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Imaging Spectroscopy as a tool for detection of nematodes, blood, skin remnants and black lining in cod fillets

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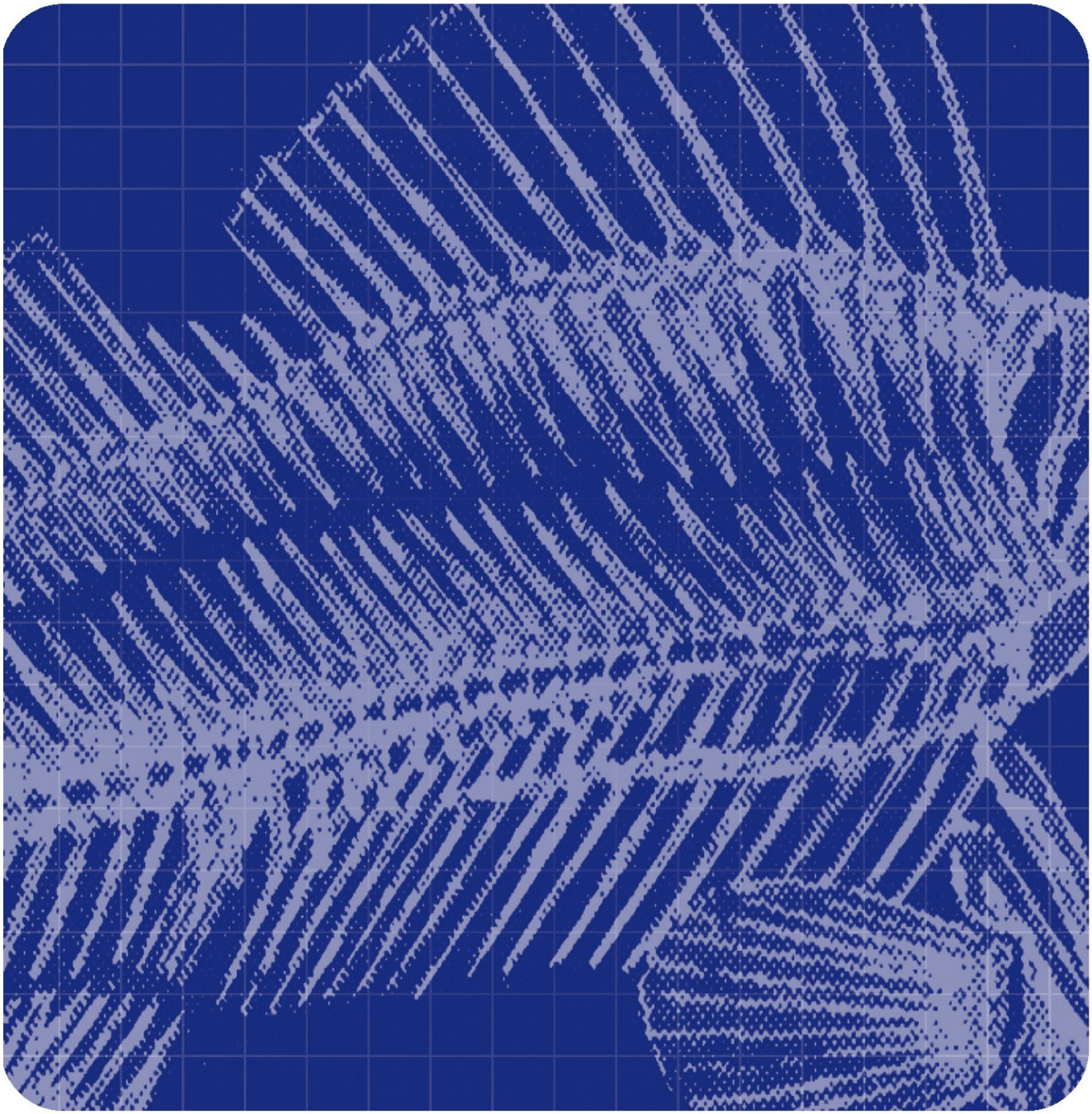

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1 INTRODUCTION

During the last decades a number of research institutes have been focusing on the problem regarding detection of nematodes in fish muscle. In this process several detection techniques have been tested and so far Imaging Spectroscopy has proven to be most efficient (Buljo *et al.* 1999, Wold *et al.* 2001). This technique makes a 3-dimentional image of the sample, both spatial and spectral resolution. This means that in each point on the surface of the sample a spectrum is recorded. Implementing the technique with the light source on one side of the sample and the detection unit on the other side ensures that the spectrum represents not only the surface but also the inside of the sample. Typically the wavelength region used represents the visible (VIS, 400-700 nm) and the near infrared (NIR, 700-1100 nm) part of the light spectrum. After recording of data a classifier is applied to differentiate between fish muscle, nematodes and so on. This classifier can be based both on the spectral and spatial information.

