

# A multivariate NIR study of skin alterations in diabetic patients as compared to control subjects

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**A group of 15 diabetic persons with different degrees of diabetes complications, including skin changes, was studied by Fourier Transform Near Infrared (FT-NIR) spectroscopy. Skin reflectance spectra were measured with a fibre-optic probe in four locations (sites): hand, arm, leg and foot. For reference, a group of 28 healthy controls was also measured. Multivariate analysis of the NIR spectra obtained shows a high potential for classification and discrimination of the skin conditions. Valuable indications for future experiments can be observed.**

*Keywords:* FT-NIR, fibre-optic probe, PCA, PLS-DA, skin changes, diabetes

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## Introduction

Persons suffering from diabetes mellitus have a number of health problems and complications that are not so common in the non-diabetic population. One of the problems is changes in the blood vessels and deterioration of nerve function, particularly in the extremities: hands and feet. Nephropathy, retinopathy, neuropathy and foot lesions are secondary complications that probably arise in diabetes patients because of high glucose levels over long periods of time. The “diabetic foot” is the most severe and also the most common complication for persons with diabetes; about 15% of diabetes patients are expected to experience foot lesions during their lifetime and the risk of needing an amputation is high in

this group. This causes a lot of personal suffering and a high cost to society.

Diabetic patients undergo slow changes in the microarteries resulting in decreased blood flow. The assumed reason for this is that long-term increased glucose levels result in tissue oedema (swelling). In the long run, these micro-oedemas give permanent nerve damage and also skin changes due to decreased blood flow. A first indication of the “diabetic foot” complication is that skin changes and ulcers develop. If the skin changes can be detected at an early stage, treatment can be initiated to prevent or delay the most severe problems. As presented in an earlier work<sup>1</sup> Multi-Frequency Bioelectrical Impedance Analysis (MFBI) of the patient’s skin can be a promising diagnostic tool. The skin changes are related to changes in structure and chemical compo-

sition and NIR spectroscopy should be able to measure them.

The measurements<sup>1</sup> were repeated with MFBIA but in addition near infrared (NIR) spectroscopy was tested as a diagnostic tool. The measurement method is non-invasive, non-destructive and fast. It requires no pretreatment of the skin. NIR FT-Raman has been used for the study of skin cancer.<sup>2</sup> A comparison of FT-IR and FT-Raman for skin studies has been described by Williams and Barry.<sup>3</sup> Clinical studies using NIR in different forms are becoming very common in the literature.<sup>4-9</sup>

The same 15 diabetic persons were recalled to the hospital four years after the first study.<sup>1</sup> In addition, a new and larger group of references (healthy controls) was studied. The diabetic patients underwent a number of clinical tests including neurological testing of the lower extremities, blood and urine chemistry and morphometric measurements. The 28 controls were healthy volunteers and were chosen for not having diabetes and matching the diabetic group in age. The typical clinical tests for diabetics were not performed on them. Table 1 gives an overview of the persons participating in this study. MFBIA and NIR were measured in addition to the more traditional clinical measurements. The MFBIA measurements are not reported in detail here. The NIR measurements were carried out in four different locations (sites): hand, arm, leg and foot (see Figure 1). The total data array of the NIR measurements is 43 objects  $\times$  1140 wavelengths  $\times$  4 locations (sites). The matrix and subsets of it are studied by multivariate methods. Outlier finding, classification and discrimination are the main results of the study.

## NIR spectrometer measurements

A Bruker Vector 22/N spectrometer was used together with a fibre-optic probe. The Vector 22/N is an FT-NIR<sup>10</sup> instrument with a Peltier-cooled InAs detector. The wavelength range is 660–2860 nm or, in wavenumbers, 15,000–3500  $\text{cm}^{-1}$ . A resolution of 4  $\text{cm}^{-1}$  was selected. The spectrometer is based on the interferometric principle using a quartz beam-splitter. The whole construction is very stable and robust, thanks to continuous checks and alignment

