

