Scattering is a general phenomena involved in spectroscopic measurements. It has been shown that the scattering in fish muscle decreases with increasing wavelength (Peturson, 1991). Scattering complicates detection of nematodes, and thus this property indicates that wavelengths in the NIR part are to be preferred to improve detection performance. However this work shows that also light in the VIS region must be included.

To decrease the number of wavelengths necessary for classification this work has focused on finding wavelengths that represents a significant contribution to the classification process. Important wavelengths are those that clearly discriminate between fish muscle and other items, or that are only influenced by the fish muscle. The last feature is important since these wavelengths can be used to compensate for varying thickness of fish fillets.

2 METHODS AND MATERIALS

2.1 Theory

The goal is to record spectral data with spatial resolution and to send this data into a classifier to differentiate fish muscle from nematodes embedded in the muscle, blood spots, skin remnants and black lining still attached to the fillet. To make sure that the spectral information is consistent compensation of interfering effects has to be carried out. Typically, effects of lamp characteristics, illumination pattern, detector sensitivity, and sample thickness should be compensated for.

Illumination from a white light source contains all wavelengths, but has normally not equal intensity at all wavelengths. In the same way the sensitivity of the detector system is not necessarily uniform with respect to wavelength. For instance the system applied in this work is not sensitive above 1100 nm. In addition the sensitivity is not uniform across the two-dimensional CCD-detector when measurements are performed the illumination pattern is optimised with respect to spatial uniformity, but still it is not uniform and has to be corrected for. In spectroscopy theory it is normal to convert the raw count spectrum, recorded in the instrument, into an absorbance spectrum using a reference spectrum. A reference spectrum is typically a raw count spectrum recorded using a standard material or just the lamp itself instead of a fish sample.

In order to calculate the absorbance spectrums for the fish sample it is necessary to perform a reference measurement before performing the imaging process of samples. The raw count reference spectrum is denoted $S_R(\underline{x},\lambda)$ where $\underline{x}$ is the spatial coordinates and λ is the wavelength. It is necessary to include the spatial coordinates since the lamp illumination is not totally uniform. In the same way the raw count spectrum is denoted $S_S(\underline{x},\lambda)$. From standard theory it is normal to assume that there is an exponential decay of the signal through an absorbing material which leads to the following definition of the absorbance:

$$A(\underline{x},\lambda) = \frac{1}{T_S} \ln \frac{S_R(\underline{x},\lambda)}{S_S(\underline{x},\lambda)}, \qquad (1)$$

where T_S is sample thickness. If sample thickness is known it is used, otherwise absorbance is defined as the product of the sample thickness and the true absorbance. As can be seen from Eq. 1 the defined absorbance is independent of sample thickness, illumination pattern, and lamp and detector characteristics.

In addition there is one other feature that must be accounted for: The integration time. Normally a larger integration time is required in the NIR region than in the VIS region to utilise the dynamic range of the instrument. In the same way the reference spectrum will not necessary be obtained using the same integration time. Experiments carried out shows that the number of raw counts is approximately linearly related to integration time. Therefore the following modified definition of absorbance will be applied.

$$A(\underline{x},\lambda) = \frac{1}{T_S} \ln \frac{I_S S_R(\underline{x},\lambda)}{I_R S_S(\underline{x},\lambda)}, \tag{2}$$

where I_R and I_S are integration time for reference and sample, respectively. In the following Eq. 2 without the thickness normalisation ($1/T_S$) is applied when results are presented.

2.2 Instrumentation

To collect data with both spatial and wavelength information an instrument build at Fiskeriforskning and the University Centre in Svalbard (UNIS) has been applied. The instrument, SpexTubeIV, is based on using a rotating mirror in front of the instrument to achieve full spatial resolution. For a fixed mirror position one line across the sample is imaged with full wavelength information. The main idea behind spectral imaging of objects may be described as follows: Firstly, light from the object must be focused by a lens or mirror to form an image at the spectrographs entrance slit plane. The resulting spectrogram is the intensity distribution as a function of wavelength and position along the slit. The diffracted slit image contains both spectral and spatial information along a thin track of the object.

Secondly, in order to obtain the object's full spatial extent, it is necessary to sample the whole object. This requires the use of a high resolution rotary element. The whole idea is to record spectrograms for each track of the object as the image at the entrance plane is moved across the slit. A front surface mirror in front of the focusing element is used in combination with a high resolution stepper motor. In fact, the use of a mirror enables us to sample target objects that are static or moving relatively to the instrument by rotating or keeping the mirror fixed, respectively.

Figure 1 shows our choice of instrumental design with a description of the involved elements. In our case the light source is positioned below the fillet resulting in a transmission measurement. Each optical element is aligned along the optical axis. In Figure 2 a picture of the SpexTubeIV instrument is shown.