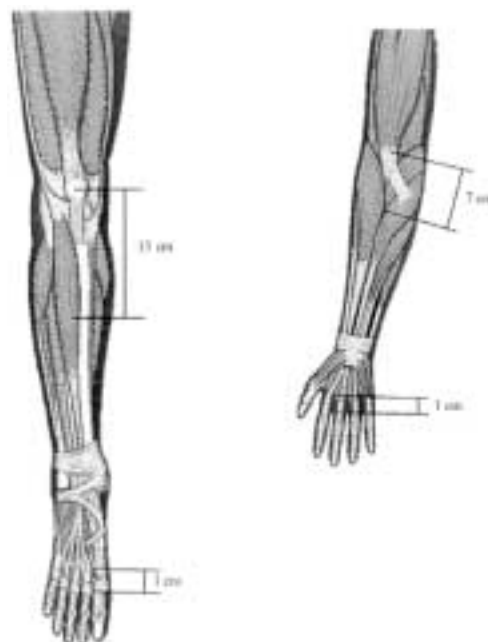


Figure 1. The different measurement sites: hand, arm, leg and foot.

based on laser beams. The given wavelength accuracy is better than 0.1  $\text{cm}^{-1}$  and the transmission accuracy is better than 0.1%. The fibre-optic probe has transmitting fibres and return fibres in the same fibre bundle. The measurement area is a disk of approximately 4 mm in diameter. The measurements were done with 32 co-added scans and three spectra were recorded for each site. The original spectra had 5964 data points and were expressed in wavenumbers versus pseudoabsorbance. Some noisy parts at the visible end of the spectra were removed, giving 5700 data pairs which were lumped together in groups of five by averaging, resulting in 1140 data points. Figure 2 shows some of the spectra obtained. The reference sample was Teflon provided by Bruker.

The fibre-optic probe is mainly used in liquids and in solid powders. The use on skin was an experiment and no information on geometry or penetration depth at the different wavenumbers was known. The measurement of the skin is via contact and is based on diffuse reflection without an integrating sphere, also called "interactance" by some authors.<sup>11</sup> The nomenclature for these measurements is not very exact.

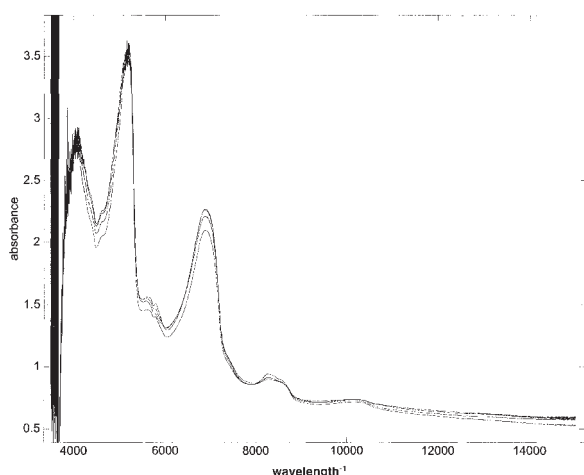


Figure 2. Some example NIR spectra given with increasing wavenumbers. The noisy part around  $3500\text{ cm}^{-1}$  was removed.

Near infrared measurements are temperature dependent. Osborne *et al.*<sup>10</sup> recommend to have a temperature interval of less than  $\pm 5^\circ\text{C}$  for the samples. No measurements of the skin temperature were made. Heating of the skin by the radiation was judged to be small.

## Clinical measurements

The study included eight male and seven female diabetic patients and 14 male and 14 female healthy controls (references). Patients are more expensive and time-consuming to work with than laboratory samples, so even though the patient and reference groups may be quite small they represent a major sampling and measuring effort. The MFBIA measurement required wetting the skin with an isotonic

Table 1. An overview of the persons participating in the study.

