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Hyperspectral imaging of blue stain fungi on coated and uncoated wooden surfaces

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ABSTRACT

Hyperspectral imaging has been applied on samples of coated and uncoated Norway spruce (*Picea abies*) to detect and quantify blue stain fungi that has colonized and grown on the surface of the samples. A clear visualization of the fungi was obtained through a Principal Component Analysis of the hyperspectral images and the amount of mould coverage could be estimated for each sample. The signal from the fungi on the hyperspectral images in near-infrared wavelengths could clearly be distinguished from water and moisture on the samples. The results indicate that NIR hyperspectral imaging can be used as a tool for assessing coverage of surface fungi and event spectral classification of the two fungal species *Aureobasidium pullulans* and *Cladosporium cladosporioides*. We suggest additional case studies both in laboratory and in outdoor environment for further optimization to encompass this method for a broader variety of fungal species and for different climatic conditions.

1. Introduction

Discolouration of coated wooden façades by blue stain fungi and surface moulds can lead to repeated cleaning and shorter maintenance intervals, which in turn increases the total cost of ownership for the wooden façades. Cost and effort of ownership is often an important factor considered when choosing a material for a building façade. More knowledge about discolouring fungi on coated and uncoated wood is of commercial interest, both with regard to objective evaluation methods and with regard to early prediction of surface performance and increased aesthetic service life of wooden façades.

The degree of mould growth on the surface of coated and uncoated wood is a measure of the substrates (wood material and/or coating) susceptibility to infestation of mould fungi and is a way of rating the substrates according to quality, treatment, time, climatic factors, workmanship, design etc. (Gobakken, 2009; Gobakken et al., 2009). Assessment of mould growth coverage implies a need for a quantification that allows objective numeric validation without considerable subjective appraisal. Visual assessment is the current state of the art for evaluating mould growth coverage on the surface of coated and uncoated wood. Several standards used for this purpose describe a rating scale often from 0 to 5 or similar where the rating goes step-wise from no growth to heavily infested or fully covered with mould fungi (i.e., BS 3900, 1989; EN 927-3, 2000; ASTM D3274, 2002; EN 152, 2011).

The objectivity of using visual assessment is often questioned (Sexton et al., 1993; Bardage, 2004; Van den Bulcke et al., 2005; Van den Bulcke et al., 2006) and there is an on-going interest and research for finding additional or alternative methods for more objective assessment. Digital image processing has been used by several scientists for assessing mould growth on coated wood with good results (Terziev, 1997; Graystone, 2002; Van den Bulcke et al., 2005; Van den Bulcke et al., 2006). Also DNA-based and spectroscopic techniques are used in several fields of research for qualitative and quantitative identification of fungal species, and have been introduced in the field of wood protection and wood durability (Siebert, 1995; Diem et al., 1999; Holm and Jelle, 2006; Alfredsen et al., 2007, 2008; Pilgård et al., 2010; Larnøy et al., 2011; Pilgård et al., 2011). SEM and FTIR have also been applied successfully to discriminate between fungi species from their growth patterns (e.g., Naumann et al., 2005; Santos et al., 2010).

All these techniques are complementary, each technique being particularly suited for observing specific parameters of the fungi. Moreover, no technique yields a complete picture of the fungi. For

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example, FTIR spectroscopy is a rapid technique with high spectral resolution, being increasingly used to detect and identify mycotoxins on food and agricultural products (e.g., Færgestad et al., 2011; Tomak et al., 2013). However, the technique offers no information regarding the location of the contaminant investigated. Measurements are either made at a single point or on a limited area on the sample that must be selected as representative for the sample.

In this work, hyperspectral imaging technology is used, a rapid, non-destructive method that provides both spatial and spectral information of an entire sample on macroscopic (centimetre) scale. Hyperspectral imaging captures and measures spectral signatures by taking hundreds of spectra, each covering, at nanometre intervals, hundreds of electromagnetic bands — scanning the sample similar to how a push broom sweeps a wide area of a floor (Goetz et al., 1985; Geladi et al., 2007). Hyperspectral imaging is a powerful technique especially when the distribution of different qualities in a sample needs to be studied. Today hyperspectral cameras are being considered in many research fields, since they can provide unprecedented capabilities for identifying properties of both living and inanimate materials (Bannon, 2009). Recently, near infrared (NIR) hyperspectral imaging has been used within the field of food industry to study different species of fungi (Williams et al., 2012), to study growth characteristics of fungi (Williams et al., 2012b) and to study fungi on oranges (Gómez-Sanchis et al., 2013).