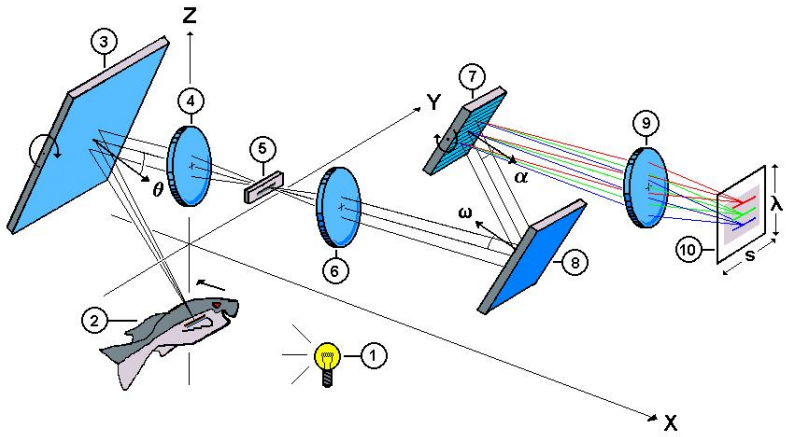


Figure 1. *A three dimensional optical diagram illustrating the main principle of the instrument. (1) is light source, (2) target, (3) scanning front surface mirror, (4) front lens, (5) entrance slit, (6) collector lens, (7) grating, (8) fixed front surface mirror, (9) camera lens, and (10) CCD imaging detector. The optical axis is parallel with the X-axis of the XYZ-coordinate system. The slit is located parallel to the Y-axis. θ is the angle between the mirror's normal and the optical axis. ω is the tilt angle of the fixed front surface mirror, equal to the grating blaze angle. α is the incident grating angle. The detector is located in the λS - plane which is parallel to the ZY - plane.*

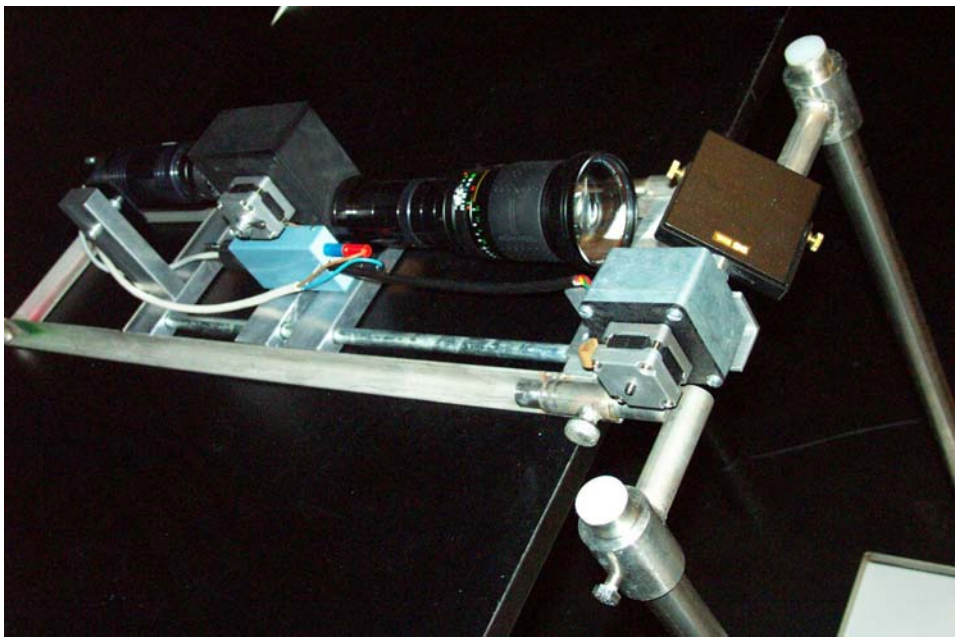


Figure 2. *A photograph of the SpexTubeIV instrument.*

2.3 Raw material

The measurements have been carried out on cod samples collected at different locations and in different seasons. Some of the samples have been measured as fresh while other have been frozen and thawed before the measurements have been carried out. A large variety of raw materials have been inspected, including embedded nematodes with a colour varying from almost white to dark brown. The nematodes have been positioned both in the belly flap as well as in the loin part, and in some cases in the surface and in other cases embedded into the

tissue. In some cases the nematodes were hidden under black lining making it difficult to see by visible inspection.

In addition to intact fillets measurements on homogenized nematodes were performed to reveal the characteristic spectra of nematodes. The nematodes were homogenized adding a small amount of water.

Appendix 1 gives more details about all the raw material that has been measured using Imaging Spectroscopy. In addition to these cod samples, recordings of saithe fillets have been performed, but they are not included in this report.