	Female	Male
Diabetic	7	8
Reference	14	14

saline solution.<sup>1</sup> The skin was not prepared in any way before FT-NIR measurement. Table 2 gives some statistics calculated on age. Matching of the patient and reference group by age was done as well as possible. There are two main types of diabetes. Children and young persons may develop type 1 diabetes. For those suffering from type 1 diabetes the immune system destroys the insulin producing B-cells in the pancreas. Type 2 diabetes is more common among older diabetics, and this is due to decreased insulin production and reduced sensitivity to insulin. The clinical measurements carried out on the diabetic patients include glucose, haemoglobin (HbA1c), oxygen saturation of haemoglobin, creatinin and ions (Na, K, Ca, Cl, phosphate). Also urine albumin excretion is measured. The clinical measurements are used as measures of the general health status of the diabetics.

The foot lesion measurements are of special importance for this study, because neural disorders and skin disorders come from the same cause. The nerve function measurements are a whole battery of objective and subjective measurements. The measurements are: arm systolic and diastolic blood pressure, arteria tibialis anterior and posterior blood pressure, toe systolic blood pressure and vibration sense threshold measurement. These measurements combined lead to an index that is subjectively deter-

Table 2. Age matching of the patients and references.

Age	Diabetes type 1			Diabetes type 2			References		
	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.
Males	59	56	53	61	57	56	57	49.5	30
Females	59	51	47				61	52.5	36

mined by a physician. This index can be called NFI (nerve function index). The patients get the status NFI = 0 (no change), NFI = 1 (slight change) or NFI = 2 (severe change). This is a qualitative measure in only three levels and therefore not very accurate. Of the female patients, three were NFI = 2, one was NFI = 1 and three were NFI = 0. Of the male patients, five were NFI = 2, two were NFI = 1 and one was NFI = 0. These classes are very small, so the construction of reliable test sets is expected to be very difficult.

## Software

The Bruker OPUS<sup>12</sup> program controlled the collection of the spectra and saved them as data files. SIMCA software<sup>13</sup> was used for the data analysis. For 3-D plotting, the program 3Drot<sup>14</sup> was used. Some data pretreatment was carried out in MATLAB.<sup>15</sup>

## Results and discussion

All the NIR data sets were pretreated with multiplicative scatter or signal correction (MSC)<sup>16</sup> and mean-centred before further analysis. The data can be analysed in an explorative manner by principal component analysis (PCA). Because the classification in diabetics and references is supervised, discriminant analysis can also be tried. For the diabetics only, matching of the PCA results with NFI data is useful. For the PLS-DA (Partial Least Squares-Discriminant Analysis),<sup>17,18</sup> pretreatment by orthogonal signal correction (OSC) was used.<sup>19-21</sup> Many score plots are shown as 3D plots with rotation.

### Differences between males and females, references and diabetics

A large data set (size 43 × 4560) was made of all references and diabetics, males and females, using results for the four sites as different variables. A schematic overview is given in Figure 3.

After pretreatment of the data by MSC and mean-centring, PCA was calculated. Some PCA results are shown in Table 3. A model of rank 5 was always

		Wavelengths			
		Arm	Hand	Leg	Foot
Objects		1,2...1140	1141...2280	2281...3420	3421...4560
Males	References	1,2,3... ...14			
Males	Diabetics	15...22			
Females	References	23,24... ...36			
Females	Diabetics	37...43			

Figure 3. The structure of the main NIR data set.

found more than sufficient. Results for components 1 to 3 are shown as a 3D score plot in Figure 4. In this Figure, reference females are marked f, reference males m, diabetic females x and diabetic males y. The first component gives the largest separation between references and diabetics and also between males and females. The separation between males and females is, however, larger than the separation between diabetics and references. The separation according to gender is due to a known difference in the skin structure.<sup>22</sup> As the first component gives the largest separation between the groups in the score plot, the first loading can be used to find out which site is most important for the separation. In the loading plot for the first component it can be seen that the foot is the most important and the arm the least important site. See Figure 5.

A closer look at the separation between males and females is given for the reference group. A 3D score plot where object numbers 1 to 14 are males and 15 to 28 females and for all sites is given in Figure 6. It is clearly the first component that separates the two groups. Two objects are, however, in wrong groups: numbers 5 and 25. There are no clinical data available for the references and there are probably outliers in the control group. Even in the healthy population differences in skin structure between individuals are present. This is a general problem with

Table 3. PCA results for the main data set and its subsets.  $R^2$  is the percentage of the sum of squares explained by the model.  $Q^2$  is the percentage of the sum of squares explained by cross-validation. It was decided to limit all models to five components by definition.

