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Lise L. Randeberg, "Hyperspectral characterization of tissue in the SWIR spectral range: a road to new insight?," Proc. SPIE 10873, Optical Biopsy XVII: Toward Real-Time Spectroscopic Imaging and Diagnosis, 108730Y (4 March 2019); doi: 10.1117/12.2504297



Event: SPIE BiOS, 2019, San Francisco, California, United States

Hyperspectral characterization of tissue in the SWIR spectral range - a road to new insight?

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ABSTRACT

Hyperspectral imaging is a generic imaging modality allowing high spectral and spatial resolution over a wide wavelength range from the visible to mid-infrared. Short wavelength infrared (SWIR) hyperspectral imaging is currently becoming an important supplement to spectroscopy in optical diagnostics due to the flexibility and adaptability of the technique. However, due to the complexity of hyperspectral data, the analysis requires a well planned approach. In this paper a simple but effective approach combining dimension reduction and unsupervised classification is suggested. Examples of *in vivo* hyperspectral data in the SWIR spectral range (950-2500 nm) from human skin bruises and porcine skin burns are presented as examples. Data are processed using the minimum noise fraction transform (MNF), and K-means clustering. K-means clustering was found to perform significantly better if applied to MNF transformed data. The classification results agree well with biopsies, spectral data and visual inspection of injuries. It is thus shown that unsupervised clustering can be a preferable technique in cases where it is challenging to use or interpret results from physics based models, or where the ground truth is lacking or not well defined. The presented results confirm that SWIR hyperspectral imaging indeed is a useful tool for optical characterization of tissue.

Keywords: Hyperspectral imaging, SWIR, Tissue characterization, Imaging spectroscopy, Image analysis, Bruises, Burn injuries

1. INTRODUCTION

Accurate tissue characterization is essential to identify markers that can be used for precise diagnostics and decision support in medicine. Several optical imaging techniques have shown clinical potential in characterizing and detecting surface near structures and anomalies. Hyperspectral imaging is one of these optical techniques showing promising results [1]. Although often referred to as a specific technique, hyperspectral imaging consists of a family of techniques sharing common features like e.g. high spectral and spatial resolution. A hyperspectral data cube consists of at least two spatial and one spectral dimension. In some cases time or depth might be added as a fourth coordinate, adding to the a multidimensionality of the data. A hyperspectral data cube can be collected using a variety of imaging geometries and -systems, ranging from simple filter based systems to sophisticated Fourier imagers and imaging spectrometers. As hyperspectral imaging is a generic technology, medical applications also covers a wide range range from high resolution microscopy to wide field imaging of larger objects [1].

Despite the variety of techniques and applications available, all hyperspectral images have a common structure containing both spatial and spectral information, which implies that they can be analyzed using the same analytic tools and algorithms. Similarities between the data cubes collected in medicine and other fields, for example remote sensing or cultural heritage, results in a larger community interested in developing good tools, making transfer learning and cross fertilization between fields feasible. Figure 1 shows the current main directions in hyperspectral image analysis. As briefly shown in Fig. 1, analytic tools vary from calculation of simple wavelength ratios to advanced statistical processing. The road to success is to chose the right tool for the task to be solved. Even simple tools such as wavelength ratios can be valuable tools to determine parameters like tissue oxygenation.

Optical Biopsy XVII: Toward Real-Time Spectroscopic Imaging and Diagnosis, edited by Robert R. Alfano, Stavros G. Demos, Angela B. Seddon, Proc. of SPIE Vol. 10873, 108730Y · © 2019 SPIE · CCC code: 1605-7422/19/\$18 · doi: 10.1117/12.2504297

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Figure 1. Generic overview of methods used for analysis of hyperspectral data.

The important decision is thus to chose the right tools, or chain of tools, to solve the problem at hand. This advise is valid both in selecting imaging system and algorithms to process data.

So far, the visible or near infrared spectral range has been the most popular wavelength range for medical hyperspectral imaging. However, over the last years, short wave infrared (SWIR) cameras and detectors have become more affordable and available. As a consequence, medical hyperspectral imaging data SWIR spectral range has increased in popularity.

