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Automatic Inspection of Cod (*Gadus Morhua L.*) Fillets by Hyperspectral Imaging

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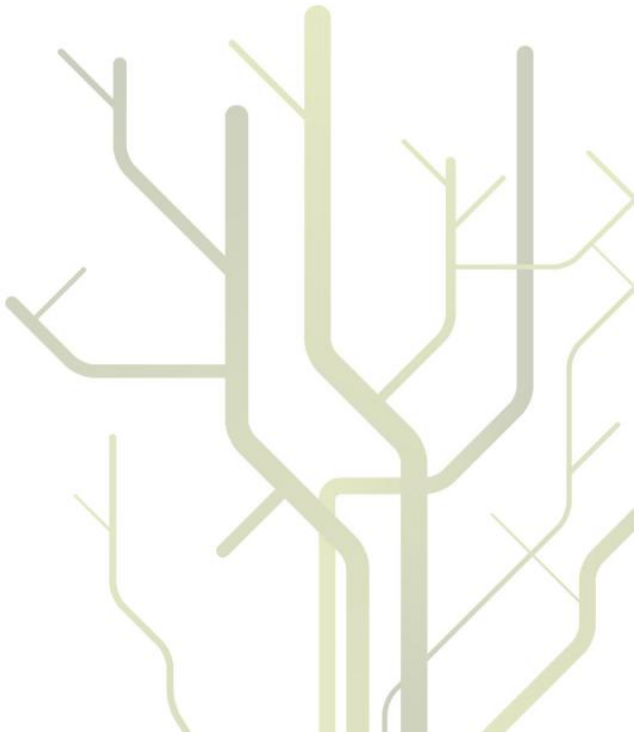

Abstract

The manual trimming and inspection of cod fillets by candling, is considered the bottleneck of cod fillet processing. The operation is both labour intensive and expensive, reported to account for as much as 50 % of the cost with cod fillet production. Due to the high labour costs in Norway, it is of great interest for the industry to optimize this process.

In this work a hyperspectral imaging system has been developed, capable of inspecting cod fillets, with or without skin, at a conveyor belt speed of 400 mm/seconds, corresponding to the industrial processing speed of one fish per second. The system is designed as proof of concept, and the algorithms are not implemented to be run in real time.

A method for segmenting a cod fillet image into the respective parts: loin, belly and tail, using the centerline as a reference system, has been developed. The method is useful for selecting standardized measurement regions on the fillet, and used for extracting data for automatic freshness assessment.

Freshness, as days on ice, can be predicted using spectroscopy in part of the visible region (450-700 nm). This can be done with an accuracy comparable to what is reported for sensory evaluation using a panel of trained evaluators. The same system is used for detecting fillets which have been previously frozen, both as whole fish and as fillets with skin. The results show a complete separation between the fresh and frozen-thawed samples. Similar mechanisms are affecting the spectra from fish stored fresh on ice, and fish that has been through the freeze-thaw cycle. The main variations seen in the spectra from cod fillets stored on ice, or frozen and then thawed, are due to oxidation of heme proteins in the muscle. This is supported by independent measurements using two different instruments, and by previous studies pointing to the visible region as the best region for freshness prediction.

Detecting objects embedded in tissue, using visible light, is difficult due to variability in the optical properties of the surrounding tissue. A method for calibrating the spectral signature from small objects embedded in translucent material has been developed. This method uses the estimated local background spectrum to calibrate the hyperspectral image, and the method is evaluated for automatic nematode detection, using the hyperspectral imaging system, at a commercial cod fillet processing plant. The local calibration method is superior to using traditional spectroscopic pre-treatment methods, and reduces both spatial and spectral variations across the image. The results from the industrial test show that the system can detect nematodes in cod fillets with a performance which is comparable or better, to what is reported by manual inspection.

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List of Publications

I. **A. H. Sivertsen**, C. Chu, L. Wang, F. Godtliebsen, K. Heia and H. Nilsen, "Ridge detection with application to automatic fish fillet inspection", *Journal of Food Engineering*, vol. 90, pp. 317–324, 2009.

II. **A. H. Sivertsen**, K. Heia, S. K. Stormo, E. Elvevoll and H. Nilsen, "Automatic nematode detection in cod fillets (*Gadus Morhua*) by transillumination hyperspectral imaging", *Journal of Food Science*. vol. 76, pp. 77-83, 2011.

III. **A. H. Sivertsen**, K. Heia, K. Hindberg and F. Godtliebsen, "Automatic nematode detection in cod fillets (*Gadus Morhua L.*) by hyperspectral imaging", *MANUSCRIPT*

IV. **A. H. Sivertsen**, T. Kimiya and K. Heia, "Automatic freshness assessment of cod (*Gadus morhua*) fillets by VIS/NIR spectroscopy", *Journal of Food Engineering*, vol 103, pp. 317-323, 2011.

These publications are referred to by their roman letters in the following chapters.

Other Contributions

During the course of the PhD research, the author has contributed to other relevant publications. These may serve as background material, but are not regarded as part of this thesis.

1. **A. H. Sivertsen**, K. Heia and H. A. Nilsen, "Device and method for contactless detection of characteristics of continuously delivered translucent products," *Patent: US20110013181*, 2009.

2. S. K. Stormo, **A. H. Sivertsen**, K. Heia, H. A. Nilsen and E. Elvevoll, "Effects of single wavelength selection for Anisakid roundworm larvae detection through multispectral imaging," *Journal Of Food Protection*. vol. 70, pp. 1890-1895, 2007.

3. K. Heia, **A. H. Sivertsen**, S. K. Stormo, E. Elvevoll, J. P. Wold and H. Nilsen, "Detection of nematodes in cod (*Gadus morhua*) fillets by imaging spectroscopy," *Journal Of Food Science*. vol. 72, pp. E011-5, 2007.

4. I. Sone, R. L. Olsen, **A. H. Sivertsen**, G. Eilertsen and K. Heia, "Hyperspectral imaging can classify fresh salmon (*Salmo salar L.*) by the type of atmosphere packaging used during storage", *Submitted to Journal of Food Engineering*.

5. K. Heia, **A. H. Sivertsen** and S. Birkeland, "Automated quality control of salmon fillets - hyperspectral imaging for on-line inspection", *The 14th International Conference on Near Infrared Spectroscopy*, Bangkok, Thailand, 9-13 November, 2009. Poster presentation.

6. **A. H. Sivertsen**, K. Heia, K. Hindberg and Fred Godtliebsen, "Automatic detection of nematodes in cod fillets by hyperspectral imaging", *The 14th International Conference on Near Infrared Spectroscopy*, Bangkok, Thailand, 9-13 November, 2009. Oral presentation.

7. **A. H. Sivertsen**, K. Heia and T. Kimiya, "Automatic differencing between fresh and frozen-thawed cod by VIS/NIR spectroscopy", *The 3rd Joint Trans-Atlantic Fisheries Technology Conference*, Copenhagen, Denmark, 15-18 September, 2009. Poster presentation.