Test subject/measured sites	$R^2$	$Q^2$
Females, males, references and diabetes		
All sites	0.770	0.684
References males and females		
All sites	0.827	0.741
Arm	0.911	0.834
Hand	0.954	0.916
Leg	0.960	0.930
Foot	0.958	0.932
Male references and diabetics		
All sites	0.827	0.696
Arm	0.909	0.811
Hand	0.956	0.913
Leg	0.964	0.932
Foot	0.941	0.871
Female references diabetics		
All sites	0.817	0.687
Arm	0.914	0.824
Hand	0.943	0.883
Leg	0.952	0.906
Foot	0.975	0.948

clinical data. For all subsequent data analysis, males and females were kept in separate groups. This makes the number of objects in each group quite small, which is a disadvantage for making test sets.

### Classification for males

Five sub-matrices were made for male objects only, one for each site and one for all sites together, where object numbers 1 to 14 are references and ob-

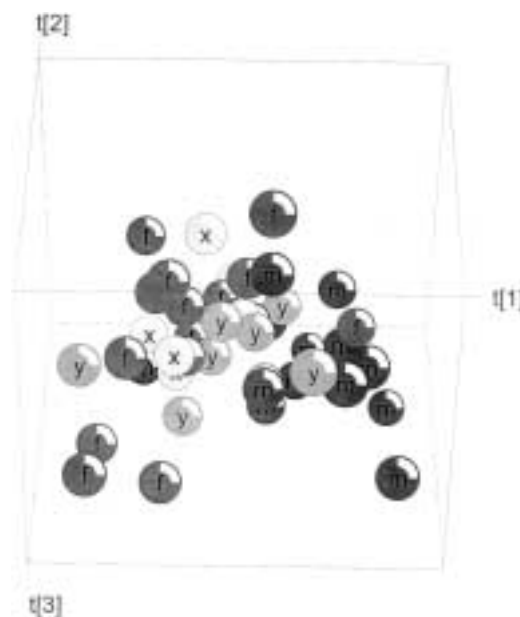


Figure 4. A 3D score plot for PCA on the whole data set of Figure 3. Components 1, 2 and 3 are used. m = male reference, f = female reference, y = male diabetic and x = female diabetic. The male and female references are best separated by the first principal component. The sizes of the spheres are used to create an illusion of depth.

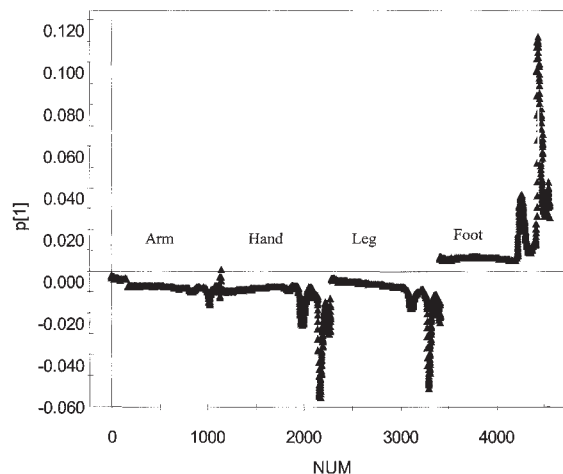


Figure 5. The first loading line plot for the PCA of Figure 4. The plot is shown with decreasing wavenumbers (increasing wavelengths) for each site.

jects 15 to 22 diabetics. Principal component analysis was calculated for all the matrices, after MSC and mean-centring (see Table 3). The results for all sites