This paper aims at showing some of the possibilities associated with medical hyperspectral imaging in the SWIR spectral range. The paper illustrates the potential of this technique sing simple, statistical image processing methods for data analysis. Data from a diffraction grating based, line scanning, push broom hyperspectral system is presented in the spectral range 950-2500 nm. The given examples are wide field imaging of a human paintball induced bruise in a human volunteer, and burn injuries in porcine skin. The paper touches upon the processing chain, and shows the effect of dimension reduction by minimum noise fraction transformation (MNF), and how it can be utilized to extract and enhance spectral signals. In addition, this paper gives an example of processing by utilizing unsupervised K-means clustering to classify burn injuries according to severity. It is shown that unsupervised clustering can be a valuable technique in cases where it is challenging to use or interpret results from physics based models, or where the ground truth is lacking or not well defined.

2. THEORY AND METHODS

2.1. Tissue optics in the short wave infrared spectral region

Several excellent publications have presented or reviewed data on the optical properties of tissue in the SWIR spectral range, see *e. g.* [2, 3]. Water, lipids, collagen and proteins are among the most important absorbers in the short wave infrared wavelength range. Wilson *et al.* [2] list water absorption peaks at 1150, 1450, and 1900 nm, lipid absorption peaks around 1040, 1200, 1400, and 1700 nm, and collagen peaks close to 1200 and 1500 nm as the most prominent spectral features in their review of optical properties in this wavelength range. Hemoglobin species exhibit a lower absorption than in the visible range, but should still be considered and taken into account [4, 5]. The scattering is less prominent in the SWIR range, and falls off with increasing wavelength [2, 6].

2.2. Experimental models

Hyperspectral data in the spectral range 950-2500 nm from skin bruises and burn injuries are included in this paper. These two data sets are described in further detail below.



Figure 2. Setup used for collecting data from bruises.

2.2.1. Bruises

Superficial skin bruises were inflicted in the ventral side of the forearm in a healthy male volunteer in his thirties (Fitzpatrick skintype III) using a paintball gun fired at a distance of 10 m. Hyperspectral data were collected using the scanning system shown in Fig. 2, which consisted of a HySpex SWIR 320 (950-2500 nm) camera (Norsk Elektro Optikk AS, Skedsmokorset, Norway), mounted on a translation stage (Standa, Vilnius, Lithuania) with a broad band halogen light source (IT2900, Illumination technologies, USA). Data were collected from the bruise 20 minutes after injury, and then every day for the first days, then every other or every third day up to day 17 after injury. The bruise data presented in this paper were collected at day two after injury. Data collected at day one and six in the same measurement campaign has previously been published in [7].

2.2.2. Burns

This experimental protocol has previously been published in [8]. A 30 kg Noroc pig (hybrid of 1/4 Duroc, 1/4 Yorkshire, and 1/2 Norwegian landrace) was used for the experiment. The animal was under general anesthesia during the entire experiment and was sacrificed after 8 hours. The animal was premedicated using 4 mg/kg Azaperone and 0.40 mg/kg Diazepam given by intramuscular injection. General anesthesia was induced with intravenous 0.04 mg/kg Atropine, 10 mg/ml Thiopental, and 10 mg/ml Ketamine. General anesthesia was maintained using a dose of 0.007 mg/kg*h intravenous Fentanyl. Intravenous antibiotics (2 g Cefalotin) was given prior to the experiment. The animal was ventilated through a tracheotomy using room air. The ventilator settings were adjusted based on blood gas readings. After the experiment the animal was sacrificed using an intravenous injection of 100 mg/kg of pentobarbital. The experimental protocol was ethically approved.

A total of 6 burns were inflicted using contact times of 1, 1.5, 2, 3, 4, 5 s. The burns were created using a brass rod heated to 100 °C in boiling water. The rod was left resting onto the skin for the given time without applying any pressure. See Fig. 3 for a photo of the burn injuries. The two burns marked with numbers 5 and 6 in the photo are the ones analyzed in this paper. These burns were created using contact times of 4 and 5 seconds, respectively.

Two 6 mm punch biopsies were collected from each burn site after sacrificing the animal. The samples were fixated in formalin and then embedded in paraffin wax before three 3.5 micrometer thick sections were cut and stained with hematoxylin and eosin and then examined under a microscope. As previously described in [8], the burn depth was classified into five anatomical levels: epidermis (1), upper (2), middle (3), or lower third of the dermis (4), and subcutis (5). Assessment of the burn extent was done according to predetermined evaluation criteria, i.e., epidermal damage, presence of sub-epidermal blisters, the highest patent vessels, the deepest occluded vessels, and depth of dermal collagen coagulation.