8. K. Heia, **A. H. Sivertsen** and S. Birkeland, "Detection of blood and melanin spots in fresh and processed salmon fillets"', *The 3rd Joint Trans-Atlantic Fisheries Technology Conference*, Copenhagen, Denmark, 15-18 September, 2009. Oral presentation.

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Chapter 1

Introduction

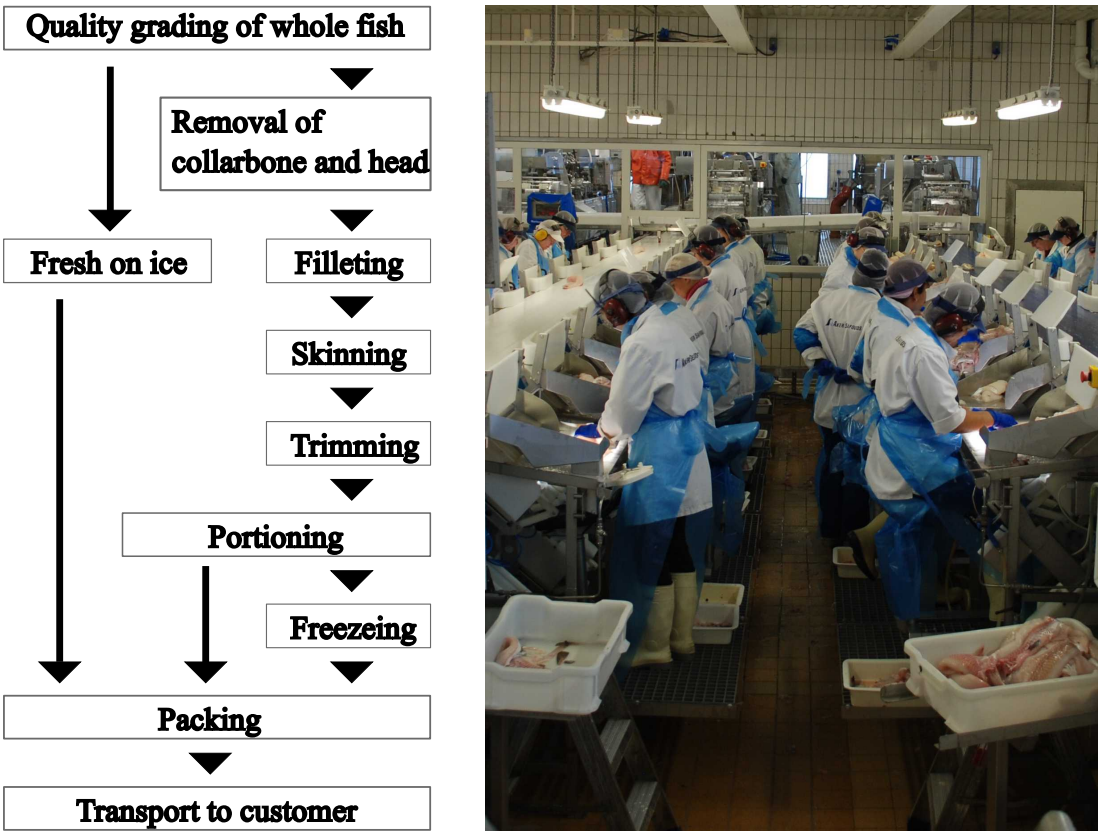
Wild caught Atlantic cod (*Gadus Morhua L.*) is the most important export commodity for the Norwegian white fish industry, with a total export value of 5.3 billion Norwegian kroners in 2010 [EFF, 2010]. Of which 25 % is exported as fresh and frozen whole fish, 7 % as fresh fillets and the rest as clipfish, salted fish, frozen fillets or stockfish.

In Norway cod processing is a highly mechanized industry where machines now can replace most of the labor intensive operations such as: de-heading, filleting, skinning, de-boning, portioning and packing. Cod intended for fresh processing is normally landed gutted with the head on. The first operation after landing is manual grading according to end product specifications and market requirements. Factors such as seasonal variations and handling; fishing gear, killing procedure, bleeding, gutting and storage, all affect the quality of the end product [Akse et al., 2005]. It is beneficial to assess the quality in an early stage of the process where knowledge regarding freshness or remaining shelf life can reduce product downgrading later in the distribution chain.

The first machine on most fillet processing lines (Figure 1.1a) is the de-heading machine with head and collarbone removal. The second step is the filleting machine, utilizing pairs of rotating knifes that cut the fillet from the backbone. If the fish is landed without head, usually frozen, there are machines that combine both collarbone removal and filleting in one operation. The next step is the skinning machine, followed by trimming, portioning and packing. Parts of the fillets are often used for frozen products, typically the loin is packed fresh, the tail is single frozen and the belly flaps are frozen in blocks used for minced or mixed products.

The manual fillet inspection and trimming (Figure 1.1b) is considered the bottleneck of most filleting lines, often performed in tempered areas, where heating of the product increases the enzymatic and microbiological degradation [Ashie et al., 1996]. It is considered the most labour intensive operation, and reported to account for as much as 50 % of the cost with cod fillet production [Bublitz, 1992].

Due to high labour costs in Norway and difficulties in recruiting workers for the hard work at the trimming stations, an increasing amount of fish harvested in Norwegian waters are frozen, and then thawed and processed into final products in other countries. As an example, Chinese fish processors bought more than 100 000 tons of At-



(a) (b)

Figure 1.1: (a) A typical cod fillet processing line. (b) The quality inspection and trimming is done manually on candling tables. The filleting and skinning machines are located in a separate room, seen in the back in the photo.

lantic cod in 2006, the majority of this being caught by Russian trawlers in the Barents sea. These were shipped to China, thawed, processed and ended up on the European marked, competing with fish landed and processed locally [Braend et al., 2006]. The cod processed in China is filleted by hand at low temperatures, resulting in a high quality end product with a high yield of the raw material. These products are approximately 20 % less expensive than similar products made in Norway, including transportation costs [Braend et al., 2006]. If one is to reverse this pattern, local fish processors must be able to deliver fresh products of supreme quality at affordable prices. Their biggest advantage is their location, between the raw material and the market, while their biggest challenge is the high labour cost.

1.1 Quality control of cod fillets

Quality features of fish fillets are commonly assessed on the basis of appearance (color and defects), texture and odour. Cutting errors from the filleting machine, blood spots due to insufficient bleeding or rough handling, skin remnants, black lining and pin bones are all defects that have to be removed or corrected for at the trimming stations. However, parasites embedded in the fish muscle is considered the major challenge since they are both difficult to spot and possess a potential threat to human health [Audicana et al., 2002].