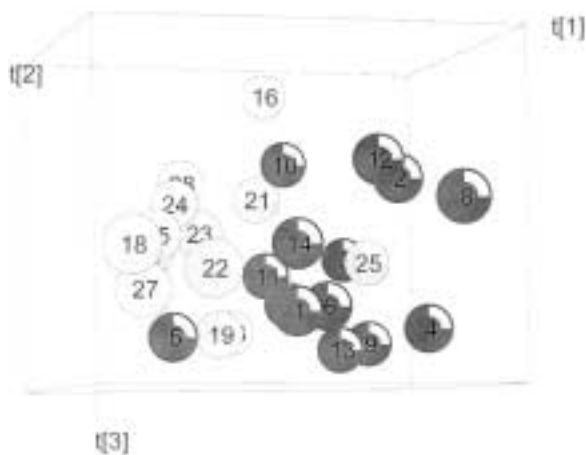


Figure 6. A 3D score plot for only references. The first principal component separates male (dark spheres) and female (bright spheres), but two outliers are recognised.

are shown in a 3D score plot in Figure 7, with dark spheres for references and white spheres for diabetics. There is a tendency towards separation between diabetics and references. One of the reference persons is among the diabetics, object number 5, the same object that was an outlier in Figure 6. The loading plot for the first component for males only shows that all sites except the arm get high loadings (Figure 8). It may also be observed that the highest loadings are for the high wavelengths.

It is an objective to reduce the measurement time, so ideally only one location should be sufficient. Figure 9 shows the results for the foot which was the best location for men. Similar plots were obtained for the other locations, but are not shown here. In all plots it is possible to see a clustering of the references and the diabetics, but subjectively the foot gives the best results.

The score plots in Figures 7 and 9 show that there is a large spread among the references. This fact and the rather small size of the group of diabetics makes it difficult to go to far-reaching conclusions.

### Classification for females

Five matrices were made with reference females and diabetic females: one for all sites and four for the separate sites. Here objects 1 to 14 are references and 15 to 21 are diabetics. A PCA was calculated for

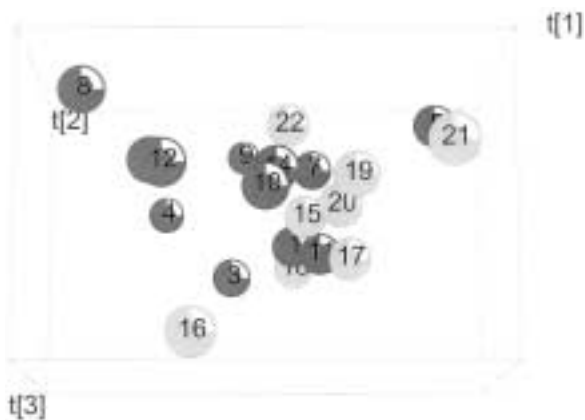


Figure 7. A 3D score plot for males only. A reasonable separation is seen between references (dark spheres) and diabetics (bright spheres) for the first component.

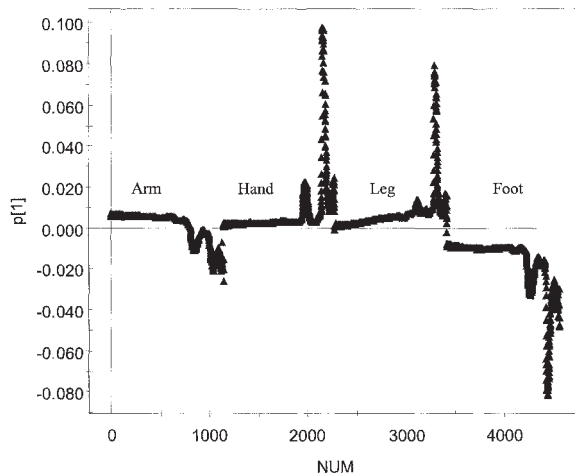


Figure 8. The loading plot for the model of Figure 7. Only the arm has small loadings.

all matrices and the results are shown in 3D score plots (see Table 3). The 3D score plot for all sites for components 2, 3 and 4 is shown in Figure 10. In the 3D score plot separation between the two classes is shown except for object 16 that seems to be in the wrong class. The separation between the two classes is mostly due to the second and third component where the references have lower score values than the diabetics. For the different sites the grouping was not as clear as for the males, however, some separation could still be seen.

