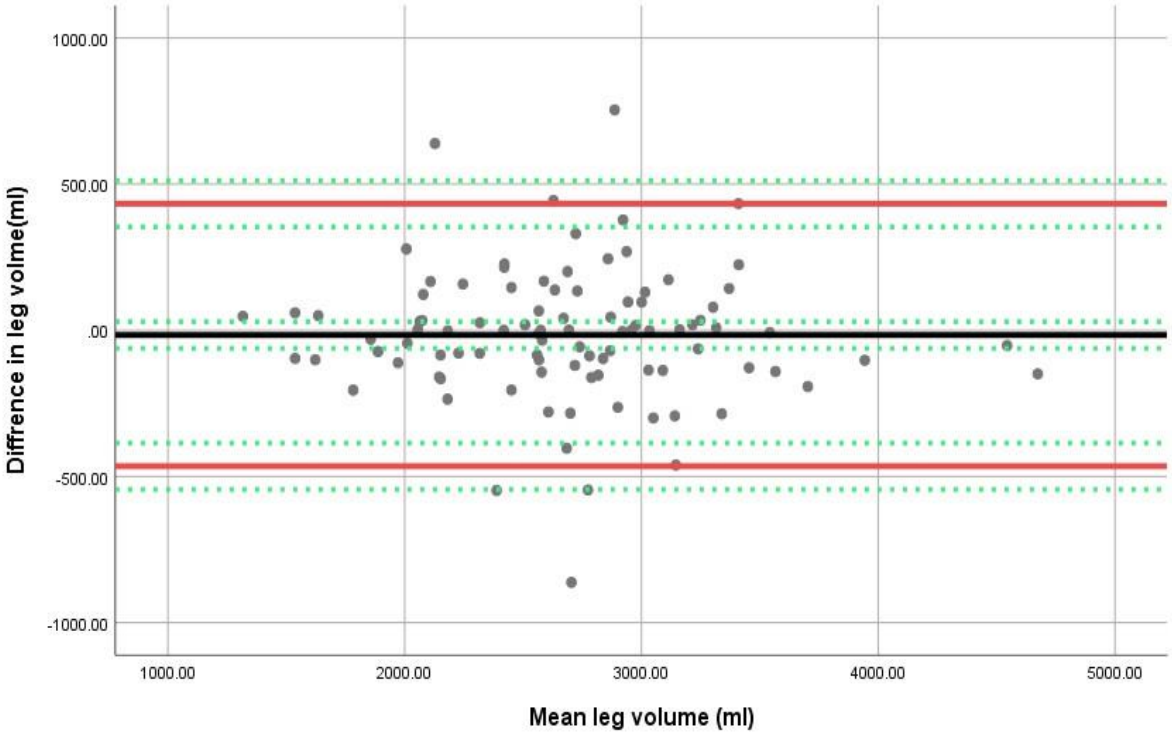


Figure 3.15 Bland-Altman plot showing inter-rater reliability for assessment of lower limb volume using the novel portable three-dimensional infrared imaging device in patients with podoconiosis.

The y-axis shows the difference in leg volume (ml) measured using the novel portable three-dimensional infrared imaging device by rater one minus the volume measured using the same method by rater two. The x-axis shows the mean of leg volume (ml) measured using the index test by rater one and rater two. Data are shown for individual patients (grey dots), the mean of the difference between the two raters (black line) with upper and lower limits of agreement (red lines) with 95% confidence intervals (dashed lines).

Paramete r	Estimate	95% CI
Mean difference	– 15.9 ml	– 61.7, 29.9
Upper 95 % LOA	433.2	353.82,512. 4
Lower 95% LOA	– 465	385.62, -544.38

CI: confidence interval; LOA: limits of agreement

Table 3.4 Bland-Altman estimate (with 95% upper and lower LOA and 95% CI) of inter-rater reliability when using the novel portable three-dimensional infrared imaging device to assess lower limb volume in patients with podoconiosis.

The mean measurement difference when the two raters used the water displacement method was 53.8 ml, (95%CI: – 120.5, 13.2), whereas it was only 15.9 ml, (95% CI: – 62.3 to 30.5) using the novel portable three-dimensional infrared imaging device.

3.*10* Intra-rater reliability

The mean ± SD values for the first and second water displacement measurements were 2694.7 ± 546.4 ml and 2675.5 ± 552.9 ml, respectively, while using the index test, they were 2682.1 ± 595.1 ml and 2735.8 ± 821.4 ml, respectively. The ICC of the water displacement technique was 0.96 (95% CI: 0.95, 0.98), whereas for the index test it was 0.70 (95% CI: 0.59, 0.79). For duplicate (average) measures the ICC of the index test was improved to 0.83 (95% CI: 0.74 to 0.88).

3.11 Discussion

The current study assessed the correlation, intra-rater and inter-rater reliability and agreement between the novel portable three-dimensional infrared imaging device and water displacement method. Studies have been conducted comparing a range of index tests with water displacement, though not specifically for podoconiosis (100-105).

Our findings demonstrated a very strong correlation between the two techniques. Good inter- rater and intra-rater reliability was demonstrated for both methods. The the novel portable three-dimensional infrared imaging device showed better inter-rater reliability than the reference standard. Though both methods demonstrated good intra-rater reliability, the water displacement method showed better intra- rater reliability. The intra-rater reliability of the novel portable three-dimensional infrared imaging device was improved when a duplicate measurement was taken. Bland and Altman plots showed large differences for some individual measurements.

In agreement with other studies compared with water displacement techniques (81, 100, 102, 105), we demonstrated a very strong positive correlation (r=0.96) between the water displacement method and the index test. However, a strong correlation between the two techniques alone does not necessarily mean the techniques are measuring the same volume. Therefore, we conducted Bland and Altman analyses to assess bias and the agreement between the two techniques. The Bland and Altman analyses, indicating little systematic difference between the volumes measured by each technique. The upper and lower limits of agreement between the techniques indicate that the novel portable three-dimensional infrared imaging device typically over- or underestimates volume by

up to 550ml. Like our study, a study conducted on validation of geometric measurements against water displacement showed an overestimation and underestimation of volume by the geometric techniques(101, 106).

According to the guidelines of Koo and Li (107), the ICC for the water displacement method could be regarded as good and that of the 3D device as excellent. Similarly, the Bland-Altman plot showed better agreement when the raters used the novel portable three-dimensional infrared imaging device than the reference standard.

The LOAs indicated a considerably larger difference when raters used the water displacement technique than the novel portable three-dimensional infrared imaging device. Concerning intra-rater reliability, both methods had 'good' intra-rater reliability according to the guideline. However, the water displacement method was more reliable than the index test, for which intra-rater reliability was greatly improved when a duplicate measurement was performed. A study conducted on validation of the perometer showed a better inter-rater and intra-rater reliability for the periometer than the water displacement method (103).

These findings have implications for clinical practice and research into leg volume measurement in patients with lymphoedema. Since this portable three-dimensional infrared imaging device could be regarded as having excellent inter-rater reliability and good intra-rater reliability it could be a promising option for the measurement of leg volume in patients with podoconiosis suffering from lymphoedema for clinical trials and monitoring of intervention overtime. There were some limitations to the study. In terms of patient selection, although some laboratory techniques were used to confirm the cause of the lymphoedema, other conditions were only assessed clinically. In addition, we did not measure patients' leg circumferences.

3.12 Conclusions

The novel portable three-dimensional infrared imaging device demonstrated strong near-perfect positive correlation with the water displacement method when used to measure leg volume in patients with lymphoedema, as well as better inter-rater reliability than the reference standard water displacement method. Repeatability was good using

a single measure and improved when the average of two measurements was used. Technically, the novel portable three-dimensional infrared imaging device is quick to use, does not require physical contact with the patient, and can be used for patients with skin lesions.

In addition, it is portable, does not require water or a continuous electrical supply, and can be successfully operated after brief training. This portable three-dimensional infrared imaging device is a promising option for the measurement of leg volume in patients with podoconiosis suffering from lymphoedema for clinical trials and monitoring of intervention overtime. Further efforts to standardize scan acquisition will be important to estimate the exact volume of lymphoedema of patients with podoconiosis.

Chapter Four: Burden of lymphatic filariasis among patients with lymphoedema

4.1 Background: Lymphatic filariasis is a parasitic disease, caused by microscopic, thread-like worms. The adult worms live in the human lymphatic system and release microscopic worms, called microfilariae, into the blood. Approximately 90% of lymphatic filariasis is caused by W. bancrofti. If the infection is left undiagnosed and untreated, it can cause Lymphoedema and, ultimately, the disfiguring and debilitating condition known as elephantiasis.

4.2 Objectives

General Objectives

The general objective of this study was to assess the burden of lymphatic filariasis among chronic patients with lymphoedema.

Specific Objectives

- To determine the prevalence of lymphatic filariasis among patients with lymphoedema
- To assess the magnitude of lymphatic filariasis morbidity
- To assess the correlates of lymphatic filariasis

4.3. Methods

Study Design and Period

The study design was a community based cross-sectional study conducted between October 2019 and January 30, 2020. All consented patients with lymphoedema who came to their nearest health post after mobilized by health extension workers in all the selected study areas were included in the survey. Since the survey was aimed at finding lymphatic filariasis cases it was biased towards selecting endemic villages.

Study settings and Sample Size

The study was conducted in Benishangul Gumuz Region, Western Ethiopia (Figure4.1). This region was selected based on the findings of a previous study conducted by Welelta S et.al. The findings of this study showed; twenty-four (24%) of all the LF cases identified were from Benishangul Gumuz region, which made it the most endemic.

Figure 4.1Map of Benishangul Gumuz Region

To select the zones and woredas, we used data on the number of lymphoedema cases in each village obtained from the zone and district health offices in the region (Table 4.1).

No	Kebele	Sample size	Remark
1	Mender 46	20	
2	Amba 11	19	
3	Amba 1	8	
4	Silga	21	
5	Amba 9	14	
6	Amba 18	8	
7	Megele 38	19	
8	Megele 33	5	
9	Otsa	12	
10	Tulu	15	
11	Mender 40	22	
12	Taji	13	
13	Togo	19	
14	Amba 13	12	
12	Mender 47	25	
13	Mender 41	12	
14	Mender 44	6	
15	Mutsa	9	
16	Mutsa saka	11	
17	Musta sirna	3	
18	Wanba	1	
19	Amba 16	12	
20	Mender 42	7	
21	Yeshishir Butuji	17	
22	Mender 48	14	
23	Yaa’a masara	15	
24	Tsore Camp	3	
25	Mustsa Kiso	6	
26	Mender 49	16	
27	Mender 43	16	

28	Wanga	12	
29	Amba 2	16	
30	Yahoha 15	7	
31	Mengele 31	9	
32	Mender 47	14	
33	Yegure	10	
34	Tsotsora	10	
35	Mimi	15	
36	Abramo	13	
37	Selga	14	
38	Ura	10	
39	Kusmengel	8	
Total		**505**	

Table 4.1 Villages in Benishangul Gumuz region where we collected data on the presence of LF

Processes into field visits:

Before we started the study, we obtained ethical clearance from BSMS Research Governance & Ethics Committee (RGEC) ref no 17/023/DAV and Wollega University institutional review board ref no WU/IRB WU-RTTVP/ 136/09.

First, we discussed and got permission from the Ethiopia Federal Ministry of Health Neglected Tropical Diseases (NTD) Department. The FMOH provided us with the kits required for this study. After getting permission from FMOH we travelled to the Benishangul regional state main city Assosa, where the regional health bureau is located. Here we discussed the details of the study and the areas in which we planned to conduct the study that sat under the bureau. We obtained permission to contact the zonal health bureaus. The zonal health bureaus allowed us to proceed with our study following discussion with the district health offices. Finally, we entered into discussions with the health extension workers (health professionals working at the primary health care unit in the selected kebeles - the smallest administrative unit in Ethiopia) for mobilization of study

participants. The health extension workers were informed to mobilize patients with lymphoedema in their respective kebeles.

Proforma, consent form and information sheet were printed in CDT-Africa, Addis Ababa University College of Health Sciences. Transportation required for the survey was arranged from CDT-Africa for the entire study period.

Participant recruitment Strategy

After the study participants were mobilized to their nearest health post by health extension workers, the purpose of the study and what participation would involve were explained to them in their local language, eligible participants were invited to give signed consent. For those who were unable to sign their names, we used their fingerprint instead.

Data Collection Process and Procedures

After obtaining consent, a small drop of blood was taken to diagnose lymphatic filariasis (Fig4.2)

Specimen Collection and Handling

1. We used the 3rd and 4th fingers and avoided fingers with rings on.
2. Then, we wiped the tip of the appropriately selected finger with an alcohol swab and we let the alcohol air dry.

Figure 4.2 Finger skin puncturing using sterile lancet (picture by Abdi Samuel)

3. After that, a sterile lancet was used to make a skin puncture just off the centre of the finger pad.

4. Wiped off the first drops of blood with a dry gauze.

5. Finally, we collected the blood from the pricked finger directly into the micropipette provided in the test kit (Fig4.3).

Figure 4.3 Collecting blood using micropipette from pricked finger

TEST PROCEDURE USED

1. We removed the test strip from the foil pouch immediately prior to use
2. Then placed the test strip in the plastic work tray, the indicator arrows pointing toward the operator.
3. Labelled the test strip with the patient result sticker
4. Using a micropipette provided in the test kit, slowly added the patient sample to the lower half of the exposed white sample pad by gently squeezing the bulb
5. Set a timer for 10 minutes.
6. Interpreted the results in the result area of the test strip 10 minutes after adding the sample.

RESULT INTERPRETATION

Positive Test Result

A positive test result produces a pink-to-purple control line in the top half of the result area of the test strip and a pink-to-purple test line in the lower half of the result area. Any pink-to-purple visible test line is positive. Results were not interpreted until 10 minutes had elapsed.

Negative Test Result

A negative test result produces a pink-to-purple control line in the top half of the result area of the test strip and the absence of a test line in the lower half of the result area. To ensure that low positive samples have had sufficient time to develop, a negative result should not be recorded until 10 minutes have elapsed from when the sample was added (Fig 4.4).

Invalid Test Result

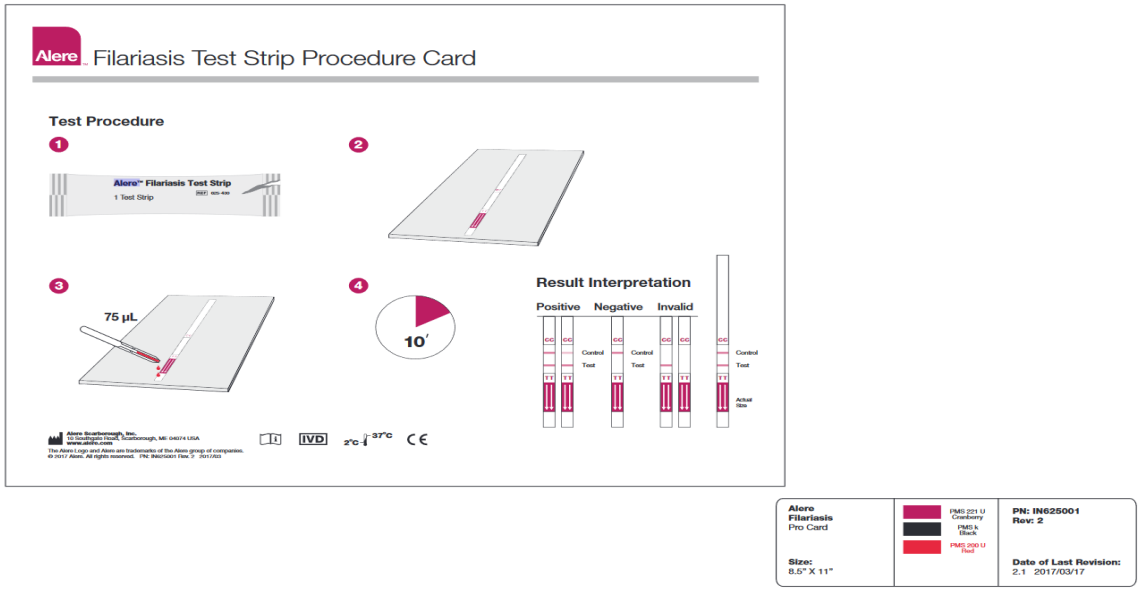


Figure 4.4 Test procedure and Result Interpretation

The test is invalid if the control line in the top half of the result area of the test strip does not appear, whether a test line is present or not.

Those study participants who were positive for FTS were referred to the nearest health center through the health extension worker in their respective village for treatment. Health education on morbidity management was provided for all the study participants at the end of their participation in the study.

Data processing and analysis

Data were entered and analysed using the Statistical Package for Social Sciences (SPSS) software version 25. The overall prevalence of lymphatic filariasis among chronic lymphoedema patients and the clinical features were calculated in percentages. The prevalence of lymphatic filariasis was calculated as the ratio of the number of patients with lymphatic filariasis to the total lymphoedema cases studied.

Chi-square tests were used to determine association of the prevalence of lymphatic filariasis with filariasis morbidity and other related factors. Binary logistic regression analyses were performed to generate odds ratios (OR) including 95% confidence intervals (95% CI) to assess relationships between the independent variables and lymphatic filariasis positive outcomes. Significance level was set at 0.05 and the confidence interval at 95%. A *p* value of less than or equal to 0.05 was considered to indicate statistical significance.

Some of the Challenges During the Data Collection

We faced huge challenges in terms of security and infrastructure (road) since the data collection sites were very remote and difficult to reach (Fig4.5 ,4.6,4.7 & 4.8).

Figure 4.5 Challenge from poor roads

Figure 4.6 Measuring the depth of the water to cross (right) and crossing through the water to reach some of our study sites (left)

Figure 4.7 Testing patients in remote area where we could not use a health facility as a site of screening due to poor infrastructure (road)

Figure 4.7 Testing patients in remote area where we could not use a health facility as a site of screening due to poor infrastructure (road)

4.4 Results of the study

Socio Demographic Characteristics of Study Participants

Between October 2019 and January 30, 2020, a survey of lymphatic filariasis was conducted among patients with lymphoedema. The survey was conducted in 31 villages of Benishangul Gumuz region, Western Ethiopia. A total of 573 study participants were recruited into this study.

The majority (360) of the study participants were female (62.8%). 243 (42.4%) of the study participants were aged over 60 years, 50-59 years accounted for 123 (21.5%) and those between 18 and 29 were 51 (8.9%). The median age of the study participants was 55 (IQR: 40 to 65) (Table 4.2).

Most of the study participants 503 (87.8%) could not read or write. All the study participants resided in rural areas. 374 (65.3%) were married, 102 (17.8%), 49 (8.6%), and 48 (8.4%) were widowed, divorced or single, respectively (Table 4.2).

The occupation of the majority, 499 (87.1%), of the study participants was farmer, 24 (4.2%) were students, both daily labourer and merchant each accounted for 6 (1%) of the study participants. The median duration of stay in the study area was 34 (IQR 18 to 34)

Characteristics	Frequency	Percentage
Sex		
Male	213	37.2
Female	360	62.8
Age group (years)		
<20	26	4.5
21-30	25	4.4
31-40	60	10.5
41-50	96	16.8
51-60	123	21.5
60+	243	42.4
Marital status		
Married	374	65.3
Widowed	102	17.8
Divorced	49	8.6
Single	48	8.4
Occupation		
Farmer	499	87.1
Student	24	4.2
Daily labourer	6	1.0
Merchant	6	1.0
Other	21	3.7
Educational status		
Can read and write	503	87.8
Can't read and write	70	12.2
Duration of stay in the village		
1-10	92	16.1
11-20	91	15.9
21-30	85	14.8
31-40	281	49.0
>40	24	4.2

Table 4.2 Sociodemographic characteristics of the study participants between October 2019 and January 2020

Lymphatic Filariasis Morbidity

44.7% (256/573) of the study participants had moderate lymphoedema, 166 (29%) had mild and 26.4% (151/573) had advanced (severe) lymphoedema (Figure 4.9).

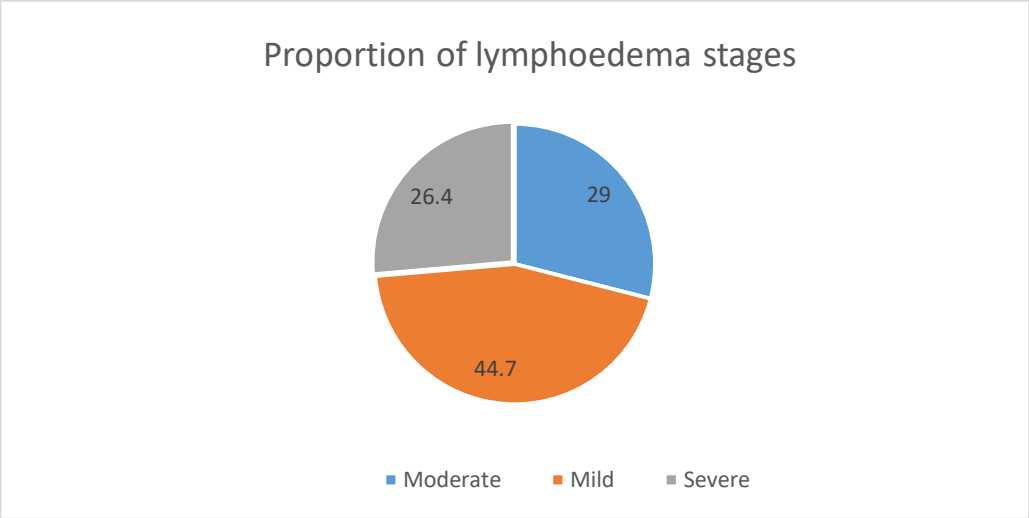


Figure 4.9 Prevalence of clinical stages of lymphoedema, Western Ethiopia, between October 2019 and January 2020

50.4% (298/573) of the study subjects had lymphoedema of both legs and 49.6% (284/573) had lymphoedema of one leg. Concerning the extent of lymphoedema, 59.3% (340/573) of the study participants had lymphoedema that extended above the knee and 40.7% (233/573) below the knee. The prevalence of hydrocele (in males) in this study was 8.5% (18/573) (Table 4.3).

Morbidity	Frequency	Percent
Leg affected		
One leg	289	50.4
Two legs	284	49.6
Extent of the leg		
Above the knee	340	59.3
Below the knee	233	40.7
Hydrocele (male only)		
Yes	18	8.5
No	195	91.5

Table 4.3 Prevalence of lymphoedema morbidity, Western Ethiopia between October 2019, and January 2020

In relation to the distribution of the clinical stages of lymphoedema according to sex, it was found that severe (advanced) cases of lymphoedema were more prevalent among women 44.6% (104) compared with men, 22.1% (47) (Figure 4.10).

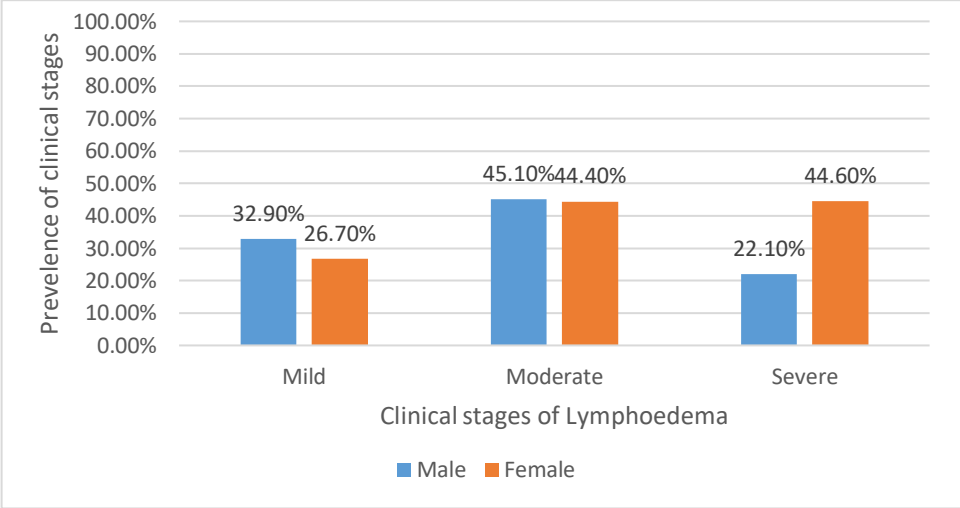


Figure 4.10 Prevalence of clinical stages of lymphoedema by sex, Western Ethiopia, between October 2019 and January 2020.

Prevalence of Lymphatic Filariasis

The overall prevalence of lymphatic filariasis among patients with lymphoedema in the study area was 13.3% (76), (95%CI: 10.6%, 16.3%). The prevalence of a positive filarial test among those with lymphoedema in the selected villages ranged from 0.0 to 100.0%. In 41.9% (13) of the villages surveyed, the positivity rate was zero. The positivity rate in Shoshor was 100% (6/6), 66.7% (2/3) in Mao komo, 50% (3/6) in Tsore camp and 35% (14/40) in Tongo (Table 4.4).

Village	Number examined	Number (%) testing positive
Amba11	45	0
Amba13	12	0
Amba16	16	4 (25)
Amba18	9	0
Amba 2	19	0
Amba 9	15	0
Hoha	10	1 (10)
Maokomo	3	2 (66.7)
Megele 31	9	0
Megele33	5	0
Megele38	21	2 (9.5)
Mender 40	30	5 (16.7)
Mender 41	17	5 (29.4)
Mender 42	7	0
Mender 43	18	2 (11.1)
Mender 44	17	1 (5.9)
Mender 46	48	5 (10.4)
Mender 47	16	5 (31.3)
Mender 48	45	2 (4.4)
Mender 49	21	5 (23.8)
Mutsa	34	5 (14.7)
Otsa	12	0
Shoshor	6	6 (100)
Silga	21	0
Tongo	40	14 (35)
Tsore camp	6	3 (50)
Tulu	21	7 (33.3)
Wanga	15	2 (13.3)
Ya'a masara	15	0
Yagure	12	0
Ye Shishir Butuji	18	0
Total	573	76 100)

Table 4.4 Prevalence of a positive filarial test among patients with lymphoedema by village, Western Ethiopia between October 2019, and January 2020.

The prevalence of lymphatic filariasis among male study participants was 18.3% (39/213) whereas it was 10.3% (37/360) among females. There were differences in lymphatic filariasis infection among male and female study participants (OR= 0.5 95%CI; 0.31 to 0.83) (Table 4.5).

Sex	Number examined	Number positive (%)
Male	213	39 (18.3)
Female	360	37 (10.3)
Total	573	76 (13.3)

Table 4.5 Prevalence of positive FTS test by sex, Western Ethiopia between October 2019, and January 2020.

When the data were analysed by age, the prevalence of a positive FTS test was higher in over sixty-year-olds (44.7%, 34) followed by those who were 41-50 years old (14, 18.4%) (Figure 4.11).

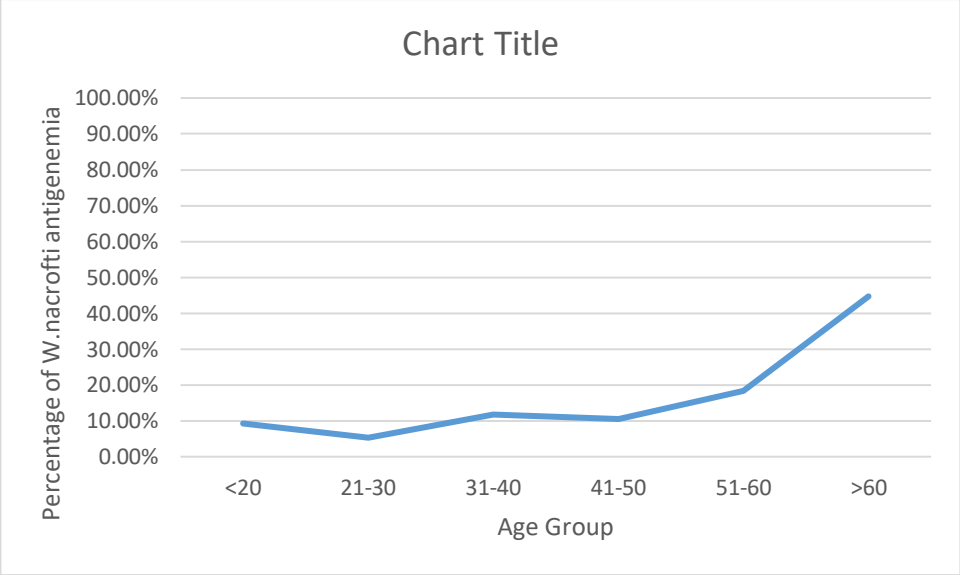


Figure 4.11 Prevalence of lymphatic filariasis infection by age, Western Ethiopia, between October 2019 and January 2020

Regarding the duration of stay in the study area, the highest prevalence was recorded in those who had stayed in the study village between 31 and 40 years (47.4%, 36), followed by those between 11-20 years (17.1%, 13). Those who had stayed between 21-30 years accounted for 14.5% (11) of the cases, those who had stayed between 1 and 10 years accounted for 13.2% (10), and those >40 years 7.9% (6) (Figure. 4.12).

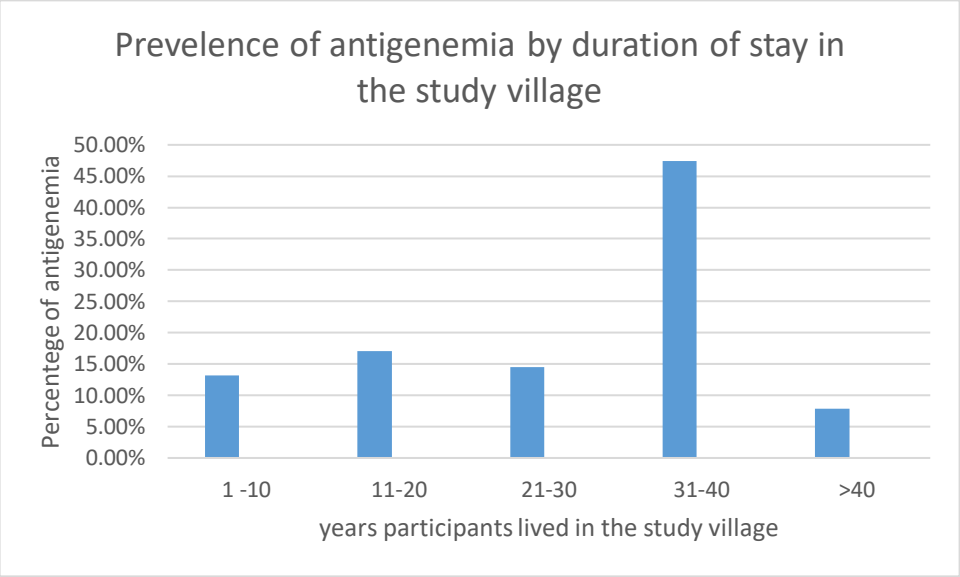


Figure 4.12 Prevalence of positive FTS test by duration of stay in the study village, Western Ethiopia, between October 2019 and January 2020.

Prevalence of positive FTS test by duration of stay in the study village, Western Ethiopia, between October 2019 and January 2020. The prevalence of positive FTS test among study participants with hydrocele was 12.5% (2), (OR= 0.81, 95% CI: 0.813, 3.61).

Among those study participants who had unilateral lymphoedema 11.1% (32) were positive for lymphatic filariasis, while the proportion was 15.5% (44) of those who had bilateral lymphoedema (OR= 1.50, 95% CI: 0.9, 2.4). Comparing study participants with lymphoedema above the knee with that below the knee, 13.5% (46) of those with above the knee swelling tested positive for LF while 12.9% (30) of those with below the knee swelling tested positive (OR=0.95, 95% CI: 0.58, 1.55). (Table 4.6).

Clinical sign	Number examined	Number positive (%)
Hydrocele (male only)		
Yes	18	2 (12.5%)
Leg affected		
One leg	289	32 (11.1%)
Two legs	284	44 (15.5%)
Extent of the leg		
Above the knee	340	46 (13.5 %)
Below the knee	233	30 (12.9%)

Table 4.6 Prevalence of positive FTS test by lymphoedema characteristics, Western Ethiopia between October 2019, and January 2020.

The prevalence of positive FTS test was 16.4% among study participants who had moderate lymphoedema, 15.3% and 13.7% among participants with severe and mild lymphoedema respectively (Figure 4.13)

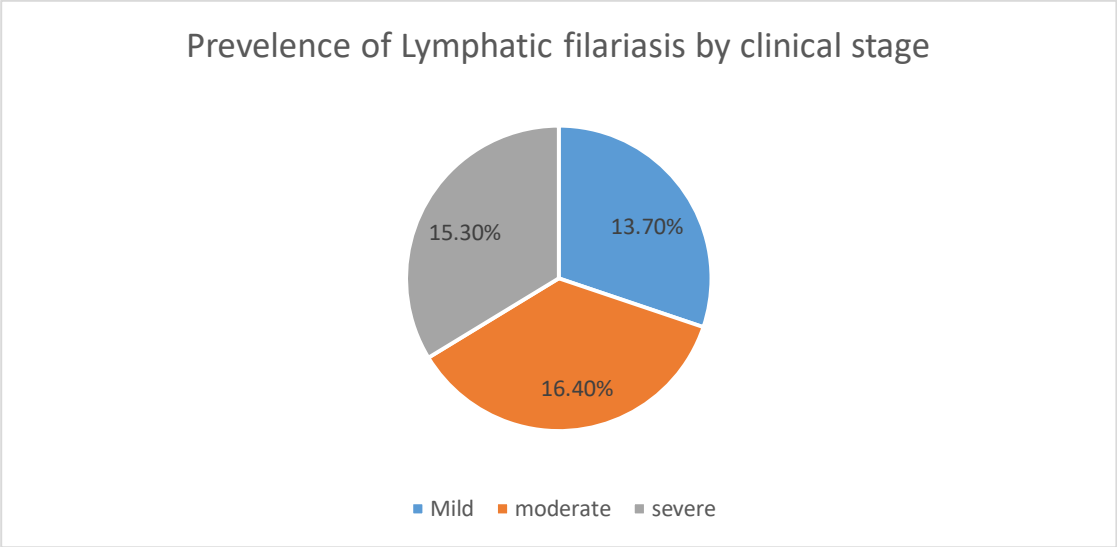


Figure 4.13 Prevalence of positive FTS test by clinical stage, Western Ethiopia, between October 2019 and January 2020.

The trend in Figure 4.14 below showed, highest proportion of W. bancrofti positivity (38.2%) was seen among study participants who had developed lymphoedema between 1 and 10 years previously and became constant and finally decreased the positivity rate among those who had developed lymphoedema for more than 40 years previously.

