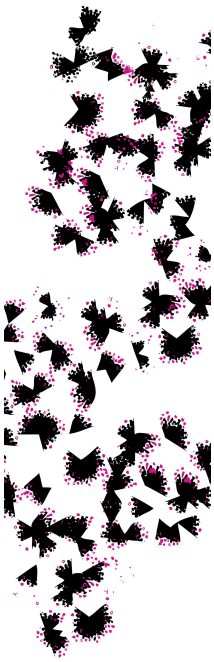

Measuring Saturation of Tissue using a Spot Spectrometer under differing conditions

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BSc Report

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Contents

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Samenvatting – Een van de complicaties die bij diabetes patiënten veel voorkomt is de zogenoemde diabetische voet. Deze wonden aan de voet genezen slechter, door een verminderde doorbloeding als complicatie van diabetes melitus.

Saturatie van de voeten kan een goede voorspeller zijn in de genezing van de wonden. De saturatie wordt gegeven door de verhouding zuurstofrijk hemoglobine en zuurstofarm hemoglobine. Een goede doorbloeding wordt gekenmerkt door een hoge aanwezigheid van zuurstofrijk hemoglobine.

De saturatie kan bepaald worden met behulp van licht; in het verslag staat omschreven hoe spotspectroscopie gebruikt wordt voor de saturatiebepaling in gezond weefsel. Deze bevindingen kunnen gebruikt worden in verder onderzoek naar het gebruik van hyperspectraal metingen in de diabetische voet.

***Abstract* —** Many diabetic patients suffer from complications of their disease. Well-known complications are the so-called diabetic feet. In diabetic feet, the blood flow in the feet is reduced, resulting in poorly healing foot ulcers. A method to measure the blood flow is using the saturation of the feet. The saturation indicates the ratio between the bounded-hemoglobin (HbO_2) and non-bounded hemoglobin (Hb).

This saturation-value is the predictor for insensitive, risky spots on the feet; that are the spots where in proportion less HbO_2 is present. To predict the change on foot ulcers, cameras / light can be used to define the saturation-value.

Goal is to find a method to define the saturation in (diabetic) feet using hyper spectral imaging. The described method, spot spectrometry, is used to define a method that reaches this goal in a more controllable situation.

Keywords—saturation, spot-spectrometry, pulse-oxymetrty, temperature, obstruction

I. INTRODUCTION

A. Background information

Approximately 3.7 percent of the Dutch population is diagnosed with Diabetes Mellitus (DM)[1]; DM is a metabolic disease that can cause an elevated blood glucose concentration.[2] DM is one of the most common chronical diseases in the Netherlands.[3] DM can be the cause of many (long-term) complications, like damage, dysfunction and failure of various organs (e.g. renal failure), blindness, vascular diseases and neuropathy. Neuropathy increases the risk of, for example, Charcot foot and foot ulcers.[4]

B. Diabetic foot

Peripheral artery disease (PAD) occurs in 50 percent of the patients with diabetes type 2 [5]. PAD is caused by arteriosclerosis and occludes the peripheral arteries. Due to neuropathy the sensitivity in the feet of DM-patients is lowered; a patient will not recognize an injury in the foot as fast as a healthy person. Also the blood-flow in, mainly, the capillaries decreases by neuropathy, thus the recovery of this injury is not provided by the body itself. Foot muscles atrophy will occur and smaller wounds can develop into ulcers and infections that can hardly recover due to the obstructed blood flow.[6]

Diabetic feet occur in 2-3% of the DM-patients.[7] Diabetic feet can be detected and treated by a wound-consultant. A wound-consultant detects potential infections and the inability of the wound to heal. A frequently used method is to define the systolic ankle pressure and toe brachial index. A predictive value for PAD is the systolic ankle brachial index (ABI) that is the systolic ankle pressure divided by the systolic pressure of the arm. PAD is likely to occur, when this ABI-value is lower than 0.90.

Diabetic feet are mostly detected in a late stadium, due to the lost sensitivity of the DM-patient. The healing of foot ulcers is difficult to predict; although some conditions can be mentioned to be related to a better healing. These are: the amount of tissue loss, presence of infection, mechanical load on the ulcer, co-morbidities and quality of care.[8]

These conditions are predictors for the healing process, together with the perfusion in the foot that is often measured using ABI-values, the systolic ankle pressure and toe brachial index or vascular imaging. Vascular imaging uses Doppler technique, to image the moving particles in the circulatory system. Vascular imaging is a more local measurement method than the earlier mentioned methods. Although, vascular imaging detects the stream rate of the blood, it cannot detect the oxygenation of the blood or give objective results to monitor the development of foot ulcers.

Different methods are currently available to treat diabetic feet. A wound-consultant can develop a care plan, which includes cleaning and monitoring of the foot ulcers. Also treatments concerning the blood vessels are used for PAD-patients; these treatments include endovascular techniques and bypass surgery. Despite the accessible methods to

treat diabetic feet, in 15-25 per cent of the DM-patients with diabetic feet (a part of) the foot has to be amputated to enforce (better) healing[7], [10]. In 2003 this concerned 728 registered patients. [11] This can be partly solved by an early detection of the ulcers and an improved monitoring the development of healing the foot ulcers.

