Chemical imaging of heterogeneous muscle foods by NIR hyperspectral imaging in transmission mode

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Abstract

Foods and biomaterials are in general heterogeneous and it is often a challenge to obtain spectral data which is representative for the chemical composition and distribution. This paper presents a setup for NIR transmission imaging where the samples are completely transilluminated, probing the entire sample. The system measures falling samples at high speed and consists of an NIR imaging scanner covering the spectral range 760 – 1040 nm and a powerful line light source. The investigated samples were rather big; whole pork bellies of thickness up to 5 cm, salmon fillets with skin and 3 cm thick model samples of ground pork meat. Partial least square regression models for fat were developed for ground pork and salmon fillet with high correlations (R=0.98 and R=0.95, respectively). The regression models were applied at pixel level in the hyperspectral transmission images and resulted in images of fat distribution where also deeply embedded fat clearly contributed to the result. The results suggest that it is possible to use transmission imaging for rapid, non-destructive and representative sampling of very heterogeneous foods. The proposed system is suitable for industrial use.

Key words: NIR spectroscopy; Near-infrared spectroscopy; hyperspectral imaging; transmission measurements; multivariate regression; chemical imaging; pork bellies; salmon fillets; heterogeneous samples; fat distribution

Introduction

Near infrared (NIR) hyperspectral imaging is becoming increasingly established as a feasible method for detailed non-destructive chemical studies of materials. One of the areas where this technology has proven to be useful is within analysis of biochemical distribution at either micro or macro level. Hyperspectral imaging is also used industrially for on-line quality control of foods and offers several advantages over conventional spectroscopy. Imaging provides spatial information and enables detection of local features of interest, such as for instance blood spots[1], parasites[2] or oxidation.[3] In the food industry it is often required to quantify the chemical composition and distribution in every product on the line. The ability to quantify and map fat in fish fillets is for instance useful for subsequent classification and sorting.[4] The same can be the case for quality grading of meat trimmings according to distribution of fat, lean muscle and connective tissue.[5,6]

Foods are heterogeneous, and measurement or imaging of the surface of the product is not always representative of the entire product. NIR hyperspectral imaging systems are usually designed for reflectance measurements, that is, measurements from the top surface of the sample. To obtain more representative spectral readings from heterogeneous samples, transmission measurement is an alternative option. The NIR region of the electromagnetic spectrum is of particular interest in this respect due to the relatively low absorption by biological soft tissues. Wavelengths shorter than 600nm are strongly absorbed by pigments in the tissue, wavelengths longer than 1300 nm are strongly absorbed by water,[7] while the 600-1300 nm spectral region is less absorbed and is often referred to the "tissue optical window".[8,9] The favorable spectral properties within this window enable relatively deep penetration of light in meat and other food products in general. Spectroscopic techniques are therefore not limited to reflection measurements only, but also transmission becomes possible.

The complete thickness of the sample can be probed when NIR radiation is transmitted from one side to the other. Transmission imaging does, however, pose some substantial challenges. Although NIR radiation is relatively little absorbed by water, the obtainable transmission depth in tissues like meat is still limited. Strong light scattering in muscle tissues will also limit penetration depth. In the case of spectral imaging, scattering can also result in blurred images with fewer details. Nevertheless, NIR transmission imaging has shown promising results when applied to mammography, where it is possible to detect internal structures and also measure oxygenation of the blood in breasts of thicknesses up to 7 cm.[10,11] Consequently, NIR transmission might be useful for samples that are heterogeneous and limited in thickness.

Transmission imaging in the visible and NIR spectral regions has been reported mainly for detection of internal defects in foods. Ariana and Lu[12] demonstrated this for whole pickles. Transmission spectral imaging has also been reported to give good results for detection of nematodes in fish fillets.[2,13] NIR transmission has also been evaluated for quantification of sugar and acids in individual blueberries, however, the imaging capability in this case was mainly utilized to detect the berries, not in the spectral analysis.[14]

A main criterion for transmission measurements is that the position of detection differs from that of the illumination. A special case of transmission, called interactance, occurs when both illumination and detection are carried out on the same side of the sample, but separated by a short distance so that the detected light has traversed parts of the interior of the product. Such a setup is used for instance to measure internal properties of fruit by NIR.[15] Interaction measurements can also be done in imaging mode and is illustrated by Wold et al.,[16] who showed that it is possible to image the distribution of liver and roe inside live crabs. In that case, the light probed approximately the upper 10 mm of the sample.

