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Towards automated detection of milk spot livers by diffuse reflectance spectroscopy

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ABSTRACT

Swine ascariasis results in milk spot livers which are manually removed from the slaughter line. In this paper, we propose and objectively assess a novel automated method for detection of milk spot livers based on digital color imaging, visible and near infrared spectroscopy and multispectral measurements. A commercial digital color camera based system provided insufficient information for reliable detection of milk spot livers with respect to different focal hepatic changes. The obtained average classification sensitivity and specificity was low, i.e. 77%. The spectroscopic and multispectral systems in the VIS and NIR spectral range performed significantly better. The obtained average classification sensitivity and specificity of milk spots was 98%. A practical, multispectral approach utilizing six LED or laser diodes with the emission wavelengths centered around 460, 600, 850, 1070, 1150 and 1350 nm was found sufficient for reliable classification of milk spot livers.

1. Introduction

Nematode worm *Ascaris suum* causes the most common swine intestinal parasitosis, ascariasis. Adult worm of length up to 30 cm inhabits the small intestine (Grist, 2007). Pigs infected with *A. suum* have depressed growth, lower feed conversion rate (Sanchez-Vazquez et al., 2010) and frequently suffer from intestinal obstruction (Grist, 2007). The infection starts by ingestion of worm eggs, which are activated by the gastric juices. Next, the larvae penetrate the intestinal wall and are carried to the liver. The migration of the larvae through the liver causes hemorrhage and chronic focal interstitial hepatitis, which can be observed as white areas that predominantly consist of connective tissue frequently referred to as "milk spot liver" (Fig. 1). Larvae then enter the blood stream and are carried to the lungs, moving up to pharynx by a combination of own mobility, lung clearing mechanism and coughing. Finally, the swallowed larvae grow to adult warms (Grist, 2007).

Prevalence of *A. suum* infection is still high and varies significantly by geographic location, e.g. the infection rate in European Nordic countries is around 10% (Roepstorff et al., 1999), 21% in Denmark (Haugegaard, 2010), from 6% to 28% in Lithuania (Petkevicius and Pereckiene, 2009) and from 29% to 40% in Africa (Nganga et al., 2008; Nissen et al., 2011). However, milk spot livers are rejected as unfit for human consumption (Gracey et al., 1999; Grist, 2007; Wilson and Wilson, 2005) and are manually removed from the slaughter line by official veterinarians. According to the Veterinary Administration of the Republic of Slovenia (Program monitoringa zoonoz in njihovih povzročiteljev za leto, 2012), almost 280,000 pigs were slaughtered in 2011, thereby, the same number of livers had to be manually examined for milk spots. While official numbers are not public, it is assumed that somewhere from 20,000 to 120,000 milk spot livers are removed each year. The time consuming, strenuous and thereby costly examination is performed by expert veterinarians. Therefore, there is a great need for fully automated and reliable methods for detection of milk spot livers. However, to the best of our knowledge, so far no studies related to the subject have been reported.

NIR and VIS diffuse spectroscopy are nondestructive contactless optical techniques increasingly used for food quality evaluation (Cen and He, 2007; Huang et al., 2008). NIR spectroscopy is considered as one of the most efficient and advanced tool for laboratory and in-line estimation of quality attributes in meat and meat products (Liao et al., 2012; Prieto et al., 2009). Furthermore, NIR spectroscopy is frequently used in a number of different scientific fields including pharmacy, chemistry (Gendrin et al., 2008), agriculture (Nduwamungu et al., 2009), oncological (Kondepati et al., 2008) and dental medicine (Usenik et al., 2012).

Besides ascariasis, the focal hepatic lesions can also be caused by other pathological conditions, like tumors and peritonitis with fibrotic adhesions. Furthermore, fat and connective tissue on the liver surface can appear similar to the milk spots making it very difficult to reliably detect the milk spots by digital color imaging systems in the visible spectral range. In this paper, we therefore propose and objectively assess a novel visible (VIS) and near

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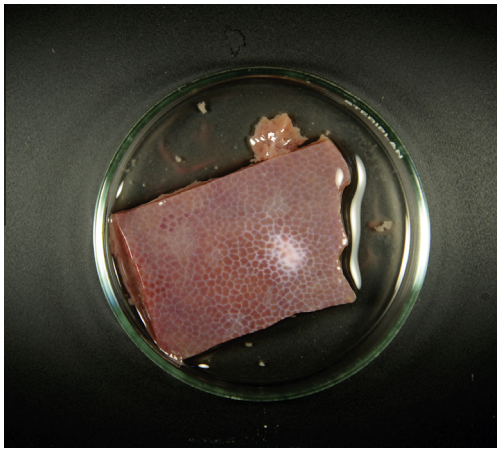

Fig. 1. Milk spot liver sample in a petri dish.

infrared (NIR) based spectroscopic system for detection of milk spot livers. Furthermore, the performance of the spectroscopic system is compared to the digital color camera based system in terms of white spot liver classification sensitivity and specificity. The presented study is the first step towards fully automated detection of milk spot livers based on the unique VIS and NIR spectral properties of the connective liver tissue (milk spots) and the unchanged hepatic liver.

