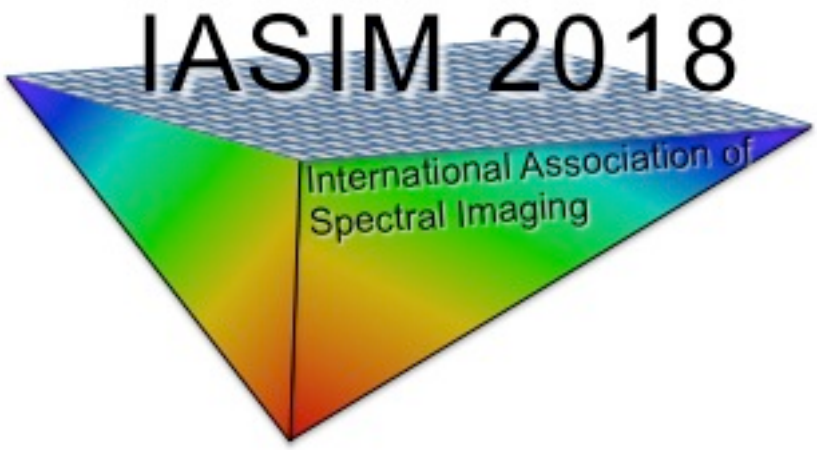

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Biomedical Spectroscopy and Imaging

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MULTIVARIATE CURVE RESOLUTION MASS SPECTROMETRY IMAGING (MSI) OF BIOLOGICAL TISSUES

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Multivariate Curve Resolution (MCR) is proposed for the analysis of Mass Spectrometry Imaging (MSI) data[1] of biological tissues. Recently, developments on ionization of samples have dramatically expanded the number of applications of MSI due to the possibility of collecting the mass spectrum for each pixel of a considered surface in a reasonable time. Using this method, both spatial distribution and mass spectral information of analyzed samples can be obtained. However, there are major drawbacks inherent to MSI related to the high complexity of the data obtained from real samples and to the extremely huge size of the data sets generated by this technique[2]. Therefore, the potential of chemometric tools in different steps of the analysis process is unquestionable, from data compression to data resolution of the different components present at each pixel of the image. The benefits of the application of the MSI method are shown for some examples consisting on data sets obtained from the analysis of biological tissues[3]. Results show that the combination of the searching and compression of the mass regions of interest (ROI) and of Multivariate Curve Resolution (MCR) in the ROIMCR method[4] allows the resolution of the spatial distribution maps of different components on the analyzed biological tissues and of their identification from their resolved high-resolution mass spectra

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EARLY DETECTION OF THE APPLE SCAB DISEASE USING HYPERSPECTRAL IMAGING

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Apple scab (ascomycete *Venturia Inaqualis*) is the main cause of stress and fruit losses in apple orchards. Its treatment is most often based on repeated fungicide applications, which is a costly and time consuming approach [1]. In this context, early, accurate and non destructive detection of apple scab infection would be an efficient solution to optimize the management of the fruit disease, reducing fungicide applications while maintaining crop quality [2].
Our study aims at exploring the potential of hyperspectral imaging for early detection of apple scab disease in leaves. Hyperspectral images of inoculated and healthy apple tree leaves were acquired daily from 2 days (D02) after inoculation until the appearance of visible infection spots (D11) using two Hyspex cameras: VNIR (400 – 1000 nm) and SWIR (1000 – 2500 nm). A chemometric classification approach, PLS-DA partial least squares discriminant analysis, was tested to discriminate infected areas of the infected leaf. The PLS-DA classification model established at an advanced stage of infection (D11) was tested on the hyperspectral images of infected and healthy leaves acquired at different infection stages.

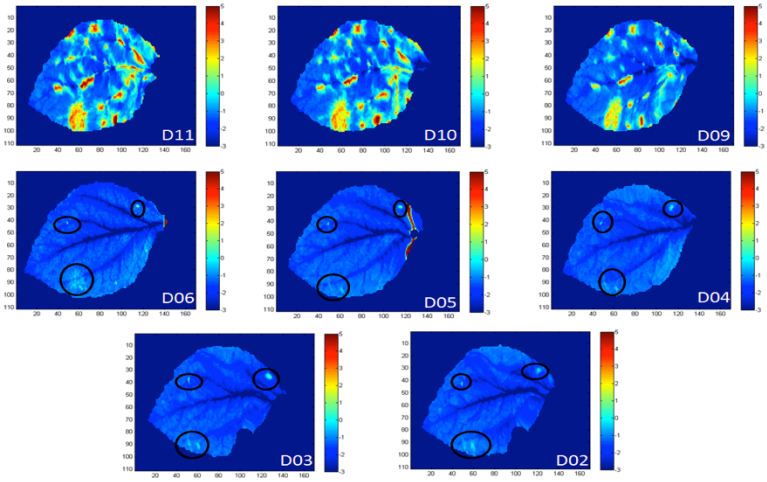

Figure 1: The scores images of the infected leaf predicted by the PLS-DA model from D02 to D11 after inoculation (black circles represent the not visible spots detected)

The study showed that good predictions can be obtained by discriminating infected areas on hyperspectral images using the PLS-DA classification model. The results suggest that the spectral range between 1000 and 2500 nm is the most suitable for early detection of scab. At early infection stages, the water absorption band at 1940 nm has the major discriminatory effect. Thus, apple scab disease affects the water content of infected leaves at early infection stages. This study demonstrates that hyperspectral imaging offers high potential as a non-invasive early detection tool of apple scab infection.

Acknowledgement: This work was supported by the French Ministry of Agriculture and the National Association of Research and Technology ANRT [Casdar project Aventuria 1412].

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CHEMOMETRICS INVESTIGATION OF ADVANCED FLUORESCENCE IMAGING DATA

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Recently, it has been demonstrated that the use of advanced fluorescence microscopy can provide high-content imaging of complex samples both at the ensemble and single-molecule level.[1,2] Obviously, data analysis plays an important role in this process. Still, state-of-the-art approaches often relies on multi-exponential fitting or pattern matching algorithms which may limit investigation possibilities.

In this work, we propose to investigate how chemometric data processing perform on the multiway data provided by advanced fluorescence imaging modalities. We present results obtained with multilinear resolution methods on spectral time-resolved fluorescence imaging to characterize the emission heterogeneity of fluorescent organic nanoparticles. Furthermore, results on biological samples (live-cell imaging) are shown. We highlight the importance of preprocessing in stochastic super-resolution fluorescence microscopy imaging. And lastly, we discuss preliminary results obtained on cells stained with different fluorophores in multi-target multispectral time-resolved fluorescence imaging.

To recap, this work gives an overview on various multimodal fluorescence imaging techniques yielding different data structures. It points at the importance of using chemometric approaches to extract a maximal amount of information.

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PATTERN RECOGNITION ENTROPY (PRE)-A NEW CHEMOMETRIC TOOL

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Pattern Recognition Entropy (PRE) is a summary statistic that is employed in understanding and comparing spectra and hyperspectral images. PRE has its roots in Shannon's groundbreaking work on Information Theory, and PRE values are a reflection of the shape and complexity of spectra. In PRE, an entire spectrum is treated as a probability distribution, where it is normalized and entered into Shannon's equation to obtain a summary statistic that characterizes it. PRE can be used as a pattern recognition tool because it is sensitive to and can differentiate between spectra with different shapes, where, as will be discussed, the spectral 'shape' is a result of contributions from all parts of a spectrum – noise, baseline, and signals. PRE was recently used to identify transitions in X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) depth profiles.[1] PRE revealed the presence of interfaces in these films, and often indicated that the first few scans in the depth profiles were different from those that follow. PRE has also been employed to identify noisy mass chromatograms to produced higher quality reduced Total Ion Current Chromatograms (TICCs) from liquid chromatography-mass spectrometry (LC-MS) data. PRE is able to uniquely quantify differences in electropherograms obtained from simulated autologous blood doping samples that are otherwise difficult to distinguish by other mathematical techniques. PRE can help automate data collection. As an increasing number of scans are collected, PRE values correlate with the increasing S/N noise ratios for the spectra. As a graphical aid in understanding PRE values, we have introduced the concept of the 'Reordered Spectrum'.[2] The shapes of reordered (sorted) spectra correlate with their PRE values and help explain them.

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PATTERN RECOGNITION ENTROPY (PRE) FOR DETERMINING SHARPNESS OF PHOTOGRAPHIC IMAGES

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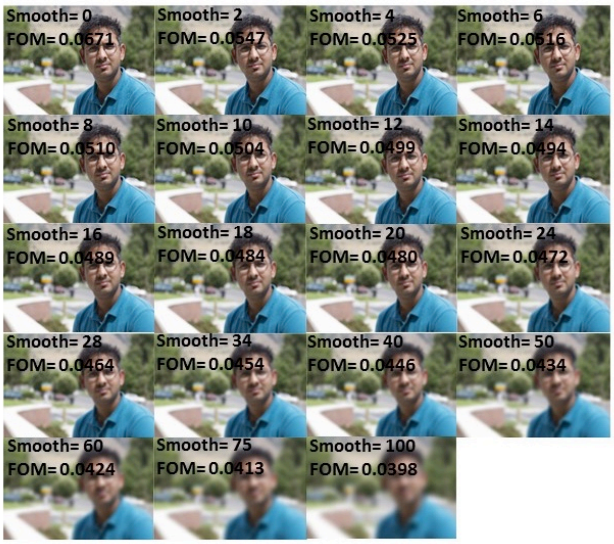
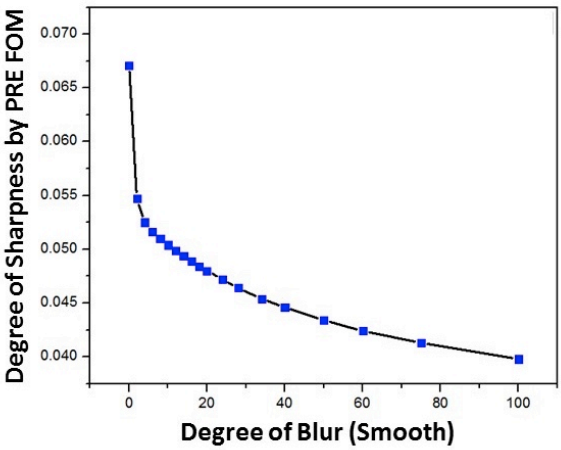
Pattern Recognition Entropy (PRE) is a new application of Shannon's entropy and Information Theory. Sharpness is a quantitative measure of the degree to which an image is in focus, i.e., a sharper image reflects higher amounts of detail. In this work, we introduce PRE as a measure of sharpness that can directly analyze and quantify colored (RGB) images. PRE is used to reduce information in the spectral channels (R, G and B) of every pixel to a single unique value. Thus, the 3-dimensional image data is reduced to a 2-dimensional PRE data matrix. The standard deviation of the values in this latter matrix constitute a figure of merit for image sharpness. That is, a blurry (out of focus) image should have pixels with similar R, G and B values, while sharper images will have pixels that stand out from their neighboring pixels. This method has been tested on a variety of sets of images including portraits and landscapes with varying focal lengths for each image, along with images that were deliberately blurred with Gaussian filters. Our algorithm has correctly identified the in focus images in all data sets analyzed. For example, the figure below shows a gradual decrease in PRE value with increased image blur. Overall, our approach is extremely fast, robust, and computationally simple, and it does not require the application of thresholds, computation of mathematical transforms, e.g., Fourier transforms, or conversion to grey scale, as do some existing methods.





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Thermal Infrared Hyperspectral Imaging for Mineralogy Mapping of Geological Outcrops

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Remote sensing systems are largely used in geology for regional mapping of mineralogy and lithology mainly from airborne or spaceborne platforms. Earth observers such as Landsat, ASTER or SPOT are equipped with multispectral sensors but suffer from relatively poor spectral resolution. On the contrary the existing airborne and spaceborne hyperspectral systems are capable of acquiring relatively narrow spectral bands, beneficial for detailed analysis of geological remote sensing data. However, for vertical outcrops those platforms are inadequate options since their poor spatial resolutions (meters to tens of meters) are unsuitable for detailed field studies. Here we demonstrated that field-based approaches that incorporate thermal infrared hyperspectral technology with about 40 nm spectral resolution and tens of centimeters spatial resolution allows efficient mapping of mineralogy and lithology of vertical cliff sections. We used Telops, lightweight and compact passive thermal infrared hyperspectral research instrument for field measurements in Jura Cement carbonate quarry, Switzerland. The obtained hyperspectral data were analyzed using Temperature emissivity separation algorithms to isolated the different contributions of self-emission and reflection associated with different carbonate minerals. The mineralogical maps derived from measurements were found to be very consistent with the expected results. Our proposed approach highlights the benefits of this type of field-based lightweight hyperspectral instruments for routine field applications such as in mining, engineering, forestry or archaeology.

In this work, we conducted mineralogy and lithology mapping of vertical cliffs in a Jura Cement quarry (http://www.juracement.ch/) at Cornaux (47 deg 2 min 20 secs N; 7 deg 25 mins E, Switzerland). This mine is essentially composed of dolomite and calcite carbonates minerals (Fig. 1 Left). In order to illustrate the feasability of hyperspectral proximal LWIR imaging to map mineralogy within a limestone quarry for calcite feedstock quality control, the Telops ground-based LWIR hyperspectral imager was deployed for this field study with the main objective of characterizing the mineralogy distribution of the mine. Our recently developed TES procedure[1] was then used to retrieve geochemical properties and the relative mineral abundances of the open mine (Fig. 1 Right).