It is shown that surface fat distribution in meat can be mapped by NIR hyperspectral imaging in reflectance mode,[17] and in the upper 15 mm layer in interactance mode.[5] The fat content of the whole meat sample can then be estimated from the probed region only, and the accuracy associated with the measurement will thus depend on the homogeneity of the sample. For heterogeneous meat, it is thus not sufficient to measure surface only, if high accuracy is required, and X-ray is now being widely used in the meat industry to measure fat content in boxes of meat as well as in e.g. pork bellies. NIR imaging can also be used on boxes of meat, and performs well when the surface layer of the meat is representative for the rest of the box.[18] On the other hand, the fat content in pork bellies is consistently unevenly distributed with a fatty layer on top of the muscle. Thus, fat classification of pork bellies is difficult using traditional NIR measurements on only one of the sides, since hardly any of the two sides will be representative for the whole belly.

To our knowledge, chemical imaging of complex foods based on NIR transmission has not been reported to date. While industrial applications involving on-line interactance spectroscopy exists,[4,5,16,18] transmission spectroscopy is mostly common in laboratory equipment, where samples can be properly prepared and presented as part of a measurement protocol.

In this paper we present initial measurements from a setup for NIR transmission imaging where the samples are completely transilluminated and an NIR imaging scanner is used to obtain spectral images. The set-up is based on falling samples, meaning rapid movement of samples and relevant for industrial use. Model samples composed of ground pork meat were used to illustrate quantitative imaging of fat distribution also when the fat was embedded inside the samples. It is also shown that promising results can be obtained for whole pork bellies as well as salmon fillets with skin.

Materials and Methods

Materials

Three different sets of food samples were prepared for the NIR transmission imaging experiment.

Ground pork meat

Three model samples were made based on two batches of ground pork meat. One batch was ground meat with a fat content of 16%, the other batch was ground back fat and held about 80% fat. The three test samples were shaped as rectangular blocks of size approximately 30 cm * 20 cm * 3 cm, composed as shown in Figure 1 A-C. In this arrangement, both the amount and location of the fat were known. Some of the fat was visible at the surface, while some of the fat was embedded inside the sample. All samples had one side with no visible high fat areas. Samples were vacuum packed in transparent plastic film to ensure that the samples were kept stable throughout the experiment.

15 calibration samples were made from the two mentioned batches of ground meat. Different shares of lean and fat were blended in samples of about 500 grams, with a broad variation in fat. The samples were made as rectangular blocks of thickness 3 cm. Fat content in each sample was calculated based on the weight ratio of the two materials. The calibration samples were used to make a simple regression model that could be applied as a reference against the spectral images from the test samples to illustrate the possibility of quantitative transmission imaging.

Pork bellies

Two fresh boneless pork bellies were collected from a slaughterhouse. The size of the bellies was approximately 50 cm * 35 cm for both, and they weighted about 6 kg. Thickness varied between about 3 and 5 cm. The outer side of the bellies consisted of the fat rich blubber covered by the smooth yellowish bacon rind. The inner side was the meat side with an uneven surface given by the anatomy of the animal (Figure 1 D).

Salmon fillets

Twenty salmon fillets with skin varying in weight from 48-438 g were obtained from 20 farmed salmon slaughtered two days before. The fillets were stored on ice before spectral measurements. After being measured they were cut in different portions (2, 3 or 4 portions depending on size), and a total of 69 portions were made with varying size, thickness and shape. Fat content was determined in each portion by low field NMR. Three example fillets are shown in figure 1 E.

Imaging spectroscopy system

The transmission imaging system was made up of three main components (Figure 2); the light source, a commercial NIR imaging scanning system (QVision 500, Tomra Sorting Solutions, Asker, Norway), and a sample holder.

QV500 is an NIR imaging system designed for daily industrial on-line spectral imaging of foods on conveyor belts. The system is designed for non-destructive determination of fat, protein and water content in ground and chopped meat and is in wide-spread use in meat processing plants. It collects VIS and NIR spectra in the range 460 – 1040 nm, sampling at every 20^{th} wavelength and thus has a spectral bandwidth of about 20 nm. Under normal operation, a light beam from an internal light source is scanned rapidly across the conveyor

belt. The field of detection is about 1 cm away from the light beam spot, so interactance measurements with typical probing depth of 15 mm are obtained, depending on the type of sample being measured. Based on the scanning and the movement of the belt, a hyperspectral image is constructed. Typical pixel size is about 5 mm*5mm. An early version of the system is described by Wold et al.[16]

In the present work, a modified version of the QV500 scanning system was used. The internal light source was turned off, and replaced with an external light source positioned on the other side of the sample, thus providing a light transmission setup.