Hyper spectral imaging is a non-invasive method to detect differences wavelengths present in the measured tissue. A spectrometer gives the intensity of the reflected light for differing wavelengths. This method could be used to define a measurement for saturation in tissue, the risk of diabetic foot ulcer development and to predict non-invasively the likelihood of healing [12]. A spot-spectrometer also measures a wavelength spectrum, of a smaller area (a spot). In this research a spot-spectrometer is used to define the saturation value.

C. Saturation

Blood is present in almost every tissue and consists of many components. One of these components are the red blood cells (RBCs). The most important constituent of RBCs is hemoglobin, with a content of approximately 15.0 g/dL blood in an adult person. This hemoglobin can bind and transport oxygen from the lungs to the tissue.[13]

The saturation describes the ratio of bounded hemoglobin (oxygenated blood) to the total O_2 capacity of hemoglobin (deoxygenated and oxygenated blood).Tissue oxygenation is related to the blood saturation, giving the saturation of a volume of tissue instead of the saturation of a volume of blood. The absorption wavelengths for oxygenated and deoxygenated hemoglobin differ from each other. Oxygenated hemoglobin absorbs more light at a wavelength of 920 nm and deoxygenated hemoglobin absorbs more light at a wavelength of 660 nm.[14] This principle is used in pulse oximetry, where the ratio is taken of the fluctuation of the intensity in these two wavelength bands.

The reflection spectrum of spot spectrometry is built up from the reflection of deoxygenated hemoglobin, the reflection of oxygenated hemoglobin and different components as melanin concentration, thickness of epidermis, and scattering properties of the tissue[12]. Every component contributes to the measured spectrum in a ratio that corresponds to the differing concentrations of the components. Finally, this is used to define the saturation that is the ratio of deoxygenated hemoglobin to the total amount of hemoglobin.

D. Goal and Outline

As previously stated, current methods for detecting and monitoring diabetic feet can measure a lot of conditions that can predict the healing of foot ulcers. Unfortunately, these methods are insufficient at the level of local blood saturation values. Hyper spectral imaging can possibly be used to define the local saturation in (diabetic) feet, as an objective method. To test this hypothesis on measuring saturation using spectrometry, the rest of this report focuses on the operations that are necessary to get saturation values from the results of spot spectrometry.

The goal of this research is to define an algorithm that finds the saturation of tissue using spectroscopy; that is built up from a value for concentration of oxygenated hemoglobin and the concentration of deoxygenated hemoglobin. This algorithm will be used to find an estimated saturation value for the pixels in multispectral imaging measurements as proposed by C. Liu[15]. The measurements are simulated on a smaller scale, using spot spectrometry. This results in a feasibility study that demonstrates the possibility to find the saturation value using spectrometry.

This method is mathematically described in Methods and Materials. Then, several experiments are performed to obtain enough results for every situation. These measurements are processed in *Matlab R2013b*, resulting in a saturation value for every measurement. The results are the plotted saturation values, these are compared with each other to define a conclusion about the method used to contract the differing concentrations and give an answer to the question if it is possible to give a measure for saturation of tissue using a spot spectrometer.

II. METHODS AND MATERIALS

A. Analysis

With a spot spectrometer, the reflection spectrum $R(\lambda)$ of a spot of the skin is recorded. The skin contains differing number of, say K, different components influencing the recorded spectrum. The components are described in this section. Every component k is represented in the skin with its own specific concentration α_k and every component k has its own, assumed known, reflection spectrum $R_k(\lambda)$. Every component contributes to the total reflection $R(\lambda)$ in a ratio that is in accordance with the ratio of its concentration.

The formula for the total reflection, $R(\lambda)$ is simplified to a function, where k_1 corresponds to

the component HbO_2 and k_2 corresponds to the component Hb. The assumption is made that all other components R_k for $k_3,...,K$ are continuous in the range of the chosen filter settings; this results in the following formula:

$$R(\lambda) \approx \alpha_1 R_{HbO_2}(\lambda) + \alpha_2 R_{Hb}(\lambda) + \alpha_3 + \alpha_4 \lambda \qquad 2.1.$$

The total reflection spectrum $R(\lambda)$, that contains the unknown reflection spectra for every component $R_k(\lambda)$, is convoluted from the raw data. The reflection of a white and a black spectrum are used; conform to the function for an absolute spectrum:

$$R(\lambda) = \frac{R_{raw} - R_{black}}{R_{white} - R_{black}} \qquad 2.2.$$

This reflection spectrum is divided into M different wavelength ranges, to simulate the filters used in hyper spectral imaging (HSI). These settings are used to find the output z_m; these filters are based on the available filters for HSI. The filters are defined as summarized in Table I [16].