Hyperspectral measurements were collected at 15 min, 3 h, 5 h and 8 h post burn in both the VNIR and SWIR spectral ranges. Information about the VNIR data and further details about the experimental protocol can be found in [8]. The present work presents data collected at 5 hours post injury. The hyperspectral data



Figure 3. Photo of burn injuries. The injuries marked with 5 and 6 are the ones analyzed in this paper.



Figure 4. Setup used to collect hyperspectral data from porcine burns. The SWIR Hyspex 320-e camera can be seen to the right in the photo.

presented in this paper were collected in the wavelength range 960-2500 nm using a Hyspex SWIR 320-e camera (Norsk Elektro Optikk AS, Skedsmokorset, Norway), see table 1 for specifications. The camera was mounted on a translation stage (Standa, Vilnius, Lihuania) and the scene was illuminated using two fiberoptic light lines coupled to a halogen light source (Illumination Technologies, Elbridge, NY, USA). A photo of the setup can be found in Fig. 4.

2.3. Data analysis

Data were analyzed using ENVI 5.5 (Harris Geospatial Solutions, Crowthorne, Berkshire, U.K.) Radiometrically calibrated data were converted into reflectance images by using a Spectralon tile (Ocean Optics, Duiven, The Netherlands). After conversion to reflectance, the data was preprocessed to remove noise. Several methods can be employed to remove noise, ranging from simple low pass filtering to state of the art techniques from remote sensing. Our group has previously tested a wide range of different denoising techniques [9, 10, 11, 12]. The preprocessing steps used in hyperspectral data analysis are shown in Fig. 5.

The minimum noise fraction transform (MNF) [13, 14], was used to extract vessel structures from the bruised skin. The minimum noise fraction transform can be used to reduce the dimensionality of hyperspectral data,

Table	1.	Hyperspectral	camera s	pecifications
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Module	SWIR 320-e
Detector	HgCdTe (320×256)
Spectral range	$0.95\!-\!2.5~\mu\mathrm{m}$
Spatial pixels	320
FOV across track	13.5 $^{\circ}$
Pixel FOV across/along track	$0.75~\mathrm{mrad}/0.75~\mathrm{mrad}$
Spectral sampling	6 nm
No. of spectral bands	256
Digitization	14 bit
Frame rate to HD	$\geq 100 \text{ fps}$



Figure 5. Preprocessing of hyperspectral images. The list of methods listed under the preprocessing step are described in [9, 10, 11, 12]

remove noise, and identify strong spectral features. The transform consists of two steps, where the first step uses the principal components of the noise covariance to whiten the noise, giving a transformed data set where the noise has unit variance and no band to band correlation. The next step calculates the principal components of the original data after noise whitening, giving a transformed image where the eigenvalues are sorted according to variance. This means that the strongest spectral contributions will appear in the first bands of the transformed image. MNF can thus be used both for noise removal and for identifying spectral features in the data.

K-means clustering was used for unsupervised classification of the burn sites and surrounding skin. K-Means clustering [15] is a technique that calculates the mean of each class and then iteratively assembles the pixels into clusters calculating the minimum distance between the pixel and the present clusters. Each iteration reclassifies the pixels according to the class mean after recalculating it. Pixels are always assigned to a class, unless a distance threshold is set, an as a consequence allowing unclassified pixels. The process continues to run until the number of pixels in each class changes less than a given threshold, or the maximum number of iterations has been reached. This method thus requires some tuning to identify the right number of classes and to some extent to find the right number of iterations. Except from that, the technique is rather straight forward to use, and as it is unsupervised, it does not require an established and well known ground truth to cluster the hyperspectral data. The K-means algorithm was in this case run using 7 classes, a with a variable number of iterations leaving it to work until the class distributions were stable. Class average spectra were then calculated. Regions of interest (ROIs) were then selected from the burn and normal skin in areas visibly different from each other, and the average spectra of these ROIs were calculated. The ROIs were chosen to match the biopsy sites as closely as possible. Class statistics were used to compute the average spectra of each class in the image. Finally, the K-means classifier was applied to selected MNF bands after transforming the image. Bands 1,2,4,5,7 and 8 were used. Bands 3 and 6 showed strong noise and artifacts and were therefore excluded from the analysis. Bands higher than 8 were too noisy to include in the analysis.