The two main types of parasites infecting Atlantic cod are *Anisakis simplex* and *Pseudoterranova decipiens*. These are often referred to as whale worm and seal worm, respectively, due to their definitive hosts. *A. simplex* is more abundant in offshore fish, whereas *P. decipiens* is more likely to be found in inshore fish [Marcogliese, 2002]. Approximately 96 % of all nematodes found in Atlantic cod are not embedded deeper than 10 mm, measured from the inside of the fillet [Hauksson, 1991]. Distribution of the two nematode species varies, where *A. simplex* is most abundant in the belly flap (93.5 %), *P. decipiens* is likely to appear in the loin and tail as well [Hauksson, 1991]. *P. decipiens* is bigger and darker, often seen as a circular shape with a typical diameter of 3 - 6 mm. Its color is mainly defined by the amount of haemoglobin in the pseudocoelomic fluid [Dixon et al., 1993]. *A. simplex* often appears pale in color with a circular shape of 1 - 4 mm in diameter. Exact identification can only be made by examining the anterior gut structure through a microscope [Olson et al., 1983].

Consuming fish infected by nematodes is generally not considered a health risk as long as the nematodes are killed by adequate cooking or frozen storage [Wharton and Aalders, 2002]. However, the increasing popularity of raw and undercooked seafood, is believed to increase the overall risk of humans being infected by live nematodes. Lately there has been an increased attention to the risk of hypersensitive reactions due to exposure of very small amount of *A. Simplex* antigen without the involvement of living parasites [Werner et al., 2011, Audicana and Kennedy, 2008]. In addition, media exposure of the nematode problem may have dramatic effects on the consumer, with a following drop in fish consumption [Fischler, 2002]. From the fish processors point of view, nematode infection represents a problem which needs to be solved.

Due to prominent scattering of visible light in the cod muscle [Petursson, 1991], nematodes are not detected deeper than 6 mm by candling [Bublitz, 1992, Hafsteinsson and Rizvi, 1987]. Both *A. simplex* and *P. decipiens* have similar absorption properties in the visible region [Stormo et al., 2007, Stormo et al., 2004], and absorb significantly more light than the surrounding fish muscle [Petursson, 1991]. However, the reported manual detection rate under industrial conditions varies a great deal and is reported in the range of 33-93 % for heavily infected fillets and 70-100 % for less infected fillets [Varga and Anderson, 1971]. One of the largest studies on manual nematode detection performance, performed over one year, in three different factories and on 22000 fillets, reports an average detection rate of 68 % [Bublitz, 1992]. Both of these studies were performed on fish from Canadian waters where *A. Simplex* rarely are found in the fillet; one study

reporting an average number of 0.038 *A. Simplex* per fillet [McClelland et al., 1983]. In the Barents sea, outside northern Norway, *A. Simplex* is abundant were as much as 96% of the fillets have been reported infected, with an average number of 6.1 nematodes per fillet [Aspholm, 1995]. No reports have been found on manual detection rate for *A. Simplex* in cod fillets under industrial conditions, but the manual detection rate by destructively slicing the fillet, is reported to only 42% [McClelland et al., 1983] and as low as 7-10 % by candling of pelagic fish [Levsen et al., 2005].

The biggest improvement in how candling is performed today, as compared to what was initially proposed by E. Hess in 1945 [Hafsteinsson and Rizvi, 1987], is the use of fluorescent lamps instead of the sun as a light source. Improving the candling efficiency by use of color filters does not improve the manual detection ability of nematodes [Power, 1958]. However, adjusting the intensity level of white light reduces human fatigue and increases productivity [Bublitz, 1992, Valdimarsson and Einarsson, 1985]. Several destructive methods for doing more accurate counting of nematodes during sample inspection or research purposes are proposed: 1) Slicing the fillets longitudinal into 13 mm thin slices [Power, 1961], 2) using pepsin, a digestive enzyme, to remove the fish muscle and count the remaining nematodes [McClelland et al., 1983], 3) compressing the fillet between acrylic plates to a thin layer of 2-3 mm in a plastic bag and count the number of visible nematodes seen under short wave ultraviolet light, after freezing [Karl and Leinemann, 1993], 4) disintegrating the fish muscle in a food processor, followed by visual inspection of diluted portions under ultraviolet light [Brattey, 1988]. These destructive methods are slow and not suited for large scale operation in an industrial environment, but can be a valuable tool for research purposes performed in the lab.

There are machines commercially available for doing part of the inspection and trimming of white fish automatically. SensorX (Marel, Gardabaer, Iceland) detects all pin bones down to 0.3 mm in diameter and can be used for assuring bone free products. The Baader 988 S (Baader, Lübeck, Germany), automatic trimming machine for salmon and sea trout, is capable of doing color grading and performing pre-programmed cutting patterns. A similar system is expected to be available for cod in the near future. Another commercially available system is the Qmonitor (Qvision, Asker, Norway), which can be used for grading wet or dried salted fish fillets based on water content and measuring fat and pigment (Astaxanthin) content in salmon fillets.

1.2 Automatic detection of parasites in cod fillets

Due to the high costs of manual inspection it is of great interest for the fish processing industry to have this operation automated. During the past decades a number of different techniques have been explored as possible industrial methods for parasite detection in whitefish. A wide range of parasites found in fish is known to exhibit a bright fluorescence in the visible region when illuminated with light at around 360 nm [Pippy, 1970]. The company Lumetech (Hellerup, Denmark) included this method as part of their pro-

cessing equipment in the early 1990s, using the setup previously patented [Jensen et al., 1984]. The method was limited to surface nematodes (down to 0.5 mm [Pippy, 1970]) and the fish had to be previously frozen. However, other features found in fish, i.e. fish scales, bones and connective tissue, also exhibit fluorescence. To our knowledge the fluorescent detection method is no longer commercially available, and Lumetech later filed for a patent using transmission in the near infrared region [Haagensen et al., 1990]. Odense (1978) suggested the use of cross polarization filters to reduce the effect of scattering in the fish muscle and enhance the contrast of deeply embedded nematodes. The technique was under development as an alternative to the standard candling table in the early eighties [McClelland et al., 1983] and later mentioned by McClelland, (2002). Collimated light has been suggested as a method for increasing the contrast of deeply embedded nematodes [Budde, 1965] and the use of a narrow beam of white light to detect nematodes has been patented [Reimer, 1989].

The BA820 Inspector (Baader-Canpolar Inc., Newfoundland Canada), utilizing direct illumination and reflection [Hearn and Reimer, 2001], claims to grade fillets based on cutting errors, blood spots, *P. Decipiens*, black lining and skin remnants. The Qmonitor (Qvision, Asker, Norway), utilising low resolution interactance imaging [Wold et al., 2006, Haugholt et al., 2005], also claims to be able to detect spots on fillets, without specifying any further. No performance reports are available regarding detection of nematodes for the these machines.