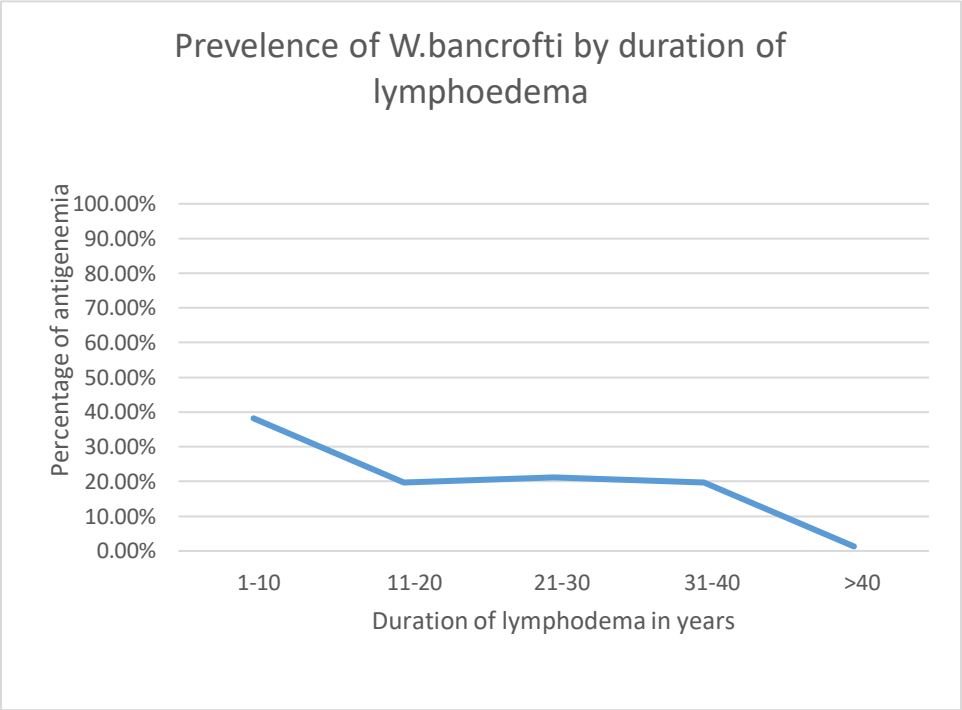


Figure 4.14 Prevalence of positive FTS test by duration of onset of lymphoedema, Western Ethiopia, between October 2019 and January 2020.

Bivariate Analysis

Bivariate logistic regression was performed to describe the univariable association between the FTS test positivity rate and, age, sex, educational status, duration of stay in the study area, duration of the onset of lymphoedema, clinical stage, clinical sign, extent of lymphoedema, leg affected, and presence of hydrocele (Table 4.7).

Variable	Category	Total examined	Positive (%)	Crude OR (95%CI)
Sex	Female	360	37 (10.3)	0.56 (0.31 to 0.83)
	Male	213	39 (18.3)	Reference
Age	>60	243	34 (14.0)	Reference
	<20	26	7 (26.9)	1.92(0.17, 1.13)
	21-30	25	4 (16.0)	1.14 (0.28, 2.6)
	31-40	60	9 (15.0)	1.07 (0.42, 2.04)
	41-50	96	8 (8.3)	0.60 (0.8, 4.02)
	51-60	123	14 (11.4)	0.81(0.65, 2.46)
Educational status	Can read & write	70	12 (17.1)	Reference
	Cannot read or write	503	64 (12.7)	0.71 (0.36, 1.38)
Clinical stage	Mild	166	20 (12.1)	Reference
	Moderate	256	36 (14.1)	1.10 (0.57, 2.16)
	Severe	151	20 (13.2)	0.90 (0.52, 1.68)
Clinical signs	Lymphoedema only	555	74 (1.3)	Reference
	Both lymphoedema & hydrocele	18	2 (1.1)	0.81 (0.81, 3.61)
Leg affected	One leg	289	32 (11.1)	Reference
	Both leg	284	44 (15.5)	1.40 (0.9, 2.4)
Extent of lymphoedema	Above the knee	340	46 (13.5)	Reference
	Below the knee	233	30 (12.9)	0.95 (0.58, 1.55)
Duration of stay in the village	>40	24	6 (25.0)	Reference
	1-10	92	10 (10.9)	0.43 (0.88, 8.49)
	11-20	91	13 (14.3)	0.57 (0.67, 5.98)
	21-30	85	11 (12.9)	0.52 (0.73, 6.87)
	31-40	281	36 (12.8)	0.51 (0.85, 6.09)
Duration of lymphoedema	>40	4	1 (25.0)	Reference
	1-10	235	29 (12.3)	0.49 (0.24, 23.53)
	11-20	122	15 (12.3)	0.49 (0.23, 24.36)
	21-30	89	16 (18.0)	0.72 (0.15, 15.58)
	31-40	123	15 (12.2)	0.49 (0.23, 24.59)

Table 4.7 Results of bivariate analysis of lymphatic filariasis and associated factors among patients with lymphoedema, Western Ethiopia between, October 2019 and January 30, 2020

Multivariable Analysis

To identify independent predictors of positive FTS test among patients with lymphoedema, multivariable logistic regression was performed (Table 4.8)

Variable	AOR	95% CI	P-value
Age	0.98	0.97, 1.00	0.083
Sex			
Female	0.48	0.29, 0.80	0.005
Educational status			
Cannot write or read	0.90	0.37, 2.17	0.808
Live in the kebele	1.01	0.99, 1.04	0.351
Clinical stage			
Moderate	1.10	0.55, 2.15	0.810
Severe	1.10	0.48, 2.33	0.893
Clinical signs			
Both legs	1.02	0.15, 6.98	0.982
Leg lymphoedema			
Both legs	1.31	0.76, 2.27	0.338
Extent of lymphoedema			
Below the knee	0.86	0.48, 1.57	0.630
Duration of lymphoedema	1.01	0.99, 1.04	0.301
Occupation			
All other	0.97	0.35, 2.68	0.949
Marital status			
Married	0.81	0.28, 2.36	0.695
Divorced	0.91	0.27, 3.11	0.879
Widowed	0.77	0.23, 2.64	0.679

Table 4.8 Results of multivariable analysis of FTS positivity and associated factors among patients with lymphoedema, western Ethiopia between, October 2019 and January 30, 2020

The multivariable analysis revealed that only sex was independently associated with FTS test positivity (p <0.005). Being female was associated with a 50% reduction in the odds of FTS test positivity (AOR= 0.48, 95% C.I: 0.29, 0.80).

To examine the effect of clustering due to village, the intraclass correlation coefficient (ICC) was calculated and found to be 0.17 (95%CI; 0.06, 0.27). To further assess the clustering effect of village, fixed model logistic regression was performed but the data did not converge and did not fit the model well since the sample size in some of the villages was small. Therefore, the robust variance estimation was used instead.

The p-value for age in table 4.7 above was 0.083, which was weak evidence against the null hypothesis. After the robust variance estimation to explore the effect of clustering due to village, the p-value for the alternative model was 0.042, the amount of evidence against the null hypothesis has only changed slightly (Table 4.9).

Variable	**AOR**	**95%CI**	**P-value**
Age	0.98	0.97, 1.00	0.042
Sex			
Female	0.48	0.23, 0.83	0.008
Educational status			
Cannot write or read	0.90	0.44, 1.82	0.763
Live in the kebele	1.01	0.98, 1.04	0.449
Clinical stage			
Moderate	1.09	0.48, 2.48	0.842
Severe	1.06	0.34, 3.25	0.924
Clinical signs			
Both legs	1.02	0.17, 6.13	0.981
Lymphoedema leg			
Both legs	1.31	0.63, 2.74	0.476

Extent of lymphoedema			
Below the knee	0.86	0.35, 2.12	0.749
Duration of lymphoedema	1.01	0.98, 1.05	0.362
Occupation new			
All other	0.97	0.34, 2.75	0.950
Marital status			
Married	0.81	0.36, 1.81	0.601
Divorced	0.91	0.30, 2.72	0.864
Widowed	0.77	0.30, 1.97	0.587

Table 4.9 Results of multivariable analysis of FTS test positivity and associated factors among patients with lymphoedema using the robust variance method, Western Ethiopia between, October 2019 and January 2020.

Discussion

This study addresses the distribution of lymphatic filariasis as assessed by positive FTS test among patients with lymphoedema. The overall prevalence of FTS test positivity was 13.3%. Age and sex were independently associated factors.

Among the study participants, those who had developed lymphoedema in the last ten years accounted for the highest filaria positivity rate and those who developed more than forty years previously were least likely to have a positive filarial test.

Overall, prevalence of lymphatic filariasis was 13.3%, which was comparable to the study conducted in Western Ethiopia (12.3%) (25), lower than a study conducted in rural tropical Guinea (25.1%, 61) but greater than a study conducted in North-eastern Tanzania (5.8%, 60)(108). This is possibly because the latter study was conducted after repeated mass drug administration (MDA), while MDA had not been conducted in our study area.

The current study descriptively indicated an increasing trend of filaria positivity rate with age, which is in line with results from other studies (43, 109-111). The increasing trend of positivity rate with age may signify long years of exposure for the mosquito bite. Among the men who reported hydrocele, 2(12.2%) were positive for microfilaria. The positivity

rate among this group was higher in the Western Ethiopia study (35.7%) and lower (1.82%), in a study conducted in Brazil (43, 112).

Gender-wise, the result of the current study showed significantly higher positivity rate in male study participants than their female counterparts, like the North-eastern Tanzania study (8.8%), (108). In the case of the Western Ethiopia study (3.9%), (43), the positivity rate was higher in males than females, though this was not statistically significant. This difference in positivity rate by gender may be due to less exposure of females to infective vectors and some studies suggested increased resistance to infection (113).

Chapter 5. Validating the DLP based NIR Spectrometry for the diagnosis and characterization of Tropical Lymphoedema in Western Ethiopia

5.1 Background

Lymphoedema develops because of damage to the lymphatic system, frequently due to cancer treatment, systemic problems, leprosy, onchocerciasis, podoconiosis or lymphatic filariasis. In the tropics the two most common causes are lymphatic filariasis and podoconiosis.

Lymphoedema can be successfully managed, and it is possible to improve patient outcomes if it is diagnosed at the earliest stage. Improved diagnosis will help to treat lymphoedema patients in a timely way with effective therapy, so a patient can return to full health and before the disease becomes complicated, disfiguring and stigmatizing. In addition, improved diagnosis is also helpful to study the disease history, drug discovery, improve patient care, clinical trials and enhance the effectiveness of therapy**.**

If not diagnosed early and left untreated, lymphoedema may lead to chronic inflammation, recurrent infection, reduced mobility, impaired function and hardening of the skin that, in turn, results in further lymph vessel damage and distortion of the shape of affected body parts (79)**.** The lymphoedema in both podoconiosis and lymphatic filariasis is reversible if diagnosed and treated early, but more advanced stages need lifelong treatment.

Currently, podoconiosis is a diagnosis of clinical exclusion based on history and physical examination. The currently available point-of-care diagnostic tests for lymphatic filariasis infection are not very sensitive in establishing filarial infection among advanced cases. Therefore, the differentiation of podoconiosis from filarial elephantiasis is limited to the use of a panel approach rather than using an accurate diagnostic technique. The aim of this study was to validate the DLP-based NIR spectrometer in the diagnosis and characterization of lymphoedema.

5.2 Objectives of the study

General objective

The general objective of this study was to validate the DLP-based NIR spectrometer for the diagnosis and characterization of lymphoedema

Specific objectives

- To determine the specificity of the DLP-based NIR spectrometer in diagnosing and characterization of tropical lymphoedema
- To determine the sensitivity of the DLP-based NIR spectrometer in diagnosing and characterization of tropical lymphoedema

5.3 Research questions

1. Can the DLP-based NIR spectrometer differentiate lymphoedema secondary to podoconiosis from that of lymphatic filariasis?
2. Can the DLP-based NIR spectrometer differentiate podoconiosis from lymphoedema of other causes (those caused by systemic problems such as renal failure, congestive heart failure etc.)?

5.4. Feasibility Study

Before embarking on the main study, we conducted a feasibility study. The aim of this was to test the DLP-based NIR spectrometer's capacity to differentiate the lower limb in podoconiosis lymphoedema from that of the healthy adult population and to determine if there were optimal sites on the leg and foot for taking readings, to train data collectors, and to calculate a sample size for the main study.

5.5 Methodology summary for the pilot study

The study was conducted in Konchi Clinic, Wayu Tuka *Woreda*, Western Ethiopia, between December 1, 2018, and January 15, 2019. Many studies suggest a sample of twenty to forty (114-116) for a feasibility/acceptability pilot study. Depending on these recommendations and considering the resources and aims of our study, we recruited thirty study participants, twenty podoconiosis patients and ten healthy controls. The study was conducted after obtaining ethical clearance from BSMS Research Governance & Ethics Committee (RGEC) ref no 17/023/DAV and Wollega University institutional review board ref no WU/IRB WU-RTTVP/ 186/09.

After explaining the purpose of the study, patients with lymphoedema were consecutively screened in Konchi Clinic when they came for their regular follow-up visits. Those who were eligible for the study were appointed to come back to stay overnight in the clinic for screening using the device being tested in the study. They were invited to give signed or finger-print consent.

Since podoconiosis is diagnosed by clinical exclusion, we collected a midnight blood sample to exclude lymphatic filariasis. To exclude lymphoedema of other causes, the participants were further screened by physical examination, and family history. Twenty podoconiosis cases were recruited into the study. Ten controls were selected consecutively among clients attending Konchi clinic for other medical services during the study period. The controls were healthy adults, who did not have lymphoedema, a family history of lymphoedema or long-term bare foot exposure and were negative for microfilariae of lymphatic filariasis.

The lower legs of all cases and controls were scanned by the NIR Scanner at ten different sites, which were anatomically identified to determine if there was an optimal area for scanning. To determine an optimal area, we used the compartmental anatomical classification of the lower leg. Anatomy of the lower leg is between the knee and the ankle. The lower leg is divided into three compartments: the anterior, the lateral and the posterior compartment. Therefore, during data collection, the lower leg of the study

participants was marked as anterior, lateral, and posterior. After that the entire length of each compartment was measured using tape (Figure 5.1)

Figure 5.1 Measuring lower leg using tape

Then, each compartment was equally divided into three parts, and we took one scan from each area. Since lymphoedema also involves the foot, these were also scanned. Anatomically the foot is classified as forefoot , middle foot, and hinge foot. The site of preference for the scan was the middle foot since this is the area where there is a large accumulation of fluid. The measurements were taken by two independent raters and each rater took duplicate measurements from each site.

5.6 Methods for the main Study

Study Design: The study was conducted to validate the diagnostic capacity of the DLP based NIR spectrometer device between October 2019 and January 30, 2020. For this study, we recruited four different groups, these were patients with lymphoedema due to podoconiosis, lymphoedema due to lymphatic filariasis, and lymphoedema due to other causes and healthy controls. The controls were people with no lymphoedema who came to Konchi clinic for other services.

Study Settings

The study was conducted in Konchi clinic Wayu Tuka District, Oromia region, Western Ethiopia, Benishangul Gumuz Regional State, Western Ethiopia and two public Hospitals namely Nekemte Specialized Hospital and Wollega University Referral Hospital in Western Ethiopia.

Nekemte Specialized Hospital and Wollega University Referral Hospital are found in the Western part of Ethiopia, East Wollega zone, in Nekemte town. They are serving as a referral centre for the western part of Ethiopia for about 11 million people . The hospitals were established in 1932 and 2009 E.C. respectively.

Konchi Clinic is a non-governmental organization clinic run by the Catholic church. It provides a range of medical services to the surrounding community and offers lymphoedema morbidity management for many lymphoedema patients. From Konchi

Clinic we collected the required number of podoconiosis cases and controls, but we extended our study area to Benishangul Gumuz region to recruit the required number of patients with lymphatic filariasis and to the two hospitals to include patients with lymphoedema of other causes.

Benishangul Gumuz region is endemic for lymphatic filariasis. The region's capital is Assosa. The region has faced major challenges in economic development, lack of transportation and communication infrastructure.

Sample Size

The sample size was calculated using the formula suggested by Hajian-Tilaki (117)as follows, considering the sensitivity of the device,

$$n = \frac{z_{\frac{\alpha}{z}}^2 p(1-p)}{d^2 \times prevalence}$$

$$n = \frac{ncases}{prevalence}$$

$$ntotal = ncases + ncontrol$$

Where n is the total sample size required, d is the margin of error, p is the sensitivity of the test and $Z_{\alpha/2}$ is the z-value corresponding to α/2 probability in each tail of the normal distribution.

Before designing this study, we ran a pilot study (n_{cases}=20 & $n_{controls}$ = 9), this 2x2 table is constructed from the pilot study result for calculating sensitivity and prevalence of podoconiosis for the target population (table5.1).

		Reference Test	
		Positive	**Negative**
Index Test	**Positive**	696	62
	Negative	72	285
	Total	768	347

Fig 5.2 a two-by-two table for calculating sensitivity of the DLP based NIR spectrometer

$$Sensitivity = \frac{true\ positive}{true\ positive + false\ negative}$$

The sensitivity of the test using the above formula was 91.8% (95% CI 89.6 to 93.7%), and prevalence of 68.0% (65.2% to 70.1%). Considering 95% Confidence intervals and a margin of error of 7%, we estimated a total sample size of 87 using the above sample size calculation formula. The number of controls required were 28 and the number of cases of lymphoedema due to each cause was 59, i.e. 59 podoconiosis cases, 59 lymphatic filariasis cases and 59 lymphoedema due to other causes were included in the study.

Source Population

The source population was all lymphoedema patients in the selected study areas, who were 18 years or older

Study Population

The study population was lymphoedema patients who were willing to be included in the study

Inclusion Criteria

For cases

Podoconiosis cases

- 18 years or older
- Negative for midnight blood film examination
- Other causes of lymphoedema excluded using the clinical algorithm

Lymphatic filariasis cases

- 18 years or older
- Positive for *W. bancrofti* microfilaria antigen or Blood film examination

Lymphoedema of other causes

- 18 years or older
- Negative for midnight blood film examination
- Excluded for podoconiosis by the clinical algorithm

For controls

- 18 years or older, without clinical lymphoedema or family history of lymphoedema.

Subject Recruitment

Recruitment of controls

The controls were selected consecutively from people attending the Konchi clinic for other medical services during the study period. They were recruited into the study if they are aged 18 years or above, have no lymphoedema, no history of lymphoedema, and no family history of lymphoedema.

Recruitment of cases

Cases were recruited into the study based on the following definition: podoconiosis case is defined as a person with lymphoedema of the lower limb present for more than 3 months for which other causes (i.e., onchocerciasis, leprosy, systemic disorder) have been excluded and who is negative for *W. bancrofti* microfilariae**.** A patient with lymphatic filariasis is defined as a patient with lymphoedema who is positive for *W. bancrofti* microfilaria. Lymphoedema of other causes is defined as lymphoedema of the leg having excluded podoconiosis by physical examination, clinical examination, and family history, and LF by negative blood film examination/FTS.

Podoconiosis Case Recruitment

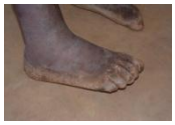
All the required podoconiosis cases for this study were recruited from the previous volume measurement study. During the volume measurement study, we developed a list of 106 podoconiosis cases, so we randomly recruited 58 of them from the list into this study.

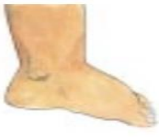
Lymphatic Filariasis case Recruitment

Lymphatic filariasis cases were recruited into the study from the prior study on burden of lymphatic filariasis among patients with lymphoedema conducted in Benishangul Gumuz Regional State, Western Ethiopia.

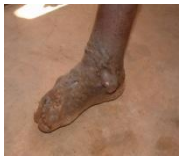
Staging of lymphoedema for lymphatic filariasis

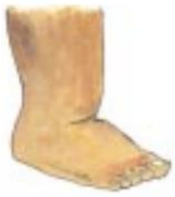
Once the participants were recruited into the study, the severity of leg lymphoedema was staged as mild, moderate, or severe(118). Patients with mild lymphoedema were categorized as those with slight, soft swelling.



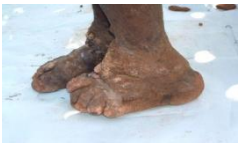


whilst moderate lymphoedema was an enlarged swelling with shallow folds.





and severe lymphoedema being greatly enlarged, with deep folds, and skin changes including mossy lesions and nodules.



In the case of bilateral lymphoedema, the leg with the higher stage was included in the study, since we evaluated only one of the legs of each study participant.

Overview and Measurement Technique using the NIR Scanner

Overview of the device

The DLP based NIR spectrometer is a device that works based on spectrometric principle. Spectroscopy is a powerful technique for recognizing and characterizing physical materials through the variations in absorption or emission of different wavelengths of light by a sample. Spectrometers measure the variation of light absorption of materials. The photo below shows the two components of the DLP based NIR spectrometer (Figure 5.2).

Figure 5.2 The two components of the Nano scan

The instructions for using the DLP based NIR spectrometer are: First, power the DLP based NIR spectrometer by connecting a micro-USB cable or an optional battery and wait for the green LED to pulse. Second, enable the Bluetooth by pressing and holding the scan button on the EVM for more than three seconds. The blue LED will light up to indicate that the Bluetooth circuits are powered and actively scanning. Once the blue LED is on, press Scan on the top-right corner of the android App to initiate a connection. The DLP based NIR spectrometer EVM blue LED will pulse to indicate that a BLE connection was established. Then, the reference and calibration data are downloaded from the EVM. When that is completed, the Start Scan button will be activated. Before starting a scan, set a filename prefix, then pressing the Start Scan button will start a scan with the selected scan configuration. Once the scan completes, the scan data is transmitted from the DLP based NIR spectrometer and plotted.

Measurement Techniques

Once the study participants were recruited into the study, all cases and controls were scanned with the DLP-based NIR spectrometer on their anterior lower leg and middle foot (Figure 5.3). The scans were taken by two independent evaluators and each evaluator took duplicate measurements from each site to check for inter-rater and intra-rater variability of the device.

Figure 5.3 Taking a scan using the DLP based NIR spectroscope

Clinical Data collection

People with lymphoedema due to systemic problems such as congestive heart failure and renal problem were collected from the two hospitals in Nekemte town namely, Nekemte Specialized Hospital and Wollega University Referral Hospital (Figure 5.4). The data was collected from inpatients and the diagnosis was transferred from the patient registry sheet.

Figure 5.4 Collecting data from patients admitted to Nekemte Specialised Hospital

Data Analysis

Repeatability of scan was measured based on Euclidian distance between repeated scans. Each Euclidean distance was calculated based on two repeated scans of the same body location and by the same rater. The Euclidean distance was then normalized against the mean of each spectrum.

Spectra were plotted using raw, normalized (0~1 range), or processed with either SNV+detrend or Savtizky-Golay 2nd derivative. Standard normal variate (SNV) and detrend is a common technique commonly used to pre-process near-infrared reflectance spectra to remove multiplicative scattering effects and slowly varying baseline shifts. The goal is to remove signals irrelevant to the absorbance features, which result in simpler and more robust calibration models(119). Savitzky-Golay 2nd derivative is another important group of techniques for pre-processing NIR spectra to remove spectral variance due to light scattering effect. The difference is that this technique applies smoothing prior to calculating derivatives, which partially offsets the detrimental effect on signal-to-noise

ratios due to derivative calculations. Spectral features related to NIR absorbance and sometimes spike noise become more prominent after taking SG derivatives(120) .

Classification Analysis was done using wavelength truncated at both ends to 997 – 1689 nm. Principal Component Analysis (PCA) with first 6 principal components retained; 5-fold cross-validation was a mechanism to train and report classifier performance by splitting the full data sets into 5 equal sized subsets. Each fold was kept out for validation of model performance while the other folds were used for training. Principal component analysis is a dimensionality reduction technique that can transform the original spectral variable space to a much smaller vector space while still being able to retain most of the embedded information. The reason to do PCA here was to reduce the dimensionality (number of wavelengths in a given spectral scan).

For the classification, we used Support vector machine (SVM). The SVM classifier is a conventional binary classification algorithm that generally works well without a significant amount of parameter tuning. It finds the optimal boundary between classes with maximum separating margins, which results in lower number of misclassifications when applied to test samples. The SVM can effectively deal with some data nonlinearity by projecting data onto high dimensional feature spaces using Gaussian kernels(121, 122).

After obtaining the results, we first plotted the data and checked the overall performance of the tests using Receiver Operating Characteristic (ROC) plots. Then sensitivity and specificity of the devices were calculated.

5.7 Results of the pilot study

Thirty (30) study participants were included in the study - twenty podoconiosis cases and ten controls. Of the twenty cases, ten (50%) were 50 years or older, and the proportion of males and females was equal. With respect to their clinical staging five of them were stage II, six stage III, three stage IV and six stage V. Of the nine controls, 6 were females and 77.8% were aged between 18 and 35 years.

Principal component analysis showed that the scanner could clearly differentiate between cases and controls. The device sensitivity and specificity were 90.6% and 82.1% respectively. The area under the curve was 0.94.

For further analysis, age was split into three groups: young adult (between 18 and 35), middle aged (35 and 50), and senior (50+ years). Age did not impact the spectrum, and neither did gender.

Study participants with higher stages of lymphoedema were more likely to diverge from those with stage II (early) lymphoedema. Clinical stage was one of the variables that impacted the spectrum.

The features that separated cases from controls (PC1 and 2) showed up more strongly at the lower leg scan sites, with the best signal on the foot. The middle foot and anterior lower third seemed to be the best candidates to distinguish patients from controls.

5.8 Results of the main study

The results of the DLP based NIR spectrometer are presented in terms of repeatability of the device and device capacity in classifying the different types of lymphoedema.

Repeatability of the DLP based NIR spectrometer

Repeatability of the device was assessed and presented for all groups of data (podoconiosis, lymphatic filariasis, other forms of lymphoedema and control groups) for all forms of pre-processings (raw, normalized, standard normal variate and SG 2nd derivative), for both raters and measurement locations (anterior lower leg and middle foot).

Control Data

Based on rescaled Euclidean distances, operator 1's and operator 2's measurements on the anterior lower leg processed using raw data indicated very similar median values. Both upper and lower whiskers looked similar. The interquartile ranges have consistent overlaps, suggesting the operators made consistent measurements.

The number of outlying measurements was higher for operator 2, but operator 1 did report a few data points that greatly deviated from the group (Figure 5.5).

On the middle foot, the operators reported nearly the same median value. Though the difference was small, the upper whisker of operator 2 looks larger. The interquartile range showed the operators made consistent measurements (Figure 5.5).

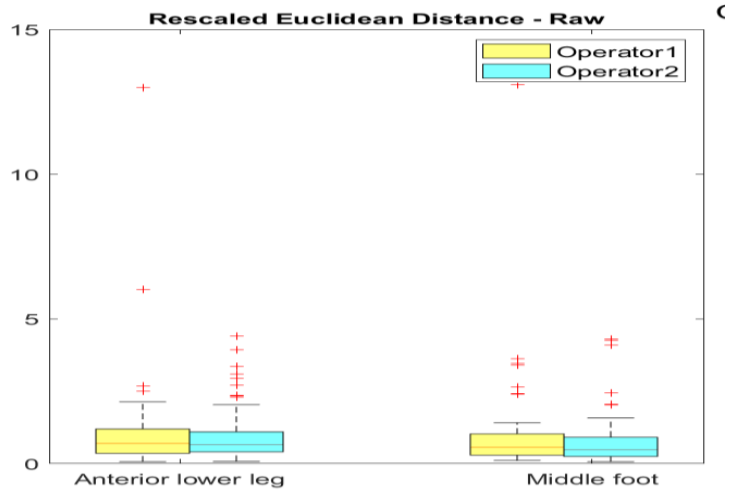


Figure 5.5 Operators' repeatability using Euclidian distance between repeated scans for anterior lower leg and middle foot based on raw control data

The results were similar for the other pre-processings; normalized, Standard normal variate (SNV)+detrend and Savitzky-Golay (SG) 2nd derivative.

The differences between mean spectra at two measurement locations were visually significant and were demonstrated consistently between operators 1 and 2. The graphs show that the patterns between operators 1 and 2 are consistent, both in terms of average values (spectral shape) and spread of the data (error band) (Figure 5.6).

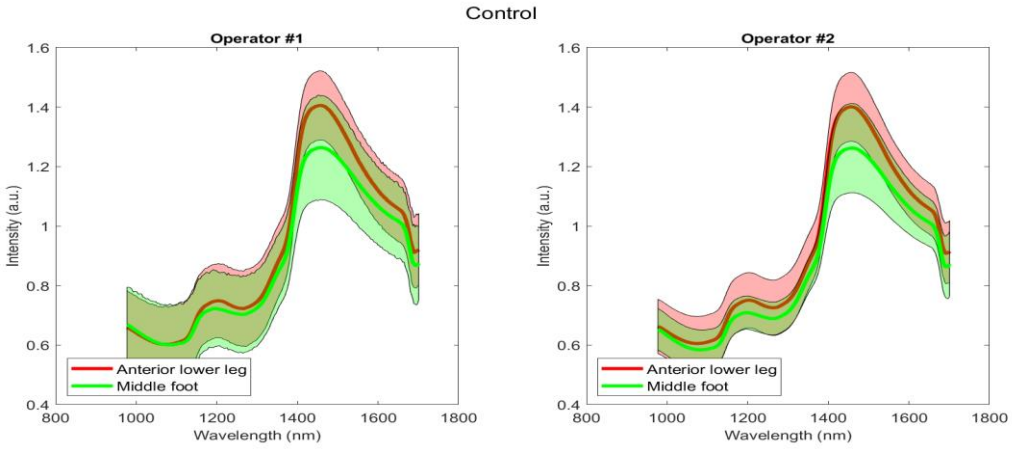


Figure 5.6 Spectra plotted using raw control data from anterior lower leg and middle foot by operator 1 (left) and operator 2 (right).

The spectra of the two operators overlapped, and visually there was no significant difference between the operators at either location. The graphs show that the patterns of difference between the measurement locations were visually significant (Figure 5.7).

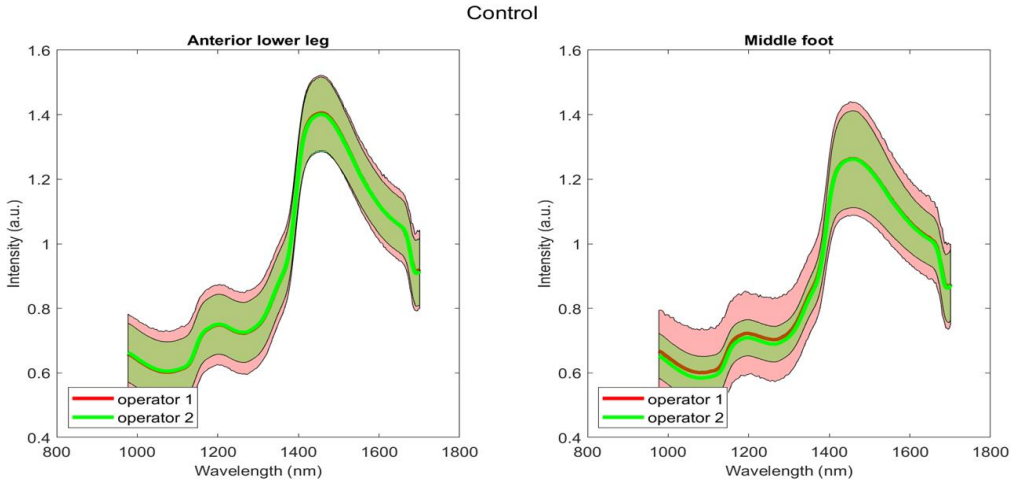


Figure 5.7 Spectra plotted using raw control data by operator 1 and operator 2 from anterior lower leg

The One-way MANOVA was performed on Principal component analysis (PCA) scores of spectra sets of same operator-different locations; and same location-different operators for raw, normalized, SNV +detrend and SG 2nd derivative preprocessing. The results showed a statistically significant difference between different locations for all pre-processing regardless of rater. There was no statistically significant difference between the two operators for any of the four kinds of preprocessing, the difference was mostly attributed to random noise (Table 5.2).

Pre-processing	**Group**	**Estimate of dimension space**	**P-value**
Raw	1	1	0.01
	2	1	0.01
	3	0	0.98
	4	0	0.91
Normalised	1	1	0.01
	2	1	0.01
	3	0	0.79
	4	0	0.98
SNV+detrend	1	1	0.01
	2	1	0.01
	3	0	0.80
	4	0	0.92
SG 2nd Derivative	1	1	0.01
	2	1	0.01
	3	0	0.94
	4	0	0.75

Table 5.2 One-way MANOVA table performed on PCA scores of spectra sets of same operator-different locations; and same location-different operators for raw, normalized, SNV +detrend and SG 2nd derivative pre-processing for the control data.

Group 1: Operator 1: Anterior lower leg VS middle foot

Group 2: Operator 2: Anterior lower leg vs middle foot

Group 3: Anterior lower leg: Operator 1 vs operator 2

Group 4: Middle foot: operator 1 vs operator 2

For the estimation of dimension, a value of 0 means the hypothesis that group mean difference is due to random chances holds true. A value of 1 means that one cannot reject the hypothesis that multivariate means lie on the same line. i.e., the spectral profiles for two measurement locations resemble each other closely.

Lymphatic Filariasis Data

Operator 1's and operator 2's measurement on the anterior lower leg processed using raw data indicated a slightly higher median value for operator 1. The range of data and the interquartile range showed more homogeneous measurements made by operator 2. These findings were quite similar for the middle foot. The number of outlying measurements were similar among the operators, but operator 1 reported one data point that deviated greatly from the group. There was no outlying measurement for operator 1 on the middle foot unlike operator 2 (Figure 5.8). The results were no different for the different methods of pre-processing.

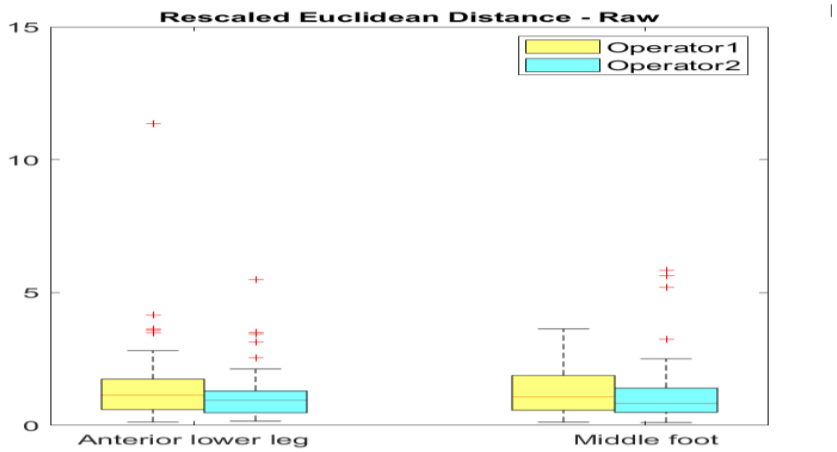


Figure 5.8 Operators' repeatability using Euclidian distance between repeated scans for anterior lower leg and middle foot based on lymphatic filariasis data.