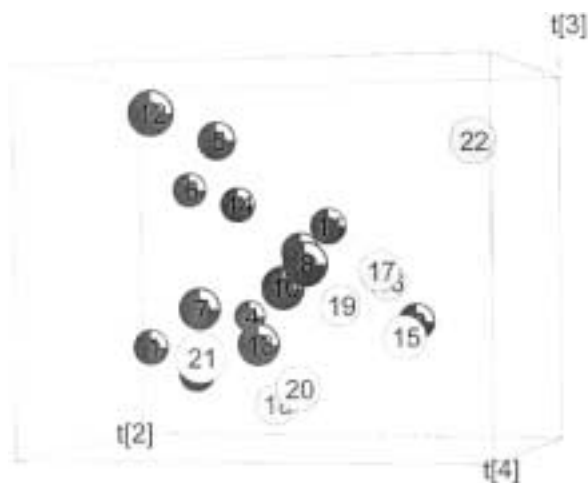


Figure 9. A 3D score plot of the PCA model for men only for the foot. The three components that showed the best results were chosen in the range  $t_1$ – $t_4$ . References are dark spheres and diabetics bright spheres.

### Supervised classification of control subjects and diabetics

Supervised discrimination with binary classes can be done with linear discriminant analysis (LDA).<sup>23,24</sup> Because there are more variables than objects and collinear variables, the usual multiple linear regression has to be replaced by PLS or a similar latent variable based regression technique. For different subsets of the data set in Figure 3, linear discriminant analysis models were built with partial least squares regression (PLS). The PLS model is:

$$y = \mathbf{X}\mathbf{b} + \mathbf{f}$$

with  $\mathbf{X}$  the NIR spectra,  $y$  the class variable,  $\mathbf{b}$  the PLS regression coefficient vector and  $\mathbf{f}$  the residual vector.

The discrimination is between patients = class 0 and references = class 1. The PLS results are shown in Table 4. One OSC component was removed for each model. The Table gives the number of components used after OSC, percentage of the sum of squares of  $X$  remaining after OSC,  $R^2$  the coefficient of determination for the model and amount correctly predicted. Testing of the models was done by leaving out references and diabetics in a test set. Two different test sets with two references and two diabetics in

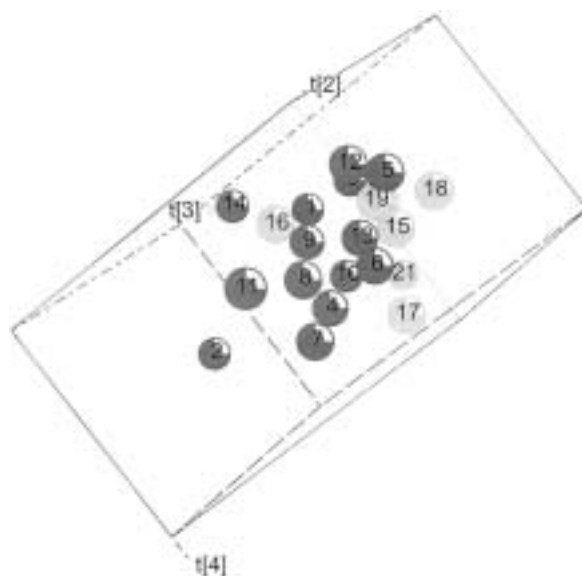


Figure 10. A 3D score plot for components 2,3 and 4 for females only. A reasonable separation is seen between references (dark spheres) and diabetics (bright spheres), but one outlier (number 16) is recognised.

each, were left out when modelling and then predicted. Interpretation of the prediction is simple. A predicted value below 0.5 means diabetic and a prediction above 0.5 means reference. Good prediction results were expected for the foot and the hand as the circulation problem is shown there first. For the males the hand and the leg did not give good prediction results. The poor prediction ability for the hand in the group of the males may be due to differences in occupational activities. One problem with measuring on the leg is that some males have a lot of hair which could give scattering or just absorb some of the emitted light that would else reflect, resulting in differences in the spectra. For the rest of the sites, seven out of eight were correctly predicted which must be considered acceptable considering that there are large individual differences among both the diabetic group and the reference group. For the females, less prediction ability was shown for the foot and for the matrix using all sites. The lesser prediction ability for the foot could be that some use skin lotion that might interact giving less good results. The lesser prediction results for the foot may have disturbed the prediction results using all sites. The

Table 4. Prediction results for the discriminant analysis by PLS-DA. The Table gives number of components used after OSC, percentage of the sum of squares of  $X$  remaining after OSC,  $R^2$  the coefficient of determination, the number of objects (out of 4) correctly predicted for each test set and the total number of properly predicted (out of 8).