The external light source consisted of two tube halogen lamps (OSRAM 64784 Haloline 2000 W), mounted in an aluminum frame, inside two parabolic mirrors to focus the light along one line on the sample. A potentiometer was used to adjust the power of the lamp. Higher light intensity was needed to obtain sufficient signals from the meat model samples, than from the salmon fillets. The high power of the lamp required rapid movement of the samples to avoid heating.

Sample movement was vertical. A tall frame (2 m high) was made with a pulley at the top. A rope went through the pulley and was connected to a horizontal rack that could be adjusted to hoist up and down. The sample was attached to the rack with metal clips so that it could be moved vertically. During scanning, the speed of the samples was close to free fall. Scanning time per sample was typically 1 sec.

The salmon fillets were measured also in interactance mode. The external lamp was switched off, and the internal lamp was used as normal for the scanner. The interactance measurements were also done on vertically moving fillets.

Image processing and data analysis

There are several ways to calibrate an NIR imaging system. In this case, we used the mean NIR spectra from each of the calibration samples (the 15 meat samples and the 69 salmon samples), and these were used to make a calibration against fat content for each of the two products. A segmentation criterion for meat/fish based on spectral characteristics was developed in order to automatically detect the sample and to extract the spectral data.
From each calibration sample we obtained an average intensity NIR spectrum (T). This spectrum was converted to an absorption spectrum (log10(1/T)) to make the data more linear. The spectral shape was affected by optical properties such as color, sample physical properties, sample distance from the scanner, as well as thickness of sample. To remove some of the spectral variation connected to these properties, standard normal variate (SNV) was applied to normalize the data; subtract the mean of the spectrum from each wavelength in the spectrum and divide by the standard deviation of the spectrum.[19]
Spectral data from the salmon portions was obtained in the following way: The spectral images were compared and aligned with the portion images (Figure 1E). The portions were then manually outlined in the spectral images by an image processing tool. The average spectrum was calculated for each portion.

Partial least squares regression (PLSR)[20] was used to make the calibration between NIR spectra and fat concentration for the two calibration sets. Full cross validation was applied to determine the optimal number of PLS factors and to evaluate the predictive ability of the models. The prediction error was estimated by the root mean square error of cross validation (RMSECV) where $\hat{y}_i$ is the predicted value from the cross validation, y_i is the reference value and i denotes the samples from 1 to N.

$$RMSECV = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}$$

The resulting regression vectors were applied at pixel level in the hyperspectral images to obtain fat distribution maps in the model samples and in the salmon fillets. To avoid overoptimistic results, portions from the fillets to be estimated were not used in the regression model. The regression vector for fat in ground pork was also applied on the pork bellies.

Principal component analysis (PCA)[20] was also used on the spectral dimension of the spectral images to investigate the main spectral components and how these were distributed in the samples.

The software The Unscrambler ver. 9.8 (CAMO Software AS, Oslo, Norway) was used for regression analysis. All image processing of multispectral images; sample segmentation, spectral extraction, spectral pre-processing, and generation of chemical images were carried out by the use of MATLAB version 7.10 (The MathWorks Inc., Natic, MA).

Reference measurements

Reference measurements for fat were conducted for all the 69 salmon fillet portions and for the lean and the fat fractions of pork meat. Five parallels from each sample were subjected to fat measurements. The average values of the parallels were used in the calibration work. The fat content was determined by low field proton nuclear magnetic resonance (NMR), using the Maran Ultra Resonance 0.5 T (Oxford Instruments, UK) equipped with a gradient probe. The

method used was “The oneshot method” developed by Anvendt Teknologi AS (Harstad, Norway).[21] Operating temperature of the magnet was 40°C and the samples were heated up to this temperature before measurement to ensure that the fat was in liquid form. The weight of the meat samples was measured and calibration was done against a reference meat sample of known weight containing 14.3% fat (SMRD 2000 Matrix Meat Reference Material, National food Administration, Uppsala, Sweden). Salmon oil was used to calibrate the instrument prior to analysis of the salmon samples.

Results and discussion

Spectral features

Figure 3*a* shows an intensity image for one channel (940 nm) of a salmon fillet. The main intensity variation in the image was caused by thickness differences. The lowest values can be seen to the upper left where the fillet was at its thickest, intensity increased towards the tail where the fillet was thinner. The thinner belly was also higher in intensity. At the tail it can be seen that the right part had higher intensity than the left part. This is due to color variation in the skin. The skin is dark on the upper part of fish and light/silver on the belly side. The dark skin will absorb more NIR light than the light skin, in particular at wavelengths close to the visible region. The high intensity at the upper part of the fillet (at the neck) was mainly due to a kind of optical reflections that appeared at the upper edge of some samples.