The difference between mean spectra at two measurement locations was visually significant and was demonstrated consistently between operators 1 and 2. The graphs showed that the patterns between operators 1 and 2 were consistent, both in terms of average spectral shape and spread of the data (Figure 5. 9).

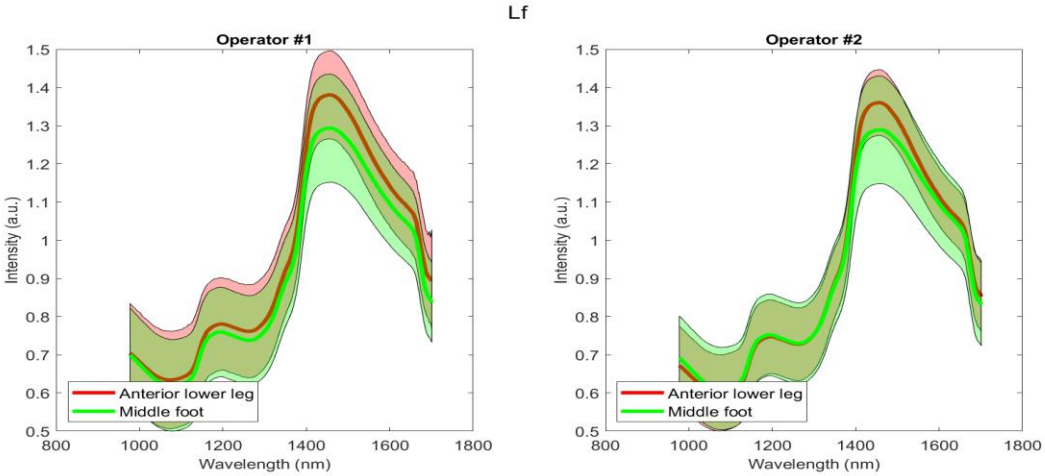


Figure 5.9 Spectra plotted using raw lymphatic filariasis data from anterior lower leg and middle foot by operator 1 (left) and operator 2 (right).

The spectra recorded by the two operators overlapped and visually there was no significant difference by operator for both locations. The graphs showed that the patterns of difference between the measurement locations were visually significant (Figure 5.10).

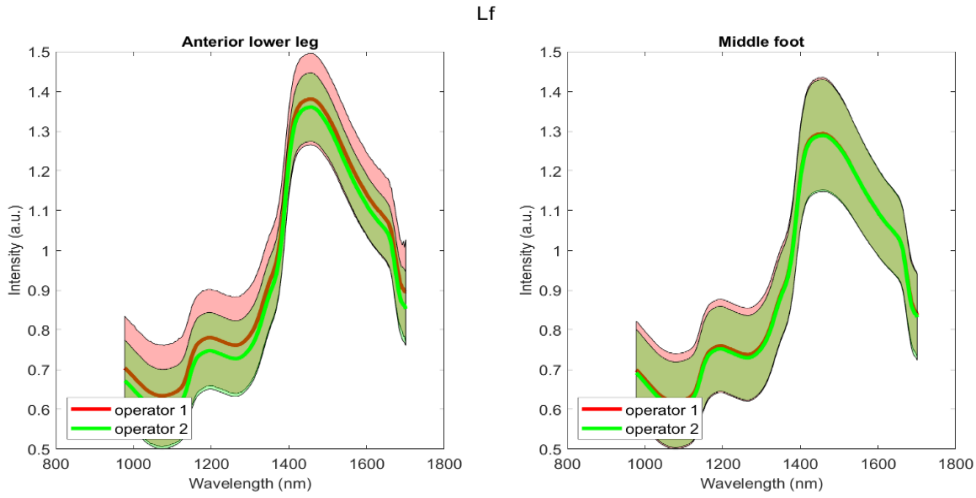


Figure 5.10 Spectra plotted using raw lymphatic filariasis data by operator 1 and operator 2 from anterior lower leg (left) and middle foot (right).

The one-way MANOVA result showed a statistically significant difference between different locations for all scenarios for both operators. There was no statistically significant difference between the two operators for any of the four kinds of pre-processing, the difference was mostly attributed to random noise (Table 5.3).

Pre-processing	**Group**	**Estimate of dimension space**	**P-value**
Raw	1	1	0.01
	2	1	0.01
	3	0	0.47
	4	0	0.97
Normalised	1	1	0.01
	2	1	0.01
	3	0	0.87
	4	0	0.29
SNV+detrend	1	1	0.01
	2	1	0.01
	3	0	0.84
	4	0	0.76
SG 2nd Derivative	1	1	0.03
	2	1	0.01
	3	0	0.64
	4	0	0.64

Table 5.3 One-way MANOVA table performed on PCA scores of spectra sets of same operator- different locations; and same location- different operators for raw, normalized, SNV +detrend and SG 2nd derivative pre-processing for the lymphatic filariasis data.

Other lymphoedema data

Similar median values were observed on the anterior lower leg between the operators. On the anterior lower leg, operator 2's data is more consistent compared to that of operator 1. The range of data and the interquartile range showed more variability for operator 1. The number of outlying measurements was higher for operator 1, but this operator reported few data points that greatly deviated from the group (Figure 5.11).

On the middle foot, the operators reported very similar median values. Though the difference was small, the upper whisker of operator 1 looked larger. The interquartile range showed the operators made consistent measurements (Figure 5.11). The results were similar for normalized, SNV +detrend and SG 2nd derivative pre-processing.

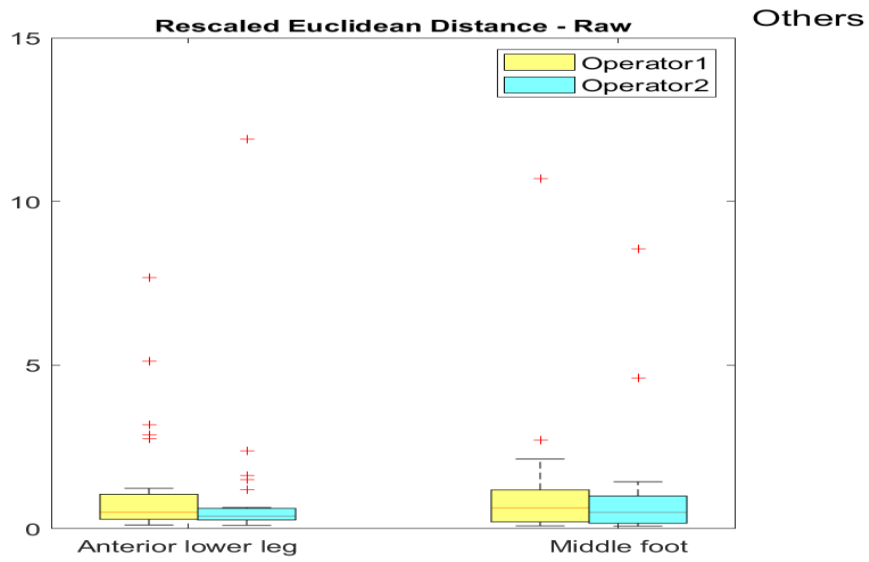


Figure 5.11 Operators' repeatability using Euclidian distance between repeated scans for anterior lower leg and middle foot based on other lymphoedema data

The difference between mean spectra at two measurement locations was visually significant and was demonstrated consistently between operators 1 and 2. The graphs showed that the patterns between operators 1 and 2 were consistent, both in terms of average values and error band (Figure 5.12).

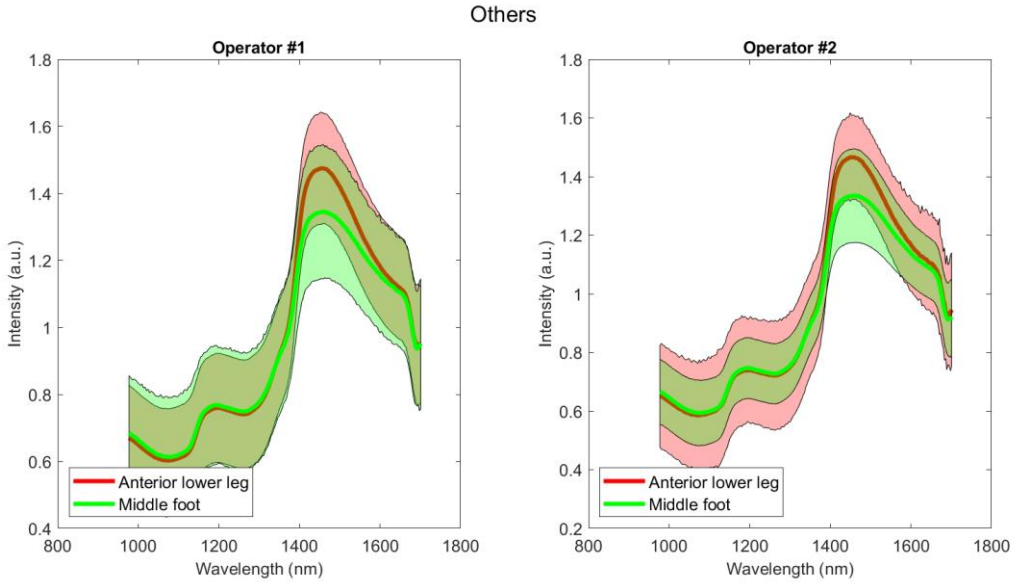


Figure 5.12 Spectra plotted using raw other lymphoedema data from anterior lower leg and middle foot by operator 1 (left) and operator 2 (right).

The two operators' spectra overlapped and visually there was no significant difference between the operators. This was consistently demonstrated at both locations. The graphs showed that the pattern of difference between the measurement locations was visually significant (Figure 5.13).

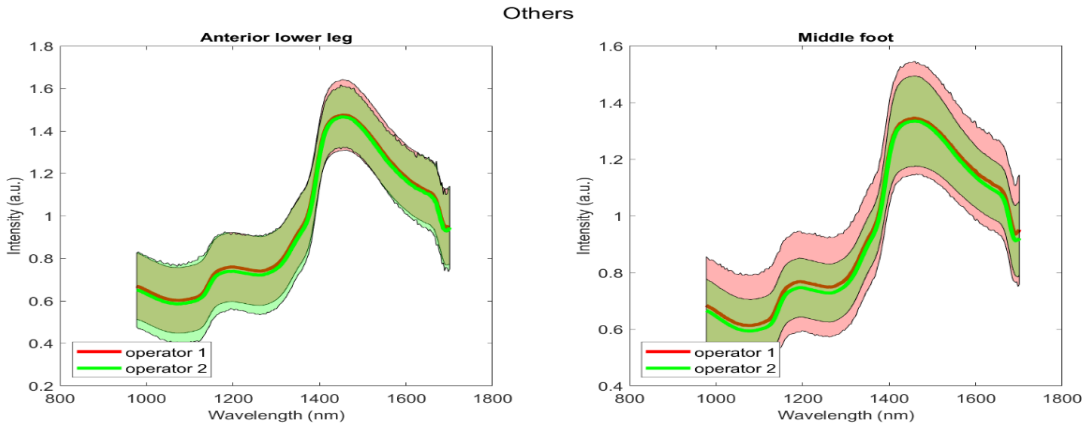


Figure 5.13 Spectra plotted using raw other lymphoedema data by operator 1 and operator 2 from anterior lower leg (left) and middle foot (right).

The one-way MANOVA analysis result showed a statistically significant difference between different locations for all scenarios for both operators. There was no statistically significant difference between the operators for any of the four kinds of pre-processing, the difference was mostly attributed to random noise (Table 5.4).

Pre-processing	Group	Estimate of dimension space	P-value
Raw	1	1	0.01
	2	1	0.01
	3	0	0.99
	4	0	0.96
Normalised	1	1	0.01
	2	1	0.01
	3	0	0.79
	4	0	0.94
SNV+detrend	1	1	0.01
	2	1	0.01
	3	0	0.73

	4	0	0.91
SG 2nd Derivative	1	1	0.01
	2	1	0.01
	3	0	0.27
	4	0	0.92

Table 5.4 One-way MANOVA table performed on PCA scores of spectra sets of same operator different locations; and same location different operators for raw, normalized, SNV +detrend and SG 2nd derivative pre-processing for the lymphatic filariasis data

Podoconiosis data

Operator 1's and operator 2's measurements on the anterior lower leg processed using raw data indicated a larger operator 1 median value. Both upper and lower whiskers looked similar. The interquartile range also looked very close between the operators, so the operators made consistent measurements. (Figure 5.14).

On the middle foot, the operators reported very similar median values. The interquartile range showed that operator 1 reported more variability in measurements. The upper whisker of operator 1 looked larger (Figure 5.14). The results were similar for normalized, SNV +detrend and SG 2nd derivative pre-processing.

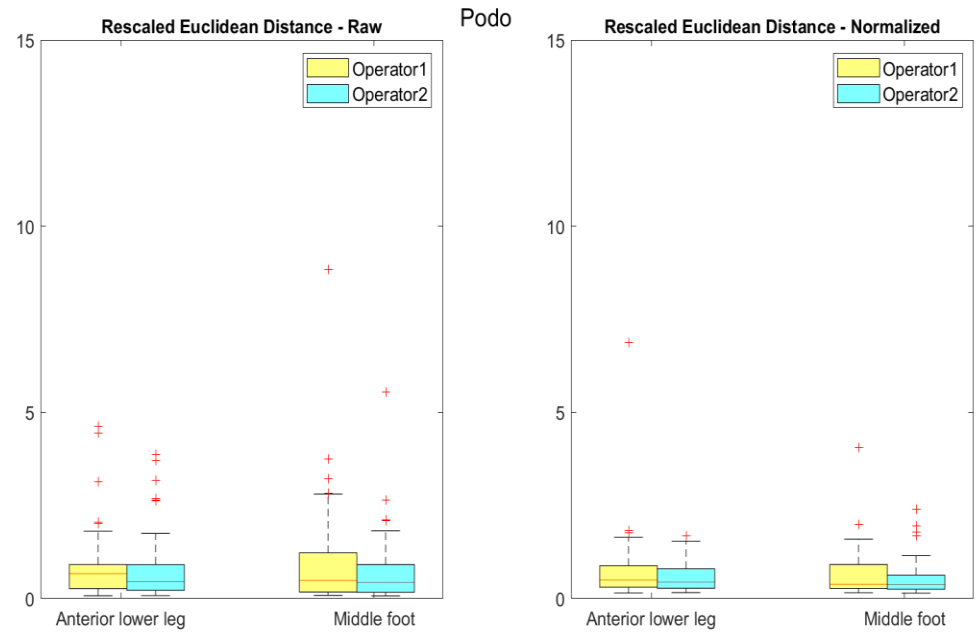


Figure 5.14 Operators' repeatability using Euclidian distance between repeated scans for anterior lower leg and middle foot based on podoconiosis data.

The difference between mean spectra at two measurement locations was visually significant and was demonstrated consistently between operators 1 and 2. The graphs show that the patterns between operators 1 and 2 were consistent, both in terms of average values (spectral shape) and spread of the data (error band) (Figure 5.15).

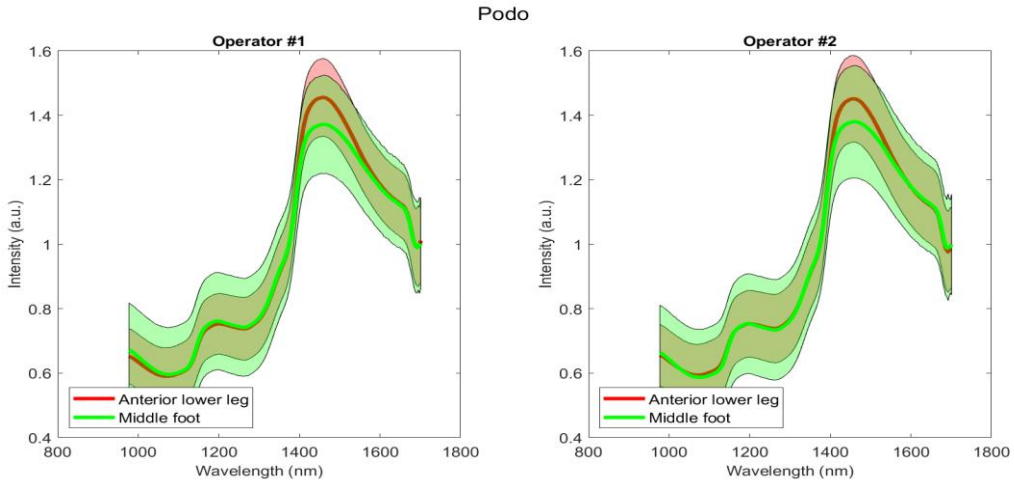


Figure 5.15 Spectra plotted using raw podoconiosis data from anterior lower leg and middle foot by operator 1 (left) and operator 2 (right).

The two operators' spectra overlapped and visually there was no significant difference between the operators at either location. The graphs showed that the patterns of difference between the measurement locations were visually significant (Figure 5.16).

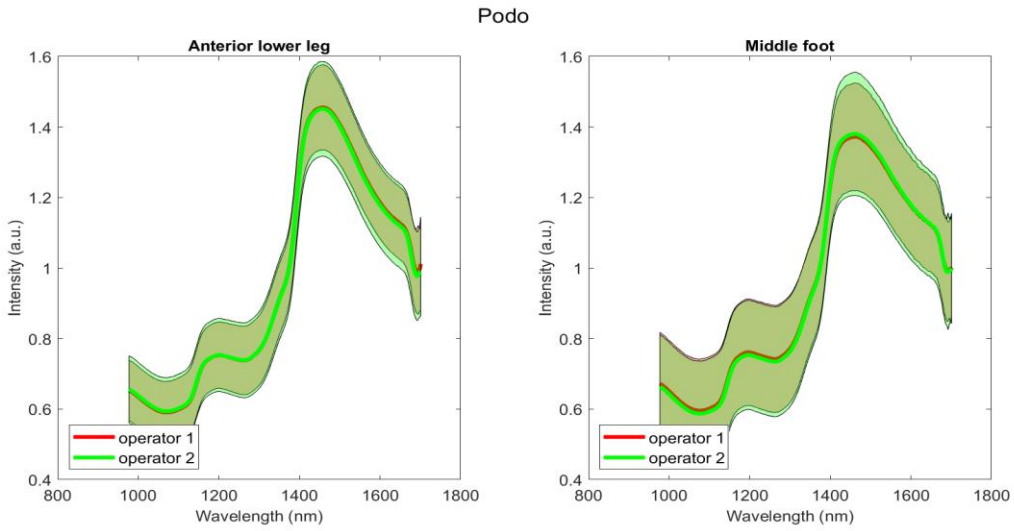


Figure 5.16 Spectra plotted using raw podoconiosis data by operator 1 and operator 2 from anterior lower leg (left) and middle foot (right).

The result showed a statistically significant difference between different locations for all scenarios for both operators. There was no statistically significant difference between two operators for any of the four kinds of pre-processing, and the difference was mostly attributed to random noise (Table 5.5).

Pre-processing	Group	Estimate of dimension space	P-value
Raw	1	1	0.01
	2	1	0.01
	3	0	0.70
	4	0	0.98
Normalised	1	1	0.01
	2	1	0.01
	3	0	0.78
	4	0	0.89

SNV+detrend	1	1	0.01
	2	1	0.01
	3	0	0.55
	4	0	0.91
SG 2nd Derivative	1	1	0.01
	2	1	0.01
	3	0	0.48
	4	0	0.94

Table 5.5 One-way MANOVA table performed on PCA scores of spectra sets of same operators- different locations; and same location- different operators for raw, normalized, SNV +detrend and SG 2nd derivative pre-processing for the podoconiosis data.

Classification Analysis

Experiments for classification analysis was performed across different categories; Control versus podoconiosis, lymphatic filariasis (LF) versus podoconiosis, podoconiosis versus lymphatic filariasis and other forms of lymphoedema; and control versus everything else; by rater, and measurement location. Sensitivity, specificity, and area under the curve for each of the categories are summarized in a table and the graphs for the ROC curve and confusion matrixes are annexed.

Control versus podoconiosis

Classification analysis based on operator and location

Operator one, Anterior lower leg

The sensitivity of the device in classifying the sample into control and podoconiosis was 0.84 and the specificity was 0.82 for mean spectra pre-processing, for normalised pre-processing the sensitivity was 0.84 and specificity was 0.75. For SNV mean spectra, sensitivity was 0.88 and 0.73 specificity, for SG-2 mean spectra sensitivity was 0.86 and specificity was 0.91.

The area under the curve (AUC) for the mean spectra was 0.898 (Figure 5.17).

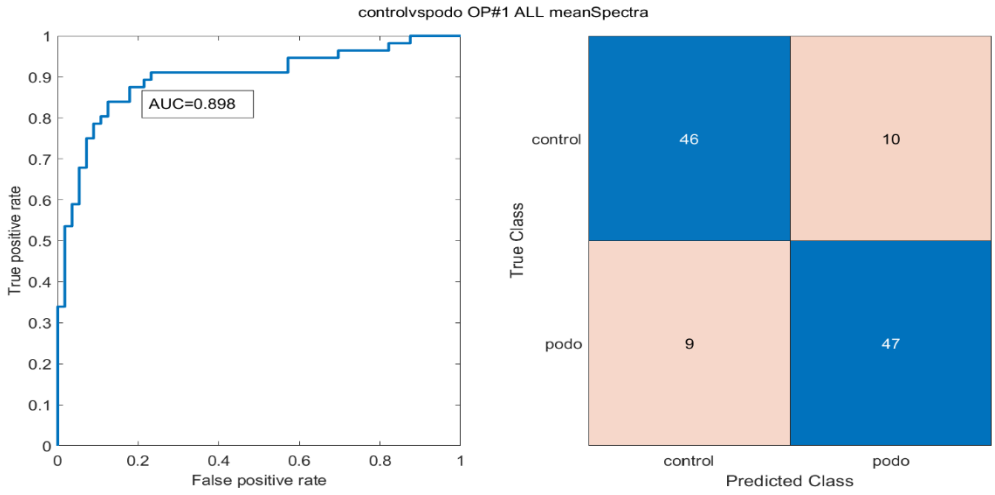


Figure 5.17 Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on mean spectra for control versus podoconiosis.

The sensitivity, specificity, area under the curve for both operators , both locations and for all the four pre-processing are provided in the table below (Table 5.6) and the ROC curves and the confusion matrices are annexed.

Operator and site	**Sensitivity**	**Specificity**	**AUC**
Operator-1 ALL			
Mean spectra	0.84	0.82	0.898
Normalised	0.84	0.75	0.852
SNV	0.88	0. 73	0.821
SG-2	0.86	0.91	0.936
Operator-1 MF			
Mean spectra	0.88	0.91	0.914
Normalised	0.77	0.75	0.858
SNV	0.88	0.88	0.882
SG-2	0.91	0.89	0.925

Operator-2 ALL			
Mean spectra	0.79	0.84	0.88
Normalised	0.77	0.71	0.846
SNV	0.88	0.82	0.852
SG-2	0.91	0.89	0.944
Operator-2 MF			
Mean spectra	0.80	0.91	0.915
Normalised	0.75	0.77	0.801
SNV	0.79	0.79	0.883
SG-2	0.86	0.86	0.942

Table 5.6. Sensitivity, specificity and area under the curve for both operators, both locations and for all pre-processing for control versus podoconiosis categories. ALL; anterior lower leg, MF; middle foot

Lymphatic filariasis versus podoconiosis

The sensitivity of the device in categorising lymphoedema into lymphatic filariasis and podoconiosis by operator one, on anterior lower leg was 0.82 and the specificity was 0.86 for mean spectra pre-processing. For normalised pre-processing the sensitivity was 0.84 and specificity was 0.80. For SNV mean spectra, sensitivity was 0.84 and specificity was 0.77, for SG-2 sensitivity was 0.84 and specificity was 0.66. On the middle foot, the sensitivity for mean spectra, normal mean spectra, SNV mean spectra and SG-2 mean spectra was 0.91, 0.93, 0.84 and 0.88 respectively and the specificity was 0.75, 0.86, 0.80 and 0.79 respectively.

For operator -2, on the anterior lower leg (ALL), the sensitivity was 0.77 based on the mean spectra, 0.86 based on normalised mean spectra, 0.84 and 0.80 for SNV and SG2 mean spectra respectively. The specificity was 0.77, 0.82, 0.79 and 0.70 based on mean spectra, normalised, SNV and SG2 mean spectra respectively. On the middle foot the sensitivity for mean spectra, normal mean spectra, SNV mean spectra and SG-2 mean spectra was 0.91, 0.93, 0.82 and 0.70 respectively and the specificity was 0.84, 0.80, 0.75 and 0.84 respectively (Table 5.7).

Operator and site	Sensitivity	Specificity	AUC
Operator-1 ALL			
Mean spectra	0.82	0.86	0.908
Normalised	0.84	0.80	0.89
SNV	0.84	0.77	0.879
SG-2	0.84	0.66	0.853
Operator-1 MF			
Mean spectra	0.91	0.75	0.885
Normalised	0.93	0.86	0.925
SNV	0.84	0.80	0.893
SG-2	0.88	0.79	0.861
Operator-2 ALL			
Mean spectra	0.77	0.77	0.823
Normalised	0.86	0.82	0.902
SNV	0.84	0.79	0.874
SG-2	0.80	0.70	0.831
Operator-2 MF			
Mean spectra	0.91	0.84	0.934
Normalised	0.93	0.80	0.924
SNV	0.82	0.75	0.86
SG-2	0.70	0.84	0.82

Table5.7. Sensitivity, specificity and area under the curve for both operators, both locations and for all pre-processing for lymphatic filariasis versus podoconiosis.

Control vs Everything else

'Everything else' means all forms of lymphoedema included in the study; podoconiosis, lymphatic filariasis and other forms of lymphoedema. In this category, all rater data were combined and varied by location only.

On the anterior lower leg, the sensitivity of the device in categorising into control or all forms of lymphoedema was 0.86 and the specificity was 0.82 for mean spectra pre-processing. For normalised pre-processing the sensitivity was 0.76 and specificity was 0.68. For SNV mean spectra, sensitivity was 0.85 and specificity was 0.80, for SG-2 sensitivity was 0.90 and specificity was 0.88. On the middle foot, the sensitivity for mean spectra, normal mean spectra, SNV mean spectra and SG-2 mean spectra was 0.89, 0.85, 0.88 and 0.89 respectively and the specificity was 0.80, 0.70, 0.79 and 0.86 respectively (Table 5.8).

Operator and site	Sensitivity	Specificity	AUC
ALL			
Mean spectra	0.86	0.82	0.905
Normalised	0.76	0.68	0.791
SNV	0.85	0.80	0.897
SG-2	0.90	0.88	0.945
MF			
Mean spectra	0.89	0.80	0.894
Normalised	0.85	0.70	0.839
SNV	0.88	0.79	0.874
SG-2	0.89	0.86	0.934

Table 5.8. Sensitivity, specificity and area under the curve for both operators, both locations and for all pre-processing for control versus everything else

Podoconiosis versus Lymphatic filariasis and other lymphoedema

On the anterior lower leg, the sensitivity of the device in categorising lymphoedema into podoconiosis, lymphatic filariasis and other forms of lymphoedema was 0.88 and the specificity was 0.79 for mean spectra pre-processing. For normalised pre-processing the sensitivity was 0.88 and specificity was 0.76. For SNV mean spectra, sensitivity was 0.96 and specificity was 0.65, for SG-2 sensitivity was 0.85 and specificity was 0.73.

On the middle foot, the sensitivity for mean spectra, normal mean spectra, SNV mean spectra and SG-2 mean spectra was 0.84, 0.93, 0.82 and 0.90 respectively and the specificity was 0.78, 0.73, 0.76 and 0.75 respectively (Table 5.9).

Operator and site	Sensitivity	Specificity	AUC
ALL			
Mean spectra	0.88	0.79	0.877
Normalized	0.88	0.76	0.857
SNV	0.96	0.65	0.848
SG-2	0.85	0.73	0.864
MF			
Mean spectra	0.84	0.78	0.874
Normalized	0.93	0.73	0.864
SNV	0.82	0.76	0.872
SG-2	0.90	0.75	0.884

Table 5.9. Sensitivity, specificity and area under the curve for both operators, both locations and for podoconiosis versus Lymphatic filariasis and other lymphoedema

5.8 Discussion

The DLP based NIR spectrometer has many functions and there are several studies that have shown its importance. To mention some, a study conducted to assess the near infrared spectroscopy for dermatological application showed, spectra could be classified according to lesion type and resulted in accuracies of 70–98% in differentiating benign from pre-malignant or malignant lesions and concluded that DLP based NIR spectrometer is a promising non-invasive technique for the screening of skin lesions (123). Another study also indicated the importance of DLP based NIR spectrometer in accurately assessing ambulatory venous dysfunction in patients with primary varicose veins (124). DLP based NIR spectrometer is also used in a rapid classification of corn varieties, and this study showed best classification accuracy up to 80% and concluded that DLP based NIR spectrometer could be used for corn variety identification (125). However, there is no study to my knowledge that is studied on tropical lymphoedema to contextualise my study with, therefore this current study discussed only its own findings.

This discussion is presented in two parts: repeatability and classification

Repeatability

For the control data, the median values of both operators were similar for both normalised and raw pre-processing on the anterior lower leg, but operator 1 recorded slightly larger median values than operator 2 for middle foot in case of both raw and normalised pre-processing. The normalised pre-processing had a short upper and lower whisker for both operators on the anterior lower leg, which means it had lower variability compared with the raw pre-processing, the measurements of operator 2 had a smaller number of outlier values for the normalised than the raw for the anterior lower leg but almost no observable improvement for the middle foot.

For the lymphatic filariasis (LF) data, the median value of the operator 1 on the anterior lower leg and middle foot for raw pre-processing was slightly larger than operator 2. Similar differences were also observed for the normalised pre-processing of the LF data.

For the other lymphoedema category data, there was a very narrow interquartile range for operator 2 on the anterior lower leg for raw data. Operator 1 made more variable

measurement in raw pre-processing compared with the normalised one both on anterior lower leg and middle foot, however operator 2's measurements on anterior lower leg were nearly similar for both pre-processing's but operator 2 made precise measurements on the normalised 1 on middle foot.

For podoconiosis data, the median value of operator 1 is slightly larger than operator 2 for the raw pre-processing, but this was not true for the middle foot. In case of the normalised pre-processing, the median values for both operators were similar.

A spectral graph was performed to examine reliability of both operators, for all categories (podoconiosis, lymphatic filariasis, lymphoedema of other category, and control) based on the four pre-processing (raw, normalised, SNV +detrend and SG2). The graphs showed, the spectra of the two operators overlapped at the same measurement locations, and visually there was no significant difference between the operators for all groups and for all pre-processing on both anterior lower leg and middle foot.

The spectral graphs plotted to visualise the patterns of the spectra for the measurement locations (middle foot and anterior lower leg) among all groups and all pre-processing generally showed a visually significant difference and these differences were demonstrated consistently between operators.

Classification

Though sensitivity and specificity must be discussed in pairs and from the same test, in this paragraph I have tried to show the best sensitivity and specificity obtained in this study. The best sensitivity was 0.96, which was found in classifying podoconiosis from lymphatic filariasis and other lymphoedema category. The best specificity was 0.93 which was obtained in discriminating control from podoconiosis on the middle foot by operator 2. As to the area under the curve (AUC), the best was 0.945 which was in discriminating control versus everything else on the anterior lower leg.

In classifying lymphatic filariasis from podoconiosis, based on all four types of pre-processing technique, the sensitivity range of operator 1 on the anterior lower leg was between 0.82 and 0.84, this showed a narrow difference in sensitivity across the pre-processing s. The same operator on the middle foot made sensitivity range between

0.84 to 0.91, better sensitivity on the middle foot than anterior lower leg. Operator 2 made a sensitivity range of 0.77 to 0.86 on the anterior lower leg based on all-pre-processing technique, which is not a large difference compared with operator 1 on same location and it was 0.70 to 0.93 on the middle foot, this 93% sensitivity was the best sensitivity obtained in classifying the lymphoedema to podoconiosis and lymphatic filariasis, it means the device was able to correctly classify 93% of the lymphoedema to podoconiosis and lymphatic filariasis. This is a promising result at this stage of the device. In terms of specificity, it ranged between 0.66 and 0.86 by operator 1 and it was between 0.70 and 0.82 by operator 2 on the anterior lower leg. It was between 0.75 and 0.86 and 0.75 and 0.84 by operator 1 and operator 2 on the middle foot respectively. The overall range of specificity was 0.66 to 0.86, which means true podoconiosis cases were wrongly diagnosed as lymphatic filariasis (14% for the 86% specificity and 34% for the 66%). The overall range of AUC was between 0.82 and 0.93, which is an excellent AUC score, even outstanding.

Here the device was assessed in discriminating all forms of lymphoedema into podoconiosis and none podoconiosis. The sensitivity range on the anterior lower leg was between 0.88 and 0.96 and it was 0.84 to 0.93 on the middle foot. The specificity range was between 0.65 and 0.79 on the anterior lower leg and 0.73 to 0.78 on the middle foot. Both in terms of sensitivity and specificity the difference was not large between the anterior lower leg and the middle foot. The AUC ranged between 0.848 and 0.877 on the anterior lower leg and 0.864 to 0.844 on the middle foot.

In discriminating podoconiosis from everything else (all lymphoedemas and control), the sensitivity range was 0.76 to 0.9 on the anterior lower leg and 0.85 to 0.89 on the middle foot. The specificity ranged between 0.68 and 0.88 on the anterior lower leg and 0.70 to 0.86 on the middle foot. The ranges for both specificity and sensitivity on both locations were almost consistent. As to the AUC, it was between 0.791 and 0.945 for anterior lower leg and 0.839 to 0.934 for the middle foot.

5.9 Conclusions and Recommendations

In conclusion, all four groups (podo, LF, control, other) demonstrated consistent patterns in repeatability evaluation; There were no statistically significant differences between raters for any combination of site and/or group. There were statistically significant differences between different measurement locations (anterior lower leg vs middle foot) for all groups, regardless of rater and pre-processing.

Regarding classification, the device was able to discriminate the control group from podoconiosis, lymphatic filariasis from podoconiosis, control from everything else and podoconiosis from all forms of lymphoedema. The sensitivity and specificity were slightly different across the groups, between anterior lower leg and middle foot, between operators and different pre-processing approaches. So, the device is promising to be used as a diagnostic tool in discriminating podoconiosis, however it needs further study on the variability using different NIR scanners and it also need to further work on optimizing the pre-processing that will be recommended for implementation in clinical practice and working on the way the device can provide results in the field setup.