3 RESULTS

Two problems have been addressed in phase 0 and 1 in this project. (1) To improve the imaging system so that the imaging spectroscopy data has high quality, and (2) apply the improved imaging system to develop a detection system for nematodes, blood spots, black lining and skin remnants.

3.1 Improving the imaging spectroscopy system

The first task to solve was to improve the performance of the SpexTubeIV instrument. We wanted to increase the instrument sensitivity, imaging speed and to achieve uniform illumination of the samples. This was achieved by introducing a new light source with improved light characteristics in the range of interest. In addition a diffuser plate was positioned in front of the light source to assure a more uniform illumination. These two adjustments improved the signal to noise ratio of the system, but did not provide totally uniform illumination.

To compensate for the non-uniformity we transform the raw count from the instrument into absorbance using Eq. 2. By use of the reference spectrogram the non-uniformity is accounted for.

Although improvements of the light source the region 800 to 1100 nm still represents a problem. The light source introduces spectral peaks in this area and therefore the analysis has focused on the wavelength range from 400 to 800 nm.

3.2 Spectral imaging and detection

By use of the improved instrumentation a lot of new samples were recorded. Characteristic spectra for nematodes are shown in Figure 3. In this figure the spectra for the nematodes are recorded using homogenized parasites. As there were no reference spectrum available for this sequence the reference were collected from the image itself next to the sample. This means that the intensity in the reference is too low and therefore a negative absorbance was observed.

The interesting features from the two spectra representing nematodes, Figure 3 (a) and (b), are that both whitish and brownish nematodes absorb light in common specific wavelength regions. The main absorbance peak is located at 420 nm and there are two smaller peaks located at 550 nm and 580 nm. In addition the brownish nematodes have a small absorbance peak round 635 nm. The absorbance spectrum for pure fish muscle, Figure 3 (c), has also an absorbance peak at 420 nm, but not the other peaks present in the nematode spectra. These findings are of value when aiming at reducing the number of wavelengths to be applied for data analysis.

Several intact samples have been imaged to look at spectral signatures for fish flesh, different nematodes, blood spots, skin remnants and black lining. In Figure 4 five different images from one cod tail is shown. Each image is based on a specific wavelength. Figure 4 (a)-(e) represents 420 nm, 550 nm, 580 nm, 600 nm and 650 nm, respectively.