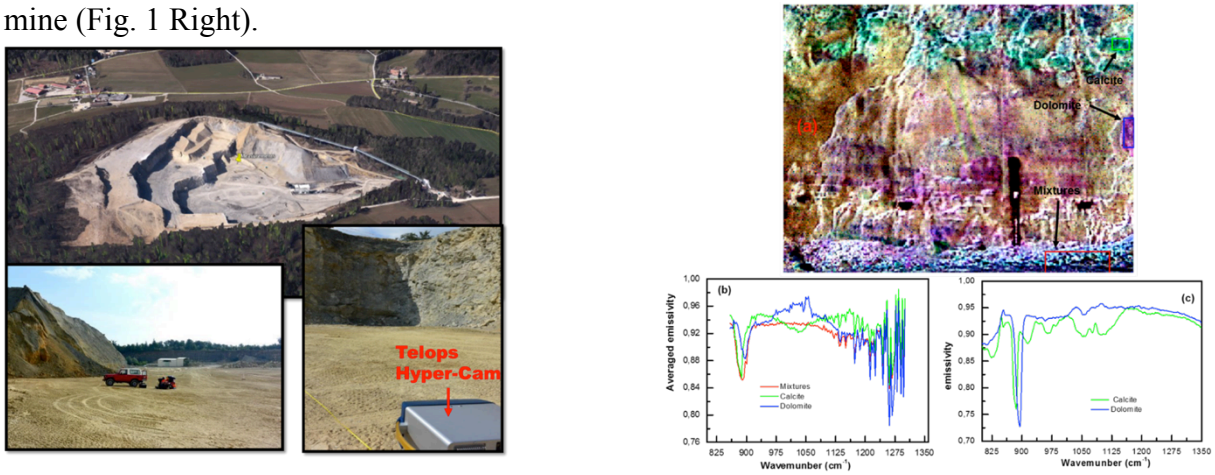

Figure 1. Left : Overview of the Jura Cement quarry, photographs of the vertical cliffs and the ground-based Telops Hyper-cam instrument. Right : Lithological map of one of the investigated wall outcrop in RGB (a) and averaged spectral emissivity on three different regions of the wall (b). The reference spectral emissivity of calcite and dolomite obtained from ASTER database (c).

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NIR-HSI FOR ASSESSING CHEESE RIPENING: HOW TO DECOMPOSE THE CONTRIBUTION OF DEHYDRATION, PROTEOLYSIS AND LIPOLYSIS

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Biochemistry of cheese ripening is considered under three general headings: dehydration, proteolysis and lipolysis. Dehydration is the clearest modification that cheese suffers during the early stages of ripening, causing crust development that will protect the cheese wheel. Proteolysis is the most complex biochemical activity that contributes to flavour development through the formation of peptides and AA and by changing the texture of cheese from the breakdown of the protein network. Lipolysis in cheese is due to the presence of lipolytic enzymes that cleave the ester linkage between a fatty acid and the glycerol moiety of a triacylglycerol; it produces free fatty acids (FFA) with chain lengths ≥C4, glycerol, and mono- and diacylglycerols. Several analytical methods are available to evaluate cheese ripening ranging from qualitative approaches, aimed at following process evolution, to quantitative approaches, which allow to quantify compounds being formed. However, regarding the spatial extent of this phenomenon, no technique was proposed yet to pattern cheese ripening, which is clearly influenced by location within a cheese and tends to evolve following a radial pathway from the centre to the borders of the cheese. Until now, a suitable sampling scheme has been the only prospective to account for such differences. Moreover, the strong inter-correlation of dehydration, proteolysis and lipolysis get more difficult the assessment of these complex biochemical reactions.

The aim of this work was the reliability evaluation of near infrared hyperspectral imaging (1000–2500 nm), to monitor not only the evolution but also the spatial extent of dehydration, proteolysis and lipolysis in cheese. First of all, a qualitative assessment of the NIR bands associated with peptides and free fatty acids was carried out on a wide set of cheese (11 different commercial cheeses), characterized by different ripening types: mainly proteolytic or mainly lipolytic. From the exploratory data analysis of commercial cheese, an in-depth understanding of the absorption bands associated with the three biochemical reaction was performed. During the second step of the experimental plan, a semisolid slicing cheese (called "Formaggetta") was followed during the early stages of its ripening, acquiring hyperspectral images daily for a total of 10 days from cheese production (three samples each day). After the deploying of suitable pre-processing methods for minimising the amount of useless information within hyperspectral data (SNV transform and mean centering), an innovative way to masking the images background was developed, taking into consideration the difference image between two key wavelengths. Principal component analysis on each interesting band was performed: 1360 – 1500 nm for assessing dehydration (combination bands of symmetric and antisymmetric O–H stretching); 1650 – 1780 nm to understand proteolysis extent (first overtone of N–H stretching); 1920 – 2205 nm for lipolysis (second overtone of C=O stretching). The brushing approach between score images and score plots, allowed to assess cheese evolution and to characterise the phenomena involved. The results indicate that the NIR-HSI approach adds spatial information in cheese biochemical knowledge allowing to deeply understand the extent of ripening not only along time but also inside each single cheese wheel.

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Caseificio Val d'Aveto is gratefully acknowledged for collaboration and samples supply.

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ANAMORPHIC IMAGING SPECTROMETER

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Pushbroom imaging spectrometers typically utilize a diffractive element, such as a diffraction grating, to map spectral information along one axis of a detector array, and spatial information along the other. Not surprisingly, often one would like to engineer characteristics of the spectral axis differently than the spatial axis. However, most pushbroom imaging spectrometers utilize circularly symmetric optics that treat both axes the same. An alternative approach uses cylindrical optics to partially decouple the two axes of the instrument, providing additional engineering parameters that can be used to increase optical throughput without sacrificing spatial resolution. Additionally, cylindrical optics fundamentally eliminate aberrations from the fore-optics. Results from an imaging spectrometer that uses cylindrical optics with be presented.

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SAMPLING IN HYPERSPECTRAL IMAGING

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Applications of hyperspectral imaging (HSI) to the quantitative and qualitative measurement of samples have grown widely in recent years, due mainly to the improved performance and lower cost of imaging spectroscopy instrumentation. Data sampling is a crucial yet often overlooked step in hyperspectral image analysis, which impacts the subsequent results and their interpretation. For example, in microscopic hyperspectral imaging, in order to obtain images in a reasonable timeframe, it is necessary to select specific regions of a specimen for imaging. This is often done on an ad-hoc basis, by visual selection by the operator, however this could lead to biased results. The sampling problem also exists for macroscopic hyperspectral imaging, where a spatially contiguous HSI data cube, or 'hypercube' can be obtained in a matter of seconds. In this case, a challenge exists in the development of calibration models, in that reference or measured values for a property of interest are not usually available at a pixel level. In conventional spectroscopy it is common to use a single bulk sample value - an average value characterizing a homogenous sample, matched to a single spectrum representing the entire sample. In the case of hyperspectral imaging, the conventional approach is to compute a single spectrum (e.g. mean or median) from the set of spectra selected within a defined spatial region of interest (ROI), but this may not always be the optimal choice. Similar problems exist in the selection of pixel spectra for the calibration of classification models. In this paper, a variety of sampling strategies for selection of spatial regions within samples and pixel spectra are presented, exemplified through a number of real and synthetic datasets. The strategies are compared in terms of the proportion of global variability captured, practicality and predictive model performance.

Acknowledgment: Funding for this research was provided by the European Research Council (ERC) under the starting grant programme ERC-2013-StG call—Proposal No. 335508—BioWater.

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INTERACTIVE EXPLORATORY ANALYSIS OF LARGE HYPERSPECTRAL IMAGES

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Interactive exploration of hyperspectral images is very important part of analysis and, often, the first step most of researchers do. Usually, it employs PCA (or similar) decomposition of an image and brushing — selection of pixels simultaneously on score density plot and on channel or score images. By excluding the selected points and consequently creating a new model for the remaining data it is possible to get rid of outliers and background or to make a local model for a particular group of pixels. Interactivity of this procedure is quite important and, therefore, it requires a powerful computer with enough RAM to operate.

However, in case of large images (from hundreds of thousands of pixels and above), the interactive exploration becomes more and more tricky as the decomposition algorithms (such as e.g. SVD and NIPALS) are getting very slow. One of the ways to solve this issue is to use probabilistic approach, which allows to reduce the dimension of dataset needed for computing PCA loadings — the slowest part of the algorithms.

In this presentation we are going to demonstrate benefits of the probabilistic approach and compare it with conventional algorithms in terms of speed and accuracy of PCA decomposition using real life examples.

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TERAHERTZ RAMAN IMAGING OF A PHARMACEUTICAL HOT-MELT EXTRUSION PROCESS

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Hot melt extrusion (HME) has been gaining popularity in the pharmaceutical industry as a way for rapid production of solid dispersions [1]. During HME, an active pharmaceutical ingredient (API) is melted and mixed with a thermoplastic polymer to form unique structure with desired API crystal forms and distributions to improve drug bio-availability and to achieve unique drug delivery profiles. HME process parameters, such as extrusion temperature, screw speed, and residence time must be controlled precisely to ensure the consistency and the product quality.

Terahertz (THz) Raman spectroscopy, which covers low-frequency region of ≤ 6 THz (≤ 200 cm^{-1}), provides important "structural fingerprint" information, such as crystal lattice or polymer structures and crystal orientation of the materials [2]. THz Raman spectroscopy has been used effectively for identifying different polymorphs of various APIs and for monitoring API crystallization and dissolution processes, both by bulk Raman measurements, as well as by Raman mapping or imaging [3]. However, there have been scant reports on the applications of THz Raman imaging for monitoring HME processes.

In this work, we demonstrate the use of THz Raman imaging for monitoring API distribution and crystallinity during an HME process. The effect of extrusion temperature and screw speed on the API distributions, API crystalline forms, and crystal sizes have been discussed.

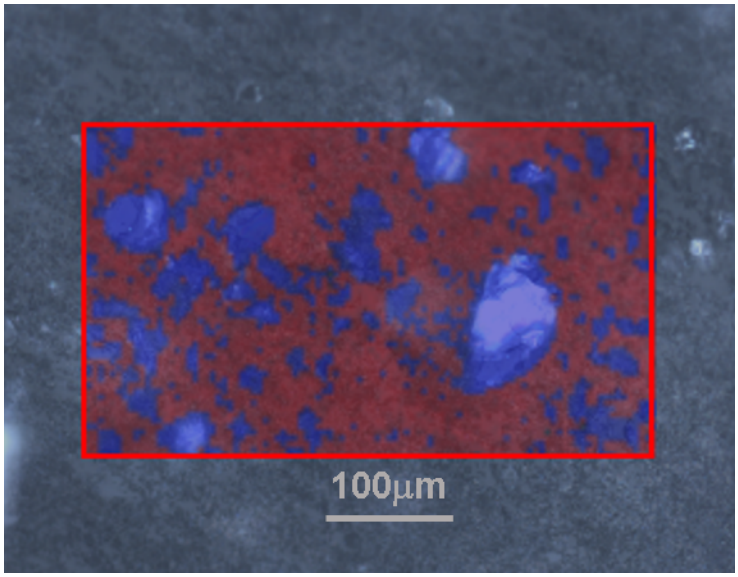

Figure 2 Superimposed Raman MCR and optical images of an HME film. Red and blue regions are amorphous and crystalline API forms, respectively.

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REMOTE SENSING POSSIBILITIES COMBINING HYPERSPECTRAL IMAGING AND MULTISPECTRAL LIDAR: CITY MAPPING OF OSLO, NORWAY

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TerraTec AS presents a unique city mapping project combining lidar at 8 pts/m^2 (1064 nm), multispectral lidar at 10 pts/m^2 (532 nm, 1064 nm and 1550 nm) and hyperspectral imaging in 474 channels (400nm – 2500 nm) with GSD of 0.3/0.7 m (VNIR/SWIR) and spectral resolution of 3.2/5.4 nm (VNIR/SWIR). Each dataset is georeferenced to absolute position with high accuracy both in plane and elevation, eliminating the need for resampling or manual co-registration. The combination of spectral and spatial high-resolution mapping with both passive and active sensors opens a whole new level of analyses and feature extraction.

We wish to contribute to and further investigate the field of spectral imaging by sharing thoughts and experience on three main topics of hyperspectral remote sensing:

1. Technical specifications of a unique dataset: combined multispectral lidar and hyperspectral orthoimagery with excellent georeferencing, including background on data capture and processing.
2. As the possible applications are nearly endless, we present an eclectic set of examples and results related to topics aiming at achieving greater urban sustainability and resilience. We will shortly describe a methodology where analysis of the data has been used to classify plant species, to identify green roofs and roof top solar panels, and to measure and classify urban surface parameters such as roughness and infiltration properties, which are important for the understanding and simulation of urban flooding. Moreover, we show how combining the two datasets allows us to conduct bathymetric depth measurements and seabed classification.

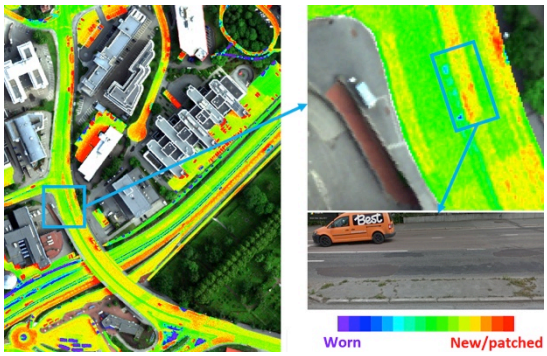

Figure 1 Identifying levels of asphalt wear.

Figure 2 Identification of green roofs.

3. Interaction with the audience: Are there methods and ideas from lab spectroscopic imaging that would be interesting to investigate with remote sensing? Sample data sets can be made available after the conference for future exploration.