Chapter 6. Testing the Zurich Instruments (ZI) Multi Frequency Digital Impedance Analyser's (MFIA) On Patients with Podoconiosis (ZI MFIA DEGITAL impedence analyser): a pilot study

6.1 Background

Podoconiosis (endemic non-filarial elephantiasis) is a noninfectious geochemical disease arising in barefoot subsistence farmers who are in long-term contact with irritant red clay soil of volcanic origins. The disease causes progressive bilateral swelling of the lower legs. Mineral particles absorbed through the skin are taken up into macrophages into the lymphatic system and result in an inflammatory process leading to fibrosis and obstruction of the vessels. This leads initially to swelling (lymphoedema) of the foot and the lower leg, which may with time progress to elephantiasis: gross lymphoedema with mossy and nodular changes of the skin.

Tropical lymphoedemas have overlapping clinical manifestations, so an early, accurate diagnostic test that will reliably distinguish the different diseases is important. This will enable the patient to be treated in a timely way with effective therapy, returning them to full health before complications are experienced. In addition, clear diagnosis is helpful to study the disease history, for drug discovery research, and enhance the effectiveness of therapy**.** Currently, clinical exclusion is the most common approach to podoconiosis diagnosis.

The Zurich Instruments (ZI) multi frequency impedance analyser (ZI MFIA digital impedance analyser) is a device under development as part of this project at university of Sussex, school of engineering and informatics. The aim of developing this device is to use this device as a point of care diagnostic technique for tropical lymphoedema diagnosis . It is based on differentiating the composition of the lymphoedema so that based on the composition we can tell the cause of the lymphoedema. However, currently the device development is at a prototype stage, therefore the aim of this pilot study was to test the ZI MFIA digital impedence analyser capacity to acquire scans and to differentiate patients with podoconiosis from those who do not have podoconiosis. The ZI MFIA digital impedence analyser is a digital impedance analyser and precision LCR meter

that sets the new standard for impedance measurements in the frequency range from 1 mHz to 500 kHz (extended to 5 MHz when upgraded). An LCR meter is a type of electronic test equipment used to measure the inductance (L), capacitance (C), and resistance (R) of an electronic component. The MFIA device used in this study is an upgraded device. The MFIA has a basic accuracy of 0.05% and operates over a measurement range spanning from 1 mΩ to 1 TΩ. It is also characterized by a high measurement repeatability and small temperature drift. MFIA comes with the LabOne user interface and with the multi frequency impedance test fixture (MFITF).

6.2 Objectives

General Objective

The general objective of this study was to test the ZI MFIA digital impedence analyser ability to acquire readings from the lower limbs of patients with podoconiosis and those who are apparently healthy and do not have podoconiosis and its feasibility and acceptability.

Specific Objectives

- To test whether the ZI MFIA digital impedance analyser can acquire readings from the lower limbs of patients with podoconiosis and those who do not have podoconiosis

6.3 Methods

Study Design

The study design was a cross-sectional study

Source Population

The source population was all podoconiosis patients attending the lymphoedema management service, and apparently healthy individuals who were companions of patients visiting Konchi Clinic during the study period in Wayu Tuka District, Oromia Region, Ethiopia.

Participant recruitment strategy

Podoconiosis patients were identified from lists of podoconiosis patients selected for the prior studies conducted using the NIR-based spectroscopy and the novel portable three-dimensional device, whereas those who do not have podoconiosis were recruited consecutively until we reached the required sample.

Study Population

The study population was podoconiosis patients who were selected from the prior patients podoconiosis list for the DLP based NIR based spectrometer and the novel portable three-dimensional infrared imaging device and consenting apparently healthy individuals.

Sample Size

For this study we recruited forty-five study participants in a 2:1 split. Thirty (30) patients with podoconiosis and fifteen (15) apparently healthy individuals without podoconiosis. The apparently healthy group were selected from companions of patients who visited Konchi Clinic during the study period.

Inclusion Criteria for selecting patients with podoconiosis

Study participants must:

- Be 18 years or older
- Have a diagnosis of podoconiosis
- Volunteer to be included into the study

Inclusion Criteria for selecting study participants who do not have podoconiosis

Study participants:

- Must be 18 years or older
- Do not have podoconiosis
- Apparently healthy
- Volunteer to be included in the study

Recruitment

Then, all the study participants were assessed using the ZI MFIA digital impedance analyzer by two independent raters, with each rater performing duplicate measurements in quick succession (the study participant staying on the bed). The aim of using two independent raters was to assess the inter-rater reliability between the methods. Taking duplicate measurements was to check the intra-rater reliability and repeatability of the method.

The following preparations and necessary precautions were strictly followed during the study

- We let all the study participants rest at the study site for at least two hours prior to the measurement, in order to avoid changes in vascular perfusion, temperature, cutaneous blood flow, vasodilation, and fluid losses
- Study participants were oriented not to use alcohol within 12h prior to measurements
- Use of diuretics was prohibited
- Study participants with renal or heart failure history were not included in the study

Study participant preparation

- Lie in the supine position for at least 5 minutes
- Ensured that their feet are not in contact with the bed frame (if in supine position) or any metal frame on which they are sitting
- Extend legs in forward direction (with no contact with each another or any other part of the body)
- Remove stockings/socks/clothes which cover the region to be tested
- Ensured that the tested region of the body (i.e. leg) should be cleaned properly (preferably through alcohol swab) and completely dried. Products such as body moisturiser can affect the results
- Removed any leg jewellery

- Ensured the tested region should be hair free for a firm electrode connection. The anatomical location(s) should be shaved (if required). For electrode positioning and frequency protocols see annex-3.

Electrode topologies: Different electrode arrangements were used to take measurements using the ZI-MFIA digital impedance analyser, depending on the leg side (left or right) and the region where diagnosis was required. We used topology - 2 for the pilot study. The different topologies are annexed for reference in the thesis (annex 20).

Data Acquisition and Management

This section discusses how the measurements were saved using the LabOne graphical user interface. In our case, the measurement acquisition was in the form of plots. The measured data was viewed in a configurable numerical display using the “Numerical Tab” of the graphical user interface.

The acquired data with the LabOne sweeper toolset was saved to CSV, ZView, MATLAB and HDF5 format.

HDF5 format was used to reconstruct the acquired data in the LabOne graphical user interface for analysis and future reference. ZView format was used for further equivalent circuit modelling with specialized software tools. CSV format was used for further analysis based on numerical data using statistical software (i.e., SPSS).

Once saved, the measurements were stored on the webserver locally within the LabOne user interface. The measured data was downloaded from the webserver and saved on cloud storage for sharing.

This pilot study was conducted in Konchi clinic, Wayu tuka District, Oromia region, Western Ethiopia. Thirty (30) patients with podoconiosis and fifteen (15) apparently healthy individuals without lymphoedema were recruited into the study.

The study aimed to assess whether the ZI MFIA digital impedence analyser could acquire readings from the lower limbs of patients with podoconiosis and from people who do not have podoconiosis. We were also interested in the device's capacity to differentiate podoconiosis cases from healthy controls, and in the inter- and intra-observer measurement repeatability. Since both lymphatic filariasis and podoconiosis are diseases that mainly affect the poor, we also tested the device's capacity using both AC and DC (solar battery, and chargeable power banks) power sources. This was intended to ensure that the device should work for those who are living in hard-to-reach areas where there is no access to electricity.

The ZI MFIA digital impedence analyser measures the electrical properties of the subject or item under observation. It assesses how well the subject or item impedes electric current flow (e.g., fat has high resistivity and blood has lower resistivity). In this study, the device helped us to detect the composition of the lower leg. Both lymphatic filariasis and podoconiosis cause lymphoedema. However, the composition of the affected limb is likely to be different, therefore, we hope this device will enable differentiation between different causes of lymphoedema.

The lower legs and feet of all study participants were assessed using the ZI MFIA digital impedance analyser by two independent raters, with each rater performing duplicate measurements in quick succession (the study participant stayed on the bed). The aim of using two independent raters was to assess the inter-rater reliability between the methods, while taking duplicate measurements enabled assessment of the intra-rater reliability and repeatability of the method. During the assessment, necessary preparations and precautions mentioned under the methodology part were taken into consideration.
Measurements were taken over four frequency groups and using two test signal amplitudes (Table 6.1). Based on data set 1 (Table 6.1(a)), four measurements were taken, using each frequency group and a 1mA test signal, and four more measurements were taken with the same frequency groups and a test signal of 10mA (table 6.1(b)) by one rater. The second rater followed the same procedure. Both raters together collected

16 measurements from one study participant. Hence, a total of 720 measurements were taken for this study from the 45 study participants. We kept a note of electrode model number and type used during data collection, to understand the properties of electrodes during measurements.

Patient 1 (Data Set 2)	
Frequency Group	**Test Signal**
Group 1: 1 kHz to 100 kHz	**10 mA**
Group 2: 100 kHz to 1 MHz	**10 mA**
Group 3: 1 MHz to 5 MHz	**10 mA**
Group 4: 10 kHz to 5 MHz	**10 mA**

(a)

Patient 1 (Data Set 1)	
Frequency Group	**Test Signal**
Group 1: 1 kHz to 100 kHz	**1 mA**
Group 2: 100 kHz to 1 MHz	**1 mA**
Group 3: 1 MHz to 5 MHz	**1 mA**
Group 4: 10 kHz to 5 MHz	**1 mA**

(b)

Table 6.1: Different frequency groups and test signals based on which data were collected from each study participant

For the data collection, we used the topology between the patella (knee) and ankle region (Figure 6.1). We placed the voltage electrodes (HPOT & LPOT) 5-10 cm on either side of the midpoint, and the current electrodes (HCUR & LCUR) at 5 cm distance of each voltage electrode (i.e., HPOT & LPOT).

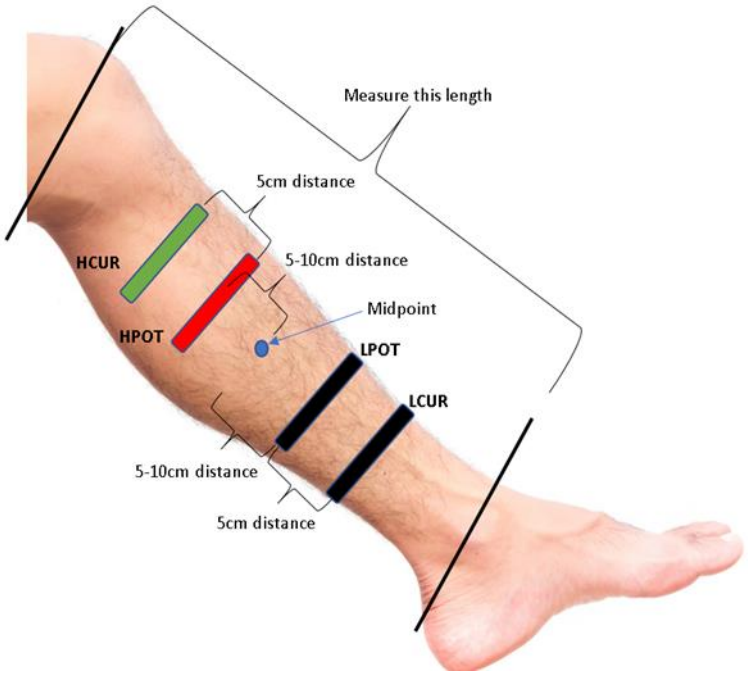


Figure 6.1: Topology used to acquire measurement from the lower leg

All the acquired data were saved to CSV, ZView, MATLAB and HDF5 formats. The HDF5 format was used to reconstruct the acquired data in the LabOne graphical user interface for analysis. ZView format was used for further equivalent circuit modelling. The CSV format will be used for further analysis based on numerical data using statistical software (i.e., SPSS) once the work is completed. The collected data were analysed by Dr Tabassum Qureshi at the University of Sussex, School of Engineering, and Informatics.

During the pilot stage, we have shown that the device was able to acquire measurements from study participants with podoconiosis and from healthy controls. The collected data could be retrieved using CSV, ZView, MATLAB, or HDF-5 formats. It has also been possible to generate reconstructions of these scans in the form of graphs. Further analyses are underway. The graphs below presented, one complete set of data from one study participant with podoconiosis using phase plot (Figure 6.2 & 6.4) and impedance plot (Figure 6.6 & 6.8) and one complete set of data from the healthy control group using phase plot (Figure 6.3 & 6.5) and impedance plot (Figure 6.7 &6.9), to indicate that we can reconstruct the collected data both from control and patients with podoconiosis for all

groups of frequency and both test signals using our new ZI MFIA digital impedence analyser.

These figures also indicated, at least descriptively, a clear difference in pattern of the graphs between the control and patients with podoconiosis. As seen in comparing Figure 6.2 and Figure 6.3, the difference in pattern between the podoconiosis phase plot frequency group1 and that of the control are typically different. Similarly, a clear difference is observed in comparing the other frequency group of the phase plot of the podoconiosis with the control in Figure 6.2 and Figure 6.3. The difference is consistently seen in the 10mA test signal. In general, there is an observable difference between the case and control data pattern for all groups of frequency and both test signals for both phase plot and impedance plot.

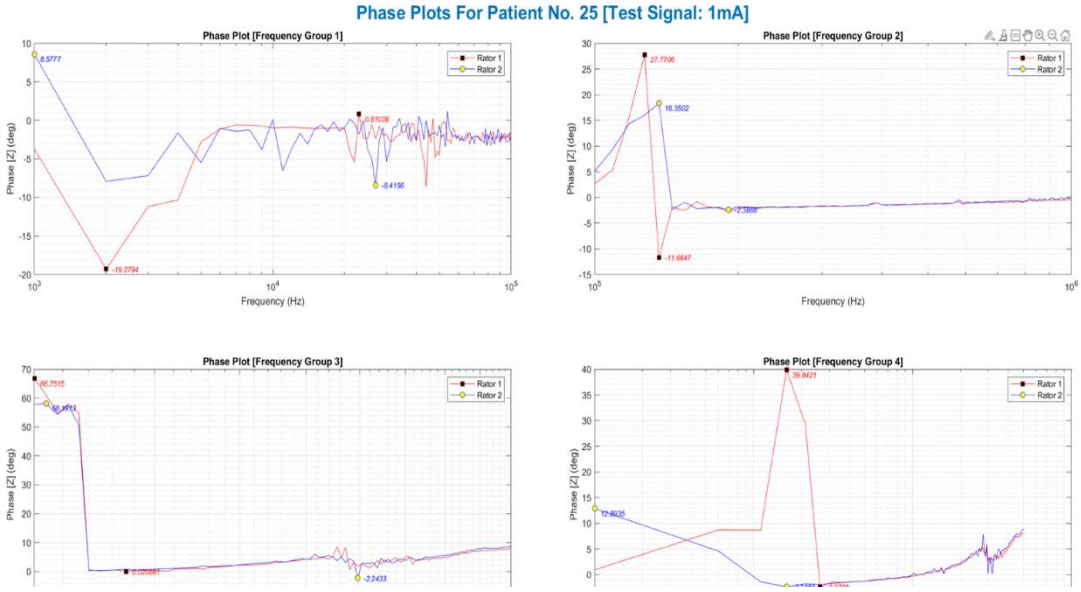


Figure 6.2 Phase plot of graphically reconstructed data of patients with podoconiosis for all the four-frequency group using test signal of 1mA

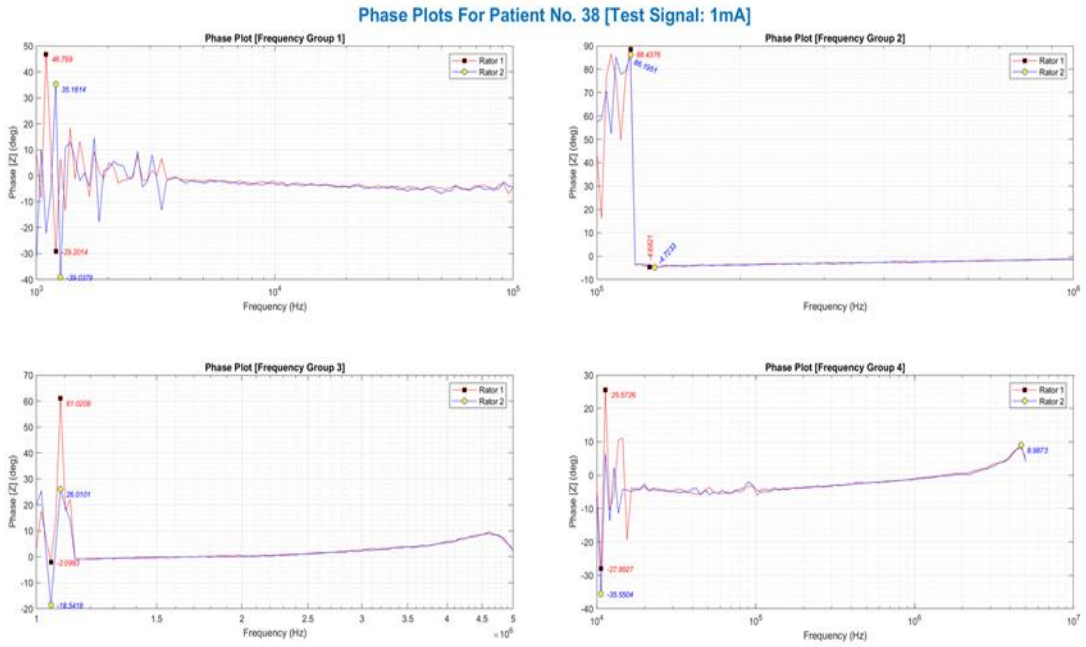


Figure 6.3 Phase plot of graphically reconstructed data of healthy control for all the four-frequency group using test signal of 1mA

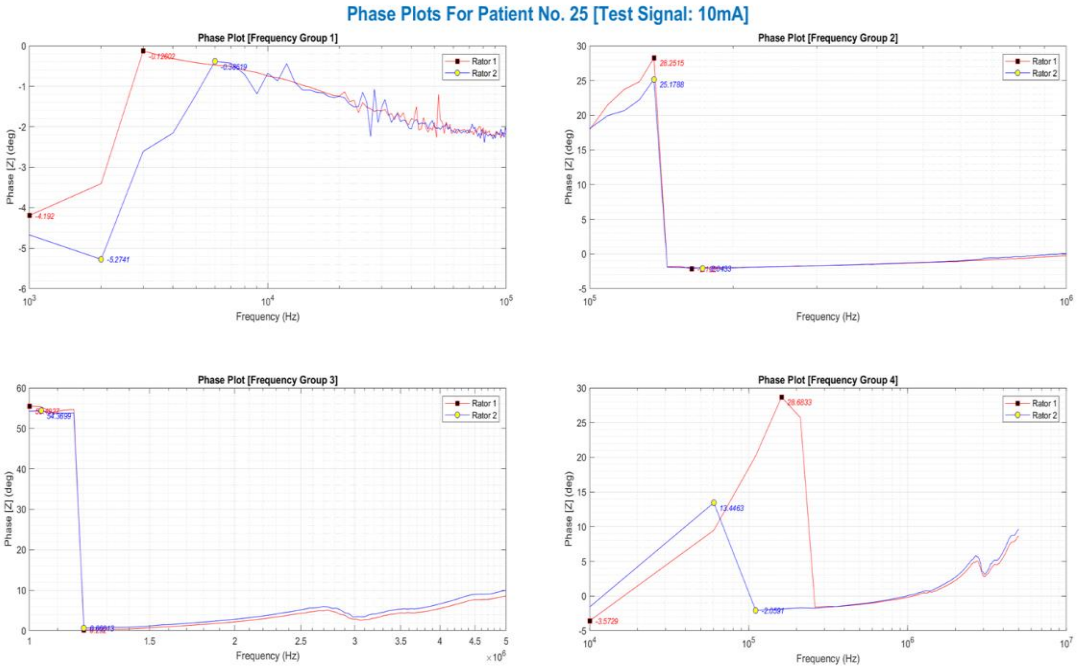


Figure 6.4 Phase plot of graphically reconstructed data of patients with podoconiosis for all the four-frequency group using test signal of 10mA

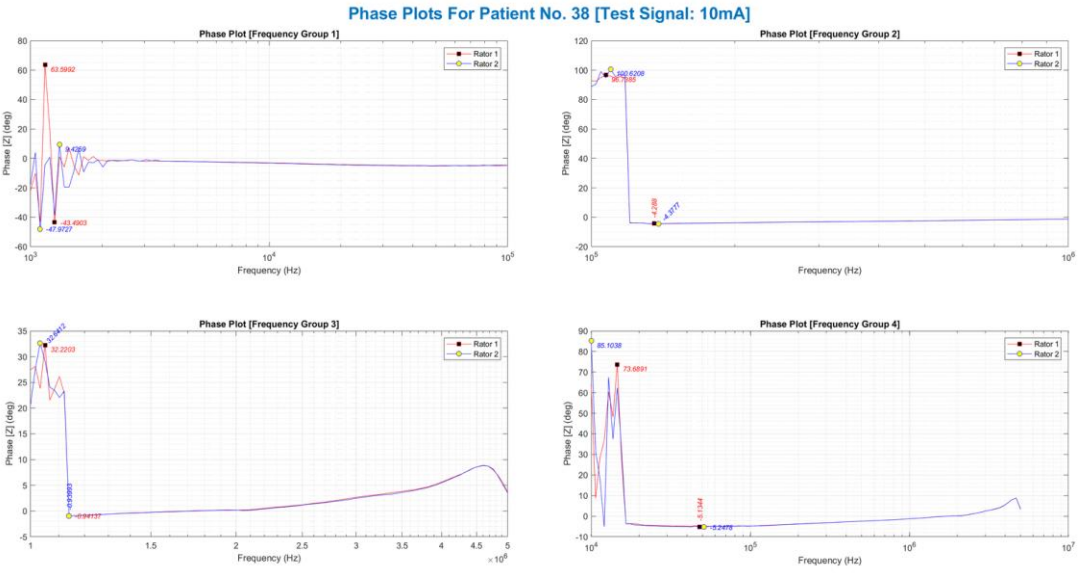


Figure 6.5 Phase plot of graphically reconstructed data of healthy control for all the four-frequency group using test signal of 10mA

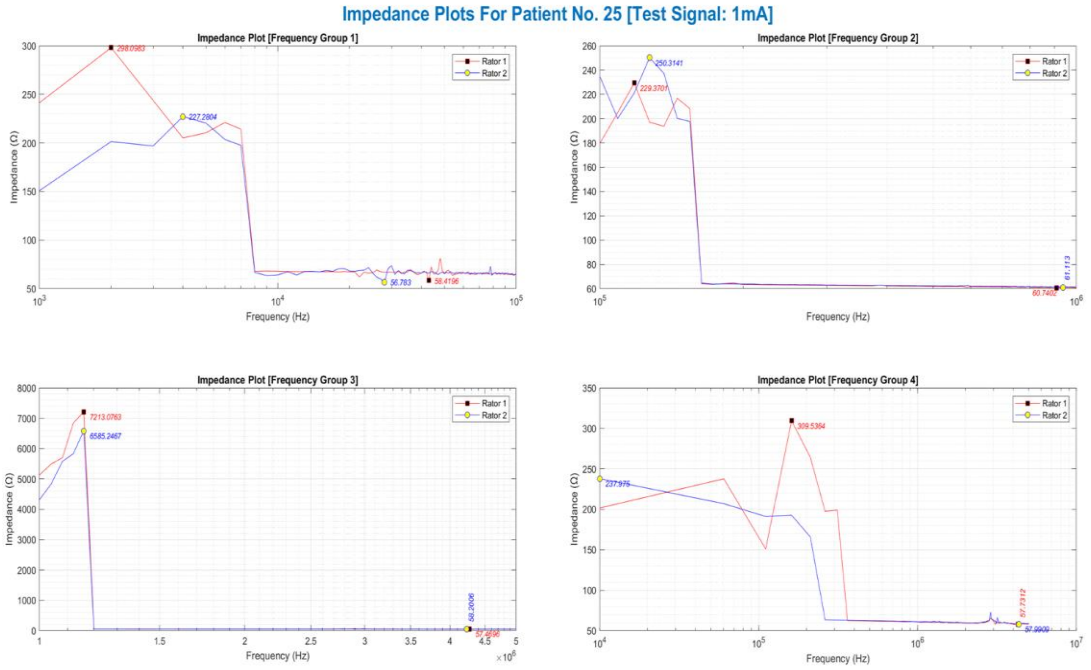


Figure 6.6 Impedance plot from reconstructed data of patients with podoconiosis for all the four-frequency group using test signal of 1mA

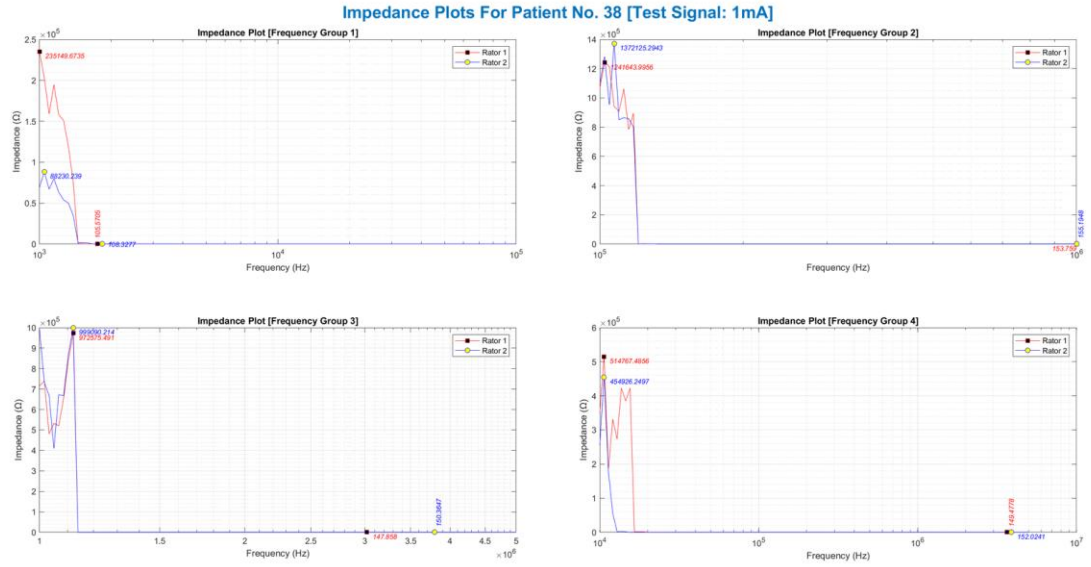


Figure 6.7 Impedance plot from reconstructed data of healthy control for all the four-frequency group using test signal of 1mA

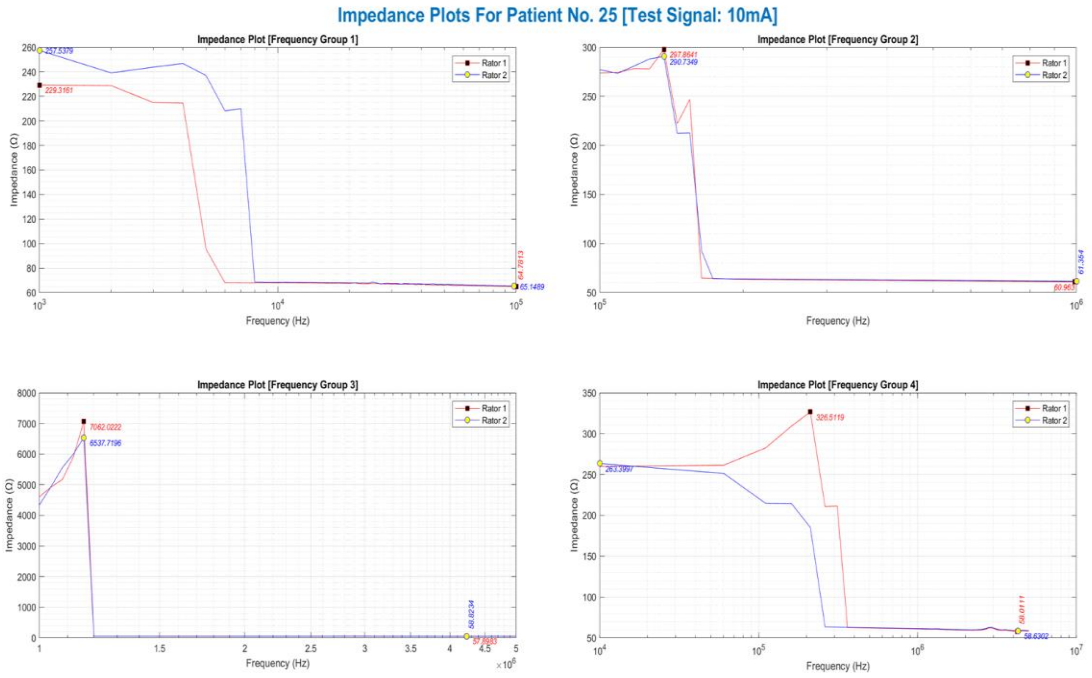


Figure 6.8 impedance plot from reconstructed data of patients with podoconiosis for all the four-frequency group using test signal of 10mA

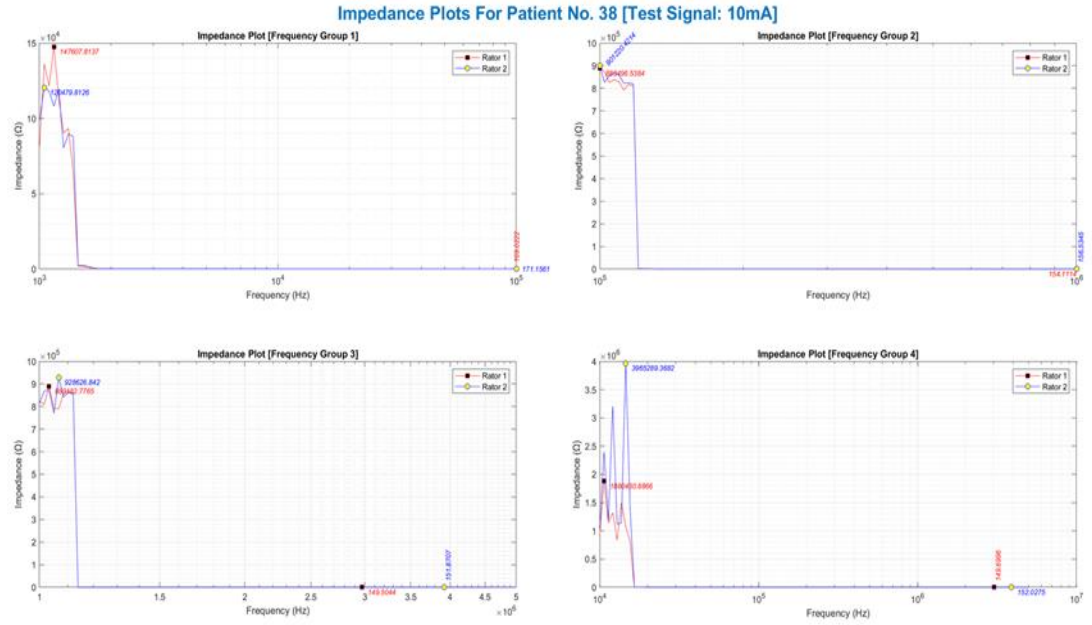


Figure 6.9 impedance plot from reconstructed data of patients' healthy control for all the four-frequency group using test signal of 10mA

Chapter 7: Discussion and Conclusions

Tropical lymphoedema is distressing, with the leg swelling causing heaviness and impairment in function, considerable stigma in society, decreased quality of life and poor marriage prospects. The two major causes of tropical lymphoedema are lymphatic filariasis and podoconiosis. This problem can be successfully managed if diagnosed and treated in the early stages.

The water displacement method is viewed as the gold standard for limb volume determination. However, it is time consuming, not portable, and unhygienic, therefore it is no longer in use in any clinical setup to my knowledge. Tape measurements of limb circumference (TMLC) are frequently used but are difficult to standardize and do not predict volume adequately for lower extremity lymphoedema.

Currently, clinical exclusion is the most common approach to tropical lymphoedema diagnosis. There is a pressing need globally to develop valid and reliable point of care diagnosis and measurement outcome measures that can be used both in clinical and research settings.

Therefore, my PhD project validated the following three instruments: the novel portable three-dimensional infrared imaging device (for volume measurement), and the DLP based NIR spectrometer and the ZI MFIA digital impedance analyzer (for diagnosis), with the ultimate aim of introducing these into the health care system.

To solve the lymphoedema volume measuring method problem, the Brighton and Sussex Global Health Research Unit team collaborated with a team at Georgia Institute of Technology, USA, Department of Mechanical Engineering to work on the novel portable three-dimensional infrared imaging device.

The novel portable three-dimensional infrared imaging device (LymphaTech, Atlanta, GA, USA) consists of a portable infrared sensor (Structure by Occipital, San Francisco, CA, USA) mounted on a tablet computer. It is a non-invasive and non-contact technology. It

works by first allowing the chip to acquire a depth image using infrared light, and then through a stage called light coding. After light coding, the chip uses an algorithm to process data from the image sensor and creates a 3D image of the scene from which it calculates the volume of the limb.

Chapter 3. Presented the results of validation of the novel portable three-dimensional infrared imaging device (index test) against the reference standard water displacement technique on 106 podoconiosis patients. All the study participants were assessed first using the index test and then by the gold standard water displacement method by two independent raters, with each rater performing duplicate measurements in quick succession. The finding showed a very strong positive correlation between the two methods ($r=0.96$, $p<0.001$). Measurements by both raters showed slightly greater variability (higher SD) when they used the water displacement method but, overall, the variabilities of each rater/method were similar. Both raters produced more reliable readings (better agreement between raters) when using the novel portable three-dimensional infrared imaging device (ICC= 0.93 (95% CI: 0.89, 0.95) than the water displacement method (ICC =0.82 (95% CI: 0.74 to 0.87)). This indicates better inter-rater variability using the index test than the reference standard. Regarding the intra-rater reliability, the water displacement technique resulted in more consistent measurement (ICC= 0.96 (95% CI: 0.95, 0.98), whereas the ICC for the index test was 0.70 (95% CI: 0.59, 0.79). The ICC of the novel portable three-dimensional infrared imaging device was improved to 0.83 (95% CI: 0.74 to 0.88) for duplicate average measures.