3. RESULTS AND DISCUSSION

3.1. Bruises

Figure 6 show an RGB image of the investigated paintball induced bruise, a gray scale overview image of the ventral side of the volunteer's arm, and spectral data from the same bruise. The numbers shown in the figure correspond to the spectra shown in the right figure (as indicated in the figure caption).

The reflectance minima observed in the spectra correspond well with the absorption peaks summarized by [2, 3] for water, lipids and collagen, respectively. However, the spectral differences are rather subtle and difficult to interpret correctly based on visible inspection of the spectral information alone. In bruised skin the most essential chromophores are usually hemoglobin and hemoglobin breakdown products such as bilirubin. [7, 16, 17, 18, 19]. These chromopores become less prominent in the SWIR spectral range, while water increases significantly. Fresh skin injuries usually experience a weal and flare reaction, followed by a biological response in several steps [20, 21, 22]. If a trauma causes a hemorrhage, but no wound, a bruise usually develops within 24-48 hours after injury [17]. However, paintball bruises are caused by light weight, fast objects dissipating their energy superficially, and thus creating a bruise that develops faster and reaches a mature stage earlier than deep injuries [19]. The initial weal and flare reaction is often followed by swelling of the tissue indicating edema (fluid accumulation). The data shown here were collected on the second day after injury, and some swelling and edema can still be expected at this stage. Despite this, it is difficult to observe swelling and edema in the spectra shown in In Fig. 6.

An investigation of the individual spectral bands expected to show water, show only weak indications of liquid accumulation. Figure 7, top left panel shows the 980 nm band from the image: only a weak shadow of the bruise can be found, while blood vessels can be seen as weak, dark lines. Non of the other individual wavelength bands did show clear evidence of water accumulation. The minimum noise fraction (MNF) transform was then applied to the data to remove redundancy in the data set and emphasize spectral features. The first two MNF bands did not contain interesting information, as they mainly show surface related structures. Band 3, 4 and 5 revealed interesting features of vessels and fluids. As the MNF transform sorts the data according to variance, these features are substantially weaker than the dominating features of the image. These MNF bands can be seen



Figure 6. Data from a paintball induced bruise on the ventral side of the forearm at day 2 after injury. The subject was a male volunteer with Fitzpatrick skin type III: Top panel: Left figure: RGB image of the measured skin bruise.; Right figure: Gray scale image of the bruise, the numbers indicate where the spectra were collected, 1 = bruise; 2 = Normal skin adjacent to the bruise; 3 = Skin above a major blood vessel; Lower panel: Spectral data from the bruise. Each plotted spectrum is the average of 15x15 pixels.

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Figure 7. Single band images and MNF bands from the paintball induced skin bruise shown in Fig. 6. Top: Left figure: Wavelength band 980 nm; Right figure: 2200 nm; Lower panels: MNF components, from left component 3, 4 and 5, respectively.

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in the lover panel of Fig. 7. Blood vessel structures can clearly be seen in band 3 and 4, while the characteristic ring of a paintball bruise can be seen in band 5. If the vessels in band 3 and 4 are compared, they appear to be in roughly the same location, but still appear different. The bruise can be seen as a weak shadow in band 4. This indicates that the observed differences might be due to either depth [23], or due to the MNF-transform picking up the spectral differences in oxy- and deoxyhemoglobin, showing more deoxyhemoglobin in the bruised area. Surprisingly, the feature shown in band 5 does not overlap with neither band 3 nor 4. This feature is therefore probably not caused by a hemoglobin or water related feature, as it then could be expected to show up in the bands showing blood vessels as well. It might be that this feature is caused by either proteins from tissue fluid or immune cells accumulating in the injured area. Further research will be needed to determine the exact cause of these observed features, as it is complicated to interpret physiological signatures derived using statistical methods [10]. Despite this difficulty in physical interpretation, it is visualized how well a simple tool such as the MNF transform are helpful to reveal "hidden" information in a data set by extracting and sorting the spectral features that cannot easily be found.