The parasites differ from fish tissue by its acoustic properties, and ultrasound has been proposed as a detection method [Hafsteinsson et al., 1989]. One problem with the ultrasonic method is the requirement of direct coupling between detector and measurement medium, and this is one reason why the technique has not been industrialized. Choudhury et al. (2002) and Jenks et al. (1996) obtained promising results by measuring the electrical conductivity in fish and parasites. The method, however, requires the fillet to be placed in a saline solution and this may be impractical in view of possible industrialization. Computer tomography and magnetic resonance imaging has also shown promising results [Heia et al., 1997], but the technology might still be to slow and expensive for industrial use.

The use of multispectral imaging [Wold et al., 2001] and later hyperspectral imaging, also referred to as imaging spectroscopy, has achieved interesting results [Heia et al., 2007, Sigernes et al., 2000]. The method requires no contact with the sample and is well suited for analyzing samples moving on a conveyor belt [Tatzer et al., 2005]. All operations normally performed with a standard vision system, such as registration of color, shape etc., can be done with a hyperspectral imaging system. In addition, several applications are already developed using imaging spectroscopy and conventional contact spectroscopy; 1) Prediction of freshness as days on ice and sensory score [Nilsen et al., 2002, Nilsen and Esaiassen, 2005], 2) Prediction of fat and water distribution [ElMasry and Wold, 2008] and 3) Prediction of ice fraction in superchilled fillets [Ottestad et al., 2009]. Combining these applications with the ability to detect defects, such as parasites and blood spots, in a single instrument at one location in the processing line, makes hyperspectral imaging a promising and versatile technology for the industry.

1.3 Objective

The main objective of this thesis is to provide the Norwegian fish processing industry with a technological solution for automatic quality control in fish fillet production. The main focus has been on cod fillet inspection, however automatic inspection of salmon fillets, using the same system, has been considered when the technology has been developed. The selection of hyperspectral imaging as the vision technology is based on previous results showing the feasibility for parasite detection under lab conditions using the Spextube IV instrument [Heia et al., 2007, Heia et al., 2003, Sigernes et al., 2000]. The Spextube IV instrument can image a 100 x 100 mm segment of a stationary cod fillet with a spatial resolution of 0.1 mm and a spectral resolution of 1 nm in the wavelength range 400 - 1000 nm. However, the data acquisition is slow, requiring approximately 90 minutes (5400 second) per sample. For a detection system to operate at industrial conditions, quality inspection should be performed at the same speed as the other operations in the fillet processing line. A typically processing speed is one fish per second, thus requiring a conveyor belt speed of 300 - 400 mm/seconds.

The specific objective for this work were to develop a new hyperspectral imaging system capable of inspecting full size cod fillets at a conveyor belt speed of 400 mm/second. Based on measurements from the new system, develop automatic methods to perform:

1. Segmentation of cod fillet images into its respective parts: loin, belly, center-cut and tail
2. Parasite detection
3. Freshness assessment using hyperspectral imaging

Chapter 2

Hyperspectral Imaging

Hyperspectral imaging, also referred to as imaging spectroscopy, is an emerging technology combining both conventional imaging and spectroscopy to attain both spatial and spectral information from an object. It was initially developed for remote sensing applications [Goetz et al., 1985], but has in the recent years gained popularity in various fields, including food safety and quality control [Gowen et al., 2007]. A hyperspectral image is generated by focusing the light collected from the sample onto a narrow slit which only passes a narrow line of the sample being imaged (Figure 2.1a). This line is then collimated onto a dispersive element, such as a transmission grating, separating the different wavelengths, before the light is focused onto a two dimensional detector array, typically a CCD detector. The effect is that a single exposure, or frame, recorded from the sample, represents a narrow spatial line across the sample. By keeping the spectrometer stationary while the sample moves on a conveyor belt, the sample is scanned line by line. Stacking the corresponding frames creates a three dimensional data cube referred to as a hyperspectral image. Each pixel, corresponding to a specific area on the sample, can then be represented by a spectrum (Figure 2.1b). Each value in this spectrum corresponds to the light intensity from the specific area on the sample at a specific wavelength. The technique is useful in the way that the power of spectroscopy is used to detect or quantify chemical constituents based on their spectral fingerprint, and imaging organizes this information into chemical maps or images. Hence, the technique is also commonly known as chemical imaging. For quality inspection of food Hyperspectral imaging is usually carried out in the VIS–NIR (400 - 1000 nm) or the NIR (1000 - 2500 nm) region [Gowen et al., 2007].

Hyperspectral and multispectral imaging are related. The difference lies in how the data is generated. Multispectral images are generated using a camera with a set of interchangeable filters, often rotating in front of the camera, and each filter has a specific center wavelength and bandwidth. This results in image bands with discrete wavelengths, not necessarily uniformly distributed across the wavelength range. Hyperspectral images are usually generated using a dispersive element, as explained above, and the resulting image bands are often uniformly distributed, with a constant or slow varying bandwidth, across the wavelength range.

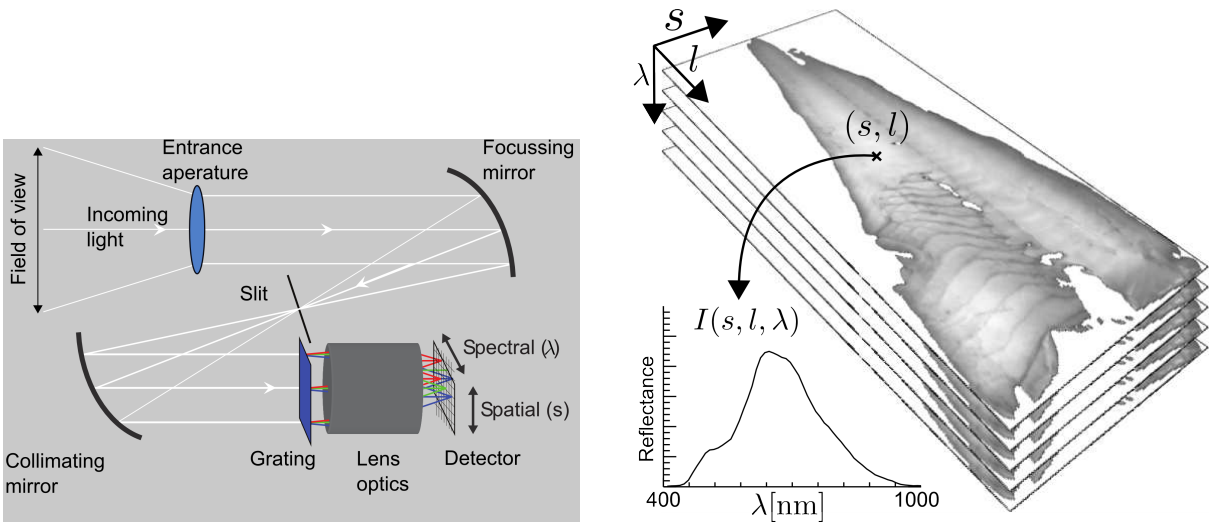


Figure 2.1: (a) The light from the spatial line is divided into its spectral band by a transmission grating, and the light intensities are recorded by the two dimensional detector (image with permission from Norsk Elektro Optikk AS). (b) In a hyperspectral image each pixel with spatial location (s, l), can be represented as a single spectrum, $I(s, l, \lambda)$, of digital numbers (DN).