In conclusion: The novel portable three-dimensional infrared imaging device demonstrated strong near-perfect positive correlation as well as better inter-rater and good intra-rater reliability. Technically, the index test is quick to use, does not require physical contact with the patient, and can be used for patients with skin lesions. In addition, it is portable, does not require water or a continuous electrical supply, and can be successfully operated after brief training. Therefore, it is promising tool for the measurement of leg volume in lymphoedema for clinical trials and monitoring of interventions over time. Based on the finding of the study, we recommend average duplicate measures to improve the intrarater reliability for practice. The next steps will be

working on making the device usable at the point of care and getting access to measurements in real time and cost-effectiveness study. Once these are addressed, we will work on getting this device into the healthcare system, specifically, the lymphoedema morbidity management program.

Chapter 4. Estimated the burden of lymphatic filariasis among patients with lymphoedema in Benishangul Gumuz region.

The overall prevalence of lymphatic filariasis among patients with lymphoedema in the study area was 13.3%. The prevalence of lymphatic filariasis infection in the selected villages of the study area ranged from 0 to 100%.

The prevalence of lymphatic filariasis among male study participants was 18.3% whereas it was 10.3% among females. Being female was therefore associated with 50% lower odds of lymphatic filariasis infection (AOR= 0.48, 95% C.I: 0.29, 0.80). The prevalence of lymphatic filariasis was higher in people over sixty years followed by those who were 41-50 years. Each additional increase of one year in age was associated with a 2% decrease in the odds of acquiring lymphatic filariasis infection (AOR = 0.98, 95%CI; 0.97, 1.00).

The highest prevalence of ICT positivity (47.4%) was recorded in those who had stayed in their village for more than 30 years. The prevalence of lymphatic filariasis among study participants with hydrocele was 12.5%. Study participants who developed lymphoedema between 1 and 10 years previously shared the largest burden of W. *bancrofti* positivity (38.2%), however the positivity rate was low among those who developed the lymphoedema 40 years or more previously.

Chapter 5. Presented the validation of the DLP based NIR spectrometer. To solve the lymphoedema diagnosis problem we collaborated with a team at Global Health Labs, Inc, Bellevue, Washington, United States of America and agreed to work on the DLP® NIRscan Nano scanner.

The DLP based NIR spectrometer is a device that works based on spectrometric principle. Spectrometry is a powerful technique for recognizing and characterizing physical

materials through the variations in absorption or emission of different wavelengths of light by a sample. Spectrometers measure the variation of light absorption of materials. The DLP® NIRscan Nano™ EVM is a high performance, affordable near-infrared portable spectrometer.

We validated the reliability and accuracy of this device. We studied its reliability and accuracy from different perspectives. The difference in repeatability and accuracy between different operators, between different lymphoedema locations (anterior lower leg and middle foot), and using different pre-processing approaches (raw, normalised, SNV+detrend, and SG2).

In terms of repeatability, all four groups (podo, LF, control, and other) demonstrated consistent patterns in repeatability evaluation. There were no statistically significant differences between raters for any combination of site and/or group. However, there were statistically significant differences between different measurement locations (anterior lower leg vs middle foot) for all groups, regardless of rater and pre-processing.

Regarding classification, overall, the best sensitivity obtained was 0.96, which was found in distinguishing podoconiosis from lymphatic filariasis and other types of lymphoedema. The highest specificity was 0.93, which was obtained in discriminating control from podoconiosis on the middle foot by operator 2. As to the area under the curve (AUC), the best was 0.945 which was in discriminating control versus all types of lymphoedema, on the anterior lower leg. The sensitivity and specificity were slightly different across the groups, between anterior lower leg and middle foot, between operators and different pre-processing approaches.

I**n conclusion:** The device was able to distinguish healthy controls from people with podoconiosis, lymphatic filariasis from podoconiosis, controls from everything else and podoconiosis from all forms of lymphoedema - and the device demonstrated good reliability. Technically, the device is portable, does not require a continuous electrical supply, and can be successfully operated after brief training. The DLP based NIR spectrometer

showed promise for use as a diagnostic tool for tropical lymphoedema, however it needs further work to standardise the sensitivity and specificity differences observed among different raters, between different pre-processing techniques and between sampling locations. It will also be important to study different devices to assess the difference in measurement between devices. Additionally, it will be important to gain access to measurements in real time before implementing it into the health care system.

Chapter 6: This is the pilot study of the ZI MFIA digital impedance analyser. This study is being carried out in partnership between BSMS and the University of Sussex, School of Mechanical Engineering to improve diagnosis of lower leg lymphoedema.

The ZI MFIA digital impedence analyser device is a digital impedance analyser and precision meter that sets the new standard for impedance measurements in the frequency range from 1 mHz to 500 kHz (extended to 5 MHz when upgraded). An LCR meter is a type of electronic test equipment used to measure the inductance (L), capacitance (C), and resistance (R) of an electronic component. The MFIA device used in this study is an upgraded device. The MFIA has a basic accuracy of 0.05% and operates over a measurement range spanning from 1 mΩ to 1 TΩ. It is also characterized by a high measurement repeatability and small temperature drift. MFIA comes with the LabOne user interface and with the multi frequency impedance test fixture (MFITF).

The aim was to assess the analyser's ability to acquire readings from the lower limbs of patients with podoconiosis and those who are apparently healthy, and its capacity to distinguish patients with podoconiosis from those without podoconiosis (apparently healthy controls).

To validate this device, we recruited patients with podoconiosis and those without. All study participants were assessed using the ZI MFIA digital impedance analyser by two independent raters, with each rater performing duplicate measurements in quick succession (the study participant stayed on the bed).

Though the ZI MFIA digital impedance analyser is in its early stage and under development, the pilot study results confirmed that the prototype was able to collect scans

from patients with podoconiosis and healthy controls. It was also possible to reconstruct these scans in the form of graphs. Currently further analyses to check the ability to distinguish patients with podoconiosis from healthy controls are underway. The prospects for this device include optimizing data collection, improving the interface (making it more user-friendly) and implementing it into the health care system.

LESSONS LEARNED

Building partnerships with the local community and government bodies is crucial. Every researcher should work in harmony with the local community and stakeholders. In our case we used community members who were routinely working on community mobilisation in other government or partner implemented campaigns such as immunisation. These people are well known in the community, the surrounding health system and government bodies. We designed the everyday activities of field research in partnership with local community members therefore, it was not difficult for us to get trust from the community as well as other concerned bodies. Our study was a bit challenging, as study participants had to stay overnight; however , engaging the locals helped us to overcome every challenge associated with this. Therefore, we highly recommend other researchers to engage locals and plan with them in their study.

Planning and preparation are key in field work research. We were planning and preparing all the necessary materials required for the studies every time before we met the study participants, therefore when study participants came , we easily and conveniently managed the data collection without creating too much inconvenience and took less of their time. Once you create inconvenience the study participants will tell each other and that may affect the study and even difficult to regain their trust once you lost it, therefore researchers shouldn't overlook the importance of pre-planning and preparation before meeting the study participants. We achieved this through training and well informing the field staff.

Commitment and courage. As you can see it from some of the pictures in the document, this project was very challenging especially during the data collection. Some of the data

collection required to convince the study participants to come and stay overnight in the study area. It requires transporting them from their home, preparing them dinner and breakfast by itself was so tough and requires commitment. Without the commitment of all of the field work team this could never happen but with commitment of all of us we made it happen. Apart from this, some of the studies were conducted in very remote settings where there is no infrastructure and high security problems but we passed through all these with commitment and courage. Therefore, a field work of a similar setup with our study is not a piece of cake, it needs commitment. But the challenges were not only challenges, they were an opportunity and experience for us, we loved it at the end.

Reflection

On reflection**,** for the Novel Portable Three-Dimensional imaging device study, I would increase the number of raters from two to three, which might impact the inter-rater reliability. I would also add circumference measurement as a reference method to compare of the index test with this technique too. Even though, it is obvious that the 3D imaging device is quicker to use than the water displacement technique, I would try to quantify the time it takes to complete measurement using both devices.

Similarly, I would increase the number of raters from two to three for the DLP based NIR scanner for improving the inter-rater reliability of the device. In addition to that, I would use two different DLP based NIR devices to explore any differences when different model devices are used.

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Annex-1. Diagnostic techniques used to diagnose lymphatic filariasis

Blood film Examination

The standard method for diagnosing active infection is the identification of microfilariae in a blood smear by microscopic examination. The microfilariae that cause lymphatic filariasis circulate in the blood at night (called nocturnal periodicity). Blood collection should be done at night to coincide with the appearance of the microfilariae, and a thick smear should be made and stained with Giemsa or hematoxylin and eosin (126, 127).

The limited sensitivity of blood films led to the development of concentration techniques (nucleopore filtration) or detection in larger quantities of lysed blood using a counting chamber (128). A diethylcarbamazine (DEC)-based provocative test was also used in some settings if night blood films cannot be done, as the treatment with DEC 'provokes' the appearance of microfilaria in the blood within 30-45 min of DEC administration, during the day (129).

Immunochromatographic test (ICT)

ICT is a highly sensitive and specific filarial antigen detection assays, both as card test (point-of-care diagnosis) and in ELISA based format are available for the diagnosis of *W. bancrofti* infection as an alternative to microscopic detection of microfilaria. This test is positive in early stages of the disease when the adult worms are alive and becomes negative once they are dead. This is used to detect filarial antigen but has low sensitivity and is unreliable for monitoring the impact of mass drug administration on disease transmission areas (130, 131).

The Alere Filariasis Test Strip (FTS)

This is a rapid diagnostic test recommended for mapping, monitoring and transmission assessment surveys (TAS) for the qualitative detection of *Wuchereria bancrofti* antigen

in human blood samples. The FTS has replaced the BinaxNow filariasis immunochromatographic test (ICT), which also detects the same antigen in blood samples (132).

Wb123 test kit

The other serologic test available is the Wb123 test kit, which is used to detect filarial antibody to the recombinant Wb123 antigen of W. bancrofti (133, 134).

DNA probes using Polymerase Chain Reaction (PCR)

These tests are of high specificity and sensitivity and can detect parasite DNA in humans as well as vectors in both bancroftian and brugian filariasis (135).

Membrane filtration method for microfilaria detection

Venous blood drawn at night and filtered through millepore membrane filters, enables an easy detection of microfilaria and quantifies the load of infection. They are usually observed in the early stages of the disease before clinical manifestations develop. Once lymhoedema develops, microfilaria is generally absent in the peripheral blood (136).

Ultrasonography

This is used to locate and visualize the movements of living adult filarial worms of *W. bancrofti* in the scrotal lymphatics of asymptomatic males with microfilaremia. The constant thrashing movement of the adult worms in their ‘nests’ in the scrotal lymphatics is described as the ‘filaria dance sign’. The lymphatic vessels lodging the parasite are dilated and this dilation is not seen to revert to normal even after the worms are killed by diethylcarbamazine administration. Ultrasonography is not useful in patients with filarial lymphoedema because living adult worms are generally not present at this stage of the disease (137, 138).

Lymphoscintigraphy

After injecting radio labelled albumin or dextran into the web space of the toes, the structural changes are imaged using a gamma camera. Lymphatic dilatation, dermal back flow and obstruction can be directly demonstrated in the oedematous limbs by this method. Lymphoscintigraphy has shown that even in the early, clinically asymptomatic stage of the disease, lymphatic abnormalities in the affected limbs of people harboring mf may occur.

Annex -2 blood film blood collection and staining procedure

Capillary Blood collection procedure

1. Label pre-cleaned frosted end slides with the participants' identifier
2. Clean the site well with alcohol; allow to dry.
3. Prick the side of the pulp of the 3rd or 4^{th} finger
4. Wipe away the first drop of blood with clean gauze.
5. Prepare both thick and thin blood film

Preparation of thick blood film

1. Place a small drop of blood in the centre of the pre-cleaned, labelled slide.
2. Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it is the size of a dime (1.5 cm^2).
3. Lay the slides flat and allow the smears to dry thoroughly

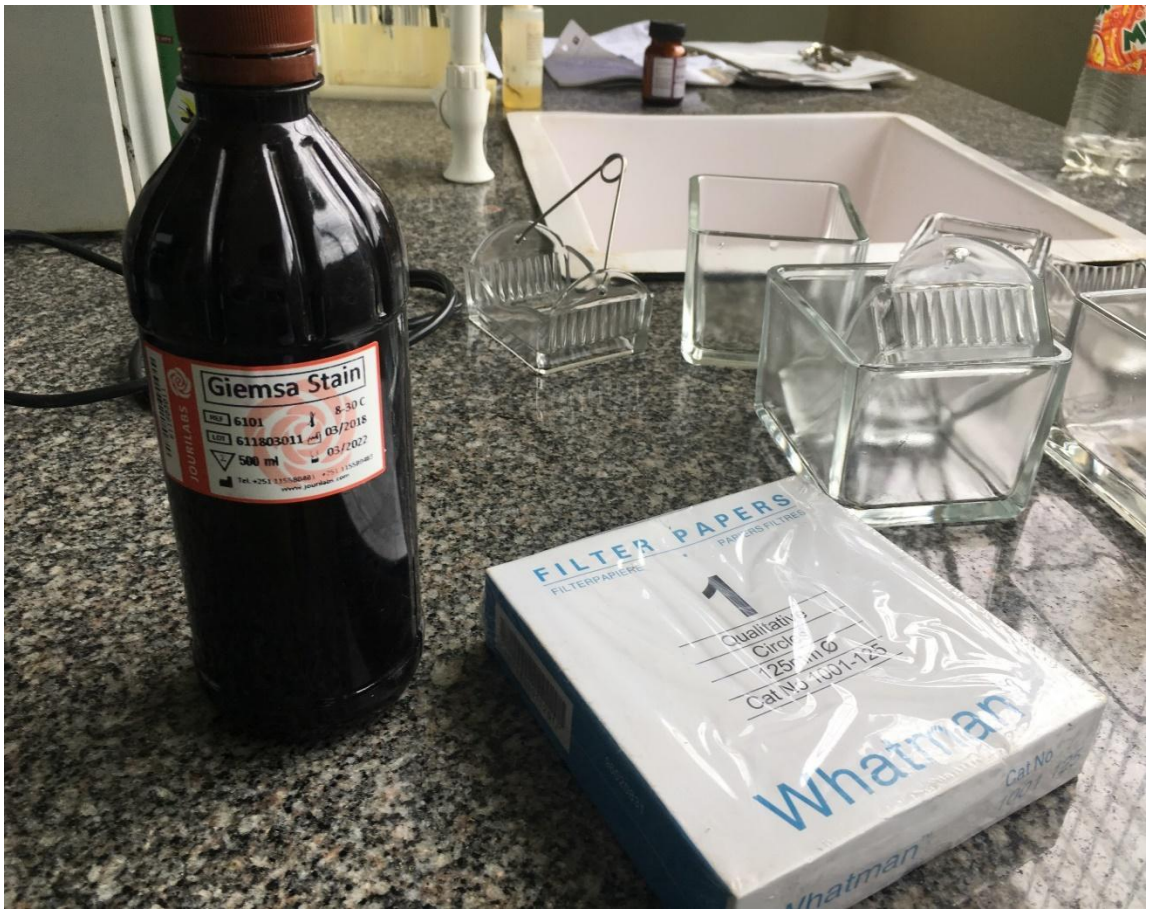
Preparation of thin blood film

1. Place a small drop of blood on the pre-cleaned, labelled slide, near its frosted end
2. Bring another slide at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the 2 slides.
3. Quickly push the upper (spreader) slide toward the unfrosted end of the lower slide.

4. Make sure that the smears have a good, feathered edge. This is achieved by using the correct amount of blood and spreading technique.
5. Allow the thin smears to dry
6. Fix the smears by dipping them in absolute methanol.

Staining blood film

1. Prepare fresh working Giemsa stain in a staining jar



2. Place slides into the working Giemsa stain 10% for 10 minutes.
3. Remove thin smear slides and rinse by dipping 3-4 times in the Giemsa buffer. Thick smears should be left in buffer for 5 minutes.

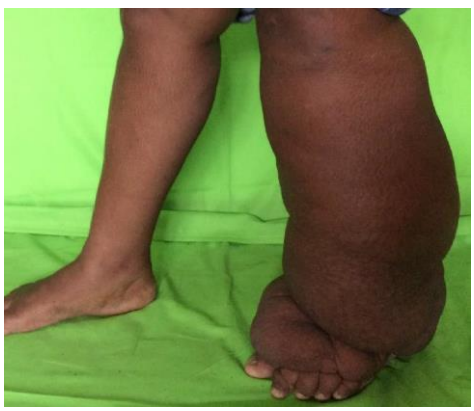
4. Dry the slides upright in a rack.

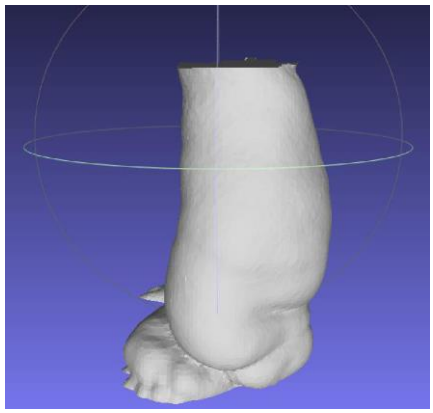
Annex-3 Procedure for the novel portable three-dimensional infrared imaging device

Patients stood in the middle of an open space with feet shoulder-width apart. They were instructed not to move during the scanning process. The rater began by standing approximately 0.85 – 1 meter in front of the patient. The rater used the sensor to position the red scanning box such that it included the entire lower leg and the scan terminated just above the knee of the patient. Once the red box was positioned correctly, the rater began the scan.

The rater performed a 360-degree rotation around the patient to capture all views of the lower leg. Starting with the first position in front of the patient, the rater then moved the scanner up and down. The rater then took one side-step in an arc around the patient and again moved the scanner up and down. The rater continued to move around the arc one side-step at a time, moving the scanner up and down at each side-step. Attention was paid to ensure the scanner was positioned to allow the maximum view of the inside of the leg, foot, and ankle because this is the area that is hardest to capture and most susceptible to poor scanning results (Figure 2.9).

Once the 360-degree rotation was complete, the rater stopped the scan. The reconstruction of the patient's legs was then visible on the screen for the rater to view (Figure 2.10). The rater rotated the reconstruction to examine all views for defects in the scan. If defects were found (visible as holes or seams in the surface of the scan, usually found on the inside of the leg), then the scan was discarded, and the process was repeated. If no defects were found, the rater saved the scan, and the process was completed.





The second part of the design involved writing a computer program to use the raw data obtained from the sensor to calculate the volume. For this study, the raters captured data from just above a patient's knees to the floor, and the imaging system software calculated limb volume from the floor to a height of 32 cm from the 3D model.

To calculate the volume of the leg in the scan, the floor points were removed from the 3D point cloud. A best-fit plane was obtained by taking the points that had a normal with an absolute y-component greater than 0.95. These points, with their normal almost parallel to the y-axis, were designated as floor points and the resulting plane was used to divide the point cloud into two components - the leg and the floor. During this process, outlier points generated by scanning errors and not connected to either component were also removed. After obtaining the point cloud consisting only of the leg, adjustments were made to ensure that 32 cm of the leg was considered for volume calculations.

This was done to be consistent with the water displacement volume measurement protocols. The final volume of the leg was calculated using surface integrals based on the divergence theorem which states that the surface integral of a vector field over a closed surface is equal to the volume integral of the divergence of that vector field over the region enclosed by that surface. To ensure that point cloud of the leg was a closed surface, a mesh fixing algorithm was used(100).

Annex-4 Electrodes positioning & Frequency protocols

The following practical considerations were made while taking measurements:

1. The injected current should not take a shortcut path. Legs should be free of other body parts and apart from each other.
2. The tested region should be still during the measurement(s).
3. The electrode cables should avoid interference. High frequency current may take a shortcut over small distances, despite electric insulation (in such cases, the resistance R_∞ & R_0 may have incorrect measured values. Hence, to avoid interference, cables should be:
    a. Free of each other.
    b. Free from the measured subject/region.
    c. Free of metal objects and earth.
4. Electrode(s) location is very important during the measurement.
    a. The voltage-detector electrodes (high potential terminal (HPOT) & Low potential terminal (LPOT)) should be in the middle of the other electrodes. See Figure 1. The topology which includes hand and leg, the voltage detector electrodes should be located on the midline between prominent bones ends at ankle and wrist.
    b. The current-injecting electrodes (high current terminal (HCUR) & low current terminal (LCUR)) should be located at least 5cm distally from the voltage-detector electrodes (HPOT & LPOT).
    c. The electrodes should be placed on the good skin surface, not on toes etc.
    d. Electrode positioning should be in the same location for all patients.
    e. Error in the electrode location may result in following consequences:
        i. **Voltage-Electrodes (V-Electrodes) wrong location:**

            Large distance between two measurement points will result in larger measured resistance and vice versa. If one or both V-electrodes are

placed distally, then the values of R_E and R_∞/R_I will be too large. Hence, it will result in low estimates of fluid volumes.

ii. Current-Electrodes (I-Electrodes) too close to V-Electrodes:

Generally, this distance should be at least 5cm. This allows even flow of current through a cross-section of the subject at the local measurement area. Insufficient distance will lead to a flow of current partially in the cross-section at the measurement area and will result in resistances that are too high. For <5cm distance, the cross-sectional area will be smaller, and the measurement is expected to be inaccurate.

5. Frequency is also an important parameter during the measurements. A wide spectrum should be covered which includes low to high frequencies. Hence, the measurements will be performed using frequency sweep for the following 4 groups:
    a. Frequencies from 1 kHz to 100 kHz
    b. Frequencies from 100 kHz to 1 MHz
    c. Frequencies from 1 MHz to 5 MHz
    d. Frequencies from 10 kHz to 5 MHz

Annex 5: Information sheet for the volume measurement study

Title of the study: Validation of a portable three-dimensional imaging system for, measuring lower limb volume of podoconiosis patients

Name of Investigators: Mr. Abdi Samuel, Prof. Gail Davey, Prof. Chris Chatwin, Prof. Abebaw Fekadu, Prof. Brandon Dixon & Dr. Kebede Deribe.

My name is ……, and I am a data collector for this study. You are invited to take part in this research, which we hope will help us to solve the problem of volume measurement among podoconiosis patients. Before you decide to be part of this study, it is important for you to understand why we are collecting this information and what participation will it involves. Please take time to read this paper carefully and discuss it with friends and relatives if you wish. Ask us, if there is anything that is not clear or if you would like more information.

Background to the study

We plan to test the portable three-dimensional scanner's capacity in measuring the lower limb swelling among podoconiosis patients. This may in future help to measure outcomes of treatment given to podoconiosis patients.

With your permission, we intend to:

1. Ask you a couple of questions about yourself;
2. Examine your legs;
3. Measure the volume of the swelling of your leg using two different techniques; the water displacement and the Novel Portable Three-Dimensional Scanner

Possible harms: We do not anticipate any harm to you from this procedure. The questions will take a maximum of one hour of your time.

Benefits: There won't be any direct benefits to you of being involved in this study, though we will compensate for the time you have given the project. We hope that in the future, other patients will benefit from better understanding of the lymphoedema volume measurement.

Confidentiality: All information which is collected about you during the research will be kept strictly confidential. The sample will link to your name but will only be identified by a code. This is just to contact you, if we find anything unexpected that needs further follow up.

Autonomy: If you wish not to take part in this study that is fine – you do not need to give a reason. If you as a patient decide not to participate, the treatment you or your family receives in future at government sites will not be affected.

If something goes wrong: If a problem arises, you can report it to the study coordinator at the address given below.

What will happen to the research? We anticipate that the results of this study will be available next year, and we hope to publish the results. You will not be identifiable in any publication.

Who is organizing and funding the research? The research is organized by the Brighton and Sussex Medical School, a UK-based University and CDT Africa, Ethiopia. It is funded by the National Institute for Health Research (NIHR) Global Health Research Unit on NTDs at Brighton and Sussex Medical School using Official Development Assistance (ODA) funding. The device, the novel portable three-dimensional imaging device, is funded by George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology Atlanta, Georgia, USA.

Contact Address: Mr. Abdi Samuel
Cell Phone: +251917813478

Annex 6. The Afaan Oromoo version of information sheet for volume measurement study

Unka Odeeffannoo

Mata duree Qo'annoo: Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa "portable 3D imaging system" jedhamuun safaruun akka danda'amu qo'annoo godhamu, aanaaWaayyu Tuqaa, Lixa Itiyoophiyaati.

Qorattootii: Obbo Abdii Saamu'eel, Pirof GeeylDeevii, Pirof. Braandon Diiksan, Pirof, Ababaaw Fiqaadu, Dr. Kabbadaa Darribe

Maqaan kiyya _____jedhama. Qayyabannaa kanaafan raga sassaaba. Qorannoon nuti gaggeessinu kun dhiitoon miillaa meeshaa haaraa kalaqame kan "the novel portable three dimensional imaging device" jedhamu kun hanga dhiitoo kanaa safaruu akka inni danda'u beekuuf qoratama. Kanaaf isin akka qoranno kana keessaatti hirmaattan afeeramtaniittu. Kanaaf unka odeeffannoo kana yaroo fudhaa dubbisaatii yoo gaaffii qabaattaniis, yoo odeeffannoo dabalataa barbaaddaniis gaafadhaa haala jiru hunda qulqulleefadha.

Bu'uura qoranichaa

Kaayyoon qayyabannaa kanaa hanga dhiitoo miillaa dhukubsatoonni podokonosisii qaban kana meeshaa "the novel portable three dimensional imaging device" jedhamuun safaruu ta'a. Kana gochuun kan inni fayyaduu, tokko qorichii dhukubsattooti kun fudhatan hagam isaan fayyadeera (bu'Aa qorichichaa baruuf), cimina dhukubichaa fi dhukubicha sadarkaale gargaraatti qooduuf fayyada.

Yoo qorannoo kana irratti hirmaachuuf eeyyamamtan:

1. Waa'ee keessan gaaffilee muraasa isiin gaafana
2. Miilla keessan sakattaana
3. Miilla keessanii meeshaale lamaan faayadamne safarra; Kuniis"Water displacement technique fi the novel portable three dimensional imaging device" jedhema.

Miidhaa qorannichi qabu: qorannoo kun miidhaa isiin irraan ga'u hin qabu. Tarii meeshaan inni tokko bishaan waan qabuuf xiqqoo isinittii tolu dhiisu danda'a. yaroo haanga daqiiqaa sa'a tokko fudhach danda'a.

Bu'aa qorannichaa: ammaan kana kallattiin qorannoo kana irraa bu'aan isiin argattan hin jiru garuu akkamiin dhukuba kana ofiin akka kunuunsiitan leenjiii sinii laanna, akkasumas yaroo isiin qor'annoo kanaaf gumaachitaniif beenyaa walgitu isnii goona. Gara fulduraatti garu isiniis ta'e warrii kaan faayidaa guddaa bu'aa qorannoo kana irraa argachu ni dandeessu ta'a.

Icciitii eeguu: qorannoo kanaaf odeeffanno isiin nuuf laattan hundi iciitiin eegama. Dhimmichiis kodiin hojjatama. Yoo firiin qo'annaa kanaa waan addaa ta'e ittiin isiin quunnamuuf itti fayyadamnna.

Mirga: qorannoo kanatti hirmmaachu dhiisuuf mirga guutu qabdu sababii hirmaachuu hin barbaadneefiis ibsa kennuun isiin irraa hin eegamu. Tajaajila isiniis ta'e maatiin keessan buufata fayyaa kana irraa argattan waliin waan walqabatus hin qabu.
Bu'anqorannoo kana irraa argamu akka xumurameen qaamoota ilaallatuuf ifa ni godhama garu maqaan keessan bifa kanmiiniyyu hin ibsamu.

Yoo rakkoon isiin mudate bilbila kanaa irratti bilbiiluun rakkoo jiru gabaasuu danadeesu. Qorannoo kana kana qopheesse Brighton and Sussex Medical School, yuniivarsiitii biyya Ingliziitti argamuuf and dhaabata CDT Africa, biyya Itiyoophiyaatti aragamudha. Baasii qorannoo kanaaf ta'u kan gargaare immoo "National Institute for Health Research (NIHR) Global Health Research Unit on NTDs at Brighton and Sussex Medical School using Official Development Assistance (ODA) funding" jedhama, yaadii qo'annoo kanaa kan namoota qorannoo kana gaggeessaniiti malee kan dhaabata kanaa miti. Meeshaan DLP based NIR scan nano device jedhamu kun kan gargaarsaan kenname dhaabata "Intellectual Ventures Laboratory, Bellevue, WA, USA" jedhamudha. Meeshaa isa "3D portable imaging system" jedhamum immoo dhaabata "George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology Atlanta, Georgia, USA" jedhamu.

Teesso nama quunnamtanii: Obbo Abdii Saamu'eel Lakk bilbilaa +251917813478

Annex 7: Consent Form for the volume measurement study

Research Title: Validation of a portable three-dimensional imaging system for measuring lower limb volume of podoconiosis patients

Name of Investigators: Mr. Abdi Samuel, Prof. Gail Davey, Prof. Chris Chatwin, Prof. Abebaw Fekadu, Prof. Brandon Dixon & Dr. Kebede Deribe	
I confirm that I have read and understood the information sheet for the study "Validation of a portable three-dimensional imaging system for, measuring lower limb volume of podoconiosis patients, Western Ethiopia". I have had the chance to read the information and ask questions about the study and am satisfied with the answers I have been given.	
I understand that my participation in this study is voluntary and that I am free to stop at any time, and I do not have to give a reason for doing so. I understand that if I ask to stop the study my medical care and legal rights will not be affected in any way.	
I understand that occasionally an external regulator or funding body may ask to look at the data to check that the study is being run correctly.	
I wish to be contacted by the research team if unexpected findings requiring follow up are identified	
I agree to take part in the above study.	

_____ _____ _____

Name of Participant Date Signature

– –
– – –

Researcher to complete:

- I have explained the information in this document and encouraged the participant to ask questions and provided adequate time to answer them.

_____________________________ __________________ ________________________

Name of Researcher Date Signature

Annex 8. The Afaan Oromo version of the consent form for the volume measurement study

Unka waliigalte

Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa" **the novel portable three-dimensional imaging device**" jadhamuun safaruun akka danda'amu qayyabanaa godhamu, anaa waayyu tuqaa, lixa itiyoophiyaati**.**

Qorattootii: ObboAbdii Saamu'eel, PirofGeeylDeevii, Pirof. BraandonDiiksan, Pirof, AbabaawFiqaadu, Dr. KabbadaaDarribe	**Mallatto itti godhaa**
Waa'eQorannoo "Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa **the novel portable three-dimensional imaging device** jedhamuun safaruun akka danda'amu qayyabanaaa naa waayyu tuqaa, lixa itiyoophiyaati godhamu," jedhu kana unka odeeffanno isaa siriitti dubise hubadheera, gaaffi gaafachuufis carraa argadheera, deebiinaaf keenanitiis itti quufeera.	
Qorannoo kana irratti hirmaanaan godhu fedhii koon akka ta'e hubadheera, yarroon barbaadeettiis dhiisu akkan danda'u beeka kana gochuufiis sababii koo illee ibsuun akkana irraa hin eegamne hubadheera. Yoon qorannoo kana irraati hirmaachu didees ta'e jalqabe garagar kute yaaliin argachaa jiruus ta'e mirgiin qabu hundi akka ittii najalaa hin bu'amne hubadheera.	
Qaami to'ataan alaas tae qaami qorannoo kana maallaqaa gargaaran odeefanno qoarnnoo kanaaf sasaabamu sirnaan deemaa jiraachuisaa gaafachuuf to'achu akka danda'aniis hubadheera.	
Qorannoo kan irratti hirmaachuuf waliigaleera.	
Waa'e Qorannoo "Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa **the novel portable three dimensional imaging device** jedhamuun safaruun akka danda'amu qayyabanaa, aanaa waayyutuqaa, lixa itiyoophiyaati godhamu. jadhamu kana unkaodeeffannoisaasiriittidubisehubadheera, gaaffigaafachuufiscarraaargadheera, deebinaafkananitiisittiquufeera.	

_____ _____ _____

Maqaa hirmaataa guyyaa mallatto

……

Unka qo’ataa qorannoo Kanaan guutamu

Yaada qorannoo unka odeefannoo kana keessa jiru sirriitti ibseeraa fi gaaffii akkagaafataniifiis jajjabeeseeran gaafiiisaanii fiis deebii ga’a kenneeraafi.

_____ _____ _____

Maqaa qo’ataa guyyaa Mallattoo

Annex 9: Information sheet for the diagnostic study

Title of the study: Testing the DLP based NIR spectrometer and the novel portable three dimensional imaging device for the diagnosis and characterization of tropical lymphoedema in Ethiopia

Name of Investigators: Mr. Abdi Samuel, Prof. Gail Davey, Prof. Chris Chatwin, Prof. Abebaw Fekadu, Prof. Brandon Dixon &Dr. Kebede Deribe

My name is ……, and I am a data collector for this study. You are invited to take part in this research, which we hope will help us to solve the problem of diagnosing leg swelling in countries like Ethiopia. Before you decide to be part of this study, it is important for you to understand why we are collecting this information and what participation will it involves. Please take time to read this paper carefully and discuss it with friends and relatives if you wish. Ask us, if there is anything that is not clear or if you would like more information.

Background to the study

We plan to test the DLP based NIR spectrometer and the novel portable three dimensional imaging device for the diagnosis and characterization of leg swelling in tropical countries. These will help us to know the actual cause of leg swelling and what to do about it.

With your permission, we intend to:

1. Ask you a couple of questions about yourself
2. Examine your legs;
3. Take measurements using two different techniques; the DLP based NIR scan nano and the novel portable three dimensional imaging device

Possible harms: We do not anticipate any harm to you from this procedure. Both scanners have been shown to be safe in a range of healthcare settings. The questions will take a maximum of 30 minutes of your time.

Benefits: There won't be any direct benefits to you of being involved in this study, though we will compensate for the time you have given the project. We hope that in the future, other patients will benefit from better understanding of the leg swelling diagnosis.

Confidentiality: All information which is collected about you during the research will be kept strictly confidential. The sample will link to your name but will only be identified by a code. This is just to contact you, if we find anything unexpected that needs further follow up.

Autonomy: If you wish not to take part in this study that is fine – you do not need to give a reason. If you as a patient decide not to participate, the treatment you or your family receives in future at government sites will not be affected.

If something goes wrong: If a problem arises, you can report it to the study coordinator at the address given below.

What will happen to the research? We anticipate that the results of this study will be available next year, and we hope to publish the results. You will not be identifiable in any publication.

Who is organizing and funding the research? The research is organized by the Brighton and Sussex Medical School, a UK-based University and CDT Africa, Ethiopia. It is funded by the National Institute for Health Research (NIHR) Global Health Research Unit on NTDs at Brighton and Sussex Medical School using Official Development Assistance (ODA) funding. The DLP based NIR scan nano device is funded by Intellectual Ventures Laboratory, Bellevue, WA, USA and the 3D portable imaging system, is funded by George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology Atlanta, Georgia, USA.