Sex	Sites	Components	OSC	$R^2$	Properly predicted	Total
Males	All	1	63.96	0.57	4	7
		2	65.34	0.74	3	
	Arm	4	38.90	0.89	3	7
		1	60.37	0.83	4	
	Hand	3	45.27	0.90	2	4
		1	49.48	0.94	2	
	Leg	4	57.47	0.78	3	6
		3	42.26	0.86	3	
	Foot	2	27.31	0.79	3	7
		3	74.89	0.79	4	
Females	All	2	58.38	0.62	4	6
		5	61.86	0.92	2	
	Arm	5	20.97	0.99	4	7
		4	24.02	0.99	3	
	Hand	5	36.22	0.98	4	7
		1	50.42	0.90	3	
	Leg	3	53.49	0.96	3	7
		3	45.28	0.77	4	
	Foot	2	39.62	0.67	2	3
		1	40.59	0.92	1	

rest of the sites, arm, hand and leg, all give seven correctly predicted out of eight.

### Comparison with NFI data

An attempt was made to build PLS models using NFI as response variable. The NFI value is only available for the diabetics. Therefore, it is not appropriate to make test sets because the NFI classes are so small. As NFI modelling by PLS was not possible, a closer study with PCA was made, this time using only the diabetes patients. Models were made by PCA after MSC and mean-centring. The results are shown as 3D score plots in Figures 11 and 12. One should be aware that the number of objects in each

data set and the number of objects in each NFI class are very small.

For the males there is only one person with NFI value 0 and this single object cannot be regarded as a group and will not be discussed any further. Separation between those with NFI values 1 and 2 can be seen for males when all sites are added together (Figure 11). A combination of the first and second component separates two groups, those in class 2 having mostly higher values in the first and second component. In the female diabetic group there is only one person with NFI 1 and therefore only those with NFI values 0 and 2 are discussed. For the matrix using all sites, separation could not be seen. When looking at

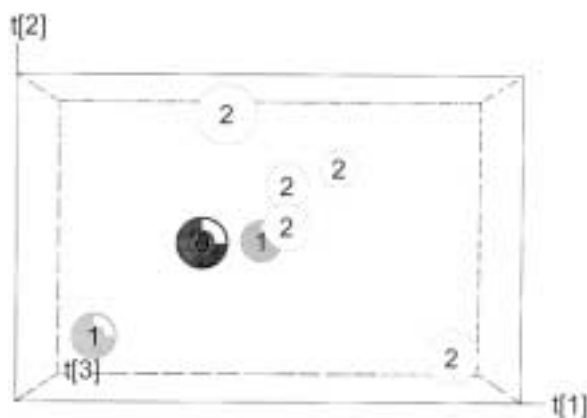


Figure 11. A 3D score plot for men, all sites with the NFI classes indicated.

the different sites the same results were found as for the males, i.e. the hand and the foot (Figure 12) gave the best separation, while no real separation was seen for the arm and the leg. The fact that the best results were obtained for the hand and the foot in both males and females underlines the assumption that the skin problems will first show at the hand and the foot. Increased capillary fragility<sup>25–27</sup> and permeability<sup>28–30</sup> in diabetic patients are well known. The presence of oedema may also impair the passage of oxygen and metabolites in the opposite direction and bring about a compression of the small vessels and contribute to the development of lesions. The presence of neuropathy impairs the capacity to vary the blood circulation to the tissue area in question. The amount of interstitial oedema evaluated by the NIR technique could therefore be of importance in the clinical work. The capillary basement membrane is normally thicker in the lower than in the upper extremities, even in non-diabetics<sup>31</sup> and the thickness increases with age.<sup>32</sup> The difference in reaction between younger and older diabetic or non-diabetic persons and between the lower and upper extremities may be explained by these anatomical differences and their variation with age.<sup>27</sup>

## Conclusions

Measurements on the hand, arm, leg and foot of patients and references were carried out with an FT-NIR instrument equipped with a fibre-optic probe.