TABLE I. OVERVIEW OF THE USED FILTER SETTINGS THAT ARE AVAILABLE FOR HYPER SPECTRAL IMAGING (HSI)

Central Wavelength (CW)	Total Bandwidth (TB)
400	50
410	10
430	10
540	10
550	40
580	10
730	30
650	25
800	25
950	25
480	10
420	10
450	10
510	10
560	10
610	10
690	10
880	10

The wavelengths are referred to as:

$$\lambda_m^0 = CW_m - \tfrac{1}{2} BW_m \qquad 2.3.$$

$$\lambda_m^1 = CW_m + \tfrac{1}{2} BW_m \qquad 2.4.$$

These intervals are used to define the following filter for the total recorded spectrum:

$$z_m = \frac{1}{|\Lambda_m|} \int_{\lambda_m^0}^{\lambda_m^1} R(\lambda) d\lambda \qquad 2.5.$$

The α_{HbO_2} and α_{Hb}, that are used to define the saturation value, are part of the formula for the recorded spectrum $R(\lambda)$. To derive the concentrations from the observed spectrum, the total integral over the reflection of one filter, z_m is described mathematically. The outcome of this filter is defined as a concentration-based summation of the defined functions of the K differing spectra:

$$z_m = \alpha_1 P_{HbO_2,m} + \alpha_2 P_{HbO,m} + \alpha_3 P_{3,m} + \alpha_4 P_{4,m} \qquad 2.6.$$

P_k is the weighted integral over the part that is an estimate of the spectrum that component k contributes to the total function for the spectrum. The functions for P_k are defined in 2.7, 2.8, 2.11 and 2.12.

$$P_{HbO_2,m} = \frac{1}{|\Lambda_m|} \int_{\lambda_m^0}^{\lambda_m^0} R_{HbO_2}(\lambda) d\lambda \qquad 2.7.$$

and for deoxygenated hemoglobin:

$$P_{Hb,m} = \frac{1}{|\Lambda_m|} \int_{\lambda_m^0}^{\lambda_m^0} R_{Hb}(\lambda) d\lambda \qquad 2.8.$$

Lambert-Beers law states that the intensity of the reflection depends on the attenuation coefficient (μ) and the penetration depth (D), resulting in the following function for the reflection:

$$R_{HbO_2}(\lambda) = e^{-\mu_{HbO_2}(\lambda) D_{eff,HbO_2}(\lambda)} \qquad 2.9.$$

$$R_{Hb}(\lambda) = e^{-\mu_{Hb}(\lambda) D_{eff,Hb}(\lambda)} \qquad 2.10.$$

Data compiled by Scott Prahl are used to define the absorption coefficient [17], [18],[19].

When assuming that the only not constant fluctuating spectrum belongs to the oxygenated and deoxygenated hemoglobin, $P_{3,m}$ is defined as a constant component a_3 and $P_{4,m}$ is defined as a constant descending or constant ascending curve.

It is assumed that the spectra $P_{3,m}$ and $P_{4,m}$ include the effect of melanin concentration, the thickness of the epidermis and the scattering properties as described in the paragraph 'Saturation' on page 3; the formulas for $P_{3,m}$ and

$P_{4,m}$ are described in formula 2.11 and formula 2.12 .

$$P_{3,m} = \frac{1}{|\Lambda_m|} \int_{\lambda_m^0}^{\lambda_m^0} c_3 d\lambda = c_3 \qquad 2.11.$$

and

$$P_{4,m} = \frac{1}{|\Lambda_m|} c_4 \int_{\lambda_m^0}^{\lambda_m^0} \lambda d\lambda \qquad 2.12.$$

Filling in $P_{3,m}$ and $P_{4,m}$ in the formula z_m results in an unknown constant c_3 multiplied with the unknown concentration constant α_3. The multiplication results in another constant, in the further method, this constant is referred to as the constant a_3.

It is assumed that the light in the certain used wavelength-range will penetrate the tissue in the same effective depth. These functions can be filled in into the formulas for $P_{HbO_2,m}$ and $P_{Hb,m}$, this is described in 2.13:

$$D_{eff,HbO_2}(\lambda) = D_{eff,Hb}(\lambda) \qquad 2.13.$$

To overview this statement a matrix $\mathbf{H}$, with the size $M \times K$ is introduced and filled with the values for $P_{k,m}$. From the above statements a vector $\mathbf{z}$, containing every z_m for $m = 1, \cdots, M$, is defined as a multiplication of matrix $\mathbf{H}$ with vector $\mathbf{a}$:

$$\mathbf{z} = \mathbf{H}\mathbf{a} \qquad 2.14.$$

With the vectors $\mathbf{z}$ and $\mathbf{a}$ depending on $k = 1, \cdots, K$ (with $K = 4$) and $m = 1, \cdots, M$ (with $M = 18$) as described in 2.15 and 2.16

$$\mathbf{z} = [z_1, z_2, \ldots, z_m]^T \qquad 2.15.$$

$$\mathbf{a} = [a_1, a_2, \ldots, a_k]^T \qquad 2.16.$$

As previously stated, the concentrations α_{HbO_2} and α_{Hb} are needed to calculate the saturation S of the measured tissue; these concentrations correspond with the values for a_1 and a_2. Using the non-negative least square estimation (LSE) method, all the values for $\mathbf{a}$ are calculated. LSE is a data fitting method that minimizes the sum of the squares of the errors made in the estimated result of the differing single equations. The constants a_3 and a_4 are not used for the concentration anymore, there these became values for the concentration multiplied with the unknown concentration constant. In the rest of this chapter these two variables are not used anymore either.

The saturation is described as a function, containing the concentration of oxygenated hemoglobin α_{HbO_2} and the concentration of deoxygenated hemoglobin α_{Hb}, in the following formula:

$$S = \frac{\alpha_{HbO_2}}{\alpha_{Hb} + \alpha_{HbO_2}} \qquad 2.17.$$

Then, the data of the spot spectrometer are cut for the exact filter settings, as defined in TABLE I. $Z(m)$ is defined as the weighted integral over the filter band's wavelengths.