Contact Address: Mr. Abdi Samuel
Cell Phone: +251917813478

Annex 10. Afaan Oromoo version of the information sheet for the diagnostic study

Unka Odeeffannoo

Mata duree Qo'annoo: Qo'annoo meeshaalee DLP based NIR spectrometer fi the 3D portable imaging system jedhamani dhiitoo miillaa sakata'uuf Itiyoophiyaatti godhamu

Qorattootii: obbo AbdiiSaamu'eel, Pirof Geeyl Deevii, Pirof. BraandonDiiksan, Pirof, Ababaaw Fiqaadu, Dr. Kabbadaa Darribe

Maqaankiyya_____jedhama. Qo'annoo kanaaf anragasassaaba. Qorannoonnuti gaggeessinu kundhiitoon miillaa meeshaa haaraa kalaqame kan "DLP based NIR scan nano and the the novel portable three-dimensional imaging device" jedhaman kun sababii dhiitioo miillaa fide sakata'uu akka isaan danda'a qo'atama. Kanaaf isin akkaqoranno kana keessaatti hirmaattan afeeramtaniittu. Kanaaf unka odeeffannoo kana yaroo fudhaa dubbisaatii yoo gaaffii qabaattaniis, yoo odeeffannoo dabalataa barbaaddaniis gaafadhaa haalajiru hundaqulqulleefadha.

Bu'uura qoranichaa

Kaayyoon qayyabannaa kanaa meeshaaleen "DLP based NIR scan nano and the 3D portable imaging system" jedhaman kun sababii dhiitoo miillaa fide kana sakata'u akka danda'antu qoratama. kana gochuun kan inni fayyaduu, dhukubi kun yaroo akka beekamu godha. Yaroon beekamuun isaa immoo yaroo yaalametu gara hamaattii hinceein ittisuu nu dandeessisa

akkasuma qayyabannaa adda addaa dhukuba kana waliin kan walqabate akka gaggeeffamu gargaara.

Yoo qorannoo kan irratti hirmaachuu feeyyamtan:

1. Waa'e keessan gaaffile muraasa isiin gaafana
2. Miillakeessansakattaana
3. Miilla keessanii meeshaale lamaan fayyadamnee safarra; Kuniis "DLP based NIR spectrometer and the novel portable three dimensional imaging device" jedhema.

Miidhaa qorannichi qabu: qorannoo kun miidhaa isiin irraan ga'u hinqabu. Tarii, meeshaan inni tokko bishaan waan qabuuf xiqqoo isinittii tolu dhiisudanda'a. yaroo hanga daqiiqaa 30 fudhachudanda'a.

Bu'aa qorannichaa: ammaan kana kallattiin qorannoo kana irraa bu'aan isiin argattan hinjiru garuu akkamiin dhukuba kana ofiin akka kunuunsiitan leenjii isinii laanna, akkasumas yaroo isiin qor'annoo kanaaf gumaachitaniif beenyaa walgitu isnii goona. Gara fulduraatti garu isiniis ta'e warri kaan faayidaa guddaa bu'aa qorannoo kana irraa argachuu ni dandeessu ta'a.

Icciitiieegu: qorannoo kanaaf odeeffanno isiinnuuf laattan hundi iciitii neegama. Dhimmichiis kodiin hojjatama. Yoo firiin qo'annaa kanaa waan addaa ta'e ittiin isiin quunnamuuf itti fayyadamnna.

Mirga: qorannoo kanatti hirmmaachuu dhiisuuf mirga guutu qabdu sababii hirmaachuu hinbarbaadneefiis ibsa kennuun isiin irraa hineegamu. Tajaajila isiniis ta'e maatiin keessan buufata fayyaa kana irraa argattan waliin waan walqabatus hin qabu.

Bu'an qorannoo kana irraa argamu akka xumurameen qaamoota ilaallatuuf ifa ni godhama garuu maqaan keessan bifa kanmiin iyyuu hin ibsamu.

Yoo rakkoon isiin mudate bilbila kanaa irratti bilbiiluun rakkoo jiru gabaasu dandeessu Qorannoo kana kan qopheesse Brighton and Sussex Medical School, yuniivarsiitii biyya Inglizii itti argamuuf and dhaabata CDT Africa, biyya Itiyoophiyaatti aragamudha. Baasii qorannoo kanaaf ta'u kan gargaare immoo "National Institute for Health Research (NIHR) Global Health Research Unit on NTDs at Brighton and Sussex Medical School using Official Development Assistance (ODA) funding" jedhama, yaadii qo'annoo kanaa kan namoota qorannoo kana gaggeessaniiti malee kan dhaabata kanaa miti. MeeshaanDLP based NIR spectrometer jedhamu kun kan gargaarsaan kenname dhaabata "Intellectual Ventures Laboratory, Bellevue, WA, USA" jedhamudha. Meeshaa isa "the novel portable three-dimensional imaging device" jedhamun immoo dhaabata "George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology Atlanta, Georgia, USA" jedhamu.

Teessoo nama quunnamtanii: Obbo Abdii Saamu'eel Lakk. bilbilaa +251917813478

Annex 11: Consent Form for the diagnostic study

Research Title: Testing the DLP based NIR spectrometer and the the novel portable three dimensional imaging device for the diagnosis and characterization of tropical lymphoedema in Ethiopia

Name of Investigators: Mr. Abdi Samuel, Prof. Gail Davey, Prof. Chris Chatwin, Prof. Abebaw Fekadu, Prof. Brandon Dixon & Dr. Kebede Deribe	Please check the box
I confirm that I have read and understood the information sheet for the study "Validating the DLP based NIR spectrometer and **the novel portable three dimensional imaging device** for the diagnosis and characterization of tropical lymphoedema in Ethiopia". I have had the chance to read the information and ask questions about the study and am satisfied with the answers I have been given.	
I understand that my participation in this study is voluntary and that I am free to stop at any time, and I do not have to give a reason for doing so. I understand that if I ask to stop the study my medical care and legal rights will not be affected in any way.	
I understand that occasionally an external regulator or funding body may ask to look at the data to check that the study is being run correctly.	
I wish to be contacted by the research team if unexpected findings requiring follow up are identified	
I agree to take part in the above study.	

_____ _____ _____

Name of Participant Date Signature

_ _

Researcher to complete:

- I have explained the information in this document and encouraged the participant to ask questions and provided adequate time to answer them.

______________________ ______________ ____________________

Name of Researcher Date Signature

Annex 12. The Afaan Oromoo version of the consent form for the diagnostic study

Unka waliigalte

Mata duree Qo'annoo: Qo'annoo meeshaaleeDLP based NIR spectrometer the novel portable three-dimensional imaging device jedhamani dhiito miillaa sakata'uuf Itiyoophiyaatti godhamu

Qorattootii: obbo Abdii Saamu'eel, PirofGeeylDeevii, Pirof. BraandonDiiksan, Pirof, AbabaawFiqaadu, Dr. Kabbadaa Darribe

	Mallatto ittigodhaa
Waa'eQorannoo "Qo'annoomeeshaaleeDLP based NIR spectrometer fi **the novel portable three-dimensional imaging device** jedhamani dhiito miillaa sakata'uuf Itiyoophiyaatt godhamu" jedhu kana unka odeeffanno isaa siriitti dubise hubadheera, gaaffigaafachuufiscarraa argadheera, deebiinaaf keenanitiis itti quufeera.	
Qorannoo kana irrattihirmaanaangodhufedhiikoonakkta'ehubadheera, yarroonbarbaadeettiisdhiisuakkandanda'ubeeka kana gochuufiissababiikooilleeibsuunakkanairraahineegamnehubadheera. Yoon qorannookanirraatihirmaachudideesta'ejalqabegaragarkuteyaaliinargachaa jiruusta'emirgiinqabu hundi akkaittiinajalaahinbu'amnehubadheera.	
Qaamit o'ataan alaas tae qaami qorannoo kana maallaqaan gargaaran odeefanno qoarnnoo kanaaf sasaabamu sirnaan deemaa jiraachu isaa gaafachuu fto'achu akka danda'aniis hubadheera.	
Qorannoo kan irratti hirmaachuuf waliigaleera.	
Waa'e Qorannoo "Qo'annoo meeshaalee DLP based NIR scan nano fi the 3D portable imaging system jedhamani dhiito miillaa sakata'uuf Itiyoophiyaatti godhamu "jedhamu kana unka odeeffanno isaa siriitti dubise hubadheera, gaaffi gaafachuufi scarraa argadheera, deebinaaf kananitiis itti quufeera.	

_____ _____ _____

Maqaa hirmaataa guyyaa mallatto

Unka qo’ataa qorannoo Kanaan guutamu

Yaada qorannoo unka odeefannoo kana keessa jiru sirriitti ibseeraafi gaaffii akka.

_____ _____

Maqaa qo’ataa guyyaa Mallattoo

S. N	Questions	Responses	Skip pattern
Part I. Sociodemographic Variables			
11	Age	_____years	
12	Sex	1. Male 2. Female	
13	Where is your permanent residence	1. Rural 2. Urban	
14	Region	1. Oromia 2. SNNPR 3. Amhara 4. Others _____	
15	Address	1. Zone _____ 2. Woreda _____ 3. Kebele _____ 4. Goti _____ 5. Gare Misoma _____ 6. Household name _____	

16	Educational status	1. Can read & write 2. Cannot read or write	
17	If your answer for question 16 above is 1, what is the highest grade attended?	_____	
18	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed 5. Other…….	
19	Occupational Status	1. Farmer 2. Daily labourer 3. Government employee 4. Housewife 5. Student 6. Merchant 7. Other……..	
110	For how long you have been living in this *kebele*?	_____(years) and ____ (months)	
Part II. Lymphoedema and its characteristics			
21	Clinical stage of the disease	1. Mild 2. Moderate 3. Severe	

22	Clinical signs	1. Lymphoedema 2. Hydrocele 3. Both lymphoedema and hydrocoele	
23	If the answer for question 22 above is lymphoedema, leg(s) affected	1. One leg 2. Both leg	
24	Extent of lymphoedema	1. Above the knee 2. Below the knee	
25	For how long you have experienced the lymphoedema ?	_____ months or _____ years	

Annex 13. A proforma prepared for the study entitled "Improving the diagnosis and measurement of tropical lymphoedema"

Participant Name _____

Identifier_____

Part III. Measurement Results (Only for Volume Measurement Study)

Rater	Right leg volume				Left leg volume			
	Water displacement		Kinect XBOX		Water displacement		Kinect XBOX	
	1st measurement	2nd measurement	1st measurement	2nd measurement	1st measurement	2nd measurement	1st measurement	2nd measurement
Rater 1								
Rater 2								

Annex 14. Volume displacement technique and the novel portable three-dimensional imaging device by two independent raters each took duplicates of measurements by both devices. Measurement Crude Data

Lower limb volume measurement data of ninety-six (96) podoconiosis patients made using water

ID	M1	M2	M3	M4	S1	S2	S3	S4
1	2760	2820	2740	2820	2887.7	2929.3	2557.0	2724.0
4	3520	3500	2520	3520	3523.1	3265.0	3298.7	3207.8
5	1920	1920	3740	1920	2447.3	2534.3	1808.2	1755.9
6	2520	2650	2280	2600	3072.0	2836.2	2802.8	3072.1
7	2900	2920	2500	2900	2563.7	2396.9	2597.6	2935.9
8	3100	3100	2460	3020	2788.5	2738.9	2587.5	3051.8
9	2580	2600	2840	2620	2691.7	1921.3	2650.6	2589.5
10	2600	2640	2780	2680	2273.1	2771.1	3135.3	3062.4
11	2320	2300	2580	2380	2090.7	2143.5	2056.9	2291.6
12	1680	1700	2880	1720	1567.1	1516.2	1507.2	1646.4
13	1900	1860	1960	1880	1489.0	1837.3	1585.1	2144.6
14	2620	2640	3420	2660	3264.0	2796.6	2509.7	2613.0
15	2520	2480	2260	2380	2501.3	2526.5	3047.4	2355.7
16	2440	2540	2740	2520	2524.1	2495.6	2377.8	2356.8
17	2580	2660	3000	2580	2559.8	2761.2	2571.0	2937.9
18	2860	2840	2060	2940	2737.0	2830.3	2824.3	2846.5
19	2620	2140	2640	2160	2065.4	2064.9	2225.1	2177.2

20	2580	2500	2440	2360	2418.2	2418.2	2418.2	2418.2
21	2820	2720	3220	2580	2768.3	2768.3	3031.7	3031.7
22	2840	2860	3480	2900	2708.1	2703.7	2869.6	2869.6
23	2680	2500	2980	2640	2981.1	2981.1	2736.3	2756.6
24	2480	2420	3180	2440	3111.0	2631.5	2733.3	2738.1
25	2200	2210	2060	2200	2192.4	2192.4	2025.4	2017.6
26	1840	1900	2700	1880	2056.2	2359.8	2053.6	2083.2
27	3080	3040	2980	3020	3049.2	3049.2	2952.7	2950.7
28	2680	2700	2740	2700	2518.0	2512.9	2498.9	2429.2
29	2580	2720	2720	2760	2994.5	3049.0	3287.5	2847.4
30	1500	1520	1500	1580	1660.1	1818.2	1609.8	1975.4
31	2500	2540	2520	2520	2703.1	2513.3	2565.1	2490.9
32	3760	3740	3740	3720	3606.5	3794.4	3798.7	4049.1
33	2280	2260	2280	2320	1916.2	1970.2	2027.2	1949.1
34	2420	2500	2500	2480	2146.3	1857.7	1868.2	2212.0
35	2320	2400	2460	2340	2467.4	2328.1	2746.6	2589.8
36	2960	2940	2840	2840	2528.2	2353.0	2312.6	2309.1
38	2540	2540	2580	2580	2324.8	2282.1	2166.7	2303.0
39	2800	2860	2880	2880	2672.0	2661.9	2504.0	2487.8
40	1820	1840	1960	1980	1841.1	1775.3	1871.6	1405.4
41	3340	2340	3420	3420	3495.5	3593.9	3636.4	3527.3
42	2200	2220	2260	2340	2796.3	2796.3	2662.0	2828.9

43	2580	2760	2740	2780	2982.8	2973.8	2966.6	3003.6
46	2620	2650	2640	2640	2516.4	2514.6	2617.5	2591.5
47	2380	2460	2440	2420	2330.5	2217.7	2304.7	2405.0
48	3280	3200	3220	3190	3626.4	3322.2	3193.1	3324.2
49	3520	3500	3480	3500	3442.0	3318.2	3299.1	3473.9
50	2880	2880	2980	3000	2482.6	2641.9	2886.0	2857.1
51	3180	3160	3180	3140	3207.6	3213.3	3271.0	3266.4
52	2400	2100	2060	2100	2599.6	2599.6	2533.0	2533.0
53	2780	2720	2700	2740	2956.1	2955.9	2956.6	2930.7
54	2980	2980	2980	3000	3266.2	3231.8	3231.8	3417.9
55	3260	3260	3200	3240	3342.0	8460.3	3263.1	3276.8
57	4280	4480	4460	4400	4599.3	4748.3	4748.3	4808.2
58	3260	3240	3240	3500	2114.4	2379.8	2662.1	2374.9
59	2860	2840	2860	2800	2892.5	2752.9	2847.5	2638.7
60	2180	2060	2060	2060	1340.4	1878.4	1292.4	1423.5
61	3420	3420	3400	3420	3200.4	3019.7	3027.2	406.5
62	3390	3340	3400	3420	2914.9	3499.5	3375.7	3699.1
63	1980	1900	1940	1940	2139.7	2158.3	2017.0	6650.9
64	2060	2060	2080	3200	1571.9	1933.9	1672.3	1562.5
65	2500	2520	2560	2580	2850.4	2822.4	2406.4	2184.1
66	2700	2620	2680	2680	2506.7	2311.6	2649.6	2283.9
69	2700	2820	2820	2800	2900.4	2908.3	3200.2	3200.2

70	2480	2540	2520	2520	2741.0	2897.0	2894.5	2758.0
71	2400	2420	2360	2360	2693.9	2691.3	2692.9	2685.6
72	3200	3200	3200	3200	3538.0	3538.0	3545.6	3541.2
73	3420	3580	3360	3340	3225.1	3227.0	3206.0	3203.3
74	3400	3380	3400	3380	3320.3	3320.3	3311.9	3344.1
75	4400	4400	4440	4420	4518.6	4517.8	4570.8	4570.8
76	2780	2800	2840	2820	3080.1	3080.1	2949.8	2951.4
77	3020	3040	3060	3020	3021.8	3023.7	3158.9	3158.9
78	3460	3460	3440	3460	3162.1	3159.8	3159.8	3156.4
79	2660	2640	2700	2680	2710.9	2711.9	2767.3	2756.0
80	2640	2660	2660	2620	2658.7	2666.6	2778.8	2779.8
81	2900	2620	2720	2740	3033.5	3029.4	3034.5	3022.2
82	3180	3100	3100	3100	2991.5	2991.5	2894.5	2894.5
83	2820	2560	2540	2560	2574.5	2574.5	2574.5	2370.4
84	2280	2260	2300	2400	2181.2	2182.9	2182.9	2171.3
85	2700	2720	2720	2700	2917.4	2917.4	2921.7	2893.8
86	3120	3080	3180	3100	2789.6	2787.6	2886.0	2886.5
87	2080	2080	2100	2100	2069.4	2055.1	2234.8	2234.8
88	2240	2220	2240	2220	2063.3	2063.3	2298.6	2302.9
89	2700	2680	2700	2720	2961.6	2964.0	3097.8	3091.0
90	2500	2560	2580	2580	2348.7	2387.3	2552.8	2551.4
91	2480	2420	2460	2420	2277.6	2279.9	2356.2	2356.9

92	1700	1700	1740	1720	2188.5	2284.0	2266.4	2266.4
94	3400	3400	3500	3480	3390.4	3390.4	3518.4	3518.4
95	2300	2320	2360	2330	2516.8	2516.8	2601.6	2599.0
96	1920	1940	1940	1900	1680.2	1682.4	1884.7	1884.7
97	3100	3120	3120	3140	3196.5	3216.3	3481.8	3481.8
99	2200	2200	2220	2180	2534.6	2307.8	2307.8	2307.8
100	3080	3060	3140	3100	2832.5	2832.5	2902.3	2895.3
101	4040	4020	4040	4000	3892.0	3903.2	3994.4	3993.6
102	2180	2120	2200	2120	2078.9	2078.9	2048.6	2050.4
103	2080	2080	2060	2100	1989.4	1991.2	2033.6	2036.8
104	2600	2600	2600	2600	2558.1	2549.1	2841.5	2681.0
106	2100	2120	2080	2100	1850.9	1850.9	1923.5	1883.7
107	2280	2200	2260	2300	2109.0	2332.5	2193.8	2211.8

Annex 15. Information sheet

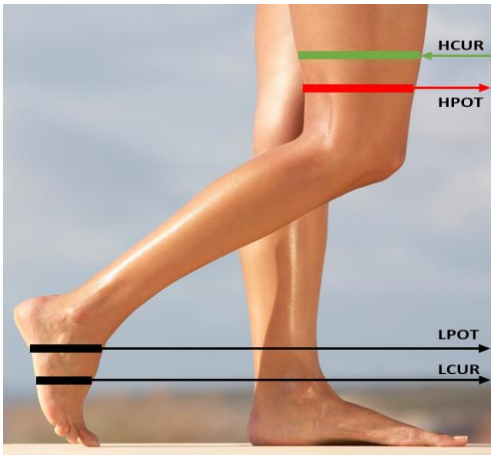
Title of Project: Piloting the body composition measuring device on patients with podoconiosis, Wayu Tuka District, Western Ethiopia.

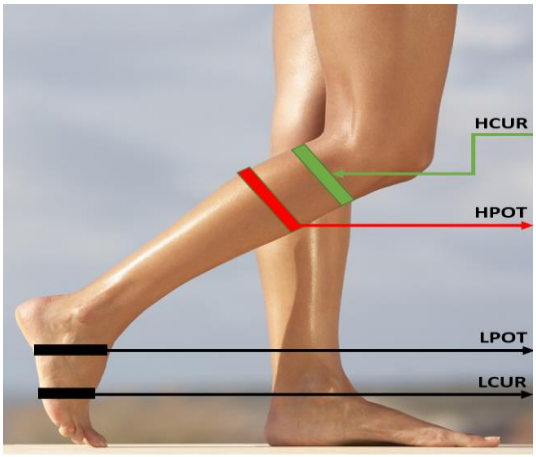
Name of Investigators: Mr. Abdi Samuel, Prof. Gail Davey, Prof. Abebaw Fekadu, Dr. Kebede Deribe, Prof. Chris Chatwin and Dr. Tabassum Qureshi

My name is ……., and I am working with a study team in Nekemte, Addis Ababa and the UK. You are invited to take part in this research study, which we hope will help us assess whether the body composition measuring device might be useful as a diagnostic tool for diagnosing podoconiosis. We are asking people with podoconiosis and people without podoconiosis to take part, because it is important that the device is checked with both groups. Before you decide whether to take part, it is important for you to understand why we are collecting this information and what it will involve.

The ZI MFIA is a digital impedance analyzer which is a body composition measuring device. It has been applied to many areas in the medical field; such as in body volume change and composition measurement, tissue identification and tissue/organ monitoring, cardiac ischemia, cryosurgery, and to monitor organs to be transplanted and also in assessing lymphedema for breast cancer patients.

Before testing with the device, you will be cordially invited to rest at the study site for at least two hours prior to the measurement, to avoid changes in vascular perfusion, temperature, cutaneous blood flow, vasodilation, and fluid losses. Then you will be requested to lie in the supine position for at least 5 minutes and remove any stockings/socks/clothes and leg jewellery which cover the tested region and your leg will be cleaned using a swab. Then, electrodes will be attached to your body as indicated in a figure1 below and finally we will perform the impedance measurement and capture the numeric data.





Electrode positioning

Please take time to read this paper carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information.

Background to the study

We plan to test the the body composition measuring device for diagnosis purposes. With your permission, we intend to:

1. Ask you a couple of questions about yourself
2. Examine your legs as stated above
3. Ask some questions about your experience once the measurement has been taken.

Possible harms: Even though a current will be passed between the electrodes, it is extremely small, and unlikely to be noticed by the participant. So, we do not anticipate any harm to you from this procedure.

Benefits: There won't be any direct benefits to you of being involved in this study, though we will provide you with soap in compensation for the time you have given the project. We hope that in the future, other patients will benefit from better understanding of the device.

Confidentiality: All information which is collected about you during the research will be kept strictly confidential.

Autonomy: If you wish not to take part in this sample collection that is fine – you do not need to give a reason. If you as a patient decide not to participate, the treatment you or your family receives in future at government sites will not be affected.

If something goes wrong: If a problem arises, you can report it to the study coordinator at the address given below.

What will happen to the research? We anticipate that the results of this immediate study will be available next year, and we hope to publish the results. You will not be identifiable in any publication.

Who is organizing and funding the research? The research has been funded by the Brighton and Sussex Medical School, a UK-based University. The research is organized jointly by researchers in Nekemte, CDT Africa and the UK.

Contact Address: Mr. Abdi Samuel
Cell Phone: +251917813478

Annex 16. Consent Form

Testing the body composition measuring device on podoconiosis patients, Wayu Tuka District, Western Ethiopia.

Name of Investigators: Mr. Abdi Samuel, Prof. Gail Davey, Prof. Abebaw Fekadu, Dr. Kebede Deribe, Prof. Chris Chatwin and Dr. Tabassum Qureshi

	Please check box
I confirm that I have read and understood the information sheet for the study '**Testing the ZI MFIA digital impedance analyser on podoconiosis patients, Wayu Tuka District, Western Ethiopia**'. I have had the chance to read the information and ask questions about the study and am satisfied with the answers I have been given.	☐
I understand that my participation in this study is voluntary and that I am free to stop at any time, and I do not have to give a reason for doing so. I understand that if I ask to stop the study my medical care and legal rights will not be affected in any way.	☐
I understand that occasionally an external regulator or funding body may ask to look at the data to check that the study is being run correctly.	☐
I wish to be contacted by the research team if unexpected findings requiring follow up are identified	☐
I agree to take part in the above study.	☐

_________________________ __________________ _______________________

Name of Participant Date Signature

_ _

Researcher to complete:

- I have explained the information in this document and encouraged the participant to ask questions and provided adequate time to answer them.

_____ _____ _____

Name of Researcher Date Signature

Annex 17. A proforma prepared for the study entitled "Piloting the ZI MFIA digital impedance analyzer on patients with podoconiosis, Wayu Tuka District, Western Ethiopia"

Identifier _____

S. N	Questions	Responses	Skip pattern
Part I. Sociodemographic Variables			
11	Age	_____years	
12	Sex	1. Male 2. Female	
13	Where is your permanent residence	2. Rural 3. Urban	
14	Region	4. Oromia 5. SNNPR 6. Amhara 7. Others _____	
15	Address	Woreda _____	
16	Educational status	3. Can read & write 4. Cannot read or write	
17	If your answer for question 16 above is 1, what is the highest grade attended?	_____	
18	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed 5. Other…….	
19	Occupational Status	1. Farmer 2. Daily laborer	

		3. Government employee 4. Housewife 5. Student 6. Merchant 7. Other……..	
110	For how long you have been living in this *kebele*?	_____(years) and ____ (months)	
Part II. Lymphedema and its characteristics			
21	Clinical stage of the disease	4. Mild 5. Moderate 6. Severe	
22	Clinical signs	4. Lymphedema 5. Hydrocele 6. Both lymphedema and hydrocoele	
23	If the answer for question 22 above is lymphedema, leg(s) affected	3. One leg 4. Both leg	
24	Extent of lymphedema	3. Above the knee 4. Below the knee	
25	For how long have you experienced the lymphedema?	_____months or _____years	

Annex 18. The Afaan Oromo version of the consent form for the ZI MFIA digital impedance analyzer pilot study

Unka walii galte

Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa” ZI MFIA digital impedance analyser” jadhamuun safaruun akka danda’amu qayyabanaa godhamu, anaa waayyu tuqaa, lixa itiyoophiyaati.

Qorattootii: Qorattootii: obbo Abdii Saamu'eel, Pirof Geeyl Deevii, Pirof. Braandon Diiksan, Pirof, Ababaaw Fiqaadu, Dr. Kabbadaa Darribe, Professor Kriis Chaatwin fi Dr.Tobassum Qureeshii	**mallatto itti godhaa**
Waa'e Qorannoo "Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa ZI MFIA digital impedance analyser jedhamuun safaruun akka danda'amu qayyabanaa anaa waayyu tuqaa, lixa itiyoophiyaati godhamu," jedhu kana unka odeeffanno isaa siriitti dubise hubadheera, gaaffi gaafachuufis carraa argadheera, deebii naaf keenanitiis itti quufeera.	
Qorannoo kana irratti hirmaanaan godhu fedhii koon akk ta'e hubadheera, yarroon barbaadeettiis dhiisu akkan danda'u beeka kana gochuufiis sababii koo illee ibsuun akka na irraa hin eegamne hubadheera. Yoon qorannoo kan irraati hirmaachu didees ta'e jalqabe garagar kute yaaliin argachaa jiruus ta'e mirgiin qabu hundi akka ittii najalaa hin bu'amne hubadheera.	
Qaami to'ataan alaas tae qaami qorannoo kana maallaqaan gargaaran odeefanno qoarnnoo kanaaf sasaabamu sirnaan deemaa jiraachu isaa gaafachuuf to'achu akka danda'aniis hubadheera.	
Qorannoo kan irratti hirmaachuuf walii galeera.	☐
Waa'e Qorannoo "Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa ZI MFIA digital impedance analyser jedhamuun safaruun akka danda'amu qayyabanaa, aanaa waayyu tuqaa, lixa itiyoophiyaati godhamu. jadhamu kana unka odeeffanno isaa siriitti dubise hubadheera, gaaffi gaafachuufis carraa argadheera, deebi naaf kananitiis itti quufeera.	☐

_____ _____

_____

Maqaa hirmaataa guyyaa mallatto

Unka qo'ataa qorannoo Kanaan guutamu

Yaada qorannoo unka odeefannoo kana keessa jiru sirriitti ibseeraafi gaaffii akka gaafataniifiis jajjabeeseeran gaafii isaaniifiis deebi ga’a kenneeraafi.

Maqaa qo’ataa	guyyaa	Mallattoo

Annex 19. The Afaan Oromoo version of information sheet for the ZI MFIA digital impedance analyzer pilot study

Unka Odeeffannoo

Mata duree Qo'annoo: Dhiito miillaa sababii podokonosisiin namatti dhufu meeshaa "ZI MFIA digital impedance analyser" jedhamuun safaruun akka danda'amu qo'annoo godhamu, aanaa Waayyu tuqaa, Lixa Itiyoophiyaati

Qorattootii: obbo Abdii Saamu'eel, Pirof Geeyl Deevii, Pirof. Braandon Diiksan, Pirof, Ababaaw Fiqaadu, Dr. Kabbadaa Darribe, Professor Kriis Chaatwin fi Dr.Tobassum Qureeshii

Maqaan kiyya _____jedhama. Qayyabannaa kanaafan ragaa sassaaba. Qorannoon nuti gaggeessinu kun dhiitoon miillaa meeshaa haaraa kalaqame kan "ZI MFIA digital impedance analyser" jedhamu kun waa'ee dhiitoo kanaa beekuuf qoratama. Kanaaf isin akka qoranno kana keessaatti hirmaattan afeeramtaniittu. Kanaaf unka odeeffannoo kana yaroo fudhaa dubbisaatii yoo gaaffii qabaattaniis, yoo odeeffannoo dabalataa barbaaddaniis gaafadhaa haala jiru hunda qulqulleefadha.

Bu'uura qoranichaa

Kaayyoon qayyabannaa kanaa waa'ee dhiitoo miillaa dhukubsatoonni podokonosisii qaban kana meeshaa "ZI MFIA digital impedance analyser" jedhamuun qorachuu ta'a. kana gochuun kan inni fayyaduu, tokko qorichii dhukubsattooti kun fudhatan hagam isaan fayyadeera(bu'a qorichiichichaa baruuf), cimina dhukubichaa fi dhukubicha sadarkaale gargaraatti qoduuf fayyada.

Yoo qorannoo kan irratti hirmaachuuf eeyyamtan:

4. Waa'e keessan gaaffile muraasa isiin gaafana
5. Miilla keessan sakattaana
6. Miilla keessanii meeshaa ZI MFIA digital impedance analyser jedhamuun sakataanee qoranna.

Miidhaa qorannichi qabu: qorannoo kun miidhaa isiin irraan ga'u hinqabu. yaroo haanga daqiiqaa 15 fudhachuu danda'a.

Bu'aa qorannichaa: ammaan kana kallattiin qorannoo kana irraa bu'aan isiin argattan hin jiru garuu akkamiin dhukuba kana ofiin akka kunuunsiitan leenjii isinii laanna, akkasumas yaroo isiin qor'annoo kanaaf gumaachitaniif beenyaa walgitu isnii goona. Gara fulduraatti garu isiniis ta'e warrii kaan faayidaa guddaa bu'aa qorannoo kana irraa argachu ni dandeessu ta'a.

Icciitii eegu: qorannoo kanaaf odeeffanno isiin nuuf laattan hundi iciitiin eegama. Dhimmichiis kodiin hojjatama. Yoo firiin qo'annaa kanaa waan addaa ta'e ittiin isiin quunnamuuf itti fayyadamnna.

Mirga: qorannoo kanatti hirmmaachu dhiisuuf mirga guutu qabdu sababii hirmaachuu hin barbaadneefiis ibsa kennuun isiin irraa hin eegamu. Tajaajila isiniis ta'e maatiin keessan buufata fayyaa kana irraa argattan waliin waan walqabatus hin qabu.

Bu'an qorannoo kana irraa argamu akka xumurameen qaamoota ilaallatuuf ifa ni godhama garu maqaan keessan bifa kanmiin iyyu hin ibsamu.

Yoo rakkoon isiin mudate bilbila kanaa irratti bilbiiluun rakkoo jiru gabaasu dnadeessu

Qorannoo kana kan qopheesse Brighton and Sussex Medical School, yuniivarsiitii biyya Ingliziitti argamuuf dhaabata CDT Africa, biyya Itiyoophiyaatti aragamu dha. Baasii qorannoo kanaaf ta'u kan gargaare immoo "National Institute for Health Research (NIHR) Global Health Research Unit on NTDs at Brighton and Sussex Medical School using Official Development Assistance (ODA) funding" jedhama, yaadii qo'annoo kanaa kan namoota qorannoo kana gaggeessaniiti malee kan dhaabata kanaa miti.

Teesso nama quunnamtanii: Obbo Abdii Saamu'eel

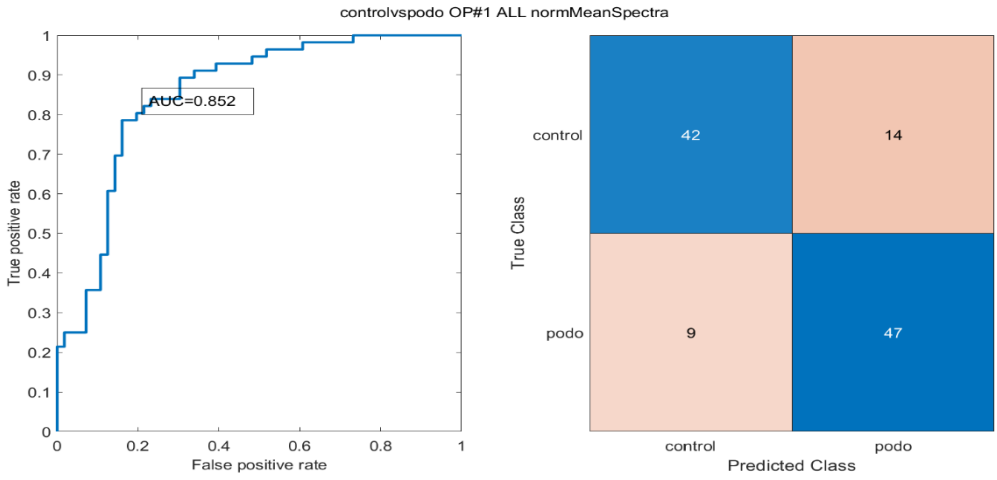
Lakk bilbilaa +251917813478

Annex 20. Area under the curve for DLP based NIR spectrometer study for different preprocessings and and study categories

Control versus podoconiosis

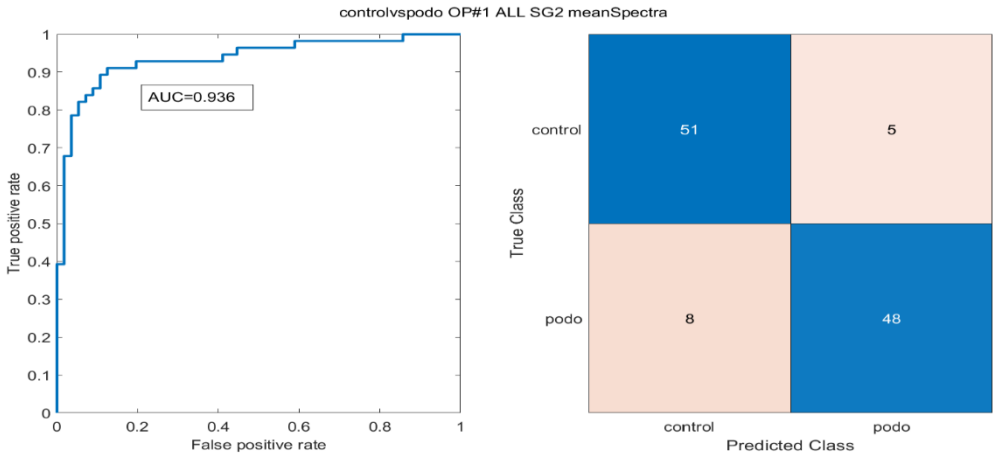
Operator one, Anterior lower leg

The area under the curve for normalized mean spectra was 0.852.