2.1 Illumination

As with conventional spectroscopy, hyperspectral imaging can be operated in various modes or configurations. For food quality measurements, the most common mode of operation is reflectance (Figure 2.2a), with 22 of 30 research papers published between 2004 and 2007 [Gowen et al., 2007] using hyperspectral imaging in reflectance mode. This mode is useful when one is interested in accurate color measurements [Wyszecki and Stiles, 2000], or detecting defects and other quality attributes visible on the surface. The illumination can be direct or diffuse, depending on the application.

Transmission, or transillumination, mode (Figure 2.2b) is potentially applicable to the detection of internal defects within foods, and has previously been evaluated for detection of nematodes in cod fillets [Heia et al., 2007].

In interactance mode (Figure 2.2c), the spectrometer and light source are both on the same side of the sample. It differs from reflectance mode in that the light source, being a narrow line of light, is focused on an area adjacent and parallel to the detectors field of view (FOV). Keeping the angle of illumination parallel to the detectors FOV is important to have a constant optical path length on the light interacting with the sample. This reduces intensity variations due to sample thickness variations, making interactance a suitable configuration for imaging biological samples such as fish fillets. Interactance also eliminates specular reflection and increases the signal received from

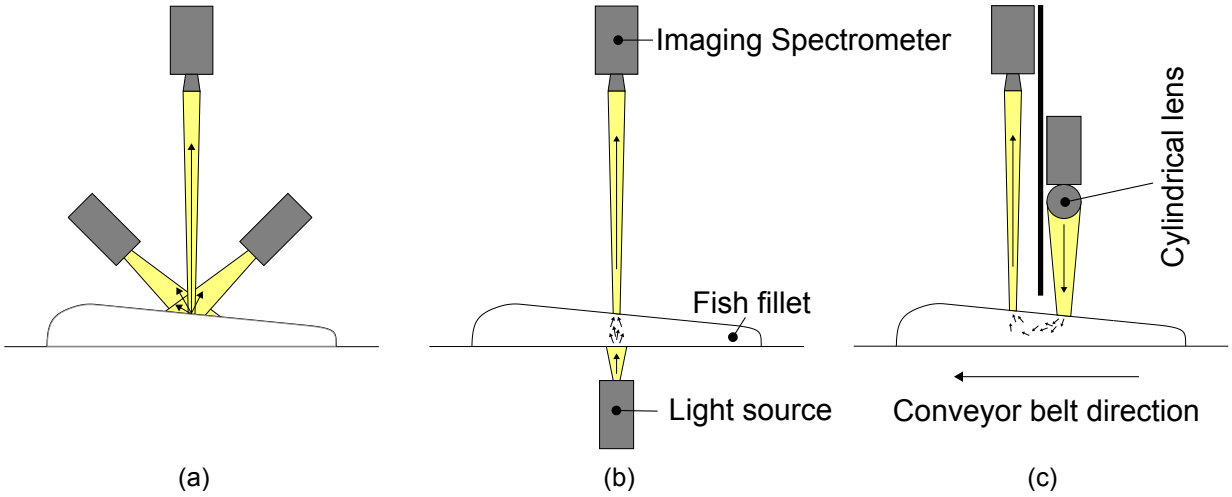


Figure 2.2: Three types of illumination setups: (a) Reflection (b) Transillumination and (c) Interactance.

inside the sample as compared to reflectance [Wold et al., 2006].

2.2 Calibration

The signal recorded by the spectrometer is affected by several factors independent of the optical properties of the sample. Correcting for these factors are referred to as calibration.

Assigning a wavelength number to pixels along the spectral dimension, λ, is referred to as wavelength calibration [McCluney and McCluney, 1994]. This is done separately from the intensity calibration, and done by recording data from a lamp, or target, with known spectral lines. Minimum two lines with known center wavelength, some distance apart, is chosen and wavelengths are assigned to the spectral pixels by curve fitting.

Assuming that a flux of photons is hitting the sensor, the total number of electrons collected during the exposure time is the sum of the photon generated electrons, $n_s(s, \lambda)$, and the combined background level, B, due to dark current [Janesick, 2001] and sensor offset. The data recorded from the CCD can be modelled as a mixed Poisson Gaussian process [Snyder et al., 1995]

$$J(\mathbf{x}) = \alpha Q(\mathbf{x}) n_s(\mathbf{x}) + B(\mathbf{x}) + \eta, \tag{2.1}$$

where $\mathbf{x} = (s, \lambda)$ is the pixel coordinate on the CCD, $\eta(\mathbf{x}) \sim \mathcal{N}(0, \sigma_\eta)$ is the Gaussian readout noise, α is the camera gain [Janesick, 2001] and $Q(\mathbf{x})$ represents the photo response non-uniformity function (PRNU). The PRNU is fixed pattern noise, and describes the individual pixels sensitivity, i.e. ability to generate electrons from photons hitting the sensor. The PRNU is usually estimated by the spectrometer manufacturer,

using an integrating sphere with known illumination characteristics, and stored as a matrix, $\hat{Q}(\mathbf{x})$, with an average value of one. The background level is estimated by averaging N frames recorded in darkness, or with the entrance aperture closed, by $\hat{B}(\mathbf{x}) = \sum_{i=1}^{N} B(\mathbf{x})/N$. A single frame recorded from the sensor is then calibrated by

$$J_C(\mathbf{x}) = \frac{J(\mathbf{x}) - \hat{B}(\mathbf{x})}{\hat{Q}(\mathbf{x})}. \tag{2.2}$$

For quantitative measurements the spectral data can be further calibrated to radiometric units [McCluney and McCluney, 1994]. This is not necessary for doing qualitative measurements, and instead it is more common to standardize the measurements using a standard target with known optical properties, such as Spectralon, Teflon or a ceramic plate [McCluney and McCluney, 1994]. An average frame for the calibration target is estimated by $\hat{T}(\mathbf{x}) = \sum_l T_C(\mathbf{x}, l)/N$, where, l is the frame number and $T_C(\mathbf{x}, l)$ is a frame recorded from the standard target and calibrated by (2.2). The standard calibrated frame is now given as

$$I(\mathbf{x}) = \frac{J_C(\mathbf{x})}{\hat{T}(\mathbf{x})}. \tag{2.3}$$

Due to the Poisson properties of the signal recorded from the CCD sensor, the calibrated image can be approximated by a normal distribution

$$I(\mathbf{x}, l) \sim \mathcal{N}(\bar{I}(\mathbf{x}, l), C(\mathbf{x})\bar{I}(\mathbf{x}, l)), \tag{2.4}$$

where $\bar{I}(\mathbf{x}, l)$ is the mean value, and $C = \alpha \,/\, \hat{T}$.