The intensity variations in the image result in correspondingly large offset variations in the NIR spectra (Figure 3*b*). Spectra from the thick part of the fillet had overall higher absorbance values than those from thinner parts. A spectrum from left tail had higher absorption than the one from right tail due to the darker skin. Spectra from thicker parts also have higher

difference in absorption between different wavelengths (higher contrast); longer pathlength gives more distinct spectral features.

The peak/shoulder at about 930 nm stems from fat (third overtone of CH stretch), the broad peak at 980 nm is absorption by water (second overtone of OH stretch), while the variation towards the visible region is much due to color variations related to skin, color and darkness of meat.[22] In the spectrum from the front part of the belly the fat absorption at 930 nm can be distinguished as a clear shoulder, while from the leaner loin the spectrum is dominated by the large water peak.

After spectral normalization, the offset variation was removed and the spectra had the same internal contrast (Figure 3*c*). The fat variation around 930 nm was then clearer. The spectra from the thin tail differ from the others by relatively strong absorption towards the visible, and this is probably because the skin contributes to a relatively larger part of the optical pathway.

PCA is an efficient way to visualize how the main spectral properties are distributed in spectral images. Figure 4 shows the spectral loadings for the first four principal components (4e) and how the corresponding scores from these components are distributed in a salmon fillet. The PCA was performed on non-normalized absorption spectra. The first component is a pure offset component and reflects the thickness of the sample. The scores from the second component is quite similar to the first and are probably related to spectral contrast; the thicker the sample is, the higher contrast in the spectra. The third component seems to some extent to separate between dark and silver part of skin. This matches with the loading spectrum, which expresses the large variation towards the visible part of the spectrum. The fourth component is related to fat content with its notable fat peak in the loadings, and the score image highlights the fat belly as well as the fat rich dark muscle along the lateral line just under the skin.

The transmission images apparently contain much information, and the spectral properties are typical for NIR spectra from muscle foods. The spectra from the pork meat samples had mainly the same properties as those from salmon and were similar to spectra obtained in interactance mode.[5,18]

Calibration

Fat content in the lean and fat pork batches were 16% and 80% of the wet weight, respectively. When these were combined in 15 different calibration samples, the fat content varied gradually from 16% to 80 %. Fat content in the 69 salmon portions varied from 6.5% to 31.5%, the mean value was 16.7% and standard deviation was 5.4.

Both data sets had a spread in fat content that was suitable for regression modelling. Table 1 summarizes the model results. The model for ground pork meat obtained high correlation between actual and estimated fat content and a prediction error of about 3.6%. The result is comparable with a model obtained with the same system in interactance mode on intact pork meat,[5] where a RMSECV of 3.0% was obtained. In that case a larger sample volume was scanned per calibration sample.

The model for fat in salmon fillet portions did also obtain a high correlation and rather low prediction error. The results demonstrate that it is possible to make quantitative models based on NIR transmission data. Variation in thickness is probably introducing some unwanted spectral variation, however, in the salmon system where thickness varied from 0.6 cm to 4 cm it was still possible to obtain a good model.

It is interesting to compare with the model obtained on interactance measurements on the same fillets. This model had lower correlation and correspondingly a higher prediction error.

The main difference between the data is that in interactance mode, only about the upper 15 mm layer was probed. It is well known that most of the fat is located beneath the skin,[23] and interactance measurements will not reach these regions where the fillets are thicker than 15 mm, while transmission measurements will capture spectral information that is representative for the full sample thickness. The interactance system is optimized to operate above a conveyor belt where fillets have a fixed distance to the scanner. In the present free-falling system there was also some horizontal movement, which resulted in variation in distance between scanner and sample. Interaction measurements might be more sensitive to such movements than transmission measurements are.

Chemical images

The regression vectors obtained by calibration were applied pixel by pixel in the hyperspectral images to estimate fat values based on the pixel spectra, as well as average fat contents for each sample. In that way images of fat distribution were constructed. The regression vectors are shown in Figure 5. Both vectors emphasizes the fat peak at around 930 nm.