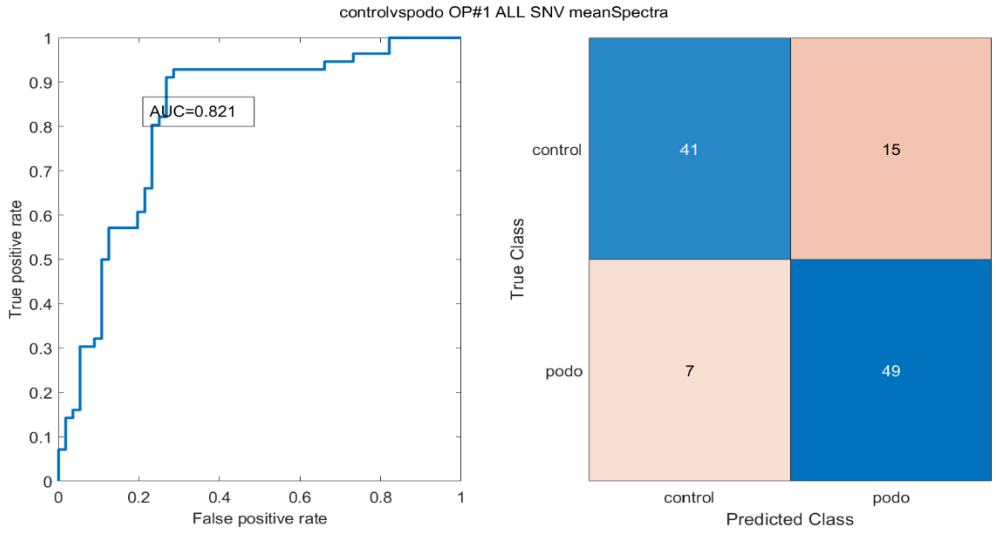
Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on normalised mean spectra for control versus podoconiosis.

The area under the curve for the SG2 mean spectra was 0.936



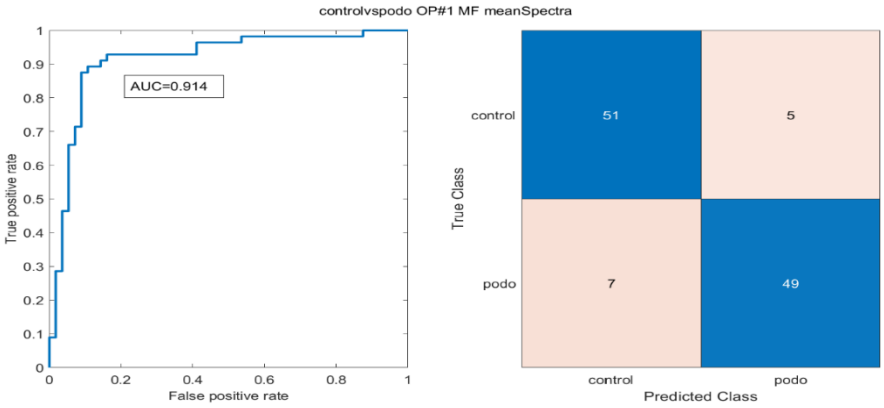
Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on SG2 mean spectra for control versus podoconiosis

The area under the curve for the SNV mean spectra was 0.821.



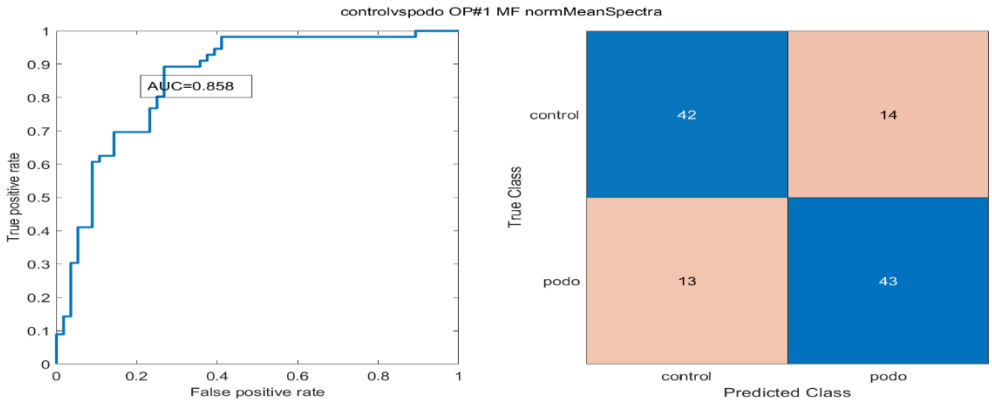
Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on SNV mean spectra for control versus podoconiosis.

The area under the curve for the mean spectra was 0.914

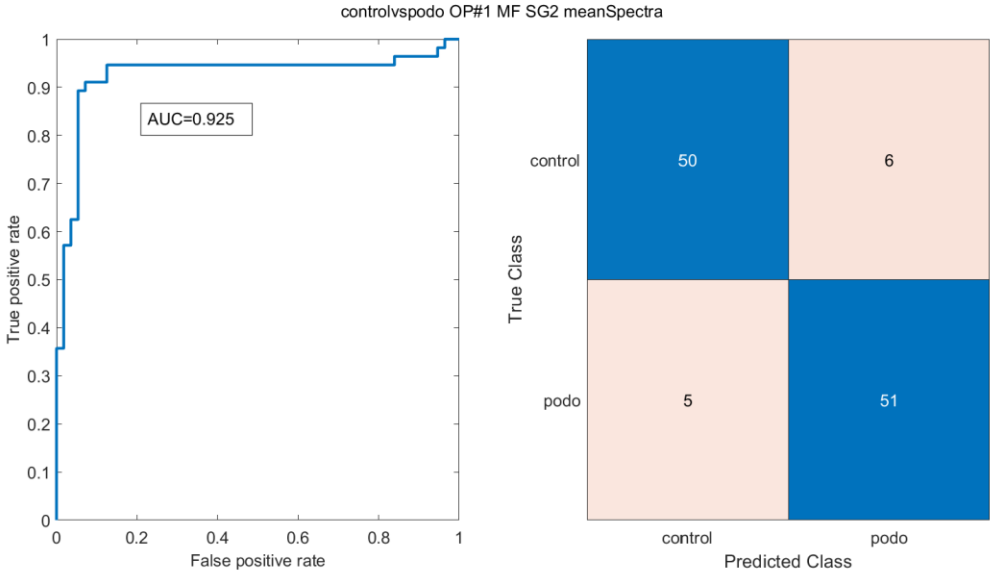


Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on mean spectra for control versus podoconiosis.

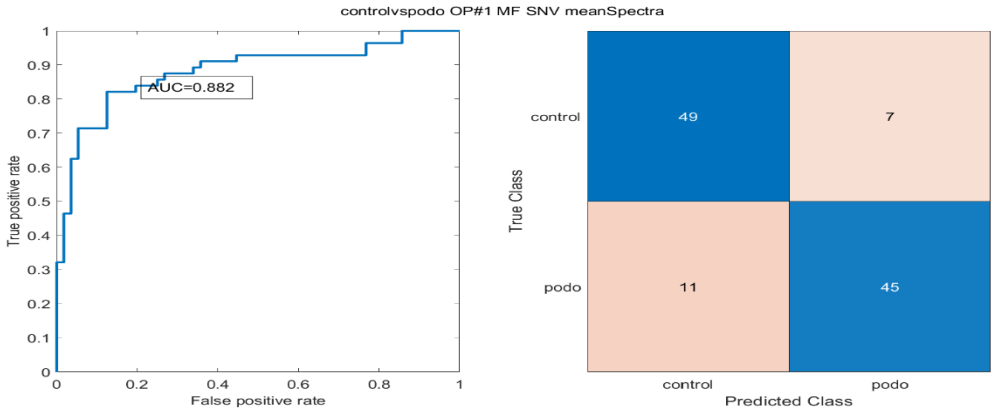
The area under the curve for the normalized mean spectra was 0.858



The area under the curve for the SG2 mean spectra was 0.925

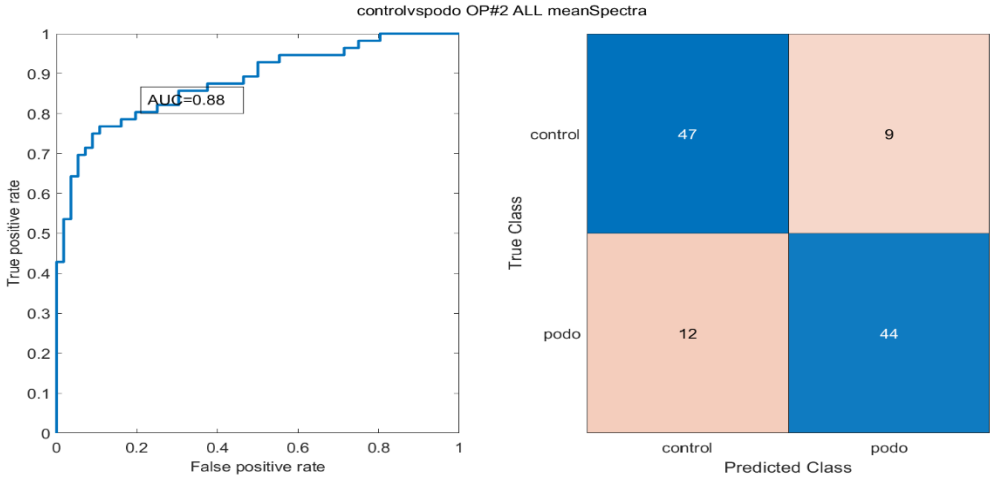


The area under the curve for the SNV mean spectra was 0.882



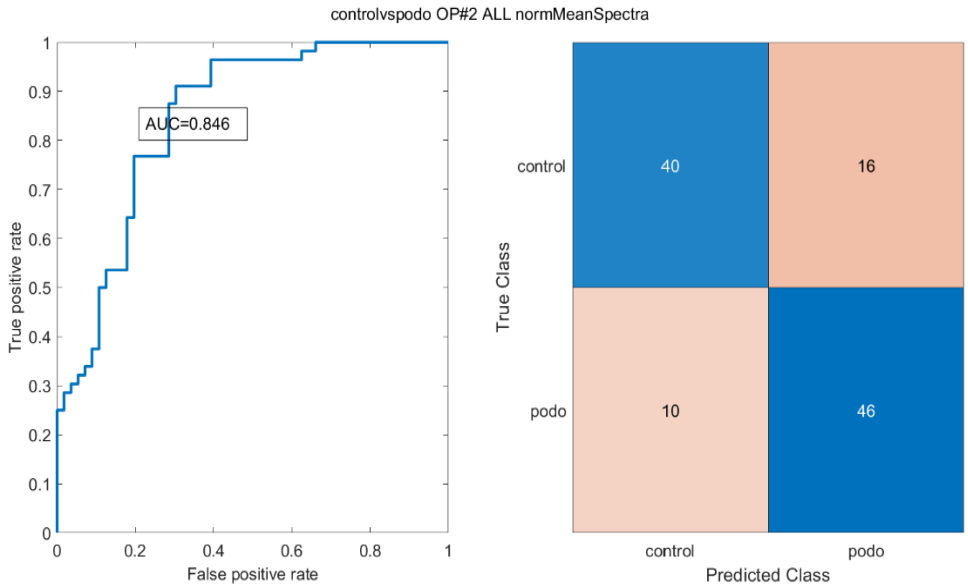
Operator two, anterior lower leg

The area under the curve for the mean spectra was 0.88



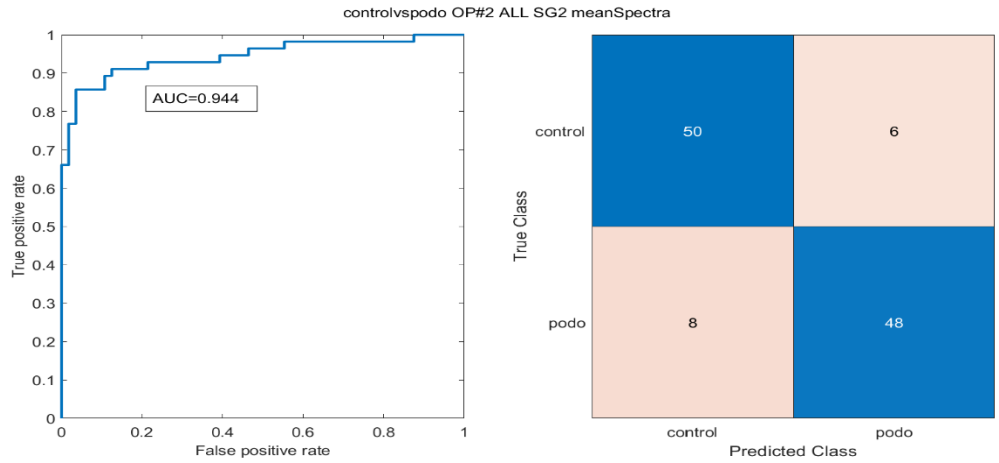
Area under the curve (left) and confusion matrix (right) for operator two from anterior lower leg based on mean spectra for control versus podoconiosis.

The area under the curve based on the normalized mean spectra was 0.846



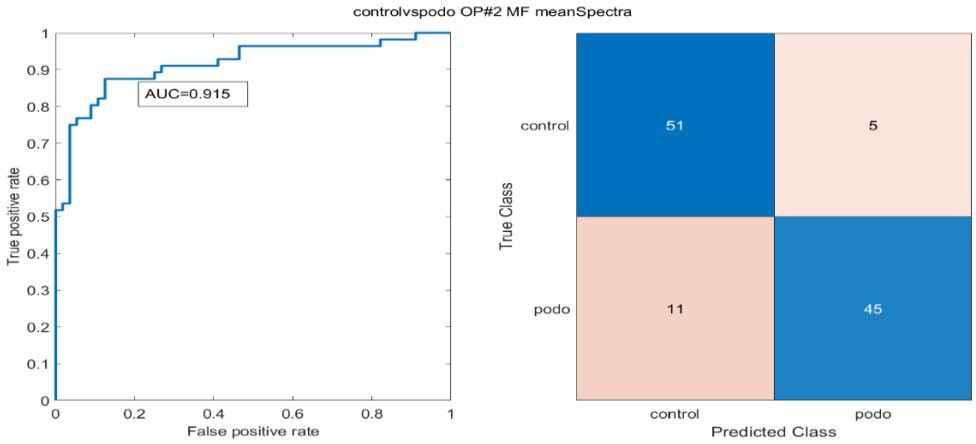
Area under the curve (left) and confusion matrix (right) for operator two from anterior lower leg based on normalised mean spectra for control versus podoconiosis.

The area under the curve based on SG2 mean spectra was 0.944

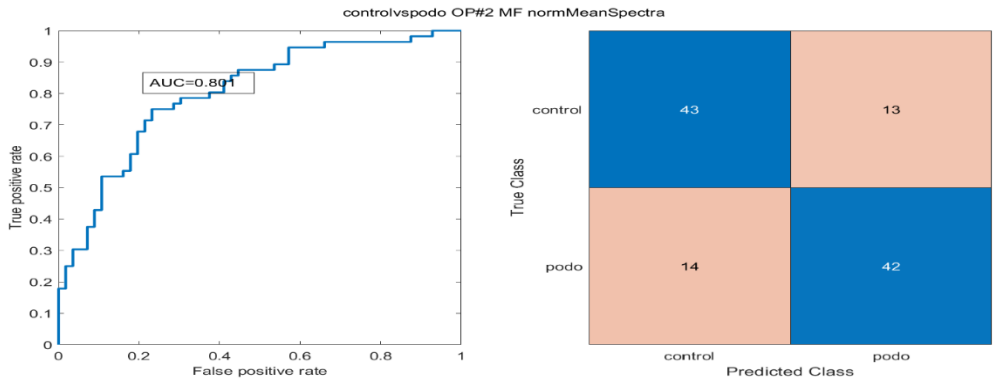


Operator two, Middle foot

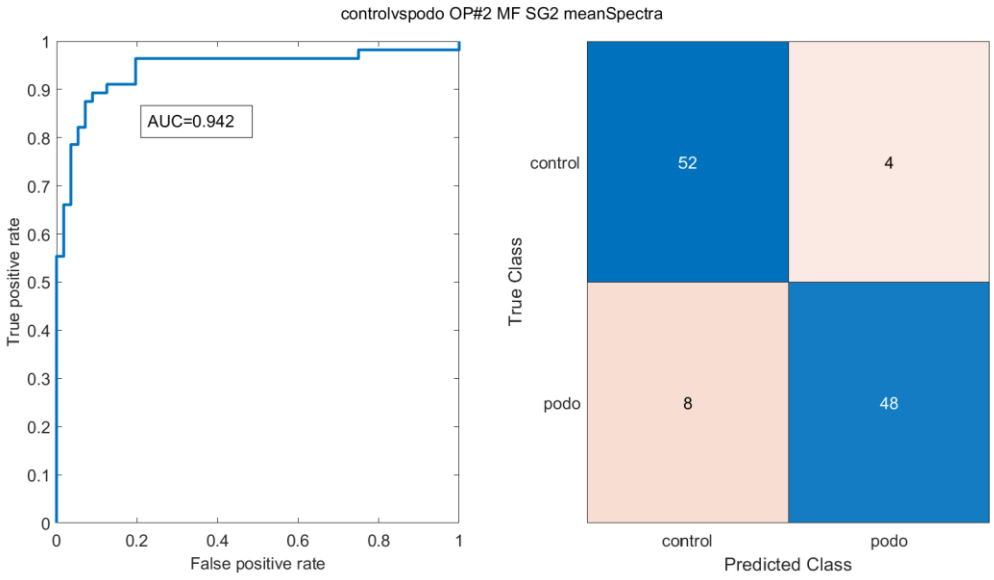
The area under the curve based on mean spectra was 0.915



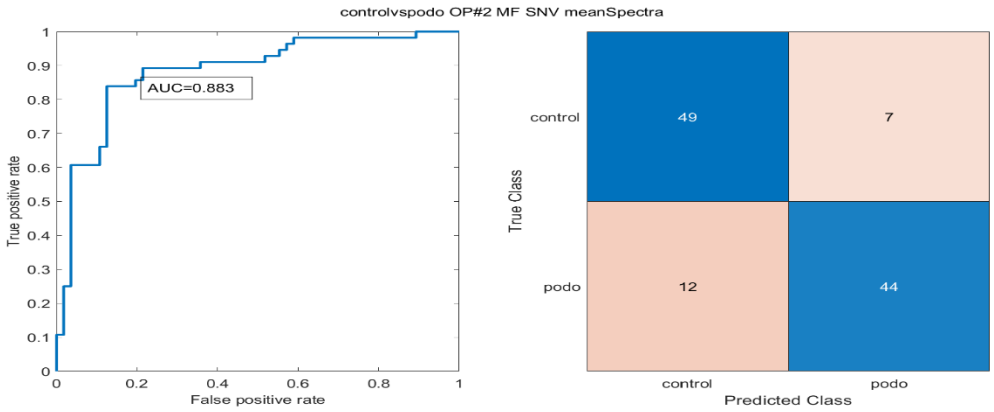
The area under the curve based on normalized mean spectra was 0.801



The area under the curve based on the SG2 mean spectra was 0.942



The area under the curve based on the SNV mean spectra was 0.883

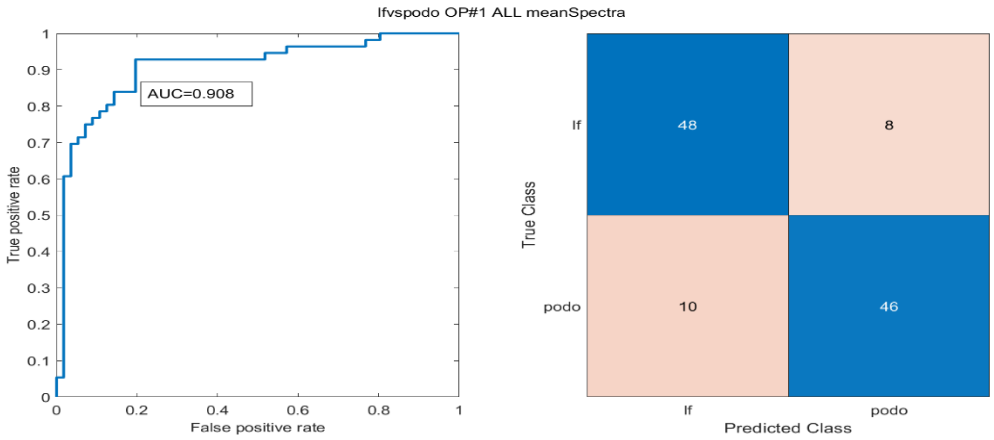


Area under the curve (left) and confusion matrix (right) for operator two from middle foot based on mean spectra for control versus podoconiosis.

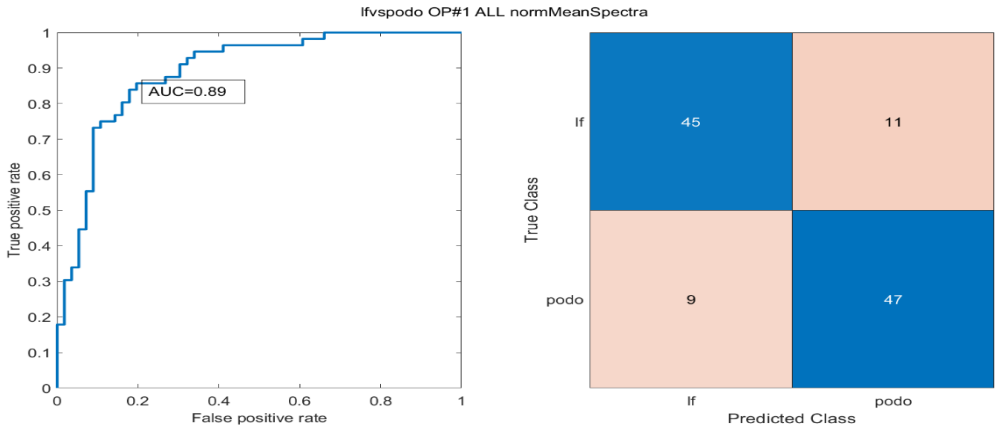
Lymphatic filariasis versus podoconiosis

Operator one, anterior lower leg

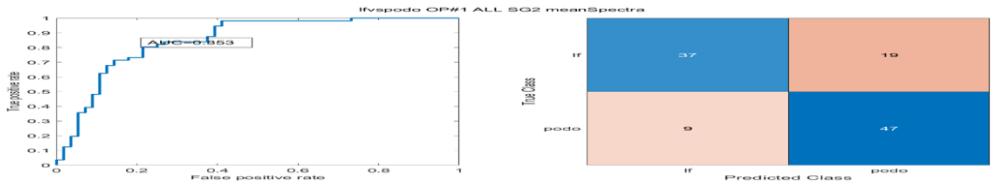
The area under the curve (AUC) for the mean spectra was 0.908



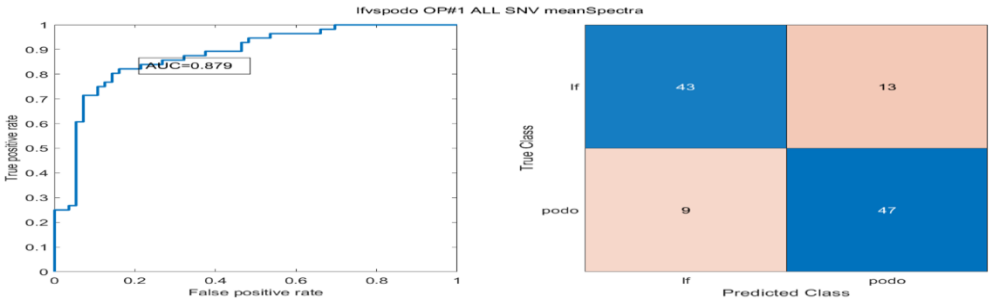
The area under the curve based on normalized mean spectra was 0.89



The area under the curve based on SG2 mean spectra was 0.853

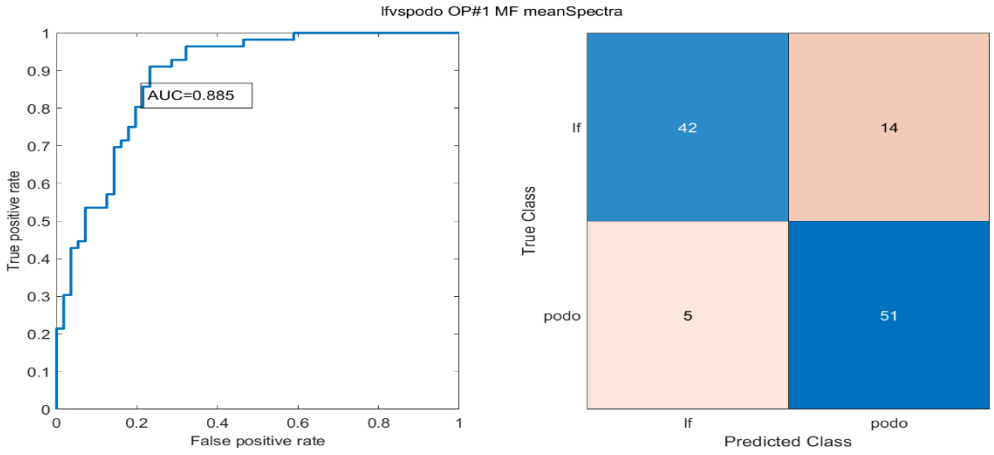


The area under the curve based on the SNV mean spectra was 0.879



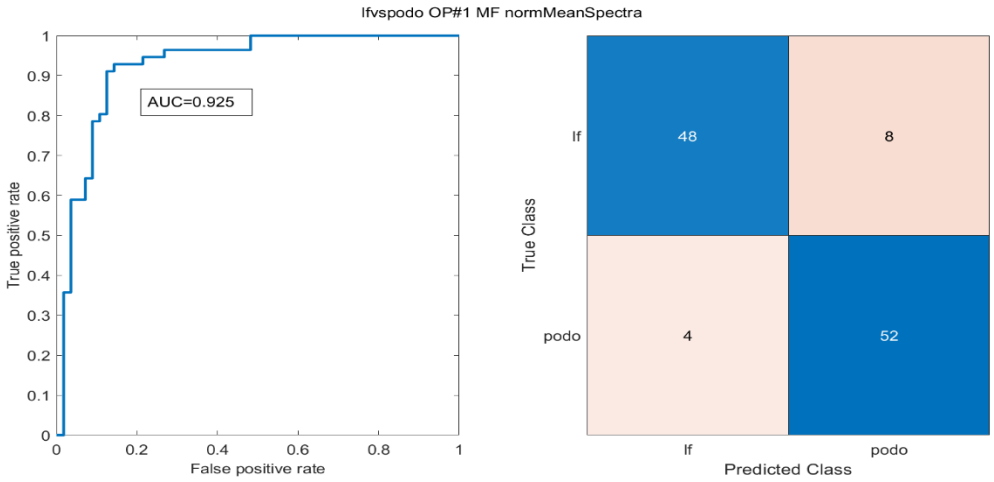
Operator one, Middle foot

The area under the curve for the mean spectra was 0.885

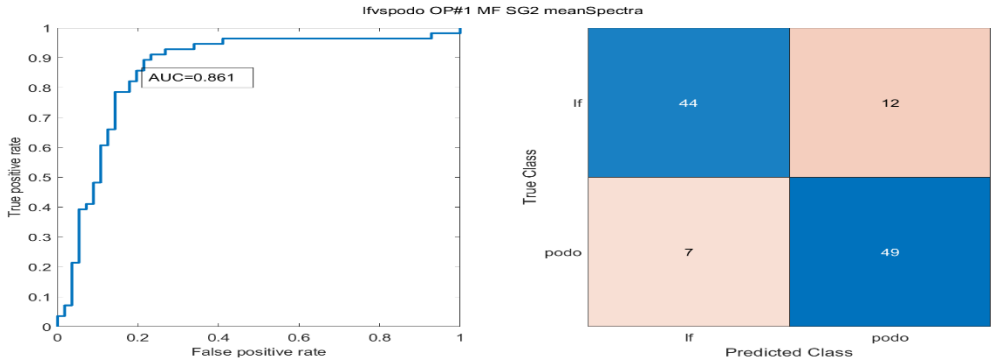


Area under the curve (left) and confusion matrix (right) for operator one from middle foot based on mean spectra for lymphatic filariasis versus podoconiosis.

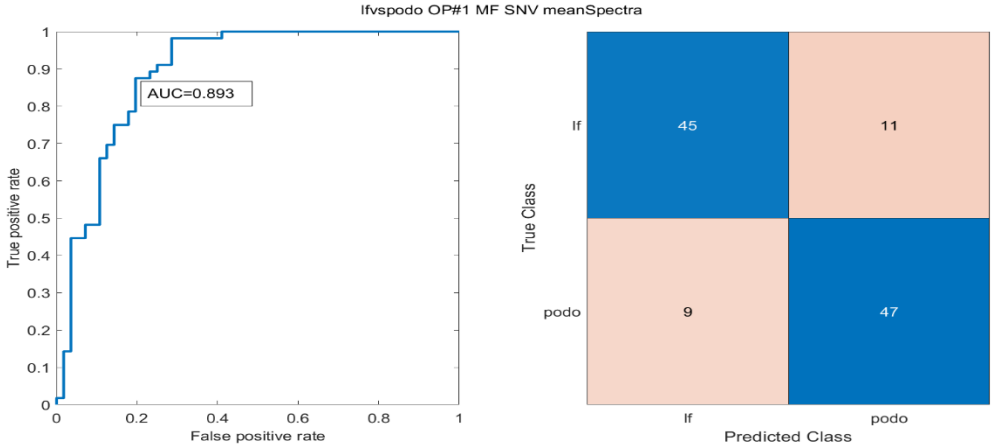
The area under the curve based on normalized mean spectra was 0.925



The area under the curve based on SG2 mean spectra was 0.861

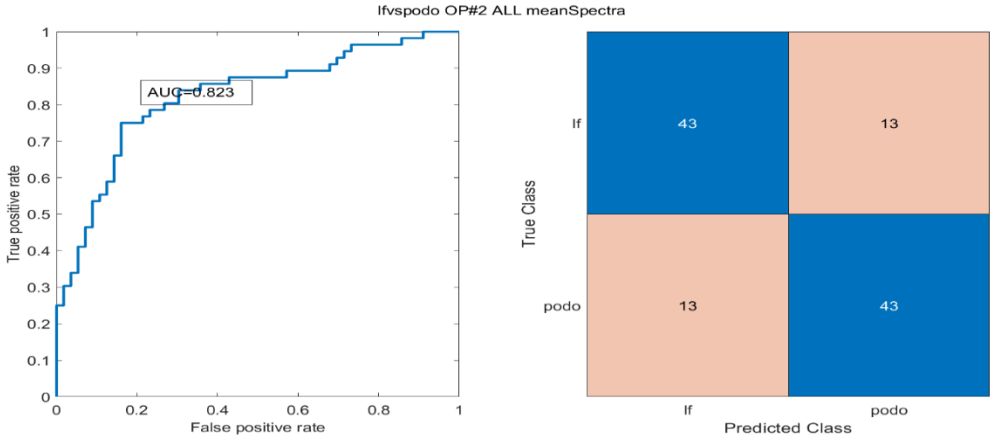


The area under the curve based on SNV mean spectra was 0.893



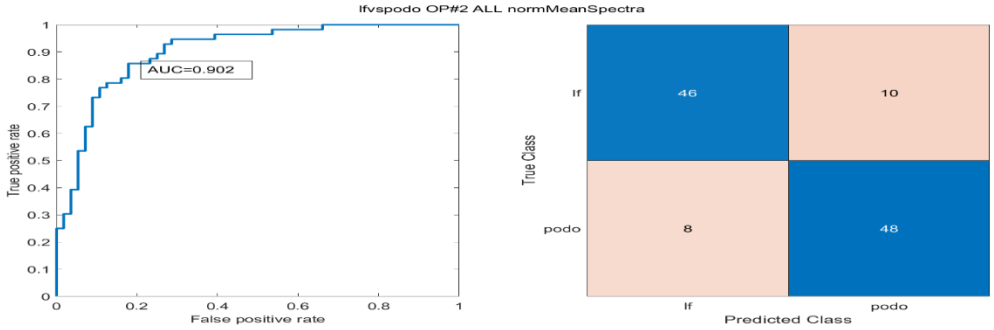
Operator two, Anterior lower leg

The area under the curve for the mean spectra was 0.823

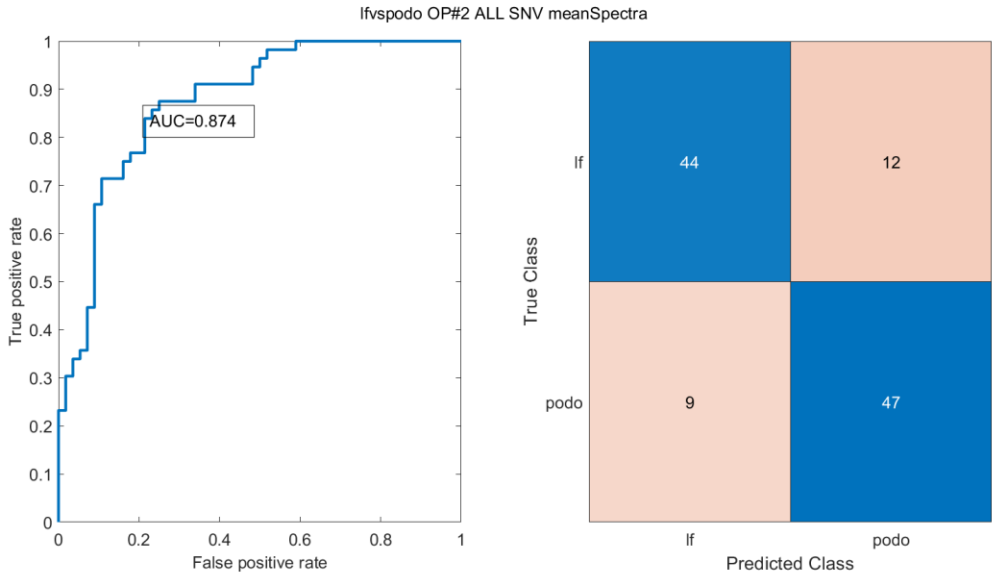


Area under the curve (left) and confusion matrix (right) for operator two from anterior lower leg based on mean spectra for lymphatic filariasis versus podoconiosis.