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RANDOMISED SIMPLISMA: USING A DICTIONARY OF INITIAL ESTIMATES FOR SPECTRAL UNMIXING IN THE FRAMEWORK OF HYPERSPECTRAL IMAGING

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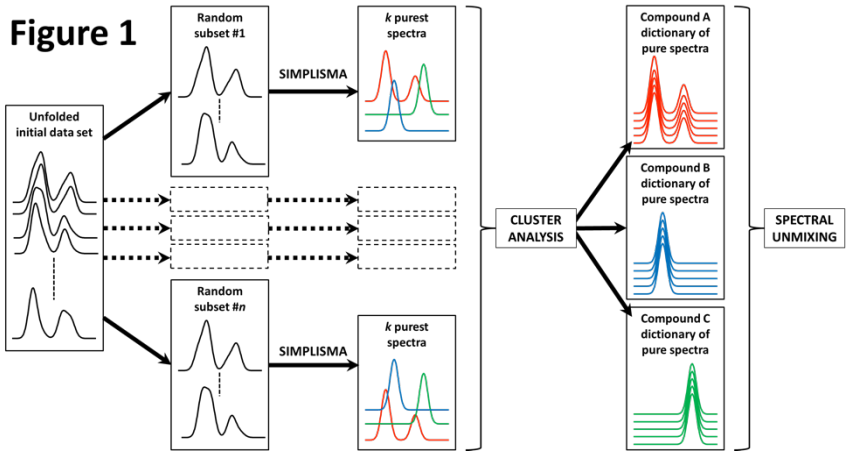
Spectral unmixing is probably one of the most important tasks for the exploration of hyperspectral data sets. Indeed it allows us to extract with no *a priori* pure spectra of all compounds present in the data cube and associated chemical maps. In other words, it is possible to identify molecules and observe their distributions over the sample region of interest without prior knowledge about the underlying physico-chemical system. In chermometrics, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) [1] is certainly the most used spectral unmixing algorithm. The reason for this is that it is a powerful and very flexible algorithm, adaptable to many different kinds of instrumental techniques, data structures and physico-chemical scenarios even beyond spectroscopic imaging. The linear mixing model is the basis of MCR-ALS driven by the assumption that a spectrum of a pixel is a linear combination of the pure spectra of all compounds present in the data set. Naturally, each pure compound of the data set is represented by a unique spectrum considering this model. Even if this assumption allows us to extract interesting information about the chemical systems, it does not take into account a possible spectral variability of each pure compound related to the changing of illumination conditions, variation of surface roughness, scattering effects or even intrinsic variability of the materials. Like most unmixing algorithms, MCR-ALS needs initial estimates of all pure compounds in the system which are refined afterwards. Given the rank of the data set, the well-known SIMPLISMA (SIMPLe-to-use Interactive Self Modeling Analysis) algorithm is often used to extract a pure spectrum for each compound of the sample [2]. In order to address the problem of spectral variability, we propose to build a dictionary of initial estimates for each pure compound using a new methodology called 'Randomised SIMPLISMA' (Figure 1). Thus SIMPLISMA is run several times on randomly subsets of the initial data cube. As a consequence, new instances of pure spectra should be extracted for each subset. Because extracted spectra suffer from a permutation problem i.e. pure spectra are not necessary ordered in the same way considering different subsets, a clustering step is finally used in order to group the candidate pure spectra into classes. In this way, each class becomes a dictionary of initial estimates for each compound. In this presentation, we will first show how we can use a dictionary of initial estimates in the final task of spectral unmixing. Considering synthetic and real spectroscopic data sets, it will be also possible to highlight interesting properties of the concept such as the generation of better quality / more representative initial estimates and potentially a better evaluation of the chemical rank which should finally lead to better extractions.

Figure 1



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REAL TIME ON LINE DETECTION OF NON PRODUCT RELATED MATERIALS (NPRM) USING HYPERSPECTRAL IMAGING AND ADVANCED DATA PROCESSING ALGORITHMS

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This study describes a method and apparatus for the real time detection and removal of Non-Product Related Materials (NPRM) during industrial processing utilizing hyperspectral imaging and analysis. Current detection method utilizes humans, optical scanners, metal detectors etc. The effectiveness of the current system can be improved. Thus we investigated the feasibility of using hyperspectral imaging system in tobacco processing to identify and remove non-tobacco materials like foam, plastic, cardboard, etc. which sometimes get into the bales of tobacco purchased from the farmers. The imaging system consists of a hyperspectral imager operating in the near-infrared region (900-1700 nm), a tungsten halogen light source to illuminate the material and a high speed computer processing unit. The hyperspectral imaging system continuously scans the sample on the conveyer belt and captures the spectral image of the sample which contains tobacco being processed and may contain impurities like foam, cardboard, plastic etc. The system works under the principle that there are subtle differences in the spectral fingerprints of different materials. We have developed an intelligent machine learning algorithm (Support Vector Machine) to identify these differences and separate the impurities or undesirable materials from the sample flow in real time on the conveyer belt. For NPRMs with size greater than 0.25 inch, the accuracy of this method is 99%. Since detection of NPRM is completely automatic, it eliminates the human subjectivity and error. This technique could also be applied to the classification and identification of common contaminants in any industrial processing system and could be used in a wide variety of industrial applications like food processing, agricultural systems, as well as other manufacturing environments.

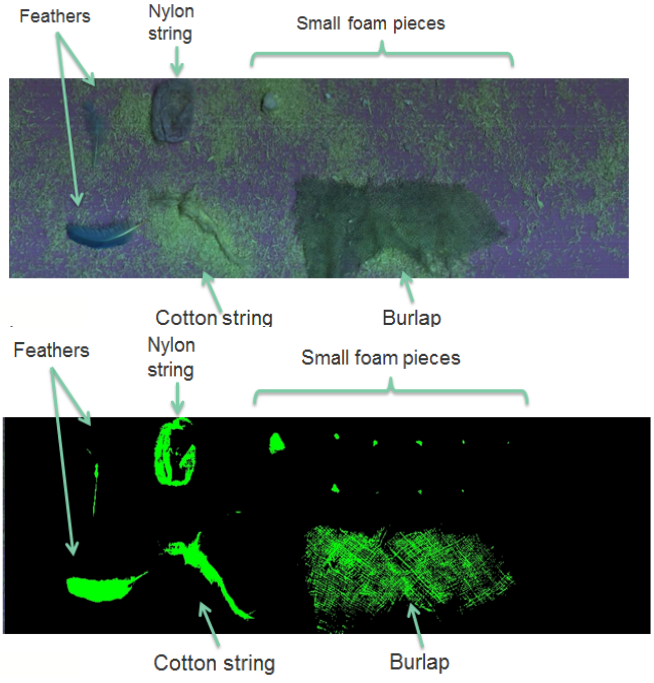

Figure 1. Comparison of RGB representation of input hyperspectral image (top) and output classification image (bottom), showing NPRMs in green

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NEAR INFRARED HYPERSPECTRAL IMAGING: A RAPID METHOD FOR THE DIFFERENTIATION OF MAIZE EAR ROT PATHOGENS ON GROWTH MEDIA

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Maize is known to be highly susceptible to the mycotoxin-producing fungi belonging to the *Fusarium* and *Stenocarpella* genus. Not only do these fungi cause destruction of the plant, they also produces harmful secondary metabolites. *Fusarium verticillioides* produces fumonisin that has been associated with oesophageal cancer in humans and rats, as well as leukoencephalomalacia in horses and pulmonary edema in pigs [1]. *Fusarium boothii* is the leading cause of Gibberella ear rot in maize ears, while *F. graminearum* s.s. was found to be the most abundant *Fusarium graminearum* species complex (FGSC) species on maize ears undergoing crop rotation with wheat. Deoxynivalenol (DON) is one of the main toxins produced by the FGSC and has been found to suppress the immune system and cause nausea and vomiting in humans. *Stenocarpella maydis* affects the yield and quality of maize and is known to produce the diplonin toxin. The current methods employed for the enumeration and identification of these pathogens include both molecular and immunological detection such as the polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) [2]. These multi-step methods are known to be tedious and time-consuming. Furthermore, specialised growth media, supplementary staining and microscopy may also be required, which extends the identification timeframe [3]. Thus, there is a need for rapid methods. The aim of this study was to differentiate between the major maize ear rot pathogens on growth media and to determining the influence of the growth age on the classification accuracy.

Eight maize ear rot pathogen isolates comprising of *Fusarium verticillioides* [MRC826, MRC 8267 and MRC8559], *F. graminearum* s.s. [M14-55], *F. boothii* [M0002, M0010 and M0100] and *Stenocarpella maydis* [SM8] were plated on potato dextrose agar in glass petri dishes, in triplicate, and incubated at 25 °C for 3, 5 and 7 days. The plates were imaged with a SisuChema short wave infrared camera, in the range 1000 to 2500 nm. Principal component analysis (PCA) was used for data exploration, after which object wise classification models were calculated with partial least squares discriminant analysis (PLS-DA). All data were treated with standard normal variate. Models were calibrated and optimised with full cross-validation, thereafter validated with independent images.

The overall classification accuracy of the PLS-DA models showed that day 5 was the best day to distinguish all the pathogens effectively. However, day 3 could potentially be used to distinguish the three major ear rot isolates, *F. verticillioides* MRC826, *F. graminearum* s.s. M14-55 and *S. maydi*s SM8, with 100% accuracy. Classification of the three *F. verticillioides* isolates on day 3 also achieved a 97% accuracy. The F1 score, i.e. the weighted mean between the predictive power of a model and the effectiveness of the model on a class basis, also demonstrated that pathogen isolates were accurately classified on day 5. NIR hyperspectral imaging can thus accurately distinguish between the major maize ear rot pathogens on day 3 or 5 depending on the pathogen, thus proving that the growth age does influence the accuracy for the differentiation of fungal pathogens.

Acknowledgement: This work is based on the research supported in part by the National Research Foundation of South Africa, Unique Grant No. 94031; the authors express gratitude to Prof Viljoen and Dr Vermaak (Tshwane University of Technology, Pretoria) for the use of their hyperspectral imaging system.

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RESOURCES FOR MULTIVARIATE ANALYSIS OF MULTICHANNEL IMAGING DATA

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Developments in Chemical Imaging, also referred to as Mass Spectrometry Imaging or Imaging Mass Spectrometry, have resulted in the generation of very large and typically complicated data sets from a wide variety of samples. Typical data sets can easily reach sizes of a few to over one hundred gigabytes. In order to process these data sets and extract information relevant to the given experiment many advanced data analysis routines have been explored and developed. Over the past decades this has resulted in the application of an alphabet soup of multivariate analysis (MVA) methods to chemical imaging data. Though there have been significant developments in the application of multivariate methods, there is still a need for educational materials to aid in helping users know when, why, and how to apply various multivariate analysis methods to chemical imaging data. There is also a need for easy to use, readily accessible tools that allow the analyst to apply these tools.

In order to address these needs, the National ESCA and Surface Analysis Center for Biomedical Problems (NESCAC/BIO) has established a web resource for multivariate surface analysis: The Multivariate Analysis Website (https://www.nb.uw.edu/mvsa/multivariate-surface-analysis-homepage). This website provides tutorials, references, links and software focused on multivariate analysis of ToF-SIMS data, however the information and software is applicable to any multichannel imaging data that is properly formatted. One important part of this website is providing free access to software tools that can be used to run various MVA methods on chemical imaging data. In this presentation, I will highlight some of the user friendly Matlab graphical user interfaces developed by NESAC/BIO (the NBToolbox https://www.nb.uw.edu/mvsa/nbtoolbox) for multivariate analysis of ToF-SIMS images (2D and 3D) and spectra. Examples will be provided for principal component analysis (PCA), maximum auto-correlation factors (MAF), multivariate curve resolution (MCR) and various tools for displaying image data and MVA results. Additional tools include functions for RGB overlays, region of interest extraction, image alignment, image filtering, image dicing, and other image analysis methods.

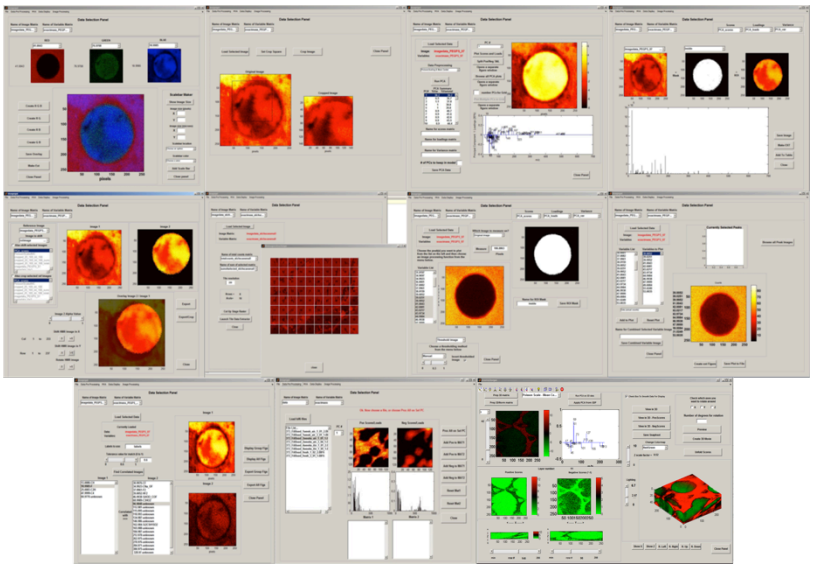

Figure 3 Screenshots of NBToolbox Image Analysis GUIs.

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HYPERSPECTRAL IMAGING ANALYSIS OF THE DRYING PROCESS OF EARLY- AND LATEWOOD

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The process of drying of a wood sample has been studied by a time series of hyperspectral images in the VNIR region (400 – 1000 nm) distributed over 159 bands. A total of 150 image cubes were obtained during a period of 21 hours. The data set was analysed through a four stage modelling procedure: 1) the measured intensity spectra were normalized by transformation into reflectance and linearized into apparent absorbance units; 2) the absorbance spectra were submitted to a simplified causal modelling of known phenomena by Extended Multiplicative Signal Correction (EMSC); 3) the high-dimensional stream of lack-of-fit residual spectra from EMSC were analysed for possible remaining systematic structures, e.g. due to unknown and hence un-modelled variation types by an adaptive bilinear modelling method; and 4) the dynamics of the various known and unknown physical and chemical variations of the drying process were assessed from their temporal developments. The concepts of the analysis with respect to known and unknown phenomena have been thoroughly described in [1]. In the present work the focus will be on the understanding of the wood properties based on these analysis, and in particular the different behaviour of early- and latewood, i.e., the growth rings in a tress due to various growth behaviour in spring/summer and autumn/winter as described in Fig. 1.

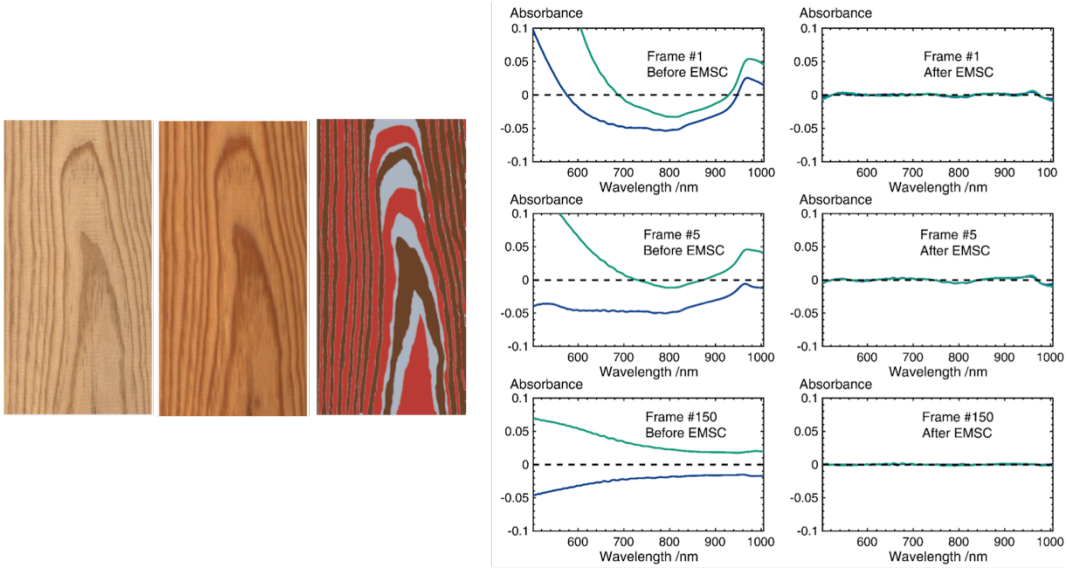

Figure 1: Left panel: From left to right, RGB image of the dry and wet sample, automatic segmentation of earlywood (red), latewood (brown) and intermediate parts that were left out of the analysis (grey). Right panel: Absorbance spectra before and after EMSC pre-processing and subtraction of reference spectrum of earlywood (blue) and latewood (green) of wet (frame #1), medium dry (frame #5) and dry (frame #150).

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HYPERSPECTRAL DARK-FIELD MICROSCOPY OF HEMATOXYLIN - EOSIN STAINED PROSTATE HUMAN TISSUE

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Dark field microscopy combined with hyperspectral imaging (HSI) is a novel optical approach that can be applied for optical material characterization down to micro- and nanometer scales. This optical modality based on acquiring the scattered light from an individual structure eliminating the background noise and yields a detailed map of surface morphology using elastic scattering spectrum for each pixel. However, relying on the transmitted or reflected light illumination leads to a limitation in optical resolution, diminished in signal-to-noise ratios, and out of focus issues.[1] This approach has great potential in bio-related disciplines due to its high contrast that opens the opportunity for identification, quantitative determination and analysis of single cells up to complex biological systems. [2, 3] Hyperspectral dark field analysis has been used in this work to investigate the manner in which the Hematoxylin - Eosin (H&E) staining interacts with prostate tissue. Dark field hyperspectral imaging was used in the visible-very near infrared range (400-1000 nm) with spectral acquisition time of 0.01 sec and 100X magnification to scan a 60x60 μm region of stained prostate tissue. Visible light delivered to biological tissue undergoes multiple scattering due to inhomogeneity of biological structures and absorption primarily in epithelial cells, fibroblast cells, sytoblasm, and blood cells as it propagates through the tissue (as shown in Fig 1) ,where a false RGB image is built using the three wavelengths bands (Blue band at 470 nm, Green band at 550 nm, and Red band at 620 nm). Initial results indicate that HSI dark fielSd microscopy provides the potential to assist, substitute, and validate traditional immunohistochemistry imaging technologies.

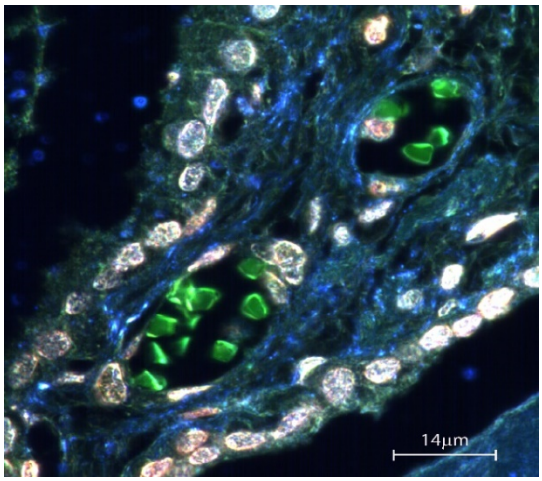

Figure 4 Dark field HSI shows a layer of epithelial cells, fibroblast (bipolar bright white), cytoplasm (blue), and blood cells (green) of H&E stained prostate tissue

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POTENTIAL OF FLUORESCENCE IMAGING AND FLUORESCENCE-RAMAN IMAGE FUSION FOR THE ANALYSIS OF VEGETAL TISSUES.

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Fluorescence imaging is a simple and highly suitable imaging technique for samples labelled with fluorophores or containing natural fluorophores in their composition, such as vegetal tissues. Classically, fluorescence imaging was solely regarded as a highly valuable visualization technique due to the high sub micrometer resolution, able to label different cellular components. For this reason, samples were usually mapped using one or few wavelength channels (typically related to one or few labelling fluorophores).

Fluorescence imaging systems have improved their versatility and working in hyperspectral mode is possible. Current systems, now equipped with white lasers, allow scanning a range of emission wavelengths or a range of excitation wavelengths, provides images formed by emission spectra or excitation spectra, respectively. More importantly, both excitation and emission ranges can be scanned simultaneously and images with a full excitation-emission (EEM) 2D landscape per pixel can be obtained. These instrumental advances allow a greater power of differentiation among fluorophores by using suitable unmixing algorithms. Thus, different data configurations and models adapted to the nature of the spectroscopic measurement acquired, e.g., bilinear models for images based on a single excitation or emission spectra per pixel or trilinear models when an EEM landscape per pixel is acquired, can be used [1].

Furthermore, fluorescence-Raman image fusion causes an additional benefit, since fluorescent and non-fluorescent compounds can be modelled simultaneously. In this particular scenario, unmixing algorithms such as Multivariate Curve Resolution-Alternating Least Squares, MCR-ALS, can work with multisets extended in the spectral direction [2,3]. To model the extended spectral mode, a hybrid bilinear/trilinear model within each spectral profile is proposed, suitable for the characteristics of each of the imaging techniques coupled.

The power of fluorescence imaging and fluorescence-Raman image fusion is shown through the analysis of real images of rice leaves.

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DEVELOPMENT OF A CLASSIFICATION ALGORITHM FOR AN EFFICIENT RECOVERY OF PLASTIC WASTE BASED ON NIR-HYPERSPECTRAL IMAGING

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Nowadays, the majority of the objects used in our daily life are made of plastic materials, such as electronic devices, shopping bags or food packaging just to cite some examples. As a consequence, an impressive amount of plastic waste is produced worldwide every year. Most of such waste is disposed in landfills or incinerators, but a considerable amount of plastics is dispersed in lands, waterways or oceans, causing severe risks for the environment. For a more sustainable use of plastics, it is therefore mandatory to identify alternative strategies for an efficient and eco-friendly plastic waste management. In this context, recycling is an increasingly used strategy, which allows to convert plastic waste into new objects and, at the same time, to reduce the consumption of virgin raw materials [1]. However, plastics include a wide range of polymers with different chemical composition and mechanical properties. Therefore, the quality of the products derived from recycled plastics strongly depends on the sorting procedure, which consists in the correct separation of the different polymers.

Recently, Near Infrared HyperSpectral Imaging (NIR-HSI) systems are increasingly used for automated plastic sorting, thanks to the possibility of characterizing the different polymers according to their chemical composition [2, 3]. However, in practical applications a key issue is represented by the implementation of fast and effective algorithms able to minimize as much possible the sorting errors.

In this context, the aim of this study is the development of a tree-structured classification model for the classification of the different plastic polymers commonly used in packaging. To this purpose, hyperspectral images of household plastic waste samples made of recyclable polymers have been acquired in the 1330-1900 nm spectral range. Furthermore, samples composed of paper and of not recyclable plastic polymers have also been considered as potential foreign materials commonly found in plastic waste. For classification purposes, an *ad-hoc* modified version of Partial Least Squares Discriminant Analysis (PLS-DA), namely Soft PLS-DA, has been used, allowing to perform at the same time classification and identification of outlier objects. In addition, the Soft PLS-DA algorithm has been coupled with a sparse-based variable selection approach to identify the relevant variables involved in the classification [4].

In order to evaluate the effectiveness of the proposed approach, the tree-structured classification model has been validated both using a test set composed of representative spectra of each material for a quantitative assessment, and at the pixel level on the hyperspectral images for a qualitative evaluation.

Acknowledgement: The authors wish to thank MUSA S.r.l. for acquiring the household waste samples and for technical support.

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X-RAY FLUORESCENCE IMAGING: A NEW TOOL FOR STUDYING CULTURAL HERITAGE OBJECTS

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Investigations of cultural heritage objects require universal and profound research techniques, in order to take into account the individual character of each work of art under study. Therefore imaging methods based on various spectroscopic techniques are an intensively developing field. Among them macro-XRF methods are still growing in popularity as a tool for studying elemental distribution. Large data sets obtained in this way combined with multivariate data analysis approach open up new possibilities in nondestructive investigation of artworks.

In this work we present results obtained using a newly developed full-field XRF imaging system, dedicated to investigations of works of art. Compared to scanning macro-XRF systems, it offers a shorter measurement time and greater safety during measurement. XRF photons from a 10 x 10 cm area are simultaneously projected on the Gas Electron Multiplier (GEM) detector [1,2] through a pinhole camera. The pinhole camera is characterized by infinite depth of field which allows one to investigate non-flat surfaces, which is not possible using any other currently used system. Particular emphasis has been placed on the application of the multivariate data analysis methods, especially various decomposition algorithms. Such approach helps to understand collected data and is aimed at performing fast and automatic identification of pigments used by the artist. It allows quick separation of data carrying relevant information from those containing noise or faulty pixels in the detector. The measurements were performed on several painting phantoms with different pigment compositions, prepared to test the system's ability to solve typical research problems related to works of art.

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APPLICATION OF NEAR INFRARED HYPERSPECTRAL IMAGING SPECTROSCOPY FOR THE ANALYSIS OF COCOA BEANS

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Cocoa is the fruit of cacao (Theobroma cacao), a species native from South America, especially from the Amazonian basin. Cocoa beans are the most important ingredient of all chocolate products.

The potential for valorisation of cocoa beans results from the combination of the cultivated cocoa variety, the cropping technique, as well as the soil and climate conditions in the region of production. Furthermore, the expression of cocoa flavor relies mainly on the post-harvest processing of the beans, which leads to the production of marketable cocoa. The main cocoa bean processing steps are: pod breaking, fermentation and drying. However, these processing steps are not necessarily all applied by the producer or they are applied using different techniques (e.g.: sun-drying vs shadow drying) or different processing time (e.g.: drying time or fermentation time). All these production parameters lead to different bean qualities available for chocolate production. According to the ICCO (International Cocoa Organization), there is a strong need for developing fast and non-destructive analytical tools allowing assessing the quality parameters and the origin of cocoa beans and their potential for production of “fine” chocolate.

According to the literature, different studies have proved the performance of the use of vibrational spectroscopy techniques for, among others, the discrimination of cacao beans according to fermentation level or variety.

The aim of this work is to prove that Near Infrared Hyperspectral Imaging can be used as a fast and reliable technique for the analysis of whole cocoa beans from the Amazon basin in Brazil in order to distinguish them according to the different parameters of post-harvest treatments namely the humidity, the drying process and the fermentation.

Acknowledgement:
This study has been conducted in the framework of the PhotonFruit project “Emergent Spectroscopic Techniques for the Quality Control and Traceability of Fruits and Fruits Based Products” (WBI/CAPES agreement, project n°3) and the program Brazilian “Ciência sem fronteiras”. This research is also funded in the framework of the project BELCOBRA "Optimisation and Application of Analytical Methods for the Traceability and the Authentication of Cocoa and Chocolate" (WBI/CAPES agreement).

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PORTABLE LWIR HYPERSPECTRAL IMAGER BASED ON MEMS FABRY-PEROT INTERFEROMETER AND BROADBAND MICROBOLOMETRIC DETECTOR ARRAY

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The fast growing consumer electronics market of connected and wearable devices is driving a wealth of new applications. Personal capability for detecting and monitoring substances part of our everyday life (food, cosmetics, drugs, etc.) by spectroscopic means will soon become a reality as a number of new miniature spectrometers are being reported. These devices mostly operate in the visible and near infrared spectral region due to the readily available low-cost detectors in these spectral regions. However, enhanced selectivity is achievable in the molecular fingerprint spectral region (7-20 µm), allowing for applications that would be difficult or impossible at lower spectral wavelengths. To this end, a compact, portable, Long-Wave Infrared (LWIR) hyperspectral imager was developed. It is based on INO's MICROXCAM-384 camera featuring a 384 x 288 pixel, 35 µm pitch uncooled bolometric broadband Focal Plane Array (FPA) and Fraunhofer ENAS' 2 mm x 2 mm aperture MEMS tunable Fabry-Pérot Interferometer (FPI). The INO's broadband FPA exhibits a Noise Equivalent Temperature Difference (NETD) lower than 25 mK (for the 8-12 µm range at 300 K, 50 fps and f/1) and a flat spectral response from 3 to 14 µm. The footprint of the hyperspectral imager is 7 cm x 8 cm x 10 cm excluding the source. The spectral resolution varies from 55 to 220 nm depending on the type of FPI used. The Noise Equivalent Spectral Radiance (NESR) is 430 mW/(m2.sr.µm) at 9 µm. Using this hyperspectral imager, spectra of various substances including polymers were recorded in the transmission, reflection and transflectance configurations. A good agreement was found with spectra obtained by applying the FPI transfer function to spectra recorded with a commercial FTIR spectrometer. The LWIR configuration of the imaging spectrometer will be described and test results presented.