Estimated fat images of the pork model samples shown in Figure 1 A-C are shown in Figure 6. Two images for each sample are shown; one based on scan with front of sample facing scanner, the other with back side of sample facing scanner. From Figure 1 it can be seen that no high fat regions were visible on any of the back sides, while the front sides exposed high fat parts. The most important result here is that the fat images looked mostly the same, independent of sample side facing the scanner. The predicted fat values at pixel level were close to the actual fat contents in the model samples. It can also be seen that the average fat contents obtained per sample were very similar irrespective of orientation. This is what we would expect from a well working transmission imaging system. The images confirm that it is

possible to make quantitative chemical images based on NIR that maps internal properties of rather thick samples.

For sample A it can be noted that that the visible fat region in the lower right on the front part was easily seen in the fat image when the front side of the sample faced the scanner. When the back side faced the scanner, this part was not possible to discern. The large rectangular fatty area in the upper left corner of sample A was visible from back side but less intense compared to front side. This effect is most likely due to light scattering. Details on the surface facing the light source get blurred as the light propagates through the sample. Details on the sample surface facing the scanner on the other side, is less obscured by light scattering. Although some of the spatial information is obscured during transmission, the chemical signals from the fat are preserved as the total estimated fat content remains the same.

Figure 7 shows a similar example with the whole pork bellies. We did not have a regression model optimized for intact pork meat. However, by using the model for ground pork it could be illustrated again that estimated average fat content was almost the same, independent of which way the belly was scanned. When the relatively flat rind side of the belly faced the scanner, the resulting fat image showed the overall variation in fat across the belly. With the meat side (as shown in Figure 1D) against the scanner, the same overall fat variation could be seen, but also details of the fat distribution on the meat side. The differences in the fat images are again due to the effect of light scattering.

Figure 8 shows estimated fat images of three salmon fillets with skin. The score images of one fillet shown in Figure 4 illustrated the huge and systematic spectral variation that is present in these samples. In spite of these variations across the fillets, the regression vector seemed to produce fat images that reflect the typical fat concentrations and distribution in salmon. The average fat values obtained for each fillet corresponded well with the reference values.

General discussion

The results confirm that it is possible, and also valuable, to perform hyperspectral transmission imaging on meat samples of thickness up to at least 5 cm. Quantitative imaging of internal chemical composition is feasible, even when samples varies in thickness or are covered with skin or rind, as with the salmon fillets and pork bellies, respectively.

Sample thickness is a main limiting factor, and at some point an increased thickness will result in signals, which are too weak or noisy to be useful. Before we made the ground pork samples of 3 cm thickness, we made an effort with similar samples of 5 cm thickness. In that case, the signals were too low and spectral shape distorted. Better signals were obtained on the 5 cm thick pork bellies, probably due to less light absorption and light scattering. It illustrates that critical thickness will vary from product to product.

Another issue is variation in thickness within the samples. It is important to adjust the lamp intensity so that signals from both thin and thick parts of the sample are applicable and not distorted by either noise or detector saturation. For the salmon fillets, for instance, the lamp intensity was adjusted to give useful spectral readings for thin bellies (0.5 cm thick) and the thicker loin part (up to 4 cm thick). Fillets measured without skin (not presented here) required less light intensity than fillets with skin.

In this work we used SNV prior to calibration to remove the largest spectral variation connected to variation in thickness. Since the signals at every pixel is heavily dependent on thickness, it would probably be possible to use this information to optimize spectral pre-processing, and also improve pixel predictions as well as the predicted average values. A thick part of a sample will have more weight than a thin part in a weighted average.

Pork bellies and salmon fillets are not ideal for a free falling sample system. The lab system was constructed for vertical sample movement for convenience. Free falling samples are used

in many optical sensor systems for food, however, transmission measurements for larger samples can also be implemented on e.g. conveyor belts separated with narrow slits.

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Figure captions

Figure 1 A-C shows front, back and cross section of model samples composed of ground pork meat. D) Meat side of one of the pork bellies. E) Example of salmon fillets cut in portions of different shapes, sizes and thicknesses.

Figure 2 Setup for transmission imaging system. It was based on vertical movement (free fall) of the samples between light source and NIR imaging scanner.

Figure 3 a) Intensity image of salmon fillet at 940 nm. Numbers in image corresponds to spectra in b) and c). b) Absorption spectra from numbered (1-6) pixels in intensity image. c) Same spectra, SNV corrected.

Figure 4 a-d) Score images for principal components 1-4 based on one single salmon fillet. e) Corresponding loadings 1 (blue), 2 (green), 3 (red) and 4 (cyan).