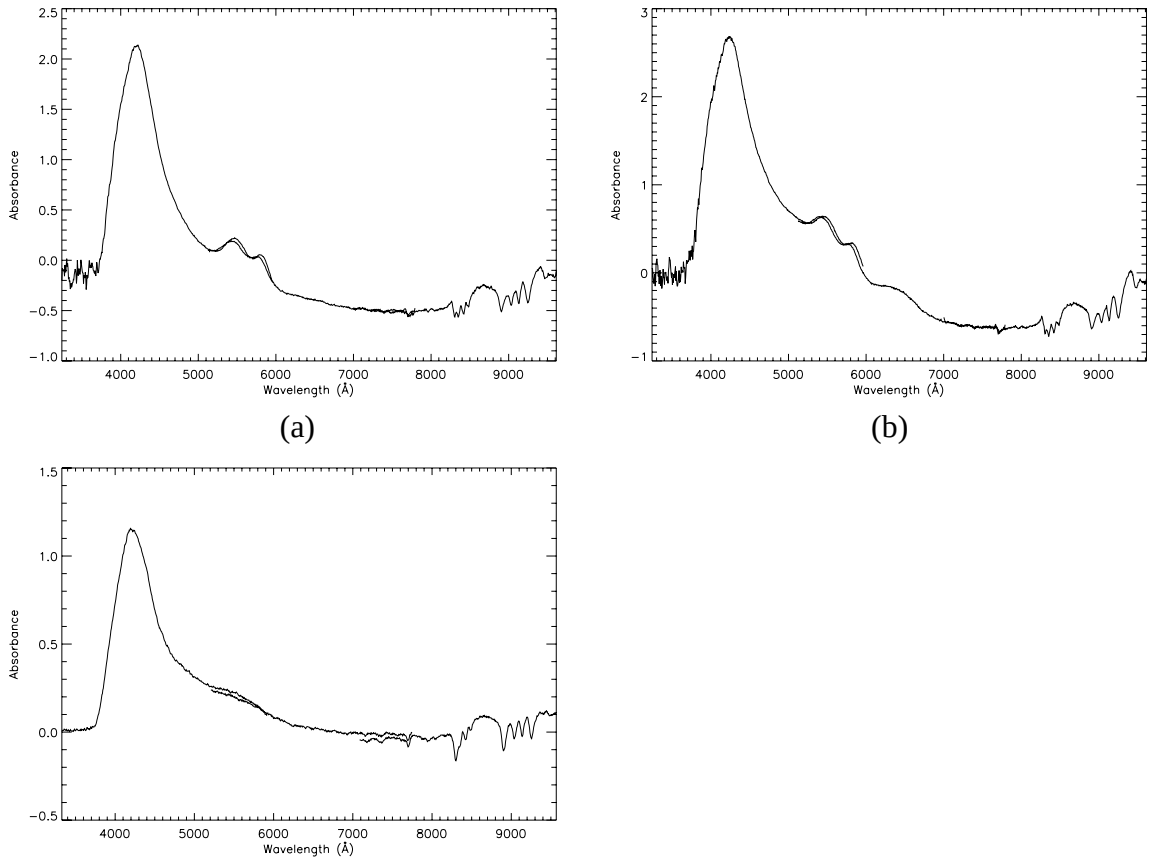


Figure 3. Absorbance spectra of homogenized nematodes and pure fish muscle. (a) Spectrum representing whitish nematodes, (b) spectrum representing brownish nematodes and (c) spectrum from pure fish muscle. Wavelength is given in Ångstrøm (Å) where 10 Å equals 1 nm.

Due to high absorbance at 420 nm for both nematodes and fish muscle, see Figure 3, the corresponding image, Figure 4 (a), appears dark. In the histogram equalised version of the 420 nm image, Figure 4 (f), one can see that there are still structures in the image at 420 nm. Nematodes are easiest to see in images based on wavelengths within the specific absorbance peaks for nematodes (550 and 580 nm). Black lining is clearly visible in all these images on the left side.

Figure 5 shows spectra from blood stains, black lining and nematodes collected from the sample visualised in Figure 4. Comparing these spectra with those in Figure 3 it is important to realize that nematode spectra collected from a sample are not “pure” spectra. These spectra are generated as a combination of cod muscle and nematodes. The same apply for blood spots and black lining.

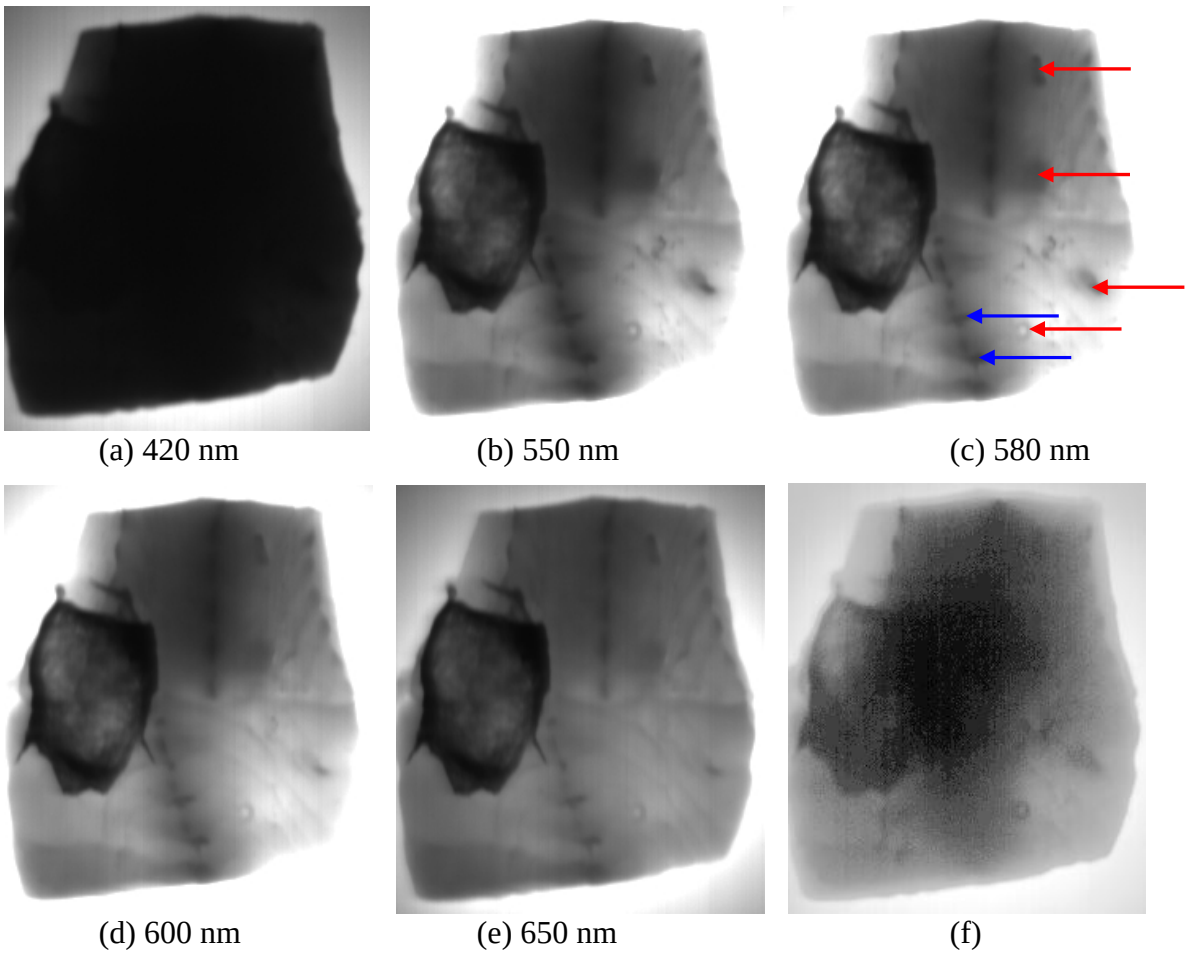
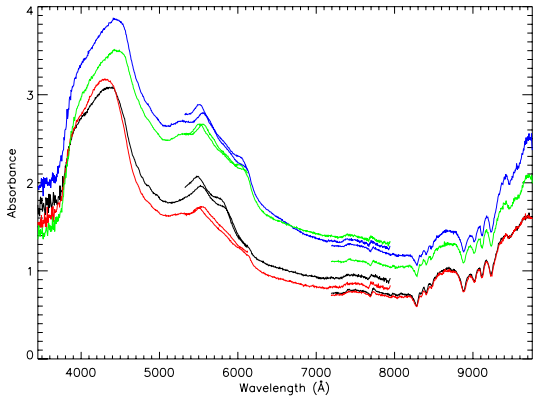
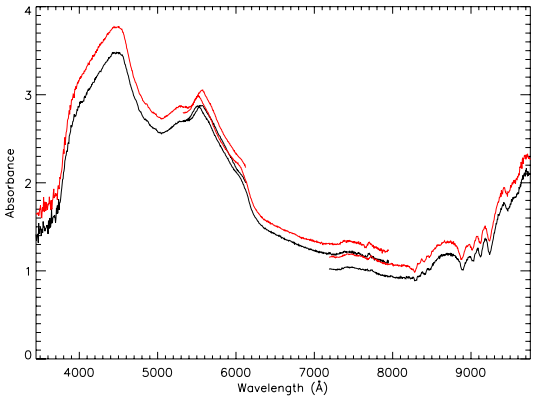