For the summation, a function P is defined as an unknown part of the total reflection. The function R is the mathematical estimation for the function P, using the Beer-Lambert law. The penetration depth is assumed to be 0.01 cm and is assumed to have an equal thickness for both oxygenated and deoxygenated hemoglobin. Using the (non negative) least square estimation, the concentration for oxygenated and deoxygenated hemoglobin can be found. The saturation S is calculated by dividing the concentration oxygenated hemoglobin by the total concentration hemoglobin, that is the summation of the oxygenated hemoglobin and de deoxygenated hemoglobin.

B. Experimental set-up

Varying measurements are performed to find a manner to describe and influence the saturation of the fingertip in a healthy person. The spectrum is measured in different situations, likely to influence the saturation in a healthy person.

Temperature will influence the saturation in a relatively big way. As shown in Figure 1, the relation between oxygen pressure and hemoglobin saturation is influenced by temperature. The affinity for O_2 is lower, resulting in a lower amount of O_2 available for tissue [20].

Another option is that less O_2 is bound after holding their breath; the hypothesis is that a healthy person cannot hold their breath for a long enough period, so the saturation will possibly not change, since holding one's breath for only 30 seconds will not influence the saturation.

The last experiment will be performed after occluding the blood flow with a sphygmomanometer band; after this occluding no oxygenated blood flow will be present in the arm, and less oxygenated hemoglobin will be measured, because the oxygen is used in the tissue. Thus, the spectrum is measured in a normal situation, after holding the breath, after heating the finger, after cooling down the finger and after obstruction of the blood flow in the arm towards the hand.

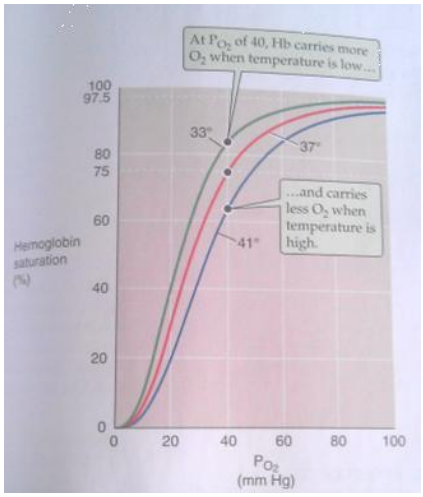

FIGURE 1: THE CURVE THAT GIVES THE RATIO BETWEEN SATURATION AND OXIGENPRESSURE, SHIFTS DUE TO THE TEMPERATURE CHANGES

C. *Materials*

- *Matlab*, version R2013b
- Spot spectrometer Setup:
  - AvaLight-HAL Tungsten-Halogen Light
  - AvaSpec-2048-USB2-UA-550-Spectrometer
  - Reflection Fiber Probe (6.5 mm diameter)
  - Skin Contact Probe Holder
  - Computer with Avasoft (for the spot spectrometer control)
- Pulse oximeter
- Timer / stopwatch
- Hot water and (ice)cold water
- Sphygmomanometer band
- Towel

The recorded skin spectra cover a wavelength range from 360 to 1000 nm. The spot spectrometer setup consists of differing parts, as can be seen schematically in Figure 2

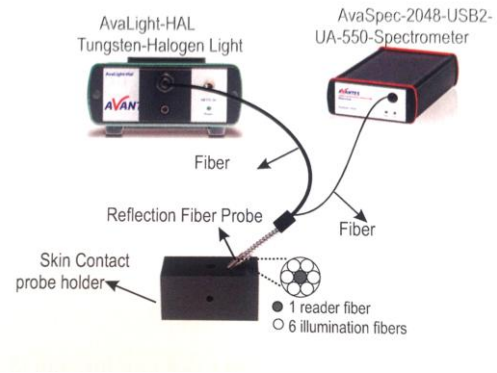

FIGURE 2 SPOT SPECTROMETER SET UP AS USED IN THE EXPERIMENTS

III. RESULTS

The first results were compared to the results the pulse oximeter gave. These results are displayed in TABLE II. TABLE III. TABLE IV. and TABLE V The pulse oximeter did not give a result when the blood flow was obstructed; this can be explained by the property of the pulse oximeter that it measures the fluctuation in the saturation value, which represents the blood flow. The spot spectrometer measures the whole spectrum of the total tissue, including the blood flow.

A. *Results compared with a pulse oximeter*

TABLE II. SPOT SPECTROMETER SATURATION RESULTS (IN %)

	Normal	Breath	Warm	Cold	Pressure
Pointing finger	90.08	89.74	89.55	89.14	87.23
Thumb	89.83	89.38	88.86	90.76	89.63

[a.] Results of the spot spectrometer, settings: integration time = 2.5 ms, D = 0.01 cm

TABLE III. PULSE OXIMETER SATURATION RESULTS (IN %)

	Normal	Breath	Warm	Cold	Pressure
Pointing finger	99	98	98	98	-
Thumb	98	98	96	97	-

[b.] Results of pulse oximeter, settings: integration time = 2.5 ms, D = 0.01 cm

TABLE IV. SPOT SPECTROMETER SATIRATION RESULTS (IN %)

	Normal	Breath	Warm	Cold	Pressure
Pointing finger	90.61	90.30	90.53	90.47	90.16
Thumb	90.73	90.45	90.89	90.95	90.39

[c.] Results of the spot spectrometer, settings: integration time = 6.5 ms, D = 0.01 cm

TABLE V. PULSE OXIMETER SATURATION RESULTS (IN %)

	Normal	Breath	Warm	Cold	Pressure
Pointing finger	98	98	98	95	-
Thumb	98	98	97	98	-

[d.] Results of pulse oximeter, settings: integration time = 6.5 ms, D = 0.01cm

The results of using two different integration times and the results of the ten performed experiments are visually displayed in Figure 3.