3.2. Burns

The burn data are used to illustrate the power of hyperspectral imaging in a case where there is a spatially heterogeneous injury. Previous work [8] has revealed that classification of burns is complicated in the visible spectral range. Burn injuries might lead to dramatic tissue changes including hemoglobin alteration, protein denaturation and coagulation of tissue. A severe burn injury leads to an altered combination of hemoglobin species due to methemoglobin formation, substantial deoxygenation due to coagulated vessels impairing perfusion in the injured area. Changes in scattering due to coagulation and denaturation of connective tissue will also occur. [24, 25, 26]. As the scattering in tissue is highly wavelength dependent for shorter wavelengths, a burn will affect the scattering parameters and thus the penetration depth in a significant manner [26]. In the SWIR range, the situation is easier due to lower and less wavelength dependent scattering, and less influence from hemoglobin species, although it should be remembered that methemoglobin has a higher absorption than oxy- and deoxyhemoglobin for longer wavelengths [2, 5].

Physics based modeling such as Monte Carlo methods or diffusion theory are popular tools to interpret spectral data, especially in the visible and short infrared spectral range, where the knowledge of the optical properties in tissue is fairly good. Data for *e. g.* hemoglobin are available and known with an acceptable standard deviation [4]. As the hemoglobin absorption is decreasing towards the infrared, it becomes more challenging to measure with an acceptable signal to noise ratio despite increased penetration depth due to lower absorption and scattering. With increasing wavelength it becomes more challenging to measure hemoglobin samples diluted in water as the water absorption becomes the dominant feature. In this case, the physical nature of the chromophore makes it more complicated to have a good and trustworthy ground truth, which again complicates modeling. Another significant challenge for physics based modeling is swelling as shown in Fig. 8.

Live animals and humans are complicated biological systems that respond to injury using a complex combination of reactions, one of them being swelling and fluid accumulation in the tissue. Swelling is a challenge for physics based models as such models often require a well defined geometrical model. Even though the skin thickness can be one of the parameters to be fitted by the model, it might be impossible if the variation is as extreme as shown in Fig. 8. Such variations will cause a problem to physics based models, especially in the case of burns where the tissue changes are highly non-linear and dramatic. The lower panels in Fig. 8 show photos of the burns investigated in this study. A difference in color can be observed across the burn. This is probably linked to the induction process, where a brass tool is heated in boiling water and then held against the skin for a given time. In this case it is expected that some water droplets were left on the tool causing a layer of water vapour between the skin and the hot tool. Such a layer of gas will reduce the heat transfer and lead to a less severe injury than expected due to the given contact time. This suggested effect is confirmed by the findings from the biopsies. The black dots in the figure mark the positions where the biopsies were taken. The numbers given in parenthesis indicate the depth of the injury given by the pathologist, details of this assessment is given in [8].

The spots marked 2-5a and 2-6a are taken from the whitish area, and correspond to deep and severe injuries reaching into the lower layers of the dermis and into the subcutis. The depth of these injuries is so large that



Figure 8. Data from the porcine model. Top panel: Substantial swelling could be observed to appear and disappear during the 8 hour duration of the experiment. This photo was taken at max swelling. The two burns seen in the back to the right are the ones analyzed in this paper; Lower panels: Photos showing the burns. The black marks show where biopsies were collected, and the number seen in parenthesis is the depth of the bruise using the scale given in the text. Another version of this photo has previously been published in [8], and the thesis of Lukasz Paluchowski (Doctoral theses at NTNU;2018:402, see http://hdl.handle.net/11250/2584982

they can be assumed to be spectrally similar (a porcine dermis is thicker than a human dermis). In reflectance measurements where the light have to travel into the skin and back again, about 10 percent of the light traveling as deep as to the penetration depth can be expected to be detected at the surface [27].

The red areas (biopsies 2-5b and 2-6b) were found to have a less severe injury protruding to the upper and middle part of dermis. As the injuries are more superficial it can be expected to detect spectral differences in these injuries. As it is labour intensive and costly to have biopsies prepared and investigated only a few biopsies could be collected from each injury. In clinical assessment of burn injuries, it might be a wish to avoid taking biopsies at all as the burn site is very vulnerable. As a consequence only a limited number of samples can be investigated, if any. In this work, two biopsies were collected from each burn. The limited number of biopsies is challenging seen from an analysis point of view as only point information is available on the ground truth.

As a first approach to understand the data material, regions of interest (ROIs) were defined based on the biopsy locations and visual inspection of a gray scale image of the burns. The locations of these ROIs are shown in the top panel of Fig. 9.

The lower panel in Fig. 9 show the average spectrum of these ROIs. The idea was to explore and visualize the spectral variation across the sample. The color of the ROI in the upper figure match the color of the plotted spectrum for each region. No quantitative assessment of the spectra was done, but a qualitative evaluation was done comparing the spectra collected in this study to the absorption peaks given in [2]. It can be seen that normal skin (maroon line) has a spectrum similar til the most severely damaged areas (yellow and green lines) for wavelengths shorter than approximately 1100 nm, and differs at the longer wavelengths, except around he water peaks near 970-980, 1150, 1450, and 1900 nm where the absorption is high in all spectra. In the spectrum from normal skin an indication of lipids can be seen around 1200, 1400, and 1700 nm. A spectral minimum can also be observed around 1350 nm in all spectra. Normal skin appears to be different from all spectra from injured skin around the collagen absorption peaks near 1200 and 1500 nm. This observation is supported by the findings in histology, confirming damage of collagen, see [8] for further details.

The spectra from the less damaged areas are divided in two groups (blue and cyan, and magenta and red lines). The reflectance is lower for the blue/cyan lines than the magenta/red lines. From the photo of the injury these areas appear as red in color, indicating that there still might be some blood circulation in the area. According to the biopsies, the red and magenta ROIs are areas with more severe damage and deeper burns. If the spectral trend from the most to the least damaged areas is considered, it seems like a higher absorption and less scattering is a positive sign for the prognosis of the burn. If the area is perfused a local inflammatory reaction might cause dilation of local blood vessels ensuring nutrition to the injured region, something witch on longer terms might promote healing. From this simple, qualitative evaluation of the spectra and from the biopsy material it can be expected to find at least three classes of variable severity in the injured areas if statistical tools are used to classify the hyperspectral data.

Identifying an optimal way of analyzing hyperspectral data becomes increasingly important in cases like this when the ground truth is either missing or limited. Limited knowledge about the ground truth effectively rules out a significant number of the tools in the toolbox, leaving statistical methods for unsupervised classification as an obvious choice. There are several such techniques available, and it is out of scope for this paper to review these techniques and their overall performance. However, this work aims at exploring the feasibility and performance of a simple unsupervised technique and compare the results with the available information from other methods. In this case a K-means classifier has been tested. All classification techniques require some degree of tuning to achieve optimal performance. In the case of K-means clustering, the most essential part is to determine a correct K, which is the number of classes the data are to be clustered into. As every pixel usually will be assigned to a class, the number of classes will determine the accuracy and performance of the algorithm. In this case the classification was repeated several times with a different number of classes to figure out the optimal number of classes are stable and the iterations should be repeated until the class means don't change anymore. In this work 5 iterations assured stable classes.

In figure Fig. 10 a gray scale image of the burns can be seen in the upper left panel. The upper right panel show the K-means result from classifying the reflectance data directly without any dimension reduction or noise





Figure 9. Spectral data from burn injuries. Top panel: Gray scale image showing regions of interest (ROIs) selected on the burn injuries. The ROIs were selected based on visual inspection of the bruise and the known biopsy sites shown in Fig. 8 The color of each ROI correspond with the color of the graph shown below; Lower panel: Mean spectra from the selected ROIs.

removal. The result show scattered classes and a significant number of single pixel classes throughout the image, the blue and green classes on the side of the burn seem to be due to uneven illumination of the scene and not differences in the tissue. Post processing algorithms such as clumping of classes were tested on this image without improving the result (image not shown).

The Left figure in the lower panel shows a K-means classification done after preprocessing the data using the MNF-transform to reduce the dimensions of the data set and thus remove redundancy. Six bands of the MNF transform were chosen based on visual inspection of the transformed image. Bands 1-8, except 3 and 6 were used. The omitted bands showed strong artifacts due to inaccurate sensor calibration (stripes). After classification the result was post processed by clumping classes to avoid single pixel classification. The classification based on the MNF transform appears cleaner and less messy than the classification presented in the upper right panel of Fig. 10, although the uneven illumination still can be seen in the classification. In the lower, right panel of Fig. 10 the same classification is shown after combining the four normal skin classes into one, and clumping the classes to avoid single pixel classification. The classification. The classification is shown after combining the four normal skin classes and one normal skin class. The data analyzed here were collected 5 hours after injury. At this time the observable swelling was reduced and the burns appeared as whitish with red patches and a red border.

The classes found cluster burn severity 4 and 5 together as expected from the spectral data and the biopsies previously discussed. The results indicate that these injuries are so deep that they cannot be differentiated using this optical technique in this wavelength range. Anyhow, these injuries are not the most interesting ones seen from a clinical perspective. The less deep burns are more of interest as it is important to identify those burns that will heal spontaneously and those that require skin transplants. The classification show two additional classes in both injury 5 and 6, but the distribution of these classes are different in the two injuries. The classification indicate that injury 6 has a region with different severity at the right edge of the burn, and at the left side of the injury, while injury 5 has this severity only around the edge of the burn. A patch of the cyan class can be seen at the leftmost edge of the image. This is an adjacent burn not analyzed in this work. From the histology it seems like the cyan class is a class with a deeper and more severe burn. This finding is supported by the biopsy material. Average spectra from the ROIs shown in Fig. 9 overlapping with the classes shown in the lower right panel in Fig. 10 contributes to confirm this hypothesis. A simple, unsupervised classification method thus seems to be able to cluster data according to spectral differences without any other prior information than the hyperspectral data itself. However, the specificity and sensitivity of a method can not be determined based on this selected example, and although these results are promising, this finding will have to be confirmed by further studies on more images.

The results show the potential for statistical processing, and shows that it is easier to understand and interpret if supported by understanding of the physics and biology of the problem in question. Statistical data will always have to be interpreted in a physiological and biological context. Such results should never be trusted if they don't appear sound and logical. The presented results show the need of proper preprocessing to achieve optimal results. One main message to be taken home is that the processing chain mus be selected based on knowledge about both the methods and the data that are to be analyzed.

4. CONCLUSION

This study presents and discusses hyperspectral imaging in the SWIR spectral range. Data from skin bruises and skin burns are used to emphasize the suitability of SWIR hyperspectral imaging for optical diagnostics of skin. The bruise data is used to illustrate how simple tools for dimension reduction and enhancement of spectral features like the minimum noise fraction transform can be used to enhance and visualize vasculature and features like fluid accumulation in a bruise. Hyperspectral SWIR data from skin burns in a porcine model are used to show the applicability of the technique to detect burn severity. Spectral data from the injured areas are investigated and compared to biopsies to reveal spectral changes correlated with burn severity. The spectral results are in agreement with the findings from the biopsies. an unsupervised method is suggested to classify the burns as he use of physics based models is complicated and demanding in a situation where the optical properties and structure of the tissue change in a dynamic manner, and where the known ground truth is missing or limited. The unsupervised technique K-means clustering does not depend on a known ground truth. It only requires tuning of the number of classes and number of iterations. K-means clustering was tested both with



Figure 10. Unsupervised classification of hyperspectral burn data collected at 5 hours after injury. Classified using K-means clustering. After tuning the classifier, the algorithm was applied using 7 classes. The classes converged after 5 iterations. Top left: Gray scale image; Top right: K-means classification based on hyperspectral reflectance, no preprocessing other than conversion to reflectance was applied. Lower Right: K-means classification of the MNF transformed image. This classification was based on MNF band 1,2,4,5,7 and 8. These bands were selected by manual inspection of the transformed data.; Lower right: Same image as in the left panel, but after post processing by clumping the classes to avoid single pixel classifications, and combining four normal skin classes (red, green, blue and yellow.). These classes did not reflect spectral differences, but uneven illumination of the scene and black text written on the pig.

and without preprocessing of the data, and turned out to preform significantly better preprocessing was applied. The classification results agree well with biopsies, spectral data and visual inspection of the injuries. It can thus be concluded that K-means clustering is a suitable and promising tool to analyze hyperspectral SWIR data from burn injuries. Further investigations are needed to be able to specify the sensitivity and specificity of the method.

ACKNOWLEDGMENTS

Thanks to Dr. Lukasz Paluchowski, Dr. Haavard Nordgaard, M.Sc. Julio Hernandez, Dr. Yi Liu and M.Sc. Asgeir Bjorgan for inspiration and for collaboration on collecting experimental data. Funding from the Norwegian University of Science and Technology and the Norwegian Research Council is acknowledged.

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