Chapter 3

Results and Discussion

Regarding quality inspection of cod fillets, parasites are considered the most important and the most difficult defect to detect. Therefore, a system intended for automatic inspection of cod fillets must consider parasites in the design process. The hyperspectral imager Spextube IV has achieved promising results for parasite detection under lab conditions [Heia et al., 2007, Heia et al., 2003]. Spextube IV has a spectral resolution of 1 nm in the range 400 - 1000 nm, and a spatial resolution of approximately 0.1 mm with a field of view of 100 mm. The acquisition time for a 100 x 100 mm sample, with full spectral range, is approximately 90 minutes (5400 seconds). The aim with the new setup is to record a 300 x 400 mm sample, comparable to a cod fillet in size, with the same spectral range and do it in one second.

Nematodes are known to absorb more light than fish muscle in the visible to near-infrared region [Petursson, 1991], and especially in the lower ultraviolet to visible region (368 - 450 nm) [Stormo et al., 2004]. Characteristic absorption peaks, located at 424, 546, 578 and 636 nm, are observed in spectra from homogenized nematodes diluted in a small amount of water (Figure 3.1). The 546 and 578 nm peaks are seen both in pale and dark nematodes and correspond with the absorption peaks of oxy-haemoglobin [Olsen and Elvevoll, 2011]. Similar peaks are seen in spectroscopic measurements of the pseudocoelomic fluid in *P. Decipiens*, were haemoglobin is recognized as the major chromophore in the nematode [Dixon et al., 1993]. The peak at 636 nm is only seen in dark nematodes and corresponds with met-haemoglobin [Olsen and Elvevoll, 2011].

Freshness of cod fillets, both expressed as days on ice and sensory score value, can be assessed by conventional contact spectroscopy [Nilsen and Esaiassen, 2005, Nilsen et al., 2002]. The visible region (400-700 nm) has given the best results, corresponding with the most important region for nematode detection. This indicates the possibility to integrate both parasite detection and freshness assessment in the same hyperspectral imaging system.

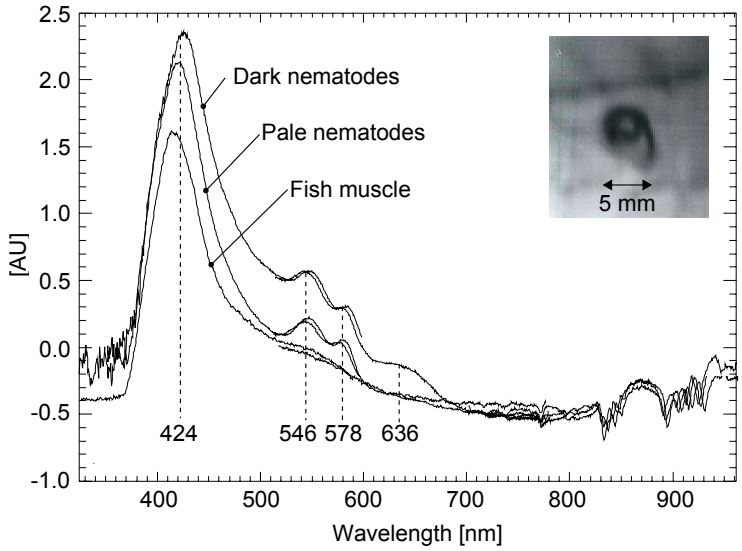


Figure 3.1: Spectra of homogenized nematodes, added a small amount of water, and pure fish muscle. The spectra were measured using Spextube IV in transmission mode (Extracted data from Heia et al. (2003)), and show broad absorption peaks at wavelengths: 424, 546, 578 and 636 nm. The image shows a nematode on the surface of a cod fillet imaged at 550 nm using the Spextube IV in transmission mode.

3.1 A hyperspectral imaging system for fish fillet inspection

Based on previous work using the Spextube IV imager [Heia et al., 2003, Heia et al., 2007] and analysis of homogenized nematodes [Stormo et al., 2004, Dixon et al., 1993], nematodes have no narrow absorption peaks in the region 400-1000 nm. The peak at 578 nm, being the most narrow peak in the nematode absorption spectrum (Figure 3.1), has a bandwidth of approximately 20 nm (based on interpolated data from Zijlstra et al. (1997)).

A system for fish fillet inspection should be versatile with regard to specie and applicability. It it thus of interest also to consider quality inspection of salmon fillets, where fat and color are important features. Fat in salmon absorbs at 980 nm [ElMasry and Wold, 2008]. For color measurements the lowest wavelength used is 380 nm [Wyszecki and Stiles, 2000]. Salmon fillets are larger than cod fillets and often wider than 200 mm. Ideally, we wanted a system covering the range 380 - 1000 nm with a spectral resolution of 5 nm, a spatial resolution of 0.5 mm and a field of view of 300 mm. This resolution and field of view requires a readout speed of 800 frames per second, corresponding to a data rate of approximately 60 MB/second. No commercial solution fulfilled these requirements, and a custom spectrometer had to be made. Due to limitations with halogen lamps (little power below 400 nm) and the CCD detector, the spectral range was limited to 400 - 1000 nm. According to given specifications the spectrometer was designed and