2. Methods

2.1. Samples

Over a period of six months, twenty-five porcine milk spot liver lobes were obtained from a local slaughterhouse. The liver lobes were taken directly from the slaughter line by an official veterinarian. Additional focal hepatic lesions were simulated by fat tissue from the back and by tendons from the digital flexors. The samples were stored in physiological solution for about 1 h, which was the time required to transport the samples from the slaughterhouse to the laboratory, where the measurements were made (Fig. 1). Each liver lobe included at least five milk spots and each sample was characterized by one digital color camera recording, one VIS and one NIR spectral measurement. In this way, we obtained around 120 RGB color measurements and spectra for each sample group (milk spots, hepatocytes, tendons and fat). The liver lobes and thereby the corresponding RGB color measurements and acquired spectra, were randomly divided into the training (13 liver lobes) and validation set (12 liver lobes).

2.2. Instrumentation

The samples were imaged under a camera stand system R1 (Kaiser, Germany) (Fig. 2a). The digital color images (resolution: 4256×2832 pixels, exposure time: 1/125 s) were acquired by a commercial Nikon D3 digital color camera using dark field illumination carried out by two halogen lamps (36 W). The employed laboratory spectroscopic system (Fig. 2b) consisted of two spectrometers. The first spectrometer comprised 2048 light sensitive pixels covering the spectral range from 250 nm to 1100 nm (Avantes, AvaSpec-2048-TEC-TF), while the second spectrometer consisted of 512 light sensitive pixels covering the spectral range from 900 nm to 1700 nm (Control Development, NIR-512L-1.7T1, 901–1685 nm). The spectral resolution of the VIS and NIR spectrometers was 2 nm and 5 nm, respectively. Both spectrometers employed a broadband halogen light source (AvaLight-Hal LS 0505003) connected to a stainless steel diffuse reflectance probe (Avantes, FCR-7IR400-2-ME) comprising six illumination and one detection fibers, illuminating and collecting the light from a circular area of 6.5 mm diameter (33 mm^2). The probe was held in a fixed position by the uniquely designed Teflon® nozzle, which prevented the surrounding light from illuminating the area under examination. The acquired color image and the spectral data were processed using the Matlab® software package (7.9, The MathWorks, USA).

2.3. Analysis and classification

It was assumed that the shape of all the focal hepatic lesions could be similar, therefore only the spectral (color) properties of the tissue were considered as the source of information for tissue classification. The color properties of the samples (i.e. hepatocytes, connective tissue of milk spots and tendons, and fat) were calculated as the average color across the entire lesion surface, defined by the normalized RGB color space. First, each color (C_c) of the recorded image was normalized according to:

$$C_{c,\{R,G,B\}} = \frac{C_{\{R,G,B\}}}{C_{0,\{R,G,B\}}}, \tag{1}$$

where $C_{\{R,G,B\}}$ are the recorded intensities of the three color channels and $C_{0,\{R,G,B\}}$ are the corresponding recorded color channel intensities obtained by the diffuse reflectance Spectralon® standard. Finally, the intensities of the three color channels were normalized to the range [0,1]:

$$c_{\{R,G,B\}} = \frac{C_{c,\{R,G,B\}}}{R_{c,\{R,G,B\}} + G_{c,\{R,G,B\}} + B_{c,\{R,G,B\}}}. \tag{2}$$

Subsets of the calculated normalized colors (c_R, c_G and c_B) formed the feature vectors for the subsequent K-nearest-neighbor (KNN) classification of the liver tissue.

Likewise, the acquired raw spectra $s(\lambda)$ were calibrated using a diffuse reflectance Spectralon® standard. The reflectance spectra $r(\lambda)$ were calculated according to:

$$r(\lambda) = \frac{s(\lambda) - d(\lambda)}{s_c(\lambda) - d(\lambda)}, \tag{3}$$

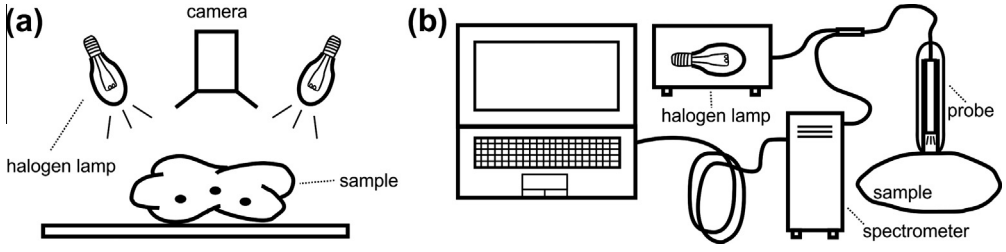

Fig. 2. Illustration of the employed digital color camera based (a) and spectroscopic (b) systems. Surface of all the liver samples included authentic milk spots and two other focal pathologies, represented by fat and tendon tissue.