In the present work, hyperspectral imaging technology was applied for evaluating the colonization of the discolouring fungi *A. pullulans* and *C. cladosporioides* on the surface of coated and uncoated wooden samples. The objective of this pilot project was to determine if fungi can be detected, localized and quantified from hyperspectral images obtained at visible or NIR wavelengths. This exercise aims at evaluating if hyperspectral technology provides additional information complementary to other existing techniques and thereby has the potential of becoming an accurate and fast method for studying the behaviour of fungi on wood surfaces.

2. Material and methods

2.1. Preparation of wood samples

The wood samples had a dimension of 50 mm × 50 mm × 2 mm and were made from Norway spruce (*Picea abies*). Two different coatings were applied to the top face of the wood samples: 1) water-borne alkyd modified acrylic paint without fungicide (Coating WB) and 2) solvent-borne alkyd paint without fungicide (Coating SB), and one set of samples were left uncoated. Twelve samples were prepared for each of the three surface finishes. The coatings were applied with an equal amount of paint on each sample before all the samples (both coated and uncoated) were acclimatized in a climate chamber of 20 °C and 80% relative humidity until equilibrium moisture content were achieved.

The samples were put on malt agar growth medium in petri dishes (one sample per petri dish). A suspension containing spores and hyphae fragments made from each of the blue stain fungi *A. pullulans* and *C. cladosporioides* was applied at a fixed amount of 500 μl to the upper face side of the samples and evenly distributed with a glass rod. Additional sample sets of each of the surface finishes without adding any fungal suspension were prepared as references. The samples were incubated at room temperature for 1, 2, 4 and 6 weeks allowing step-wise mould growth coverage (spares mould growth, intermediate mould growth and heavy mould growth) for each of the surface finishes.

2.2. Equipment for imaging

Hyperspectral imaging of the wood samples was carried out by using two cameras, both from Specim, Oulu Finland. A Si CCD camera (Janesick, 2001) records in the visible/NIR wavelengths (400 nm–1000 nm) distributed on 800 spectral channels, and a Mercurium Cadmium Telluride (MCT) camera (Norton, 2002), is sensitive in the NIR wavelength region (1000 nm–2500 nm) distributed on 256 channels (see Fig. 1 for experimental setup). One dimension of the detector is used for the spectral separation and the other for imaging one of the two spatial directions so that one line is recorded each time with a spectrum in each pixel. The second spatial dimension is obtained by moving the camera over the sample using a translation stage. In average it takes 5 s to scan the sample. The spatial resolution of the setup was approximately 130 μm and 200 μm for the visible and NIR camera respectively. A 99% reflecting white reference bar was included in all the recorded images in order to correct for variations in the light source. The total cost of the hyperspectral imaging setup with the two cameras for research applications is approximately €1,30,000. Measurement with this equipment is expected to reveal information on what type of spectral sampling and resolution that is necessary for studying fungi on wood surfaces. This information can further be used to define equipment for measurements at other research institutions or for commercial applications.

2.3. Data processing

Hyperspectral image files are composed of several spectrally resolved 2D-images of the sample, also called a hypercube (Burger, 2006). The structure of the hypercube **X** is (M × N × λ) where the M and N axes represent spatial information and the λ axis correspond to the spectral pattern, illustrated schematically in Fig. 2. The quality of the raw measurements was affected by instrumental variations and varying light conditions, thus preprocessing of the data was required. Subtraction of dark frames was performed to correct for pixel to pixel variations in the detector, and a flat field division was conducted using the white reference bar to correct for variations in the light source. Before the exploratory analysis, images of four different samples with the same surface finish were combined to mosaic images (2 × 2). Three of the samples in each mosaic image had fungal growth in different stages caused by either *A. pullulans* or *C. cladosporioides* and one sample was without fungal growth (clean sample). An RGB image of the mosaic of

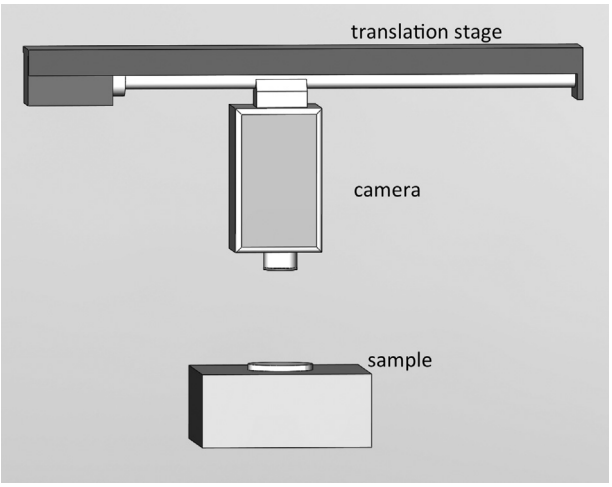

Fig. 1. Schematic illustration of the hyperspectral camera setup.