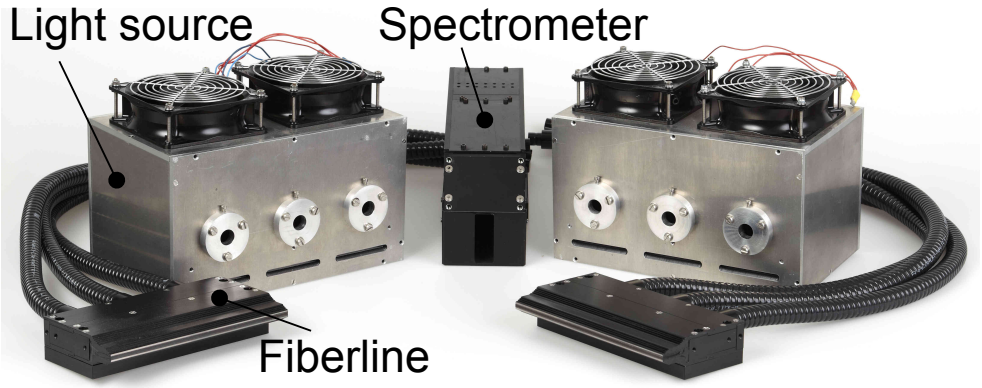
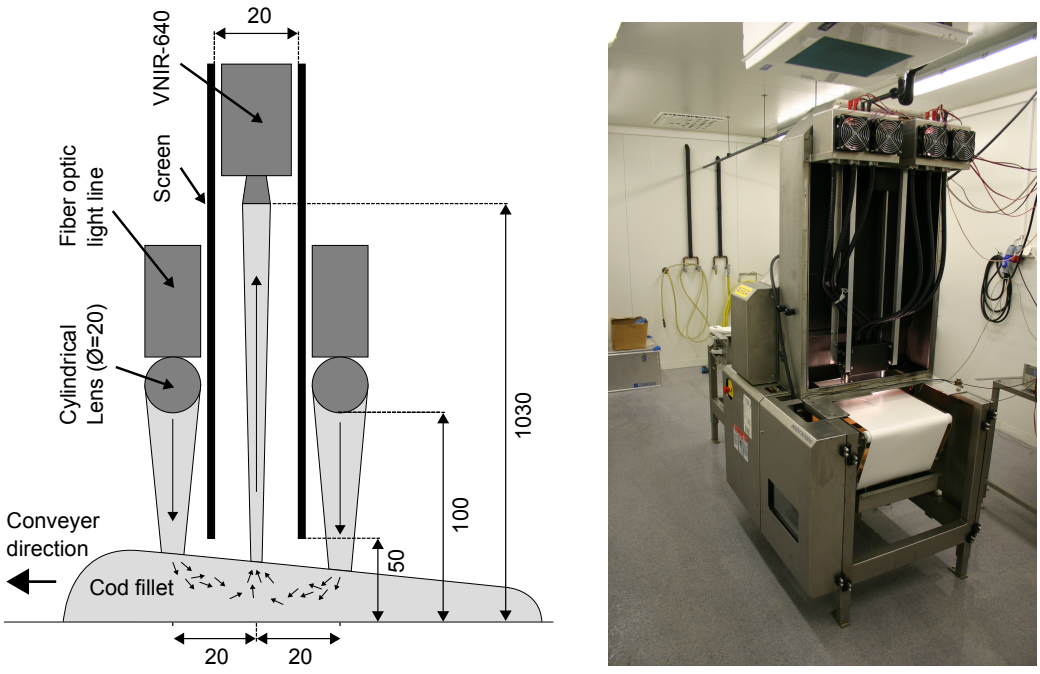


Figure 3.2: Custom made hardware, developed for automatic quality control of cod fillets at a speed of one fillet per second (Photo: Audun Iversen, Nofima AS).

built by Norsk Elektro Optikk AS, and is now commercial available (VNIR-640, Norsk Elektro Optikk AS).

In the preliminary fillet inspection test a standard fiber optic illuminator (21 DCL, Stockeryale, NH, USA) was used (Paper I). To get sufficient signal from the fish sample when running the hyperspectral imaging system in interactance mode, the frame rate had to be lowered to 70 frames/second, corresponding to a conveyor belt speed of 35 mm/second. To increase the speed, more light was required. A fiber line, combining three fiber bundles into one line, was designed and custom made. In addition, a custom made light source, with three 21 VDC 150 W halogen lamps with aluminium reflectors, was made to power the fiber line. All custom made components are shown in Figure 3.2. This system was evaluated under lab conditions for nematode detection using transillumination (Paper II). Preliminary tests showed that the system still could not be used at the required conveyor speed of 400 mm/second. To achieve the required speed, a new interactance setup was implemented. The new setup consisted of two identical fiber lines positioned on both sides of the spectrometer field of view (Figure 3.3a), doubling the amount of light focused on the sample [Sivertsen et al., 2009]. In addition to increased light intensity, using two lines instead of one made it possible to obtain a signal from both the tail and the neck region. This was a problem encountered with the one line configuration in Paper I. The spectrometer was also adjusted, by doubling the slit width and reducing the frame rate to 400 frames/second. This resulted in a resolution of 1 mm along the conveyor belt and a spectral resolution of 10 nm. The new system is implemented on a conveyor belt (Figure 3.3b), and has been evaluated for automatic freshness assessment (Paper IV) and nematode detection under industrial condition (Paper III).



(a) (b)

Figure 3.3: (a) A sketch showing the design and dimensions in mm, of the hyperspectral imaging setup for cod fillet inspection, and (b) a photo of the machine with the front cover removed. The light sources are connected to the two fiber lines through the black fiber cables seen in the photo.

3.2 Paper I - Segmentation

The software analyzing the imaging data from a machine vision system for cod fillet inspection, must be able to detect the defects and make a decision based upon the location and the type of defect. Segmentation of a cod fillet image into its respective parts: loin, belly, center-cut and tail, requires a spatial reference system which is robust to rotation and warping of the fillet. This is the motivation for applying the centerline as a reference system for the segmentation of cod fillet images and the topic addressed in Paper I.

The centerline, consisting of veins and arteries cut off during filleting, is always visible in cod fillets due to the high haemoglobin content. It runs from the tail to the neck region, and is most visible in the tail. For thick fillets, the loin has a tendency to cover the centerline in the neck region. The proposed method uses the band ratio 715 nm/525 nm to enhance the contrast between the haemoglobin rich area of the centerline and the surrounding muscle. Based on this ratio image, the centerline is located in the tail region of the fillet and tracked from tail to neck using the proposed method referred to as directional average ridge follower (DARF).

Problems with initializing the ridge follower were solved by first searching backwards from 15 % to 0 % of the total fillet length, and then searching forwards to the

neck region. The accuracy of the ridge follower was improved by using a dynamic bandwidth in calculating the local average along the fillet. The method was evaluated on 80 industrially processed cod fillets sampled from the processing line, between the filleting machine and the trimming stations. The results show that the centerline can be detected with an accuracy of 1 mm up to 77 % of the total fillet length. The error increases rapidly towards the neck, with a typical error of 4 mm.

This method can be used as a tool for locating the various fillet parts: loin, belly flap, center cut and tail. The centerline can also be used for locating the pin-bone area, which can be a useful tool for robotic portioning of the fillet and removal of the pin bones. Based on the location of the centerline, standardized measurement points can be selected on the fillet (Paper IV) and statistics based on location of detected defects as a function of position on the fillet can be made (Paper III). The method is currently being tested on salmon fillets, where it will be used for selecting standardized measurement points for color and freshness.

3.3 Paper II - Parasite detection by transillumination

Parasites are considered the major challenge with quality control of cod fillets, and currently every single fillet is inspected manually for parasites on candling tables. This is the bottleneck for the cod fillet processing industry, were halting the flow of products in room temperature during inspection and trimming, increases the microbiological and enzymatic degradation of the products. Being able to automatically separate fillets that require manual trimming from fillets that can be sent directly for portioning and packing, would reduce the labour costs and increase the end product quality. This is the motivation for developing an automatic inspection system for cod fillets, and Paper II and III are focused on automatic parasite detection.

Previous reports have indicated an improvement of the contrast of embedded objects in tissue using cross polarization or a narrow beam of light. This was investigated in paper II where transillumination hyperspectral imaging was combined with both a narrow line of light and cross polarization. The results indicated no improvement in using either of the two techniques.

Transillumination requires high intensity light for penetrating the thickest fillet samples. This creates a problem around the edges of the sample, and when no sample is present, between the light source and the spectrometer. The high intensity light saturates the sensor, and internal scattering in the spectrometer interfere with areas on the sensor corresponding to areas on the fillet. This was solved by tilting the light source, so that the direct light entering the spectrometer was minimized.