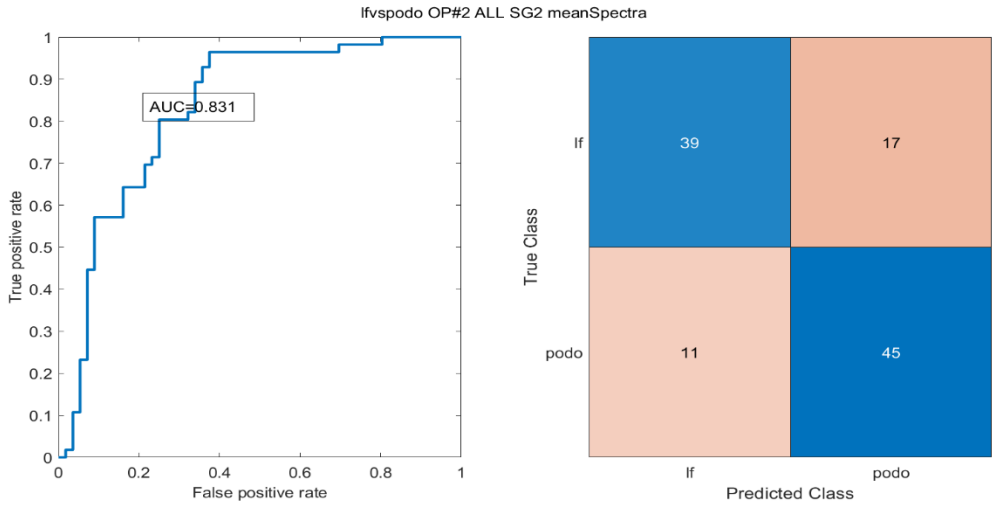
The area under the curve based on normalized mean spectra was 0.902



The area under the curve based on SNV mean spectra was 0.874).



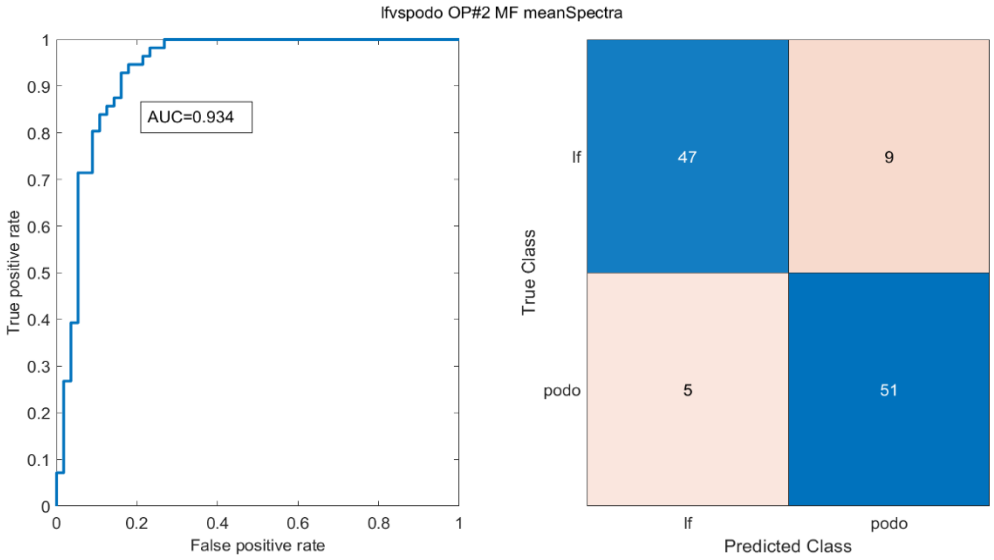
The area under the curve based on SG2 mean spectra was 0.831



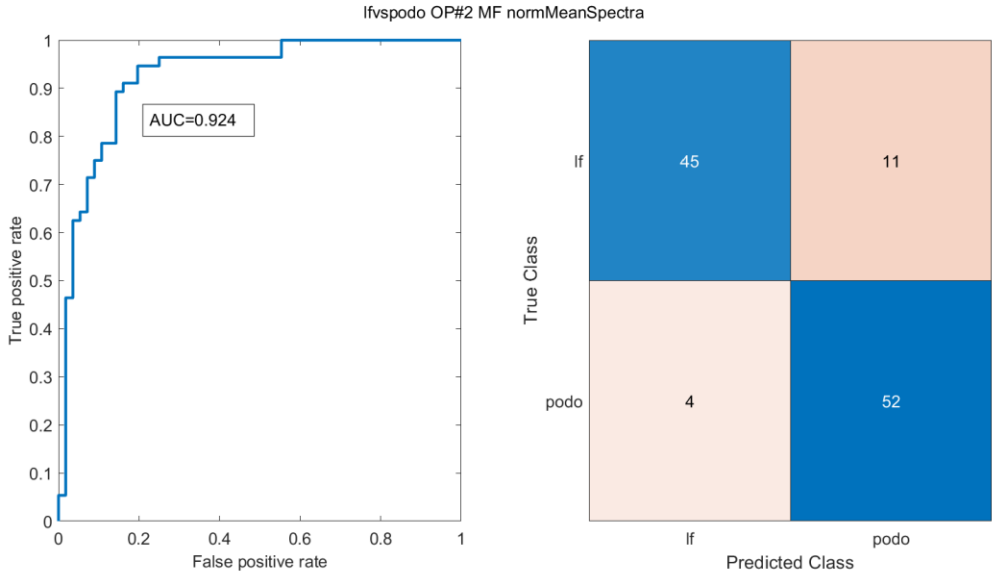
Area under the curve (left) and confusion matrix (right) for operator two from anterior lower leg based on SG2 mean spectra for lymphatic filariasis versus podoconiosis

Operator two, Middle foot

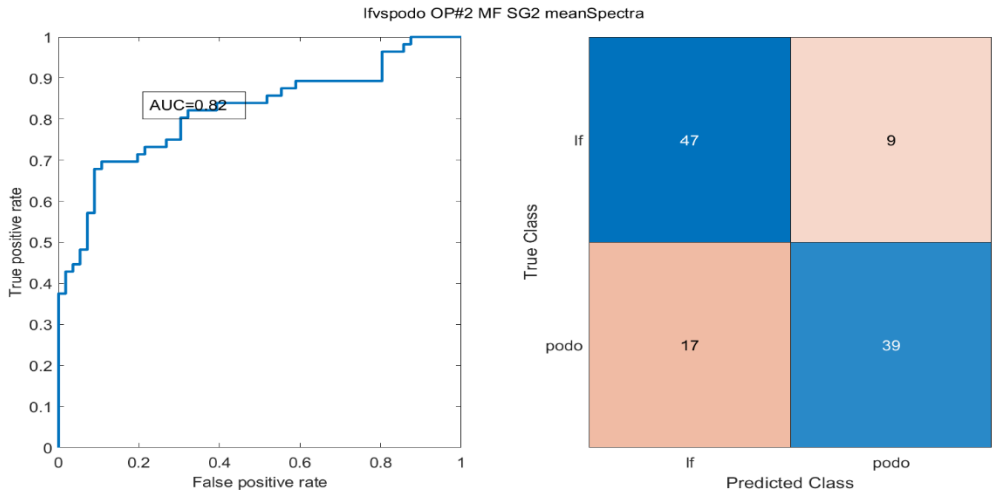
The area under the curve based on mean spectra was 0.934



The area under the curve based on normalized mean spectra was 0. 924

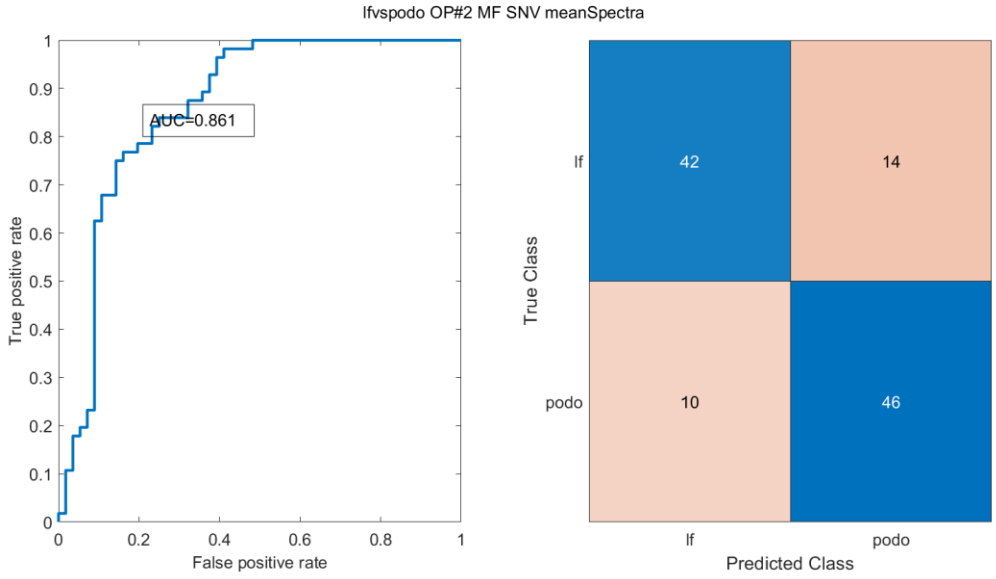


The area under the curve based on SG2 mean spectra was 0.82



Area under the curve (left) and confusion matrix (right) for operator two from middle foot based on SG2 mean spectra for lymphatic filariasis versus podoconiosis.

The area under the curve based on SNV mean spectra was 0.861

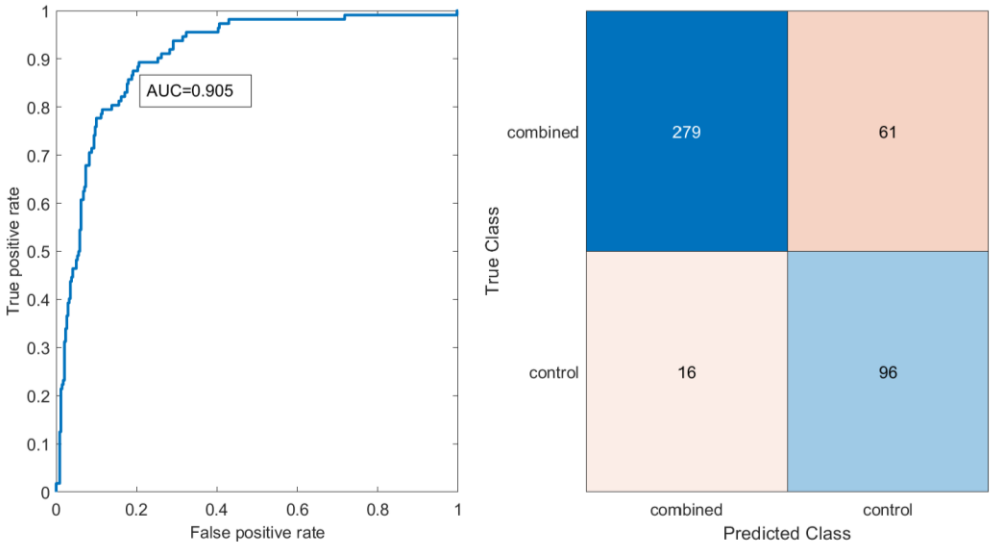


Area under the curve (left) and confusion matrix (right) for operator two from middle foot based on SNV normalised mean spectra for lymphatic filariasis versus podoconiosis.

Control vs Everything else

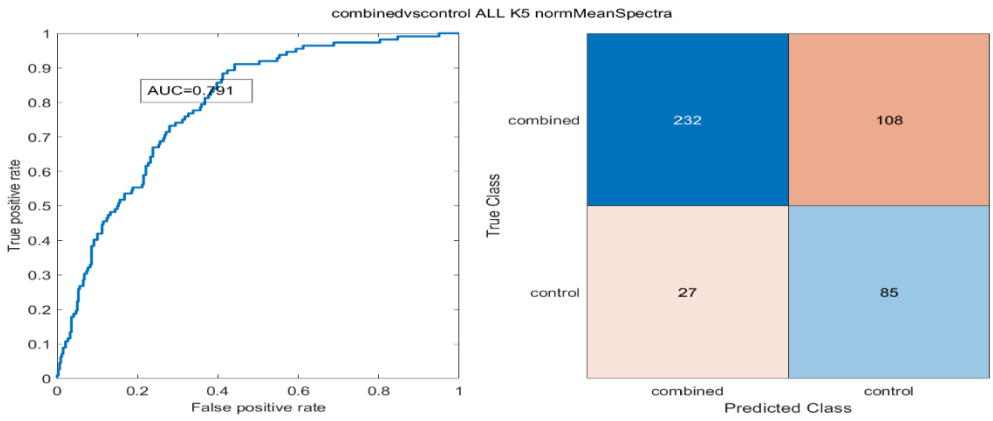
Anterior lower leg

The area under the curve (AUC) for the mean spectra was 0.905

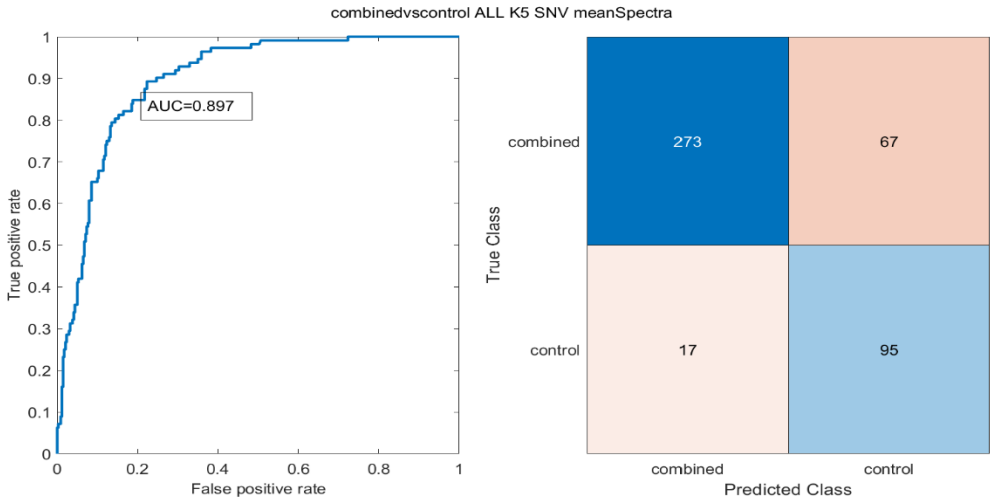


Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on mean spectra for control versus everything else.

The area under the curve (AUC) for the normalized mean spectra was 0.791

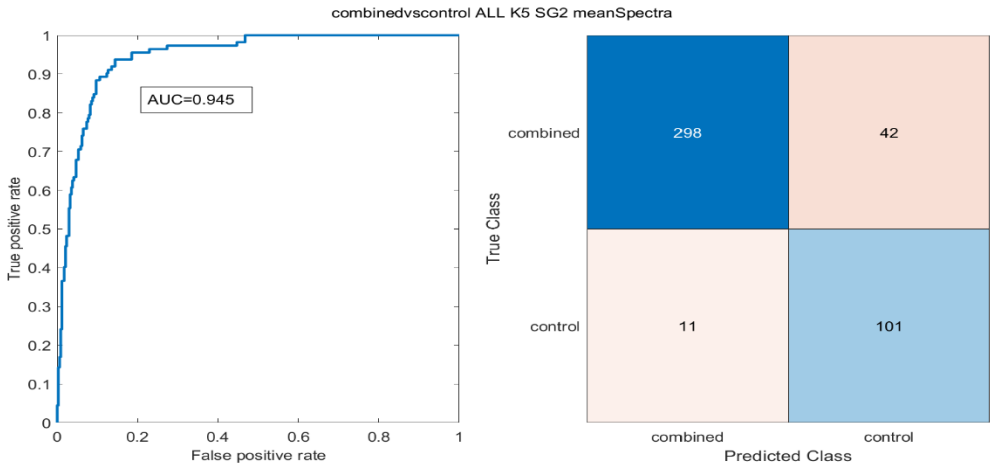


The area under the curve (AUC) for the SNV mean spectra was 0.897



Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on SNV mean spectra for control versus everything else.

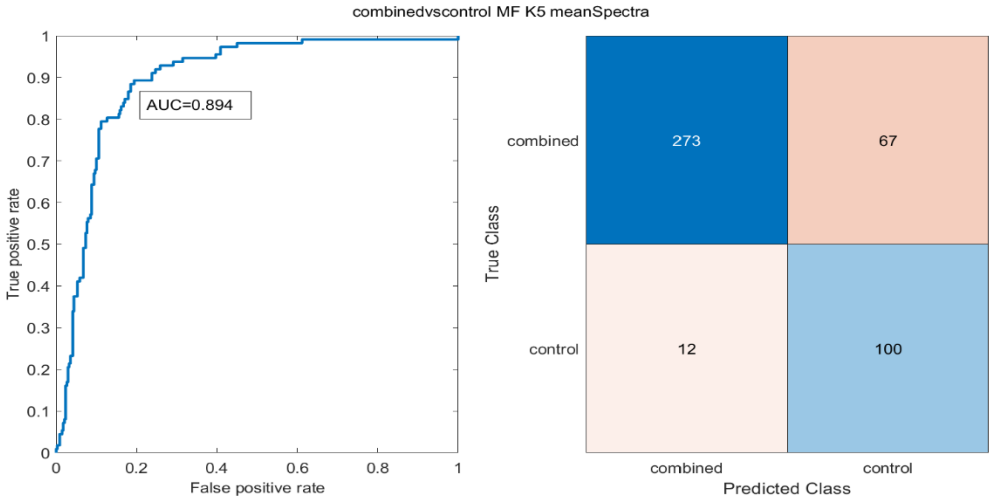
The area under the curve (AUC) for the SG2 mean spectra was 0.945



Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on SG2 mean spectra for control versus everything else.

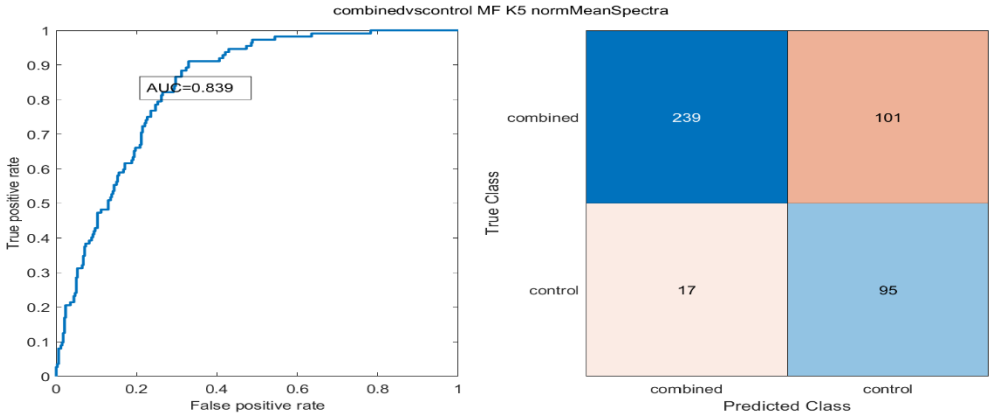
Middle Foot

The area under the curve for the mean spectra was 0.894

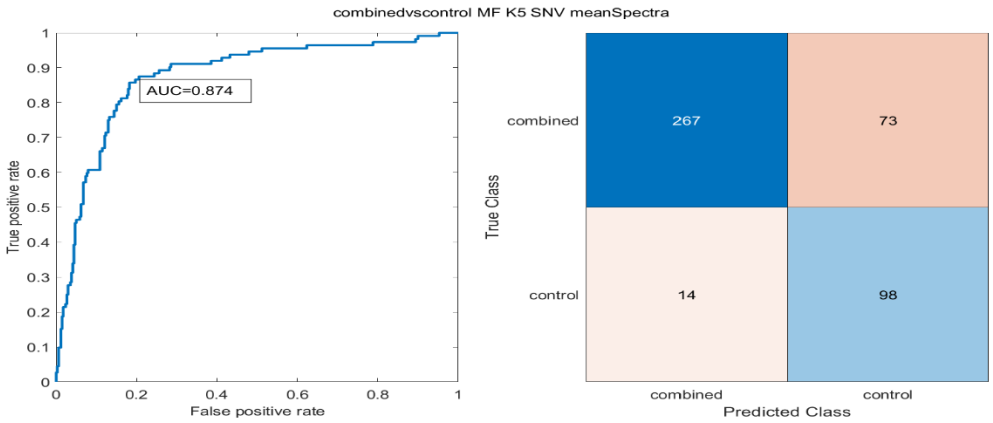


Area under the curve (left) and confusion matrix (right) for operator one from middle foot based on mean spectra for control versus everything else.

The area under the curve for the normalized mean spectra was 0.839

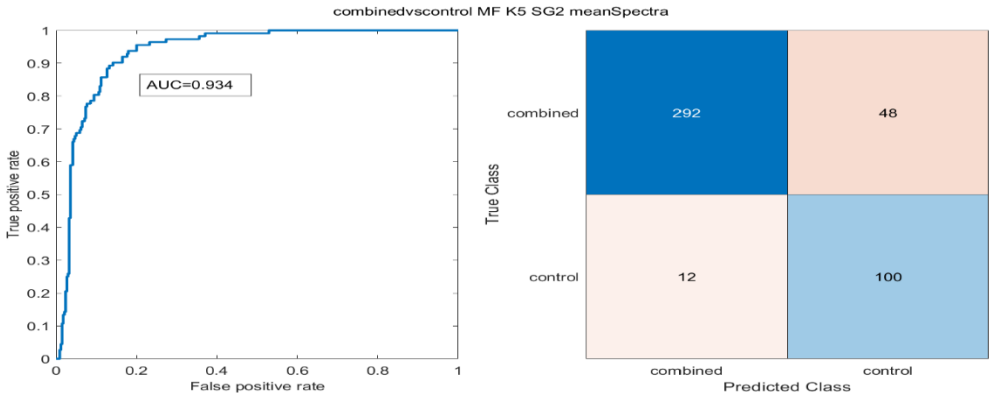


The area under the curve for the SNV mean spectra was 0.874



Area under the curve (left) and confusion matrix (right) for operator one from middle foot based on SNV mean spectra for control versus everything else.

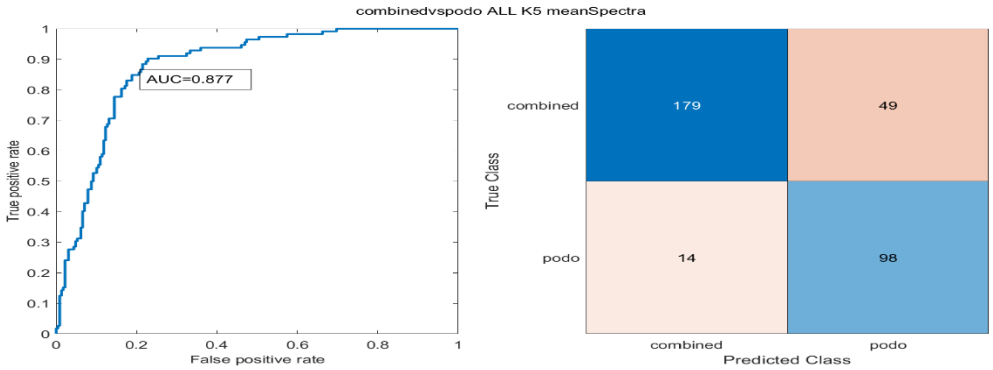
The area under the curve for the SG2 mean spectra was 0.934



Podoconiosis versus Lymphatic filariasis and other lymphoedema

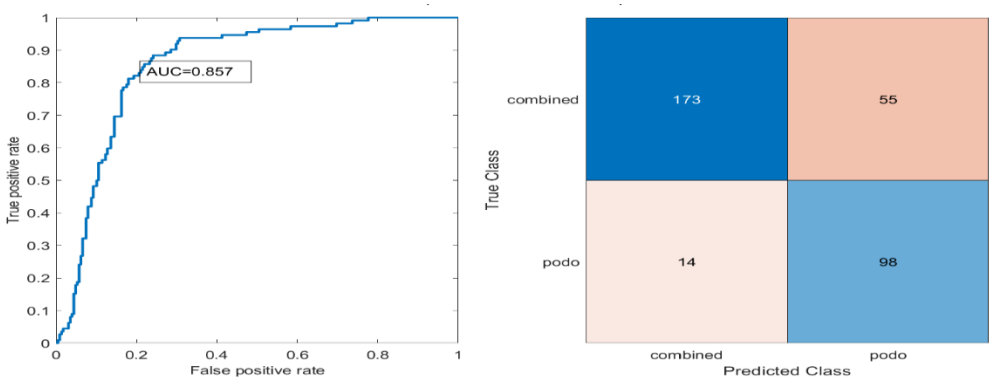
Anterior lower leg

The area under the curve (AUC) for the mean spectra was 0.877



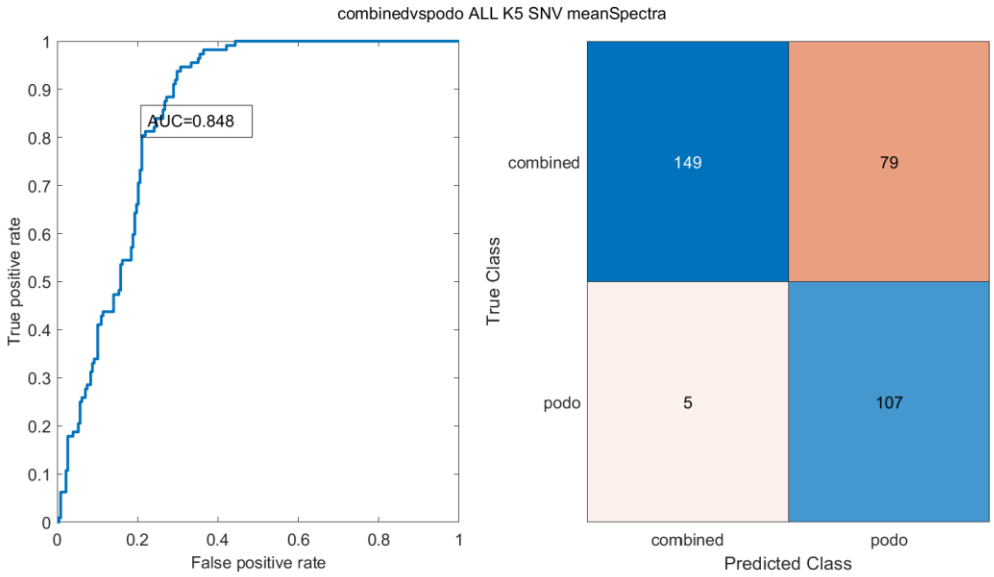
Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on mean spectra for podoconiosis versus Lymphatic filariasis and other.

The area under the curve (AUC) for the normalized mean spectra was 0.857



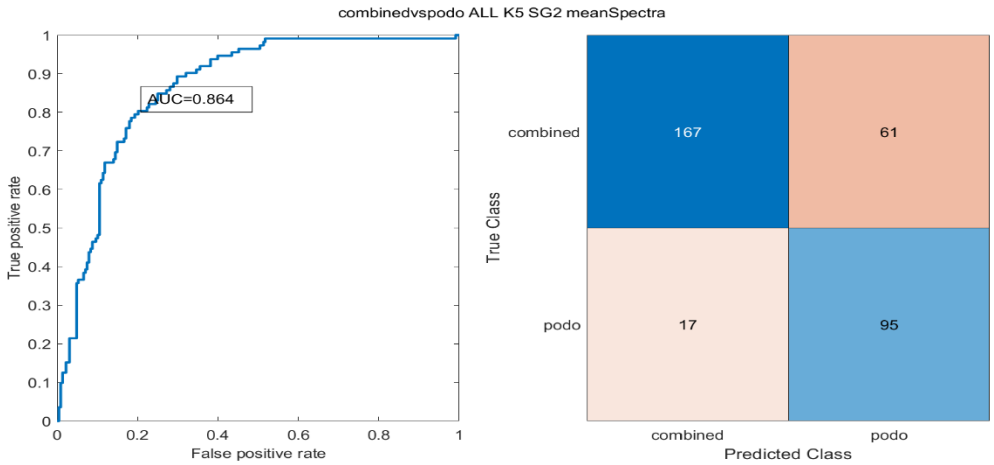
Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on normalised mean spectra for podoconiosis versus Lymphatic filariasis and other.

The area under the curve (AUC) for the SNV mean spectra was 0.848



Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on SNV mean spectra for podoconiosis versus Lymphatic filariasis and other.

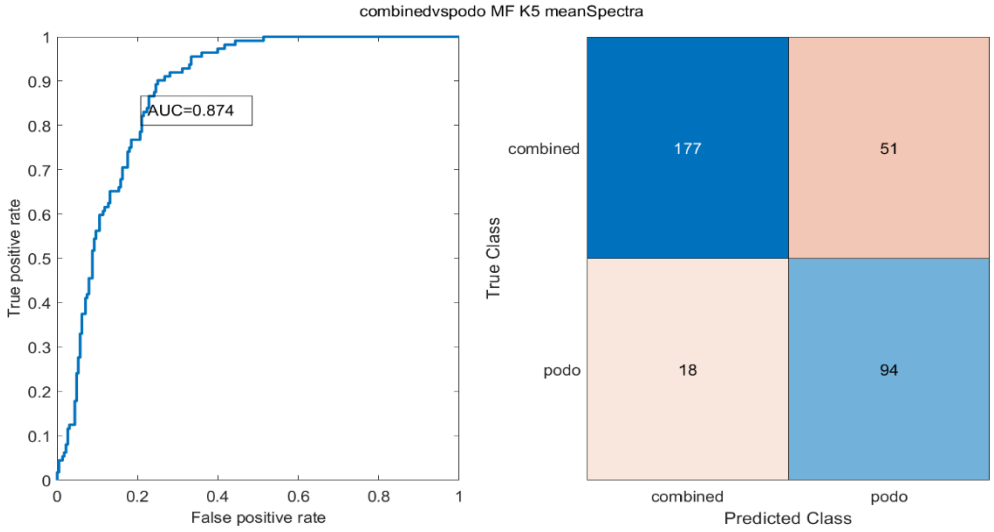
The area under the curve (AUC) for the SG2 mean spectra was 0.864



Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on SG2 mean spectra for podoconiosis versus Lymphatic filariasis and other.

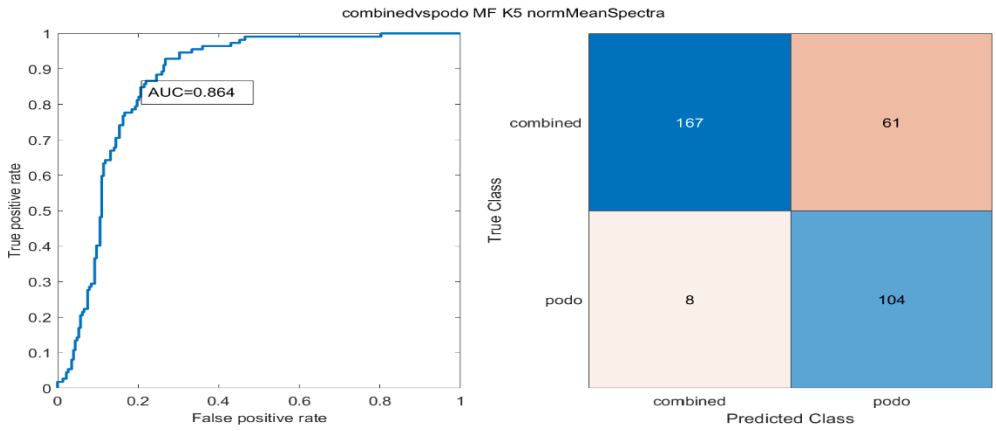
Middle foot

The area under the curve based on mean spectra was 0.874



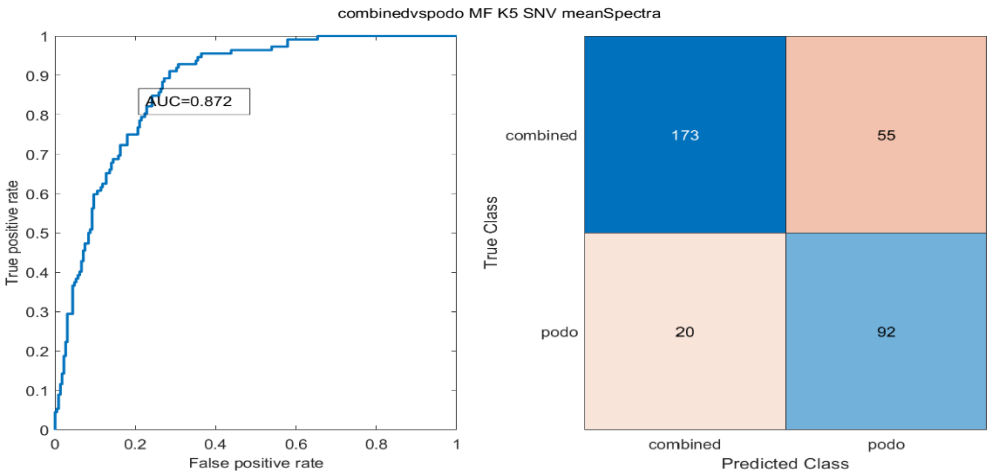
Area under the curve (left) and confusion matrix (right) for operator one from lower leg based on mean spectra for podoconiosis versus Lymphatic filariasis and other.

The area under the curve based on normalized mean spectra was 0.864



Area under the curve (left) and confusion matrix (right) for operator one from anterior lower leg based on normalised mean spectra for podoconiosis versus Lymphatic filariasis and other.

The area under the curve based on SNV mean spectra was 0.872



The area under the curve based on SG2 mean spectra was 0.884