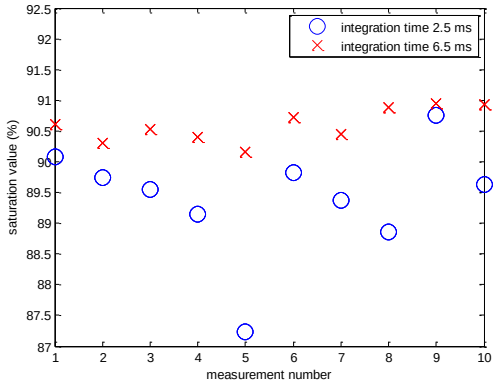

FIGURE 3: RESULTS OF THE SATURATION USING A SPOT SPECTROMETER IN 20 EXPERIMENTS; 1: NORMAL SITUATION, 2: HOLDING THEIR BREATH, 3:WARM, 4: COLD, 5: OBSTRUCTING THE BLOOD FLOW. 6-10 ARE THE SAME SITUATIONS RESPECTIVELY MEASURED IN THE THUMB, INSTEAD OF THE POINTING FINGER.

B. Results after different situations

The experiments that are described the chapter Results are performed on the fingertip. Measurements 1 and 6 give the normal saturation value, 2 & 7 give the value after holding the breath, 3 & 8 after warming the finger, 4 and 9 after cooling down the finger and 5 & 10 after obstructing the blood flow. The first five measurements are performed on the pointing finger; the last five measurements are performed on the thumb. Every experiment is repeated five times for both fingers. The second result of measurement number 4 did not give a reliable saturation value; there the concentrations were too small to perform a good mean square error operation and a *Matlab* error occurred.

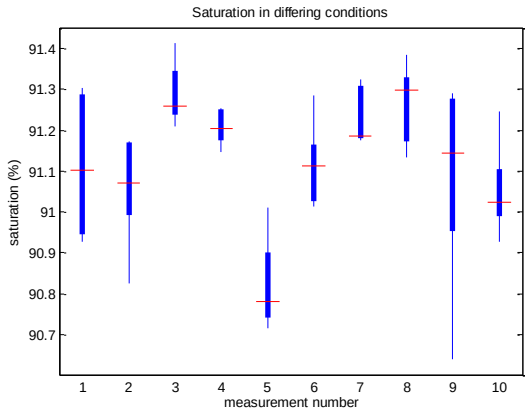

FIGURE 4: SATURATION IN DIFFERING CONDITIONS. 1: NORMAL SITUATION, 2: HOLDING THEIR BREATH, 3:WARM, 4: COLD, 5: OBSTRUCTING THE BLOOD FLOW. 6-10 ARE THE SAME SITUATIONS MEASURED IN THE THUMB, INSTEAD OF THE POINTING FINGER.

C. Results after blood obstruction

In the following figures the saturation value over time is displayed for five measurements. The measurements started after 10 seconds, in that 10 seconds the blood pressure band was pumped until the blood flow was obstructed completely. Every 2 seconds a frame was shot, and for every 2 seconds a saturation value is calculated. After 30 frames, the blood pressure band is released and the blood flow in the arm was recovered to the normal blood flow.

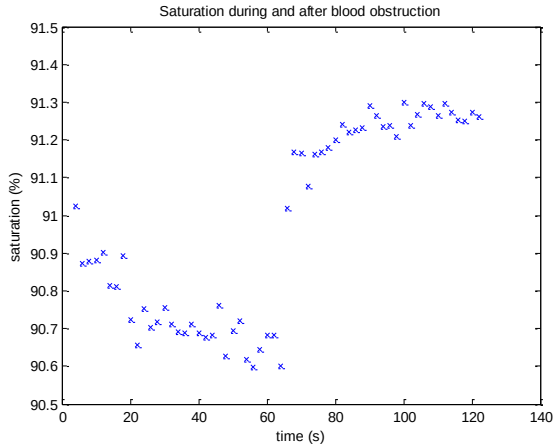

FIGURE 5: SATURATION DURING AND AFTER BLOOD OBSTRUCTION (AFTER 60 S.), ONE CONTINUOUS MEASUREMENT

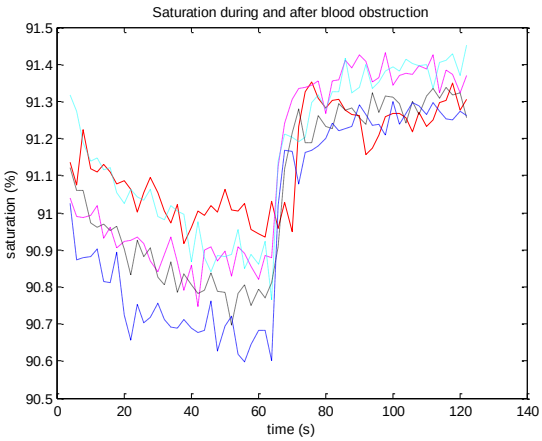

FIGURE 6: SATURATION DURING AND AFTER BLOOD OBSTRUCTION, FIVE MEASUREMENTS

D. Results for differing thickness settings

Thickness of the skin is a factor that influences the reflection spectrum of the spot spectrometry. The thickness of the skin can be influenced by, for example callus, which is present on the foot sole. The saturation is calculated for the six spots on the foot that are indicated in Figure 8, results are displayed in Figure 8.