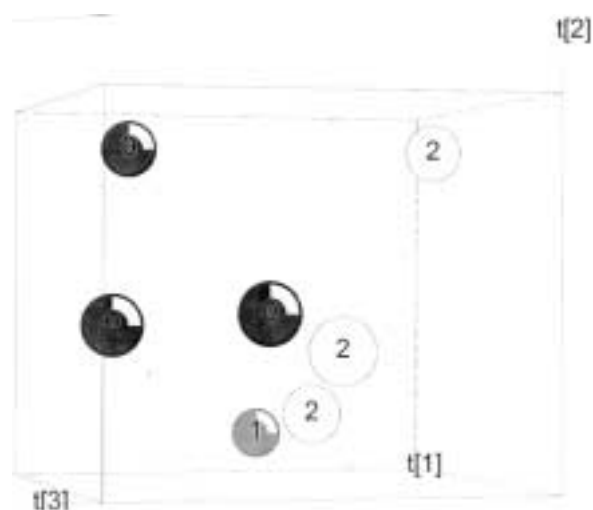


Figure 12. A 3D score plots of the PCA model for women for the foot only, with NFI classes indicated.

The instrument used was found to be very robust and stable in operation. Multivariate analysis of the NIR spectra after MSC pretreatment and using PCA gave clusterings that were useful. First, it was found that splitting the data in men and women was necessary. After that, clustering in references and patients was detected in 3D score plots. Figure 6 shows that healthy men and women have different skin and Figure 4 shows that skin deterioration blurs the difference. For the results of separate measurement locations, the hand and foot values were most promising. The agreement between the clustering in the score plots and the NFI (neural function index) was reasonable. Attempts were made at modelling other clinical measurements using the NIR data, but they gave little result. A suspected dependence of the NIR results on ion or glucose concentrations, or other urine and blood values could not be confirmed. From this it must be concluded that the changes in skin composition and structure are a major factor in the differences shown. The preliminary success of the unsupervised clustering led to the test of PLS-DA as a supervised classification technique. The classification rates found were satisfactory given the low number of objects available in each class.

The references showed an unexpected large spread and even some outliers. In this study no clinical data was collected for the references and this makes it difficult to give a correct judgement of their

results. As the females and males had to be divided into different groups, it is necessary to measure a much larger group of diabetics with different NFI for both males and females. For future research, also a more detailed interpretation of loading plots should be included. This may lead to a better selection of wavenumbers and a better understanding of what structure or substance in the skin that is responsible for the differences.

There have been skin studies of fat<sup>32</sup> using a fibre-optic probe in the wavelength range 700–1100 nm, showing high correlations between skin fat and total body fat measured with a number of classical techniques. The authors found an influence of water and fat peaks on the signal in the 700–1100 nm wavelength range. In the present study, the wavelengths below 1200 nm hardly influence the multivariate models. One may ask how deep the different wavelengths penetrate in and under the skin, and what portion of the radiation is returned. The observed signals may describe a mixture of skin and muscle composition. This is a subject for future studies.

Near infrared measurement is fast, non-destructive and non-invasive and has shown to give good separation between healthy persons and those suffering from diabetes. It may be a promising technique for screening for diabetes-related skin changes. The patients and references were also measured by MFBIA and these results are useful for unsupervised and supervised classification,<sup>1</sup> but they will be published separately. In the future, larger patient and reference groups will be studied and the references will also require some form of clinical investigation. It is very important to have enough members in each class (male, female, patient, reference, NFI class) for making test sets. Because diabetes is a chronic disease, patients have it for many years. Therefore there is good reason to have long-term studies of each individual patient, showing how the skin deteriorates from healthy to visibly diseased.

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