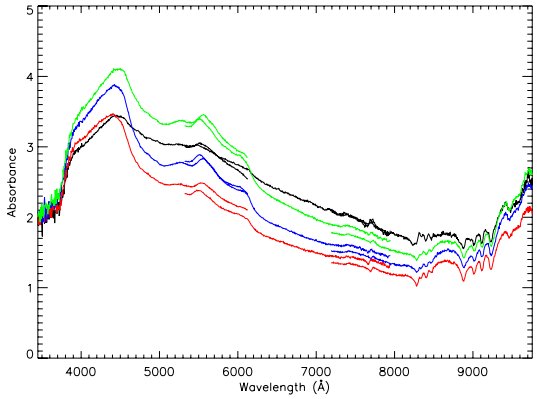
Figure 4. Images of one cod fillet containing nematodes, blood spots and black lining obtained at 5 different wavelengths, (a)-(e), and histogram equalized version of the 420 nm image, (f). Red arrows denote nematodes, while blue arrows denote blood spots. The parasites are located on the surface and at depths down to 5mm.



(a) Spectra from four nematodes.



(b) Spectra from two blood spots.



(c) Spectra from four locations on black lining.

Figure 5. Spectra collected from the sample shown in Figure 4. (a) Four different nematodes, (b) two different blood spots and (c) four locations on the black lining.

As can be seen from Figure 5 there is some variation in between spectra of the same feature. For instance in Figure 5 (a), the nematode spectra, the overall shape is the same, but absorbance level varies quite much between different nematodes. These findings are also verified in spectral recordings performed with other types of instrumentation at Matforsk.

Multivariate analysis was used to analyse the different data sets. In Figure 6 and 7 principal component analysis (PCA) is used too see if it is possible to separate nematodes, black lining, blood spots and pure fish muscle. In the PCA analysis shown in Figure 6 all wavelengths between 400 and 800 nm were applied. In this case it was possible to separate nematodes (Kve), black lining (Sva), blood spots (Blo) and fish muscle (Fis) as indicated with the added ellipses when principal component (PC) 2 and 4 were plotted.

The PCA visualised in Figure 7 is only based on five wavelengths, 510, 550, 580, 670 and 720 nm. Plotting PC 2 and 3 shows that the fish spectra (Fis) and black lining spectra (Sva) can be separated from the other spectra, see the ellipses in Figure 7. Nematodes (Kve) and blood spots (Blo) are on the other hand mixed. This illustrates the complexity of the system: What is clearly identified as nematodes by eye inspection is not that easily recognised by artificial identification.