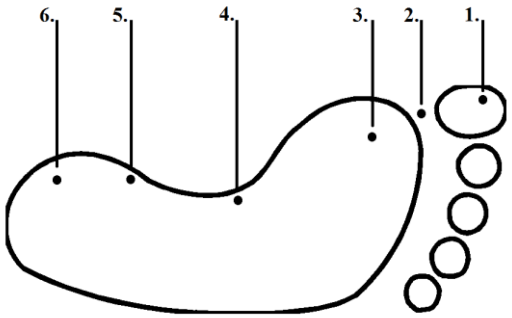

FIGURE 7: MEASUREMENT PLACES ON THE FOOT SOLE

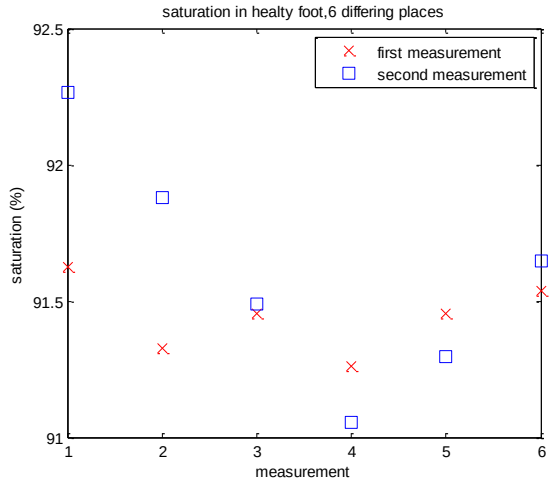

FIGURE 8: SATURATION IN DIFFERENT PLACES IN A HEALTHY FOOT, POINTS CAN BE FOUND IN FIGURE 7

The result of the pointing finger in five situations, with a differing thickness setting from 0.1 to 0.001 cm is shown in Figure 9. These thicknesses are manually set in the *Matlab* script, the same measurement is used for every manually set thickness. The real thickness of the penetration depth in the fingertip is unknown.

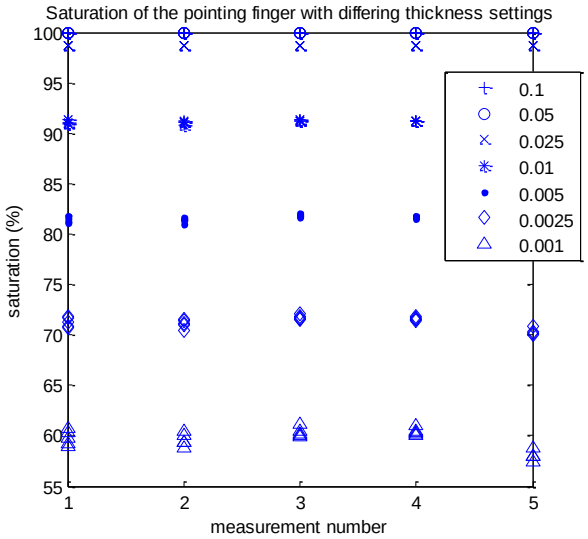

FIGURE 9: SATURATION FOR DIFFERING SCRIPTED THICKNESS SETTINGS, THE SAME MEASUREMENTS ARE USED FOR THE FOLLOWING SITUATIONS: 1: NORMAL SITUATION, 2: HOLDING THEIR BREATH, 3:WARM, 4: COLD, 5: OBSTRUCTING THE BLOOD FLOW

IV. DISCUSSION

A. Script

In the script, the assumption is made that the other two components, k_3 and k_4, can be simplified to a constant component and a constant ascendant or descendant component. This makes the mathematics easier and the results seem to match the hypothesis, although, it would possibly be better to make a more elaborate model to define all the components separately to see if the results differ from the results of this simple method.

The measurements are performed on a healthy person. The different experiments did influence the saturation value, although the more controlled the saturation value, the more reliable results are found for the experiments. Recommendation is to use a phantom to control the saturation of the so called skin for further research. The assumption for the penetration depth plays a big role in the experiments, as can be seen in Figure 9.

To obtain the results that are presented in this report, all the available filters are used. It is possible that amount of the used filters and their characteristics will influence the outcome of the

calculations. Recommended in further research is to elaborate on the influence of the used filters.

B. Measurements

The first measurements were performed with an integration time of 6.5 seconds; this was the ideal integration time calculated by the program. It was later discovered that this integration time caused clipping in some of the experiments. A smaller integration time is used in the rest of the measurements; this small integration time is more variable over time.

For measurements on the foot sole, the callous is causing the spectrum to contain a lower and flatter amplitude. Therefore, there is a possibility that the spot spectrometer cannot be used in further research for the measurements on the foot sole, because the power of the lamp is too low. Thus a possible option is to use a more powerful lamp to overcome the barrier of the callous.