Figure 5 Regression coefficients for fat in salmon fillets (dashed) and ground pork (solid)

Figure 6 Images of predicted fat distribution in model samples A-C (shown in Figure 1) measured with front and back side facing NIR scanner. Estimated average fat content indicated above each image.

Figure 7 Images of predicted fat distribution in two pork bellies (A and B) measured with front and back side facing NIR scanner. Estimated average fat content indicated above each image.

Figure 8 Images of predicted fat distribution in salmon fillets with skin. M: Measured average fat content by reference method, P: Estimated average fat content by NIR imaging.

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Table I Regression results for PLSR calibrations for fat. # PLS factors: Number of ltent variables used in the model, R: correlation between estimated and measured fat content, RMSECV: Root mean square error of cross validation.

Data	# PLS factors	R	RMSECV (%)
Pork meat	5	0.98	3.67
Salmon transmission	4	0.95	1.68
Salmon interactance	3	0.89	2.41

Figures

Figure 1

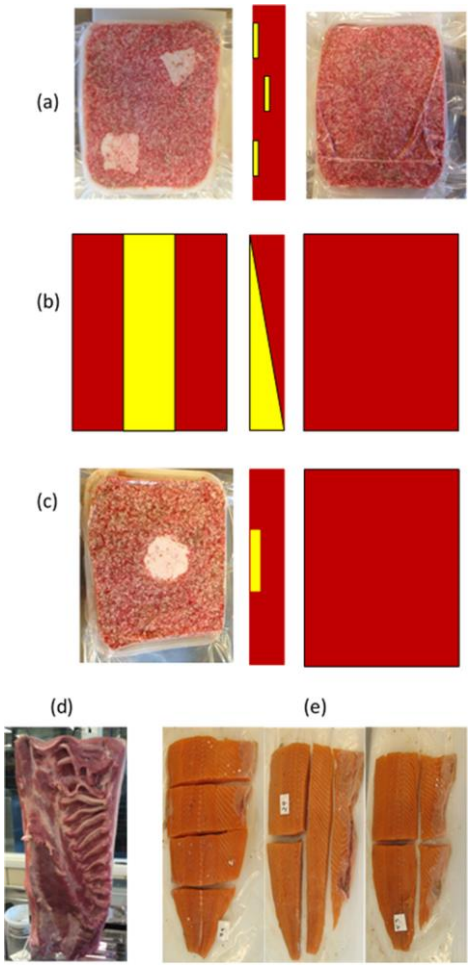


Figure 2

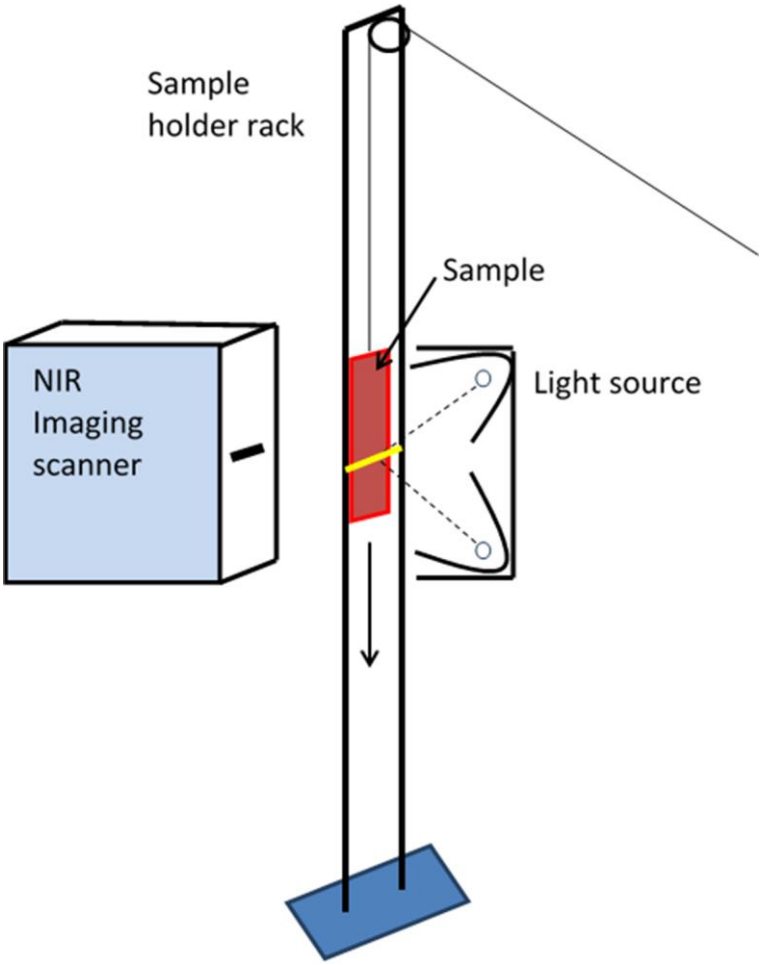

Sample
holder rack
Sample
NIR
Imaging
scanner
Light source

Figure 3

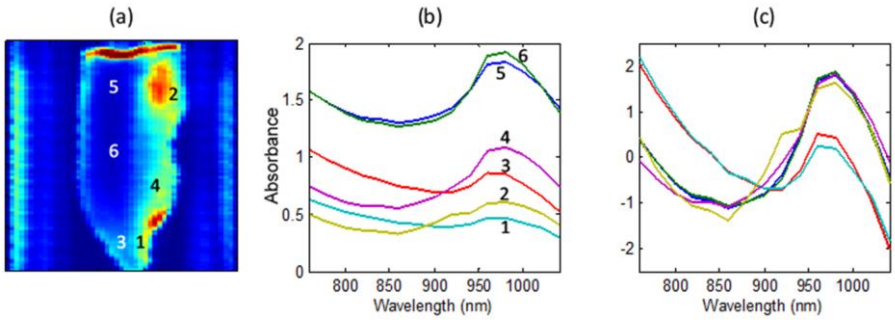
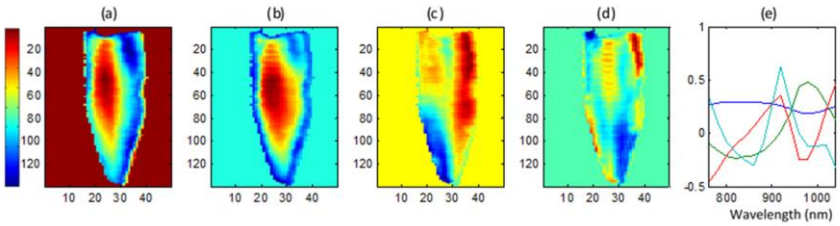


Figure 4


(a)
(b)
(c)
(d)
(e)
20
40
60
80
100
120
10 20 30 40
1
0.5
0
-0.5
800 900 1000
Wavelength (nm)