The transillumination setup was evaluated for nematode detection in a pilot test on untrimmed, industrially processed fillets. The conveyor belt speed was 25 mm/second during the test. The reported detection rate was 58 % of all nematodes (N=922), with a detection rate of 71 % and 46 % of the dark an pale nematodes, respectively. These results are better than what is reported for manual detection of *P. Decipiens* under in-

dustrial conditions. The false alarm rate was high, with one or more false alarms in 60 % of the fillets.

3.4 Paper III - Parasite detection by interactance at industrial speed

Continuing the work from Paper II, the aim of this study was to get the hyperspectral imaging setup to operate at a conveyor belt speed of 400 mm/second, and evaluate it for automatic nematode detection in a commercial cod fillet processing plant.

The spectrometer was upgraded by doubling the slit width, hence reducing the spatial resolution from quadratic pixels of 0.5 x 0.5 mm to rectangular pixels of 0.5 x 1 mm, which also reduced the spectral resolution from 5 nm to 10 nm. A new interactance system was built, using one fiber line on either side of the spectrometer field of view. This doubled the light intensity on the sample, and the imaging system could now operate at the required speed. The data analysis was not implemented in real time, but were done at line in batches of 10 fillet images.

The heterogeneous structure of fish muscle, leading to variation in the light scattering properties, is a major contributor to the spectral variations observed across the fillet. This will influence the spectral characteristics of the light interacting with an embedded nematode, and the spectral signature observed from the nematode will be directly affected. To reduce these spectral variations, a method for calibrating the spectral signature of a nematode, by the estimated local background spectrum, was developed. The local calibration method reduces the number of spectra needed to be classified by 89.6 %. For one or more false alarms in 60 % of the fillets sampled after the trimming station, the Gaussian maximum likelihood classifier detects 70.8 % and 60.3 % of the dark and pale nematodes, respectively. This is an improvement over what is previously reported using a higher resolution instrument on a slow moving conveyor belt under lab conditions (paper II), and comparable or better to what is reported for manual inspection under industrial conditions.

For an automatic nematode detection method to become accepted as an alternative to manual inspection, accurate numbers for manual detection performance under industrial conditions are needed. The existing studies are all performed in Canadian waters. These reports does not differentiate between pale or dark nematodes, and it is likely that they reflect the detection rate for *P. Decipiens*; hence mainly dark nematodes. To get a more accurate estimate for the manual detection rate in Norwegian fish fillet plants, similar studies should be performed in Norway. This is a potential topic for further studies.

3.5 Paper IV - Freshness assessment at industrial speed

Due to the low price, frozen-thawed fillet products from low cost countries are gaining popularity in some European markets. To prevent unfair competition by false labelling, differentiating fish on freshness as days on ice or whether it has previously been frozen is an important authenticity issue in the seafood sector. Control of labelling or documentation of quality with respect to freshness and frozen-thawed is possible only if there are fast and reliable methods for doing so at control points or during a production flow. Previous work has shown that conventional spectroscopy using a contact probe, can be used to predict freshness as days on ice and as a Quality Index Method (QIM) sensory score value. These reports both pointed to the visible region as the best region for freshness prediction. The benefit of online freshness assessment is the possibility to grade fillets more accurately by their quality attributes and expected remaining shelf life. Being able to assess freshness by a handheld probe on single fish could be a valuable tool at control points in the distribution chain, and omit the need for a panel of trained personnel performing sensory evaluation on batches of fish. This is the motivation for evaluating freshness using hyperspectral imaging and a handheld probe in paper IV.

The handheld probe was designed to match the hyperspectral imaging setup with regard to optical path length. The idea was to get independent, but comparable, measurements from two different instruments supporting the same hypothesis. Using the segmentation method developed in Paper I, standardized measurement locations were automatically selected. Spectra from these locations were extracted and regression models were built. The results showed that freshness as days on ice was predicted with an accuracy of 1.6 days on individual fillets using four wavelengths only. This is comparable to what is achieved using QIM with 12 trained panellists on three successive fillets.

The instrument was also evaluated as a tool for discriminating between fresh and frozen-thawed samples, and a full separation between the fresh and the frozen-thawed samples was achieved.

Oxidation of haemoglobin and myoglobin, during freezing-thawing and cold storage on ice, explains most of the variations seen in the visible region of the spectrum. This is supported by previous findings showing that the visible region is the most important region for freshness prediction.

The effect of variable blood content in the fillet, due to bad bleeding or gear marks, might affect the results. This is an important question to address in future studies.

Chapter 4

Conclusion

During the work of this thesis a hyperspectral imaging system has been developed. The system, having a field of view of 300 mm, a spatial resolution of 0.5 x 1.0 mm and a spectral resolution of 10 nm in the range 400 - 1000 nm, is capable of inspecting cod fillets, with or without skin, at a conveyor belt speed of 400 mm/second. The system is evaluated as a tool for automatic cod fillet inspection, where the main focus has been on automatic parasite detection and freshness assessment.

A novel method for segmenting a cod fillet image into its respective parts: loin, belly, center-cut and tail, has been developed. The method, utilising the fillet centerline as a robust reference system, is used for selecting standardized measurement regions for automatic freshness assesment. The segmentation is also used for counting the number of nematodes detected in the various parts of the fillet, and thus indicating the severity of the defect.

For parasite detection, a method for calibrating the hyperspectral images using the local background spectrum has been developed. This method is superior to using standard pre-treated spectra in terms of detection rate and false alarm rate. The method was evaluated under industrial conditions at a commercial cod fillet processing plant. The results showed a detection rate of 70.8 % and 60.3 % of dark and pale nematodes respectively. This is comparable, or better, to what is expected for manual inspection. The downside is the high false alarm rate, with 60 % of the fillets, sampled after the trimming stations, having one or more false alarms. Even though the false alarm rate is high, there is a potential in reducing the amount of fillets needing manual inspection. A clear benefit with this system is that is can be used on fillets with skin. The manual detection rate for nematodes in fillets with skin is previously reported to 25 %.

Both using the hyperspectral imaging system and a handheld probe, the freshness, measured as storage days on ice, could be predicted with an accuracy of 1.6 days. Based on the spectra, the samples could also be classified as either fresh or frozen-thawed. Oxidation of haemoglobin is believed to be the main reason for the changes observed in the visible region of the spectra during storage on ice or the freezing-thawing cycle.

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Chapter 5

Paper I - IV

Paper 1:
Ridge detection with application to automatic fish fillet inspection

Paper 2:
Automatic Nematode Detection in Cod Fillets (*Gadus Morhua*) by Transillumination Hyperspectral Imaging

Paper 3:
Automatic nematode detection in cod fillets (*Gadus Morhua L.*) by hyperspectral imaging

Paper 4:
Automatic freshness assessment of cod (*Gadus morhua*) fillets by Vis/Nir spectroscopy

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