The thickness of the skin influences the results, as displayed in Figure 8 and Figure 9. In figure 8 the fluctuation of the saturation can be found for different places on the foot sole. Figure 9 shows that a lower scripted thickness will give smaller saturation values.
The setting with the highest penetration depth, give the results most close to 1. It is recommended to find out the effect of the callus and the thinner skin parts on the penetration depth for differing wavelengths of the light. The penetration depth has to be a more reliable and more stable factor in the measurements.

The probe was placed in the block by lying the block on the table and placing the probe in the slanting hole until it touched the table. In Figure 10 it becomes clear what the influence is on the distance to the probe tip for the saturation values. In this measurement the probe tip is slowly released from the block by hand, halfway the saturation value dropped, possibly because the probe slid a bit towards the skin instead of releasing it from the skin.

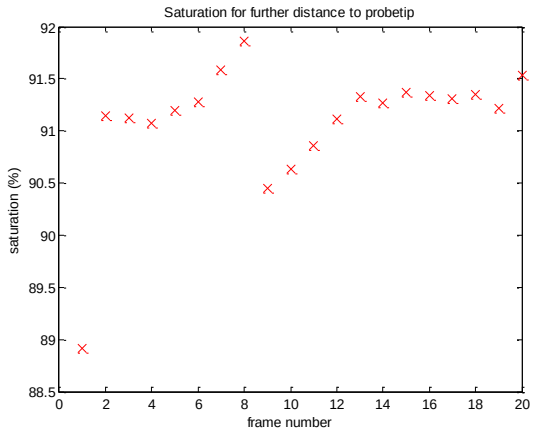

FIGURE 10: RELEASING THE PROBE TIP FROM THE FINGER TIP IN TIME

The warming up and cooling down of the tissue was performed by holding the finger in the hot or cold water. Water reflects light in quite a different way than in air, and if the finger is not dry enough, it would be possible to break the light and in that way influence the spectrum of the spot spectrometer. Therefore, the following saturation values were calculated for four differing fingers in a wet en dry situation, as can be found in Figure 11.

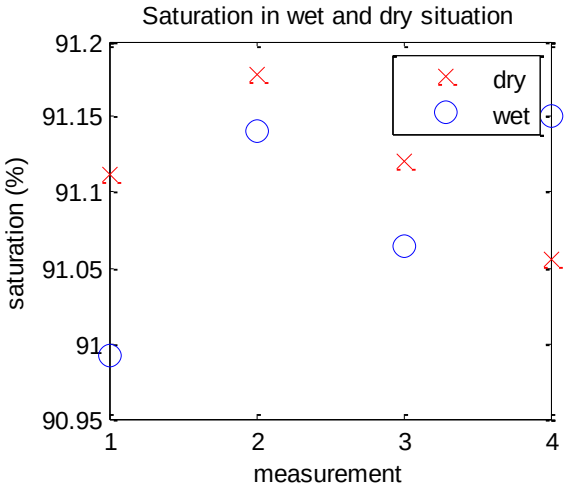

FIGURE 11: SATURATION VALUE FOR FOUR FINGERS IN DRY AND WET SITUATION

During experiments there was no fluctuation in the power of the lamp, the warming up time was fifteen minutes. The spectra of the measurements are not mutually comparable: since the pressure that is applied on the probe in the block that influences the amplitude of the reflection spectrum, as can be seen in Figure 12. The pressure on the capillaries is likely to influence the saturation, as the blood flow is obstructed. The spectrum can only be compared with itself, because of this fact that is noticed during the measurements. The results on a higher and lower pressure on the probe tip are showed in Figure 13; in this figure, only once was the medium pressure performed. Medium

pressure was too hard to perform between the soft and hard pressure in the rest of the measurements.

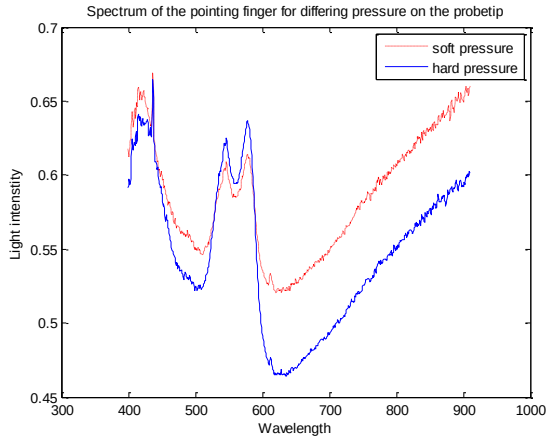

FIGURE 12: THE DIFFERENCE IN SPECTRUM WHEN A SOFT AND HARD PRESSURE IS PERFORMED ON THE TIP OF THE SPOT SPECTROMETER

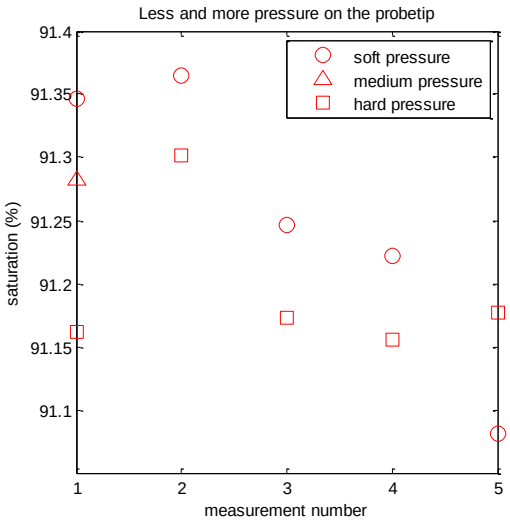

FIGURE 13: SOFT AND HARD PRESSURE ON THE PROBE TIP

With the results achieved with the in previous chapters described script, the saturation values of the measurements with the spot spectrometer can be found.