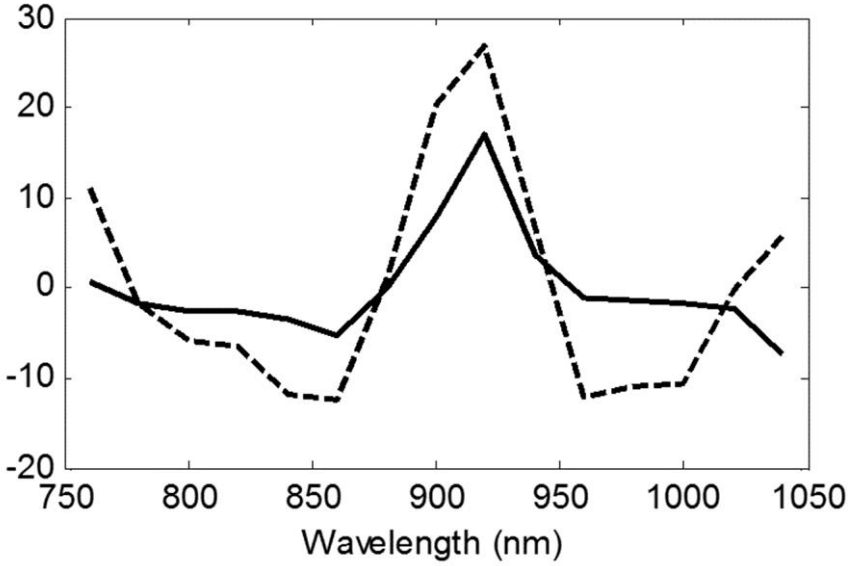


Figure 5

Figure 6

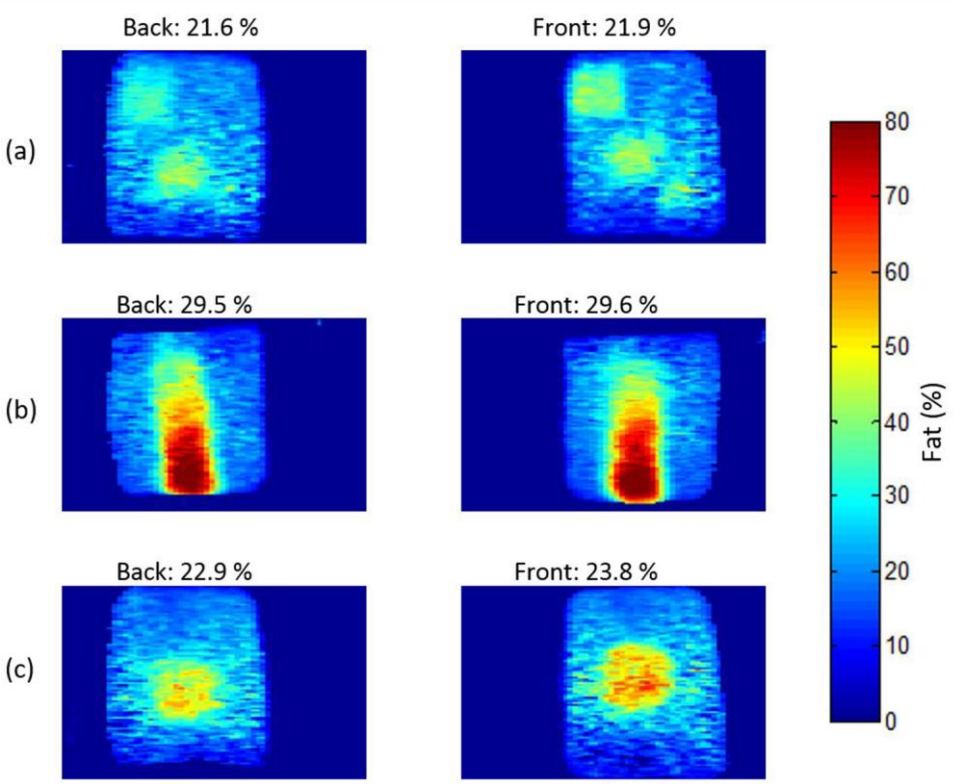

Back: 21.6 %
Front: 21.9 %
(a)
Back: 29.5 %
Front: 29.6 %
(b)
Back: 22.9 %
Front: 23.8 %
(c)
80
70
60
50
40
30
20
10
0
Fat (%)

Figure 7

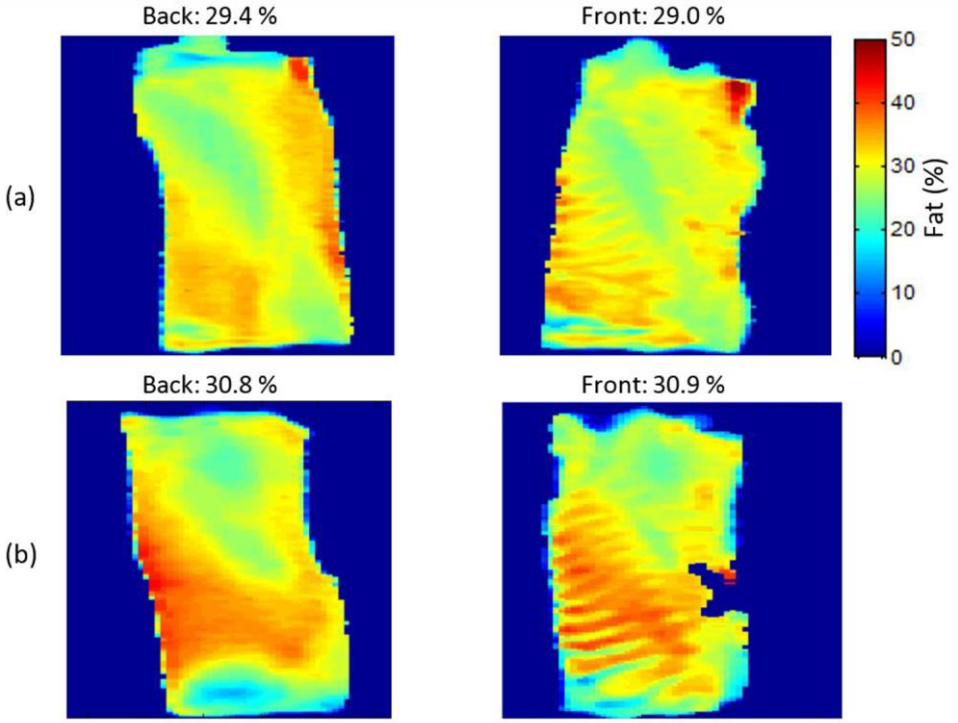


Figure 8