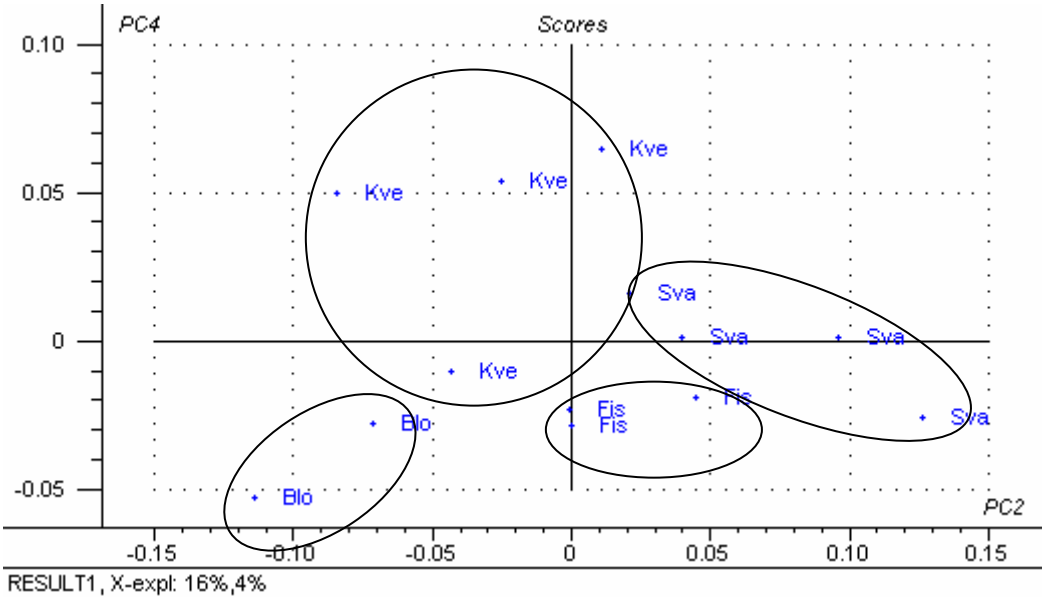


Figure 6. Principal component analysis (PCA) of spectra from the sample shown in Figure 4. Principal components two and four separates nematodes (Kve), blood stains (Blo), black lining (Sva) and fish muscle (Fis) when all wavelengths between 400 and 800 nm is used.

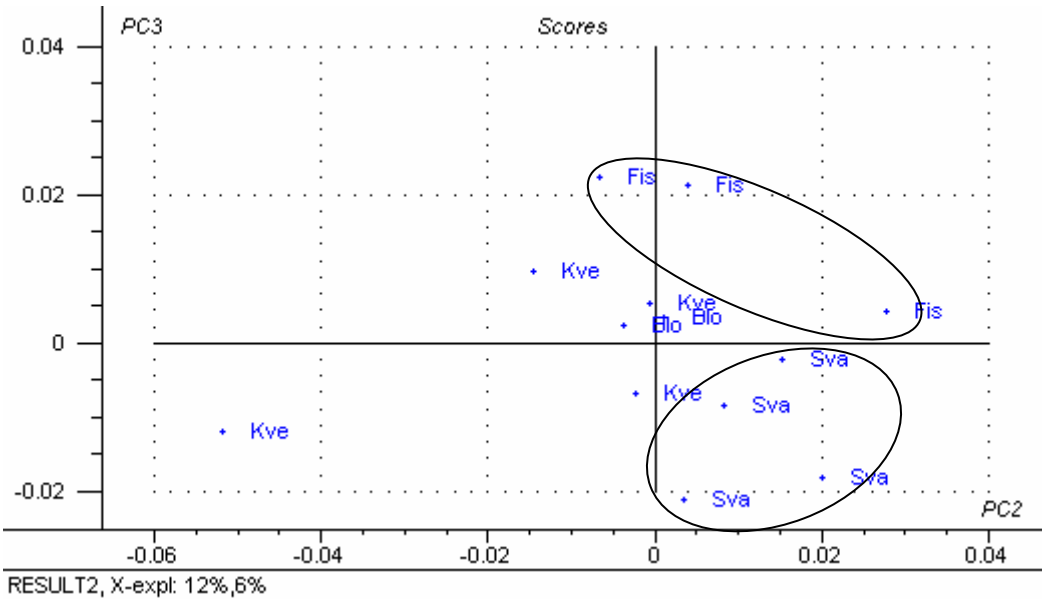


Figure 7. Principal component analysis (PCA) of spectra from the sample shown in Figure 4 using only 5 different wavelengths, 510, 550, 580, 670 and 720 nm. The following components were analysed: Nematodes (Kve), blood stains (Blo), black lining (Sva) and fish muscle (Fis).

4 DISCUSSION

Spectra from homogenized nematodes shows two absorbance peaks that are not present in fish muscle. They were located at 550 and 580 nm, see Figure 3. These two wavelengths are important to include in a classification procedure.