C. Pointing finger or thumb

The measurements of the pointing finger slightly differ from the saturation values of the thumb, as can be seen in Figure 4. There seems to be more variation in the saturation values of the pointing finger in different situations. An explanation for this observation can possibly be found in the differing thickness of the tissue on the pointing finger compared with the tissue on the thumb. The thickness is further elaborated in paragraph IV.B: Measurements.

D. Breath

There is no matching result after 30 seconds holding their breath. This is explained by the remaining oxygen present in the blood. Therefore, holding their breath for only 30 seconds will probably not influence the saturation of one of the fingers.

E. Warm / cold

Cooling down the fingertip does not result in an evident difference in saturation, compared with the normal measurement. Warming up the fingertip will result in a slightly higher saturation value in the fingertip. More oxygenated blood will be present in the fingertip, due to the warming; also, some redness is visible for the eye after warming up with water.
There is a possibility that water will reflect the light in the spot spectrometer in a different way than dry skin, therefore the fingertip is dried very carefully. Some additional measurements are performed to find out if the wetness of the fingertip will really influence the saturation-value. 4 measurements are performed on 4 differing fingers (pointing, middle, ring and pinky). The four fingers are measured again when wet. The results are shown in Figure 11; there the measurements are performed only once, a small point can be made that there is a possibility that a wet finger will give slightly lower saturation values. To prove this expectation, more research is needed.

F. Obstructing the blood flow

While obstructing the blood flow, the pulse oximeter won't give any results any more. There the pulse oximeter measures the saturation of the pulsating blood flow and the total blood flow is obstructed. According to the results of the saturation in differing conditions (Figure 4), there is a possible lower saturation measured with the spot spectrometer after obstruction. To further validate this observation, an additional experiment is performed; measuring the saturation with the spot spectrometer during and after the blood obstruction.

These results, shown in Figure 5 and Figure 6, show a decreasing saturation line during the obstruction. The lowest saturation is found just before releasing the machete. After release, the saturation value peaks to a slightly higher value than the value measured when the measurement started. The value measured at the beginning of the measurement is not the same value as a normal saturation value; a possible explanation for this observation is the ten second delay before the measurement starts. These ten seconds are used to pump the blood pressure measurer, but in the real case the machete is pumped in less than ten seconds, the saturation will in the first

measurement be lower than in the normal situation. This observation is corresponding to the hypothesis that obstructing the blood flow causes a lower saturation value. The described script seems to give a value for saturation in this case.

G. The probe tip

The distance from the probe tip to the skin seems to have an influence on the calculated saturation value. The larger the distance between probe tip and tissue, the higher the saturation value will be. This is probably caused by the light that is scattered and absorbed by the block in which the probe is held. Also, the pressure that is performed on the probe tip seems to have a small influence on the saturation value, as shown in Figure 13. It is recommended to find out what the influence is of the pressure on the probe tip, or to perform further measurements in a dark room, applying no pressure on the probe tip and use a set distance between tissue and probe tip.

H. Spot spectrometer vs pulse oximeter

As mentioned before, the pulse oximeter did not give a value for the obstructed arm. For all other circumstances two measurements are performed to compare the spot spectrometer with the pulse oximeter. The results are shown at paragraph III.A: Results compared with a pulse oximeter. Thickness of the tissue is chosen as D = 0.01 cm Overall, the points where the pulse oximeter gives a higher saturation, the script / spot spectrometer gave an higher saturation value as well. But the absolute values do not correspond; this can be explained by the chosen tissue-thickness, as described above. In the second series of measurements the pulse oximeter is not used.

V. CONCLUSION

Light can be used to define a value for the saturation in tissue. The concentration of the components oxygenated hemoglobin and deoxygenated hemoglobin are used to find a measure for saturation. Two other components that are included in the estimated spectrum, are defined as a constant and a constant descending component. The ratio between the oxygenated and deoxygenated tissue gives a measure for saturation.

The saturation is influenced by warming and cooling of the skin, holding the breath and obstructing the blood flow. These experiments gave fluctuating results for the saturation value of the tissue, measured with the spot spectrometer. The most reliable method could be the obstruction of the blood; giving a considerably lower saturation after a minute of obstruction and a considerably higher saturation after releasing the band; this effect can be found in paragraph III.C: Results after blood obstruction of this report.

The spectrum is obtained using a spot spectrometer, measuring in a total range from 174 to 1100 nm. The ideas behind this research have to be proved by using a phantom and the conditions of the measurements have to be specified in a natural way. Nevertheless, there is a possibility that this research is a small step towards measuring the saturation using hyper spectral imaging and a maximum of 18 differing filters, that is a possible elaboration on the diagnosis and treatment of diabetic feet.

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