Keywords: Hyperspectral imaging, Mid-IR imaging, microbolometer, MEMS Fabry-Pérot, nondestructive testing, chemical compounds analysis, spectroscopy

100-word text summary: This paper reports on the development of a compact, portable LWIR hyperspectral imager based on a MEMS Fabry-Perot interferometer and INO's microXCAM-384 equipped with a broadband 384x288 pixel, 35 µm -pitch uncooled bolometric detector. The broadband responsivity of the latter allows for the recording of spectra in the 3 to 14 µm wavelength region with uniform detectivity. Spectra of various polymers and substances were recorded in transmittance, reflectance or ATR modes. A good agreement was found with those simulated by applying the FPI sampling function to spectra recorded with a commercial FTIR. The LWIR imaging spectrometer will be described and its applications presented.
https://www.ino.ca/en/products/hyperspectral-camera-microxcam-384i-hs/

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WAVELET COEFFICIENTS AS ROBUST FEATURES FOR ANALYSIS IN HYPERSPECTRAL IMAGES

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In a measured spectrum signals of different origins are superimposed. In addition, the spectrum in hyperspectral images changes depending on the viewing angle of the surface of a sample and its scattering properties. Therefore, the absolute value of a spectral band often can not be used directly, and data preprocessing methods are required for further analysis.

The presented approach of a pre-processing and feature extraction method has the idea to decompose a measured spectrum into its elastic and inelastic scattering parameters. Elastic scattering effects caused by particle size and micro structures can be described by Mie or Rayleigh scattering. Elastic scattering effects can therefore be approximated by a smooth polynomial contribution in the spectrum. Inelastic scattering or, more accurately, absorption bands can be approximated by a bell shaped function like a Gaussian. For the separation of the two processes different methods of chemometrics and signal processing are combined.

The core of the method is a wavelet transformation of the spectral data to describe the absorption bands by wavelet coefficients, which are called features in the following. A Spectral analysis based on these features is comparable to the use a combination of optical bandpass filters. Thus, the features determined can also be used to make quantitative statements about existing ingredients in a sample. By relative consideration of the features, multiplicative influences in the spectral signature can also be reduced.

The presented method automatically leads to a dimensional reduction of approx. 90%. A great advantage is that even with the use of different sensors usually identical features are determined. This allows a simple transfer of analysis models as opposed to methods based on, for example, the score values of a principal component analysis.

On the basis of a laboratory experiment, the presented pre-processing and feature extraction method is explained in more detail. For this purpose, powdery samples with different granularity are compared. The experiment shows that nearly identical features can be obtained for samples of the same substance despite different scattering properties.
In addition, a one-class classifier for the detection of impurities in herbs by pyrrolizidine alkaloids is shown. Using selected features, possible changes in NH-absorption can be observed. This can e.g. be used in a sorting system for bulk materials.

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IDENTIFICATION OF *CAMPYLOBACTER* WITH HYPERSPECTRAL MICROSCOPE IMAGING

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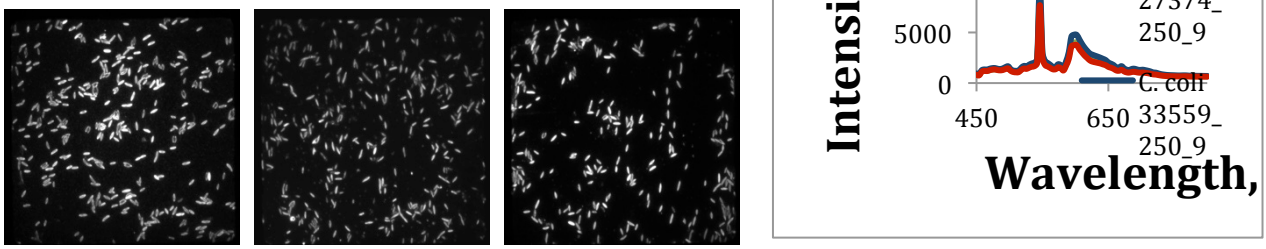
Keywords: Food safety, Rapid detection, Campylobacter, Poultry, Label-free

Campylobacter is the major group of bacteria responsible for foodborne gastroenteritis in humans worldwide. Most of the cases of *campylobacteriosis* have revealed their poultry origins so that various detection methods have been employed from the farm to processing levels. Especially *Campylobacter jejuni* is the most common cause of bacterial foodborne illness in the United States. The Center for Disease Control and Prevention (CDC) estimates that *Campylobacter* causes approximately 845,000 illnesses in the US each year. Specifically, over 6,000 cases of *Campylobacter* infection were reported in 2009. Also, European Food Safety Authority (EFSA) estimates the cost of *campylobacteriosis* in the EU to be approximately EUR 2.4 billion per year so that food safety is focused on controlling *Campylobacter* infections. Current methods for isolating and detecting *Campylobacter* from foods are culture-based techniques using several selective agars designed to isolate *Campylobacter* colonies. In addition, several immunological and molecular techniques are commercially available for the detection and identification of *Campylobacter*. However, most significant drawbacks of current methods are laborious for process, time-consuming for results, and expensive to use. Recently, hyperspectral microscope imaging (HMI) has demonstrated the potential to differentiate gram-positive from gram-negative bacteria with high accuracy (>99%) and also identified *Salmonella* serotypes (>95%) as well as *Staphylococcus* (>94%). Herein we expand HMI methods we developed to identify *Campylobacter* serovars. The objective of this research is to develop rapid methods to identify and detect *Campylobacter* serotypes with hyperspectral microscope imaging using scattering intensity from live bacterial cells.



(a) (b) (c) (d)

Figure 1. Hyperspectral microscope images (1000x) and their spectra from three *Campylobacter* serotypes (a) *C. coli*. (b) *C. jejuni* (c) *C. fetus* and (d) spectra at 250 ms and 3.5% gain

Acknowledgement: The authors thank Dr. Nasreen Bano, QSARU of USDA, ARS for bacterial sample preparation for the research.

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Hyperspectral Imaging of Minerals in the LWIR: The Use of Laboratory Reference Measurements for Field Exploration.

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Hyperspectral Imaging has evolved in the last few decades as a method for remote detection and identification of earthen materials. Hyperspectral imaging identifies the spectral signatures within an individual pixel, utilizing the reflected / emitted energy of one or multiple materials and comparing these to a spectral library. The Telops Hyper-Cam LW (longwave series) is an infrared hyperspectral imaging (HSI) spectrometer that is configured to collect data in the spectral range 7.7 to 11.8 μm (1300-850 cm^{-1}). We recently conducted a field experiment using the Hyper-Cam to collect data of 24 minerals and background materials to determine how well it is able to detect and distinguish the pure minerals and mineral mixtures remotely with varying angles (25°, 35°, and 45° relative to the ground), temperatures and diurnal effects. Prior to performing the field experiment, the reflectance spectra of the 24 samples were measured in the mid-infrared using a Fourier transform infrared spectrometer (FTIR) equipped with a gold coated integrating sphere; these spectra were used as data in the reference library. To investigate the solar illumination angle as well as observe effects on detection for the angle relative to both the sample and the sun, a plywood frame with sample holders was built that could be tilted to vary the observation angles. Statistical methods such as classical least squares (CLS) and multivariate curve resolution (MCR) were applied to the data to aid in discriminating the pure minerals and mineral mixtures from one another.

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Hyperspectral Imaging and Analysis of Plant and Geological Materials using Multiple Spectroscopies (Cameras)

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Hyperspectral imaging can provide detailed chemical information for each pixel within an image and therefore can be an excellent tool for a variety of research and screening applications. The success of these applications will be dependent on the type of spectroscopy specifically used. In addition, some applications may be improved by the use of multiple spectroscopies/cameras consisting of independent information. Also, during the discovery phase it may be important to test multiple spectroscopies to understand the best camera platform. We have developed a multi-camera imaging platform consisting of 5 different spectroscopies (VNIR, SWIR, MWIR, LWIR and Fluorescence). The sample is interrogated while being conveyed through the platform where suitable light sources illuminate the sample for the various cameras in a push-broom collection modality. We have found this technique to provide valuable information when looking at plant material and geological mineral deposits (drill core samples). Figure 1 shows the hyperspectral imaging results from a corn plant using three different spectroscopies. The analysis of these images used an internally developed software package (KemoQuant™) which allows for the co-localization of pixels from the various different spectroscopies and provides side-by-side comparison and interrogation of up to 5 different spectral images. There are 3 main multivariate analysis tools available in KemoQuant: Multivariate Curve Resolution (MCR) [1], Classical Least Squares (CLS) [2] and Principal Component Analysis (PCA). Our fast and effecient MCR algorithms can produce realistic and meaningful spectral components and intensities due to the non-negativity constraints placed upon the alternating least squares algorithms. Also, MCR can be a powerful technique when dealing with unknown biological and geological samples because it can provide quantitative analysis of the image data without the need for standards, and it can discover all independently varying species present in an image, even those with no *a priori* information. MCR is our main work horse when extracting spectral components from images where the spectral components are unkown. Once the spectral components are determined by MCR or the spectral components are be obtained through pure material, CLS can be used to rapidly produce quantitative images. In this presentation we will demonstrate this technology and the analysis approaches using plant and geological mineral samples.

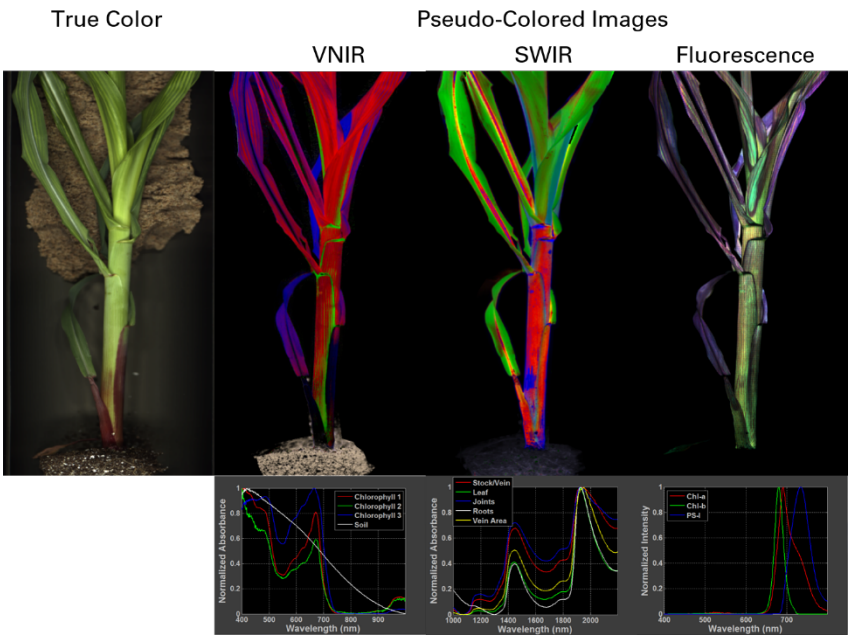

Figure 5 – Hyperspectral imaging of a Corn plant. True-colored image in comparison with reconstructed images colored by MCR and CLS spectral components shown beneath each spectroscopy.

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RECONSTRUCTION OF EXPLOSIVE-CONTAMINATED, OVERLAPPED FINGERPRINTS VIA LASER-INDUCED BREAKDOWN SPECTROSCOPY

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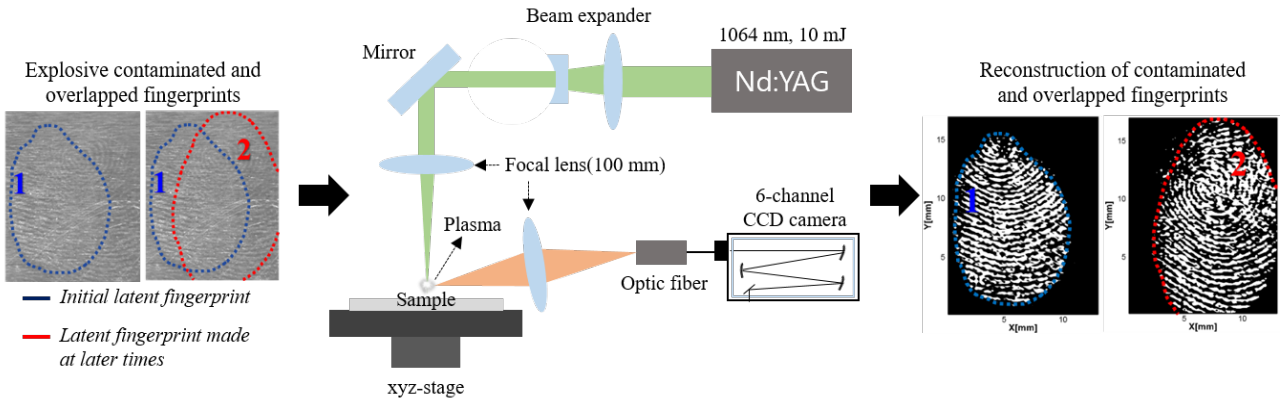
This research proposes the attempt to reconstruct explosive-contaminated, overlapped fingerprints using atomic spectroscopy as fingerprint at crime scenes can offer a viable clue in identifying a suspect. Laser-induced breakdown spectroscopy (LIBS) is an analysis for atomic emission which can be applied to investigate the chemical composition in forensic science [1]. In this research, a series of laser spectrum from explosive-contaminated fingerprint are investigated using LIBS. The study is concerned with the precise identifcation of the explosive content and at the same, the chemical reconstruction of two-dimensional image of each contaminated fingerprint. The chemometric methods are applied to the LIBS data to separate the overlapped fingerprints containing varying explosive traces.



Acknowledgement: This work was financially supported by grants from the Korea National Research Foundation (NRF-2014M1A3A3A02034903, NRF-2016R1D1A1A02937421) through IAAT at Seoul National University.

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KEY QUALITY PARAMETERS IN HYPERSPECTRAL CAMERAS

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Top level specifications for hyperspectral imaging systems (such as number of spatial pixels, number of spectral bands, F#, frame rate, weight and dimensions) may at first sight seem trivial to compare. However, as in the case of consumer cameras (in which the number of megapixels, F# and zoom range do not tell you very much about how well the system actually performs) the previously mentioned top level specifications are insufficient to thoroughly describe a hyperspectral system.

The lack of a common industry standard for measuring and documenting hyperspectral instruments, may thus often lead to requirement specifications which do not discriminate properly between instruments when it comes to the more subtle technical parameters that really count more than the top level specifications. In many cases, price therefore becomes the main discriminant and for an advanced scientific instrument, this may not always give the best decision from a user perspective.
We present an extensive set of performance parameters relating to the instrument itself that are not covered by a standard technical specification of a hyperspectral imaging system. We also discuss calibration and test procedures followed by examples of important operational and practical aspects e.i. the keystone effect and its influence on classification results.

The ambition with this talk is not to describe how system characterization should/must be done, but rather emphasize the importance of it and provide some useful insight and guidance, thus enabling a well-informed purchase decision and further usage of the hyperspectral imaging systems.

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INNOVATIVE SOLUTIONS FOR DATA ANALYSIS OF HYPERSPECTRAL DATACUBE ACQUIRED ON HISTORICAL PAINTED OBJECTS

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Visible hyperspectral imaging (HSI) is a fast and non-invasive imaging method that has been adapted by the field of conservation science to study painted surfaces [1]. By collecting reflectance spectra from a 2D surface, the resulting 3D hyperspectral data cube contains millions of recorded spectra. While processing such large amounts of spectra poses an analytical and computational challenge, it also opens new opportunities to apply powerful methods of multivariate analysis for data evaluation. With the intent of expanding current data treatment of hyperspectral datasets, and solving the problem of nonlinear unmixing of hyperspectral reflectance data acquired on painted works of art, two innovative data analysis approaches have been recently developed. First, a data driven approach for data reduction and visualization that uses a statistical embedding method known as t-distributed stochastic neighbor embedding (t-SNE) successfully provides a non-linear representation of spectral features in a lower 2D space [2]. Second, a nonlinear unmixing approach using overdetermined pigment dictionnary combined with supervised classification and two-constant Kubelka-Munk theory efficiently determines pigment concentrations within the pixel.

The efficiency and limitations of the proposed methods for painted surfaces from cultural heritage will be presented and discussed through the study of laboratory prepared paint mock-ups, and medieval French illuminated manuscript, part of the Isabella Gardner Museum collection (Boston, USA).

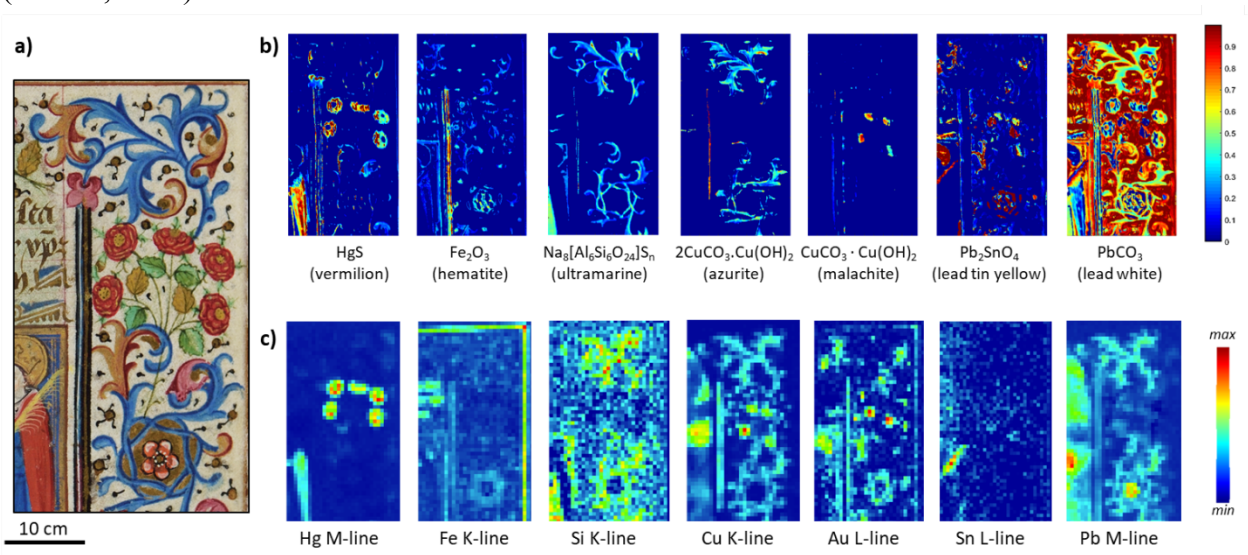

Figure 6: a) Visible picture of the area analyzed; b) Distribution maps of vermilion, hematite, ultramarine, azurite, malachite, lead tin yellow, lead white pigments extracted from hyperspectral datacube using second proposed approach; c) Elemental map distribution of mercury, iron, silicon, copper, gold, tin and lead from XRF scanning of the same area confirms pigment identification.

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MCR-ALS OF HYPERSPECTRAL IMAGES WITH SPATIO-SPECTRAL FUZZY CLUSTERING CONSTRAINT

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In recent years, ever-increasing attention has been paid to the resolution of hyperspectral images in the Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) [1] context; in fact, MCR-ALS has the advantage of providing both spectral and spatial information, so it can be used to visualize the spatial distribution of pure components belonging to a sample simply starting from their spectral signatures, but the range of constraints applicable to hyperspectral images is limited. Under this perspective, the aim of the present work is to exploit the spatial information obtained from Fuzzy Clustering, which was previously considered in the resolution of monochromatic images [2,3], in the MCR-ALS resolution of hyperspectral images. Consequently, the possibility of introducing it into the ALS routine has been studied: it was decided to define an algorithm that would allow calculating the membership function of each pixel of a hyperspectral image through a fuzzy clustering procedure that combines spatial and spectral information. This procedure defines a first probability for each pixel of belonging to the various clusters based on the spectral information only, then this probability is updated depending on the spatial distribution of the membership functions. in this way, the probability that a pixel belongs to a cluster will become higher if one or more of its neighbouring pixels are likely to belong to the same group.
In practice, the number of clusters is set to be equal to the number of MCR components and, at each iteration, the membership functions are calculated for each pixel based on the estimate of the concentration maps. These membership functions are then used as weights to calculate a new estimate of the concentration maps to be used in the next iteration of the ALS algorithm. The procedure is repeated until the algorithm converges. To verify the possibility of introducing Spatio-spectral Fuzzy Clustering as a constraint in the iterative MCR-ALS algorithm, this process was applied to two different samples, a HeLa cell sample and an oil-in-water emulsion, which both gave satisfactory results; in the first case, MCR-ALS together with spatio-spectral fuzzy clustering helped to resolve the shape of the cell more clearly than with only the non-negativity constraint, while in the second case it allowed to separate the interface component in two different contributions in accordance with the chemical composition of an emulsion with two surfactants.

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REDUCING COMPLEXITIES OF CULTURAL HERITAGE PRESERVATION THROUGH LINKED DATA ANALYSIS

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Keywords (3-5): cultural heritage, spectral imaging, spectroscopic, chemometrics, data fusion

Cultural heritage materials are often fragile, unique and have little documentation about their history and hence what is best for their preservation. Challenges of cultural heritage materials therefore necessitate a focus on non-invasive analaytical techniques with a large component of the investigation being a forensic-like approach to recreate the history of use, the impact of various environmental parameters and provenance. The results of these analyses are then used to determine degradation mechanisms and how best to preserve these historic items for future generations. Cultural heritage materials include a range of material types and go from antiquity to yesterday – historic and modern polymers, paper and parchment as susbtrates, inks and colorants and the media used to capture and record our history, photographic processes. Other collections include glass, metal, wood, ceramics, textiles, magnetic tape, wax cylinders and other sound and storage media. Paper and parchments are prepared and manufactured in many different ways with different sizings, trace metals that can become corrosive at specific levels in both papers and pigments, pigments having a range of binder materials, all impacting the way materials degrade and the decisions needed to stabilize and reduce loss of cultural information, and historically, all requiring sampling for accurate characterization and identification. The Preservation Research and Testing Division (PRTD) at the Library of Congress have created a scientific reference sample collection – the Center for Library Analytical Scientific Samples (CLASS) – with a collection of all the materials found in heritage items. These reference materials have been analyzed with a range of instrumentation and analytical techniques – both non-invasively and through destructive samples – to provide baseline data that can then be correlated with properties found in historic collection items, and with the destructive testing and accelerated aging, can then be used to predict longevity and the impact of various environmental conditions. In addition, often many rare collection items can only be in the research labs for short periods of time, so a *go-team* of staff has been created to efficiently collect a diverse range of complementary data from the specific collection item in that time. These portable and lab-based instruments include multispectral imaging (reflectance, transmitted and raking) as the baseline *mapping* of the object, Fourier Transform Infrared spectroscopy (FTIR) – diffuse and reflectance, X-Ray fluorescence spectroscopy (XRF) point and linescan, Fiber Optic Reflectance Spectropscopy (FORS), 3D fluorescence spectroscopy, Raman spectroscopy and other specific techniques that best relate to the material type, and micro-sampling with size exclusion chromatography (SEC) has been correlated to loss of mechanical properties to predict impact of treatments. As a further preservation component, extensive testing of volatile organic compounds (VOCs) with thermal desorption gas chromatography mass spectroscopy (TD-GCMS) to quantify and identify damaging emissions from building and storage materials, Detecting changes due to exposure of historic documents and objects to various environmental conditions, treatments and the assessment of changes during exhibition of light-sensitive materials is critical for preservation and monitoring deterioration or changes due to these conditions assists in understanding the degradation mechanisms. Multivariate image analysis, chemometrics and data fusion processing techniques have been used to analyse these datasets using Solo+MIA, ImageJ, Matlab and ENVI software. Examples of linked spectroscopic datasets will be discussed for a range of historic documents including 15th century Block books, unique historic comic book hero's, 21st century political cartoons, historic pigments and sound recordings.

Acknowledgement: PRTD staff and Library of Congress curatorial staff.

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HYPERSPECTRAL IMAGING AND CHEMOMETRICS. PAST, PRESENT AND… FUTURE?

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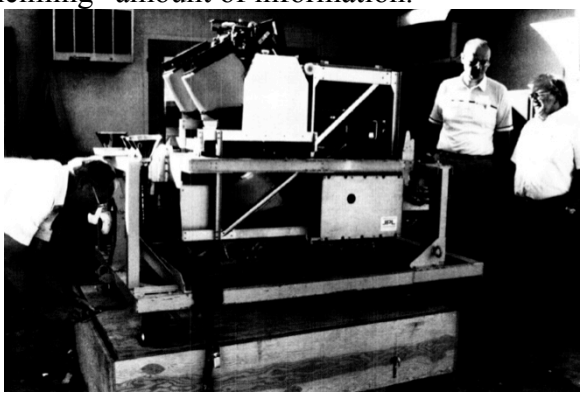
Being in the present, learning from the past, is the only way to reach a wonderful future.
This sentence is mine (even though is not that original). But it represents something that is especially relevant in hyperspectral imaging. We can find many papers published recently with the sentence "Hyperspectral imaging is a new methodology … bla bla bla… that together with new data analysis… bla bla bla…". Even in my own papers we can find it! As example, it is the first sentence that we can read in the first paper I published dealing with hyperspectral imaging [1].
Nothing could be further from the truth. Acknowledging that the (apparent) real explosion of hyperspectral imaging came when the first devices where implemented in the laboratory in the middle 90's, the reality is that the first manuscripts, or scientific reports published using multispectral or hyperspectral machinery together with advanced data analysis methods can be found in the late 70's / early 80's. Take a look, for instance, at the image in this abstract. It is taken from a report made by NASA of the AVIRIS multiband camera made in the early 80's. This camera was able to measure at 4 wavelengths (including NIR) and NASA started to generate algorithms able to handle that "overwhelming" amount of information.



In this presentation we will make a journey that will start in the past of hyperspectral imaging, where we will see the first applications and first algorithms employing hyperspectral machinery. The journey will pass by the main milestones and achievements in both machinery and algorithms, until we arrive to the start-of-the-art in hyperspectral imaging. Parental advisory: It is not a boring review. We will see and discuss many applications.
Then it comes the future. And there, I can already tell you the outcome. We will need faster and cheaper (especially cheaper) sensors, together with faster and more reliable algorithms able to handle more and more information. But the interesting point, and profiting that this is the last talk of the conference, I will encourage you to start an open debate to discuss what, how, where (and how much) we need from hyperspectral machinery and chemometrics in the future.

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MAPPING AND MONITORING METAL CONCENTRATIONS IN ALASKAN THERMOKARST LAKES

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Traditional water quality management practices rely on the spatial extrapolation of point samples, gathered through grab samples or instrumentation, to model the hydrochemistry of entire water bodies. Sampling excursions can be labor-intensive and are restricted to safely accessible locations within the waterbody. This multi-year project demonstrates the effectiveness of pairing point-samples, reflectance spectra, and hyperspectral aerial imagery to accurately map the concentration of metals (arsenic, barium, manganese, and iron) in thermokarst waterbodies in the North Slope Burrough, Alaska.

In 2014, a multi-year, multi-company monitoring protocol was established by Satelytics, SLR Consulting, and an unnamed client to map the above chemical constituents in thermokarst waterbodies. A Lake Sampling Device (LSD) was used to collect water samples, spectrometer data, and GPS points at various sampling locations. In 2014, a plane was flown concurrently with sampling to collect hyperspectral imagery. Spectra collected in the field were used to develop a classification of bottom types in addition to calibrating the aerial imagery used to map the given constituents. Multiple preliminary algorithms were developed from the calibrated aerial imagery for each metal to determine the influence of bottom type on the predicted metal concentration. A single algorithm for each metal was refined until an accuracy reflecting the laboratory accuracy was reached (parts per billion concentration). Each algorithm was applied to the calibrated hyperspectral aerial imagery to map the concentrations across all waterbodies in the region.

Subsequent sampling in the past three years has yielded an ever-growing database of metal concentrations, field-collected spectra, and bottom-type definitions. The field collected spectra are being resampled to model various satellites, including Landsat 8 and WorldView-3, and paired with the hydrochemistry from water sampled concurrently to develop appropriate algorithms for ongoing monitoring.

Results are presented to the client via satelytics.io, a web-based software-as-a-service. Each concentration map as well as natural-color imagery is available to users for use. The satelytics.io platform will be demonstrated as part of this presentation. Users can view concentrations for single-pixels (2-meter x 2-meter) as well as the distribution and mean values of a user-selected areas allowing decision makers to more accurately understand the complex geo- and hydrochemistry of monitored sites.

The use of single-point water samples, backpack spectrometer data, and aerial hyperspectral imagery has proven to be a solid foundation on which to build a fully remote monitoring regimen using satellite imagery of varying spectral resolutions. This lessens the risk for field personnel working in adverse and very remote locations while also increasing the spatial extent of concentration maps.

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COMPARING CAPABILITIES OF HYPER- AND MULTISPECTRAL IMAGING FOR MAIZE DEFECT CLASSIFICATION

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Introduction: White maize is a staple food in the diet of millions of South Africans. Maize is graded to ensure safety and quality, and to determine market price. Legislation bases grade allocation solely on the amount of defective material present in a maize sample, as determined by visual inspection. Routine industry applications of spectral imaging have the potential to overcome the high risk of human error in manual classification processes, such as in grading. The viability of using spectral imaging for maize grading by separating sound maize from the 13 defective materials classes has been investigated. Two main categories of spectral imaging are currently used in food research, namely hyperspectral imaging, where instruments are expensive and more suited to detailed research and laboratory applications, and multispectral imaging, which is simpler, and thus offers faster acquisition times and lower cost of the camera. The classification capabilities of both techniques have been compared in this study.

Materials and methods: Chemometric classification models were developed using 13 classes of naturally occurring defective kernels encountered at the commercial South African Grain Laboratory (SAGL), Pretoria. Images of the defective kernel classes and the sound maize class were acquired using a hyperspectral imaging instrument including a Xenics short wave infrared (SWIR) XEVA camera and an ImSpector N25E spectrograph in the range 1118 to 2425 nm (209 spectral points), and a VideometerLab2 multispectral instrument in the range 375 to 970 nm (19 spectral points). Object-wise principal component analysis (PCA) with was calculated in Evince v2.7.10. SNV and mean-centring were applied to all models, and Savitsky-Golay transformation (2nd order polynomial, 1st derivative, 15 points) was applied to the hyperspectral data only. An object-wise two-way PLS-DA model between each class and sound maize was calculated with full cross-validation. Objects were calculated using the average spectrum of each pixel in a maize kernel. PLS-DA prediction maps were generated.

Results and discussion: Of the 13 hyperspectral imaging based classification models, 8 achieved 100% classification accuracy, while the 5 remaining classes only predicted one kernel incorrectly per validation set of 60 kernels i.e. either only one false positive or negative (98%). The R^2 and Q^2 values were generally quite high, ranging 0.780 to 0.995 and 0.754 to 0.994, respectively. The prominent peaks included 1219 and 1476 (associated with starch), 1941 (associated with protein), and 2117 (associated with moisture). The use of the multispectral imaging instrument also lead to 8 out of 13 models achieving 100% classification accuracy, although the specific classes were not the same. However, the remaining 5 classes tended to perform poorly, with *Diplodia* fungal damage kernels encountering 6 misclassified kernels per validation set of 35 (83% classification accuracy). The R^2 and Q^2 values were again quite high, except the *Diplodia* damaged class (0.459 and 0.351, respectively). Wavelengths related to red, pink and blue carotenoid and anthocyanin pigments (505, 525, 570 and 590 nm), and NIR wavelengths 890, 940 nm (associated with fat) and 970 nm (associated with water) were identified as important.

Conclusion: Hyperspectral imaging achieved better classification overall, but was not rapid enough and too expensive for current on-line industry implementation. Multispectral imaging is simple and quick, ideal for implementation in industrial settings. However, the multispectral system available for this study was pre-built and did not incorporate any of the same wavebands as the hyperspectral instrument, and classifications with consistently high accuracy were not achieved. For improved results, it is recommended to investigate building a multispectral instrument according to the prominent wavelengths identified in the hyperspectral imaging research.

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IMAGE PROCESSING CONSTRAINTS IN MCR-ALS. NICE IMAGES, BETTER RESOLUTION?

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Hyperspectral imaging (HSI) is a tool used to create a visual image of chemical samples while simultaneously providing information about its chemical composition or properties. The basic unit is the chemical map, a grayscale image that carries intensity information of only a single wavelength, and its goal is to gain an understanding of the structures and processes of this sample by investigating these images for the different wavelengths. Dealing with complex mixture samples, different methods can be applied such as Multivariate Curve Resolution – Alternating Least Squares (MCR-ALS) [1] and with the recent proposal to adapt the MCR-ALS framework slightly for HSI [2], image processing algorithms can be introduced as constraints during the analysis. The approach allows a considerable additional flexibility during the resolution of the spectral problem by inputting information that links pixels together.

In this work, we present a range of different spatial constraints and show their influence on the obtained distribution maps and spectral profiles of the components present in the mixture. In general, the true spectra can hardly ever be uniquely obtained from real experimental data, but the chemical maps can give a good indication about the correctness of these profiles; a picture says more than a thousand words and this is no different for spectral decomposition of chemical mixtures. On the other hand, pushing the solution into a direction in which the chemical maps are more attractive and visually pleasing, the spectral profiles will not remain unchanged. We rationalize this approach on simulated data and show the results obtained on hyperspectral imaging covering Raman, near-infrared and infrared spectroscopy data in which spatial constraints prove to be advantageous for both decomposition directions.

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NEAR INFRARED, RAMAN AND FOURIER TRANSFORM HYPERSPECTRAL IMAGING TO STUDY DAIRY RESIDUES ON SURFACES

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Milk is a complex emulsion of fat and water with proteins (e.g. caseins and whey), vitamins, minerals and lactose dissolved within. Milk has an important role in the human diet and is consumed in liquid form or within dairy products [1].The purpose of this study is to investigate the interaction between surfaces used in dairy processing with milk products using hyperspectral imaging. A combination of Near Infrared (NIR), Fourier Transform Infrared (FT-IR) and Raman hyperspectral imaging were used to achieve this goal. From previous studies it is possible to predict the type of information that each technique can provide. Fat micelles affect light scattering in NIR spectroscopy [2]. A clear signal for fat and protein is evident using Raman spectroscopy [3] and FT-IR spectroscopy provides information on lactose [4].

In this study the Raman wavenumber range was 190-3900 cm^{-1} using an edge laser for excitation at 785 nm. The laser was, set to 5% power (power measured at source was 306 mW) and 5 s acquisition time was selected for each spectrum. Images were obtained using a 10 x 0.25 NA objective lens. FT-IR spectra were acquired over the range 475-7500 cm^{-1}. Each spectrum was a mean of 16 acquisitions with a total acquisition time of 5 s per spectrum in reflectance mode. An aperture size of 150 x 150 μm and step size 150 μm was used. NIR images were collected in reflectance over the range 880-1720 nm using a push-broom system (speed:20 mm/s, area pixel around 320 x 320 μm). Aluminium and Stainless steel types 304 and 316 were chosen as candidate surfaces due to their widespread use in food production [5]. In order to characterise the signal of fat and general proteins in dairy residues, real samples of dairy products exhibiting a range of these characteristics were selected. Spectra of dried samples of whole, skimmed, protein and butter milk were compared with the signal obtained from butter and whey protein. The spectroscopic information collected was not only affected by the chemical signal of the milk composition, but also by surface related signals, evident in baseline and multiplicative effects. The study and interpretation of these effects can be used to investigate the interaction between surfaces and dairy residues. In addition, the combination of the spectral information with spatial information can improve data interpretation in terms of characterising spatial variability in the selected surfaces.

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P01
THE EFFECT OF IMAGE PROCESSING CONSTRAINTS ON ROTATIONAL AMBIGUITY IN MULTIVARIATE CURVE RESOLUTION OF HYPERSPECTRAL IMAGES

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Hyperspectral imaging refers to the collection of a spectrum for each pixel in the image of scene. It is now being used in many different fields of industry and research; and the number of applications keeps growing. The main interest of hyperspectral imaging is that it combines spatial and spectral information about a sample or a scene. However, to extract this information, spectral unmixing of some kind has to be performed. Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is one of the techniques that can provide pure spectra and distribution maps of different components in the samples [1]. MCR-ALS is very useful and probably the most commonly used technique in chemistry for this purpose. However, as for any two-way factor analysis technique, it inherently suffers from non-uniqueness of the pure component decomposition, known as rotational ambiguity. Rotational ambiguity can be alleviated and sometimes totally avoided by a judicious use of the data structure or by incorporating well-suited constraints [2].

In this contribution, we aim to visualize and investigate the reduction effect of some image processing constraints on the extent of rotational ambiguity in MCR-ALS of hyperspectral imaging data [3, 4]. For this purpose, Borgen plots of three component simulated systems are used. Different spatial information, such as 2D-Gaussian fitting (hard-modeling), image segmentation and sparse image recovery are investigated. In each case, the extent of rotational ambiguity is evaluated. We discuss how these spatial constraints can reduce uncertainty and improve results accuracy. Hard-modeling, and to a certain extent sparse image fitting, can lead to unique solution, but their applications of spatial hard modeling constraint in real scenario can be questioned. Image segmentation, on the other hand, is more widely applicable, and its application result in less ambiguous solutions. Lastly, a couple of real examples such as remote sensing data sets and FTIR imaging are taken.

To recap: we visualize rotational ambiguity before and after applying constraints and discuss the importance of using proper and chemically meaningful constraints.

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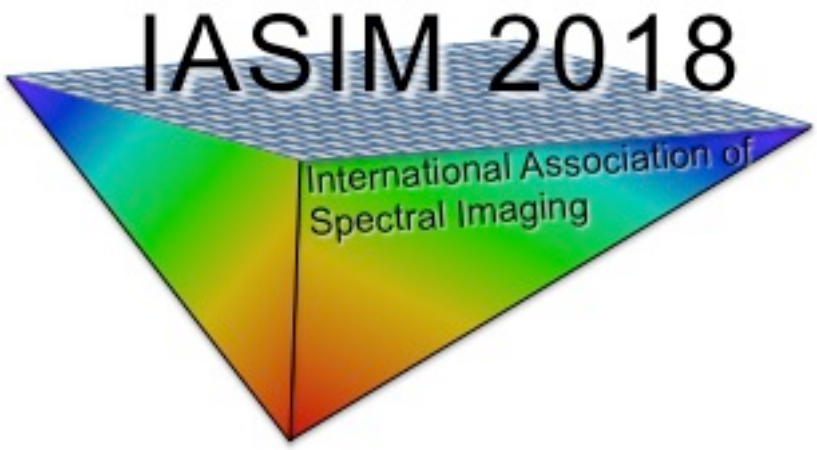

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P01
THE EFFECT OF IMAGE PROCESSING CONSTRAINTS ON ROTATIONAL AMBIGUITY IN MULTIVARIATE CURVE RESOLUTION OF HYPERSPECTRAL IMAGES

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Hyperspectral imaging refers to the collection of a spectrum for each pixel in the image of scene. It is now being used in many different fields of industry and research; and the number of applications keeps growing. The main interest of hyperspectral imaging is that it combines spatial and spectral information about a sample or a scene. However, to extract this information, spectral unmixing of some kind has to be performed. Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is one of the techniques that can provide pure spectra and distribution maps of different components in the samples [1]. MCR-ALS is very useful and probably the most commonly used technique in chemistry for this purpose. However, as for any two-way factor analysis technique, it inherently suffers from non-uniqueness of the pure component decomposition, known as rotational ambiguity. Rotational ambiguity can be alleviated and sometimes totally avoided by a judicious use of the data structure or by incorporating well-suited constraints [2].

In this contribution, we aim to visualize and investigate the reduction effect of some image processing constraints on the extent of rotational ambiguity in MCR-ALS of hyperspectral imaging data [3, 4]. For this purpose, Borgen plots of three component simulated systems are used. Different spatial information, such as 2D-Gaussian fitting (hard-modeling), image segmentation and sparse image recovery are investigated. In each case, the extent of rotational ambiguity is evaluated. We discuss how these spatial constraints can reduce uncertainty and improve results accuracy. Hard-modeling, and to a certain extent sparse image fitting, can lead to unique solution, but their applications of spatial hard modeling constraint in real scenario can be questioned. Image segmentation, on the other hand, is more widely applicable, and its application result in less ambiguous solutions. Lastly, a couple of real examples such as remote sensing data sets and FTIR imaging are taken.

To recap: we visualize rotational ambiguity before and after applying constraints and discuss the importance of using proper and chemically meaningful constraints.

References:

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P02
CONTRAST ENHANCEMENT AND TOTAL VARIATION IMAGE PROCESSING CONSTRAINTS: COMPLEMENTARY APPROACHES FOR MCR-ALS OF HYPERSPECTRAL IMAGES

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MCR-ALS [1] has demonstrated to be a valuable tool to resolve hyperspectral images (HSI). However, traditional MCR-ALS analysis of an HSI requires the unfolding of the hyperspectral data cube into a two-way array, which causes the loss of information on adjacency between pixels. In such cases, exploiting the spatial structure of HSI to improve the corresponding MCR-ALS solutions is unfeasible [2]. Several approaches have been developed to overcome this issue. The angle constraint (also known as contrast enhancement constraint) proposed by Winding et al. [3, 4] takes advantage from the duality of the spectral and spatial domain to enhance the selectivity of one of the two. Alternatively, a refolding step can be added in the least squares loop of the MCR-ALS decomposition [2]. This allows image processing constraints like sparsity, segmentation or total variation [2, 5-7] to be employed for improving the visualisation of regions of interest with sharp edges.

The aim of this work is to compare the outcomes resulting from the MCR-ALS analysis of hyperspectral images when contrast enhancement and total variation constraints are applied. Three simulated and a real dataset are here investigated, with the aim of exploring different experimental scenarios in which their effect is tested. The resolution of each dataset is discussed and the pros and cons of the two approaches are highlighted. Particular attention is given to how overlap among components (both in the spectral and spatial domain) can affect the possibility of achieving a physico-chemical meaningful MCR-ALS solution. Overall, contrast enhancement led to more *orthogonal* MCR-ALS components: for this reason, it may represent an optimal approach when such components are scarcely overlapped in one of the two aforementioned domains (e.g. overlapping distribution maps but independent spectra). On the other hand, for datasets clearly structured in the spatial domain, total variation was found to be able to retrieve even largely overlapping components.

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P03
ITERATIVE TARGET DETECTION FOR DETECTION AND CLASSIFICATION IN HYPERSPECTRAL IMAGES WITH AN APPLICATION TO A LANDSAT 8 IMAGE OF LAKE CHELAN, WA USA

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Classical least squares (CLS) is the tool of choice for detection and classification in hyperspectral images because often target spectra are known but reference values for each pixel are rarely available. Generalized least squares (GLS) is a weighted CLS model used to suppress clutter signal (interferences and noise) while enhancing minor target signal. (GLS is also known as the matched-filter.) An iterative target detection approach exhibits synergy between GLS and the extended mixture model (extended least squares, ELS) to further improve discrimination. The approach is relevant for chemical imaging, medical imaging and remote sensing. An example is shown for a Landsat 8 image of Lake Chelan, WA USA. GLS was used iteratively in a hierarchical approach to classification and GLS combined with ELS was used to further split a single class that was otherwise difficult to classify.

The first task used GLS iteratively to create global classes in the image associated with Water, Green (orchards, vineyards and lawn), Bare Earth, three types of forest, Road, Buildings and Other (corresponding to no specific class). The second task used GLS/ELS to split Class Green into signal attributable to lawn (e.g., associated with the municipal golf course) from cherry orchard (and other agricultural land including vineyards and other orchard types). Both objectives were complicated by the presence of a large number of pixels associated with water, forest, rough terrain, dry scrubland, homes, roads and buildings. The local GLS/ELS model showed good results that were verified in many cases using ground truth.

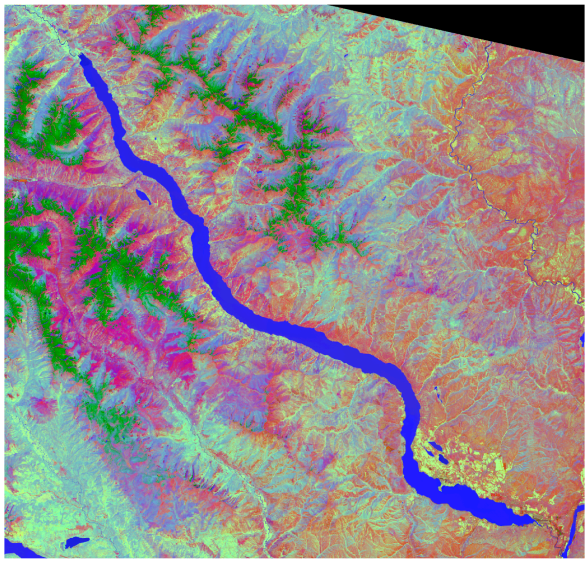

Figure 1 PCA Image of Lake Chelan Landsat8 – False color image of components 1, 2 and 3.

P04
REAL TIME AIRBORNE GAS DETECTION USING THERMAL HYPERSPECTRAL IMAGING

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Gas leaks and air pollution sources present to a certain extend health, safety and environmental risks. A history of crisis management in the Upstream has shown the value of efficient and accurate tools for detecting gas leakages and/or the characterization air pollution agents. Knowing about the existence of a leak or the existence of an environmental thread is not always enough to launch a corrective action. Additional critical inputs such as the leak source, the chemical nature of the gas cloud, its direction and speed and as well as the gas concentration must most of the time be gathered in a short amount of time to help securing the hazardous areas. Most of the time gas identification for gas leaks surveys or environmental monitoring purposes involve explosives and/or toxic chemicals. In such situations, airborne measurements present particular advantages over ground based-techniques since large areas can be covered efficiently from a safe distance. In this work, we present our recent results on real time airborne gas detection up to 4600 feet above the ground using thermal hyperspectral Imaging technology. The Fourier transform technology used in the longwave (8-12 mm) hyperspectral camera on an airborne platform allows recording of airborne hyperspectral data using mapping and targeting modes. These two acquisition modes were used for gas imaging a ground-based ethylene, Methanol and acetone gas release experiment. Real time quantitative airborne chemical images of the three gas clouds were obtained paving the path toward a viable solution for gas leak surveys and environmental monitoring.

P05
COMBINATION OF MALDI IMAGING MASS SPECTROMETRY WITH CHEMOMETRIC TOOLS FOR INVESTIGATION OF TUMOR HETEROGENEITY

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Tumor heterogeneity could help to explain, why some patients that initially respond positively to a cancer drug eventually relapse, often with new tumors that no longer respond to the therapy. Understanding tumor heterogeneity is therefore a key factor in cancer science. Differences between cells types within a tumor tissue can have profound implications during the diagnosis and treatment of cancers [1-2].

Matrix-assisted laser-desorption ionization-Mass spectrometry imaging (MALDI-MSI) is very well suited to study molecular distribution patterns within CRC tissues. The aim of this study is to demonstrate that the combination of MALDI Imaging mass spectrometry with chemometric tools allows investigation of tumor heterogeneity and identification of relevant tumor populations within the cancer tissues. With this purpose and as a preliminary study, human colon adenocarcinoma cell lines sensitive HCT116 (S) and resistant HCT116-SN50 (R) to irinotecan (chemotherapy drug) were used as a model of experimental tumors. Clonogenic tumor xenographs were generated by subcutaneous injection of both unique cell line on athymic mice whereas a model of heterogeneity was created injecting various mixture of (R) and (S) HCT116 clones. Tumors were then collected, sliced, and analyzed by MALDI Imaging mass spectrometry. Potential discrimination of R and S populations will be tested by classification methods such as Least Squares Dicscriminant Analysis (PLS-DA), k-Nearest Neighbors (kNN) and Soft independent modelling by class analogy (SIMCA)

Acknowledgement: This work is part of the BEST Postdoctoral Program, funded by the European Commission under Horizon 2020's Marie Skłodowska-Curie Actions COFUND scheme (Grant Agreement no. 712754) and by the Severo Ochoa program of the Spanish Ministry of Science and Competitiveness (Grant SEV-2014-0425 (2015-2019)).

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P06
HSI-NIR: SIMPLY A SURFACE-ANALYSIS TECHNIQUE? INSIGHTS ON PENETRATION DEPTH AND TRANSMISSION MODE

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Hyperspectral imaging is usually considered as a surface analysis method. Nevertheless, electromagnetic rays may penetrate in depth, inside the upper layers of the sample, including information from the inner parts in the recorded spectra. Moreover, such an effect depends on the matrices analysed and on the specific wavelengths at which spectra are recorded.
In the present study, penetration depth was evaluated for a near infrared hyperspectral imaging system working in the 1000–2500 nm spectral range, at 8 nm spectral resolution (HSI-NIR SWIR3 camera equipped with a LabScanner 40x20).
To the aim, a chessboard background was assembled using two polymeric materials characterised by different total reflectance values (about 0% and 90%, respectively) and with different and characteristic spectral profiles. The use of a geometrical pattern, in comparison with uniform backgrounds, adds spatial information to the penetration depth evaluation. Images were recorded placing samples (sliced cheese), both sliced at different thickness and wedge-shaped, between the source and the chessboard background.
Univariate and multivariate image analysis was carried out in order to highlight both spatial and spectral features, allowing to evaluate the penetration depth. First of all, a region of interest (ROI) within the hyperspectral image, including a representative portion of the sample was selected. Then, principal component analysis (PCA) was carried out, reducing data dimensionality and removing non-useful information to confirm the level of penetration on the basis of score images.
On the basis of these outcomes, in many practical applications, hyperspectral imaging should be regarded as a transflectance approach, depending, of course, on the physico-chemical characteristics of the matrix, such as the chemical composition and the physical microstructure, and on characteristics of the electromagnetic radiation involved (wavelengths and intensity).
A further interesting topic concerns the possibility of acquiring hyperspectral images in the transmission mode. To this aim, an in-house instrumental setup was adopted, allowing to transmit electromagnetic radiation across samples. Particular strategies for focusing and normalising image intensities were developed. Transmittance images obtained on samples of biological interest allowed capturing important chemical information on the internal structures of biological tissues, complementary to information acquired in the reflection mode.

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P07
THE HIGH-POWERED EYE OF HSI-NIR: A DECISIVE TOOL IN FORENSIC SCIENCES

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During the last decade, there has been an increasing interest in the development of customised analytical methods based on non-destructive chemical imaging in several fields of application. This is of particular interest when it is important to spatially visualise the results as an unequivocal proof of a crime and without damaging the evidences, because further investigations could be required during legal inquiries. For these reasons, the present work aims at developing a reliable method for the application of hyperspectral imaging in the near infrared region (HSI-NIR) to detect biological traces on the crime scene; in this way, a screening procedure is proposed to detect areas of interest in which collecting micro-samples for applying the conventional more targeted DNA analyses. In more detail, this work will give a fundamental contribute to forensic scientists in identifying biological traces directly on specimens, recognising the type of trace (e.g. blood, urine, semen and their mixtures as well as food traces) regardless of the material constituting the collected evidences (e.g. paper, glass and cloth).

HSI-NIR is one of the newest technologies developed in the field of NIR spectroscopy; it is based on the interaction between radiation – in the region of the electromagnetic spectrum between 1000 and 2500 nm – and the sample, to obtain chemical information about its composition, thanks to the spectroscopic absorption of characterising compounds. The advantages of this approach are exploited when samples to be evaluated are characterised by a non-uniform distribution of chemicals inside the whole matrix. In this situation, the validity of traditional chemical analyses is strictly dependent upon the design of a correct sampling plan; the extent of sampling required to account for such a variability of distribution is necessarily quite large and the method of collecting samples is also critical. All these limits become serious when data collected have to be used for drawing conclusions at the service of law. In fact, sampling limitations leave an analytical crack in which crime may act.

Often, HSI-NIR equipment setup is not directly applicable, since optimisation steps are required, in terms of both instrumental parameters and image acquisition; furthermore, the acquisition of images is not sufficient for extracting reliable information from samples in a robust way. For these reasons, in this work, HSI-NIR is supported by a proper chemometric strategy particularly suited to account for the spatial information, allowing to define a customised method for the specific application.

P08
DEVELOPMENT OF A HAND-HELD HIGH RESOLUTION HYPERSPECTRAL IMAGING CAMERA

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HinaLea Imaging developed a unique low-cost high-resolution hyperspectral reader platform to read and decode objects labelled with its microscopic silica tags which are edible memories sparsely embedded in a wide variety of objects. These readers can be readily adapted to Hyperspectral Imaging and offers a unique cost, size, spatial-resolution and spectral-resolution combination.

Although their application space is broad, these instruments can revolutionize the space of sample analysis. Whereby most current instruments analyze the bulk properties of samples, the new imager cannot only analyze spectral content with spectral resolutions approaching that of state-of-the-art spectrometers but can also provide high spatial resolution.

At the heart of the imager is an optical engine using HinaLea Imaging's proprietary Fabry-Perot Interferometer and embedded technology which has been adapted into a mass-manufacturable solution. This optical engine can be adapted to other applications and instruments. HinaLea Imaging is currently collaborating with and is looking for new collaboration partners for harnessing the power of this technology to new application areas.

Figure 1: HinaLea Imaging's Solid-State Tunable Filter Production and the Model 4100H

P09
PROS AND CONS OF IMAGE TEXTURE ANALYSIS ON HYPERSPECTRAL DATACUBES: THE FLOUR CASE-STUDY

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Surface texture is an important feature for evaluating physical nature of analytical samples, allowing to highlight differences between matrices with a very similar chemical composition. To measure such a property, several algorithms of image texture are available in literature and are typically applied on mono-layer images, usually the grey scale ones.

The aim of the work is to compare and evaluate the ability of three different approaches of image analysis on hyperspectral data; the fact that these data are organised in three-dimensional data cubes may constitute a limiting factor in applying image texture algorithms directly on the hypercube. In more detail, two algorithms typically applied on mono-layer images are taken into consideration: GLCM (Grey Level Co-occurrence Matrix) and AMT (Angle Measure Technique), while for multivariate approaches an algorithm proposed by Bharati e MacGregor and based on PCA was considered [1]. Different types of data compression, such as integral images and PCA, were applied to extract the information related to physical properties of the samples, before performing the procedures under study.

In the flour case study, images of 5 types of flour (durum wheat flour, strong flour – Manitoba, wholemeal, plain flour and soft flour) were recorded by a hyperspectral camera SWIR3 (SPECIM Ltd, Finland), working in the spectral range 1000-2500 nm. These images were submitted to combinations of data compression and image texture algorithms to distinguish between the five commercial types of flour.

This work allows to deeply evaluate the reliability of well-known image texture approaches on HSI-NIR images; pros and cons of each procedure were highlighted.

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