Looking at absorbance spectra from a fillet with nematodes, black lining and blood spots clearly show that they are different. The nematode spectra have absorbance peaks around 550 and 580 nm as was found for homogenised nematodes. Blood spots and black lining also have peaks in that region, but the spectral shape is different. It is important to note that the absorbance level can vary a lot as seen for the nematode spectra in Figure 5 (a). In this case this is not due to sample thickness, but more likely due to nematode orientation.

Using all wavelengths between 400 and 800 nm as input to a PCA analysis showed that based on principal component 2 and 4 it was possible to separate between nematodes, blood spots, black lining and fish muscle. This were visualised in Figure 6 by adding ellipses.

Since a future on-line instrument most likely will be limited to a lower number of wavelengths, feature selection will become more important. Reducing the number of wavelengths to five it was still possible to separate nematodes, blood spots and black lining from fish muscle, but nematodes and blood spots were mixed (Figure 7). If fillets after automatic inspection are sent for manual trimming mixing blood spots and nematodes might not be critical. Two of the selected wavelengths corresponded to nematode absorbance peaks, while to other three wavelengths were selected from areas without specific absorbance peaks. More effort should be spent on selecting the last wavelengths more carefully.

5 CONCLUSIONS

Based on imaging spectroscopy it is possible to distinguish between nematodes, blood spots, black lining and fish muscle. It is also possible to reduce the number of applied wavelengths from a full spectrum down to a number between five and ten wavelengths and still obtain the wanted separation.

A number of interesting wavelengths for differentiating between nematodes, blood spots, black lining and fish muscle are present in the VIS region. The wavelengths 550 nm and 580 nm have significantly higher absorption for nematodes than for fish muscle, and hence should be included in a detection system.

Due to scattering in fish muscle surface nematodes are easiest to detect than nematodes embedded deeply into the muscle (6 mm).

PCA analysis of the absorbance spectra shows that it possible to separate nematodes, blood spots, black lining and fish muscle. To improve the classification other procedures that utilise spatial information should be applied.

Based on the results achieved so far, it is realistic to start the implementation of an industrial test system during 2004.

6 REFERENCES

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APPENDIX 1

Table 1: Listing of cod samples where each sample number is one or several samples. Each sample is represented as a hyperspectral 290x290 image with 1470 bands. Nematode depth and filet thickness are measured in millimeters.

Sample Number	Sample description
1	Frozen-thawed belly flap with six white naturally embedded nematodes. Nematode depth {1,1,1,1,1}, filet thickness = {6,6,5,6,5,4}.
2	Fresh belly flap with four light-red nematodes, placed on the surface. Nematode depth {0,0,0,0}, filet thickness = {6,6,6,6}.
3	Fresh belly flap with five light red to brown nematodes, placed on the surface. Nematode depth {0,0,0,0,0}, filet thickness = {12,12,9,9,7}.
4	Six nematodes (light red to dark brown) and one piece of white muscle, placed directly on the diffusor plate.
5	Homogenised light red nematodes in a quartz cuvette.
6	Homogenised dark red nematodes in a quartz cuvette.
7	Homogenised 50/50 mix of light and dark-red nematodes in a quartz cuvette.
8	Five different frozen-thawed belly flaps, with five naturally embedded nematodes (white to red in colour), one blood spot and a region of brown muscle. Nematode depth {3,2,1,1,1}, filet thickness = {7,7,7,6,6}.
9	Three different frozen-thawed belly flaps, with five white naturally embedded nematodes, three blood spots and a region of brown muscle. Nematode depth {2,1,1,0,0}, filet thickness = {4,6,5,6,6}.
10	Two different frozen-thawed belly flaps, with seven naturally embedded nematodes (white to light red in colour) and a large region of black lining. Nematode depth {2,1,1,1,1,0,0}, filet thickness = {9,10,8,6,6,7,9}.
11	Three different frozen-thawed belly flaps, with ten white naturally embedded nematodes and five blood spots. Nematode depth {0,0,1,2,2,2,0,0,0}, filet thickness = {6,6,6,6,6,6,6,6,9,7}.
12	One thick belly flap with eight naturally embedded nematodes (white to red in colour) and one blood spot. Nematode depth {4,3,0,2,0,8,9,4,13}, filet thickness = {15,13,14,13,14,10,11,15,14}
13	Two different loins. One is from a starved cod and is extremely white in colour with a soft texture. The other loin has a natural white colour with a firm texture. Four large red nematodes are embedded in the loins. Two blood spots and a region of black lining are also present. Nematode depth {3,1,5,3, }, filet thickness = {11,11,10,10}
14	Eight days old ice stored loin with five naturally embedded nematodes (red to dark brown in colour). Nematode depth {3,3,3,3,9}, filet thickness = {25,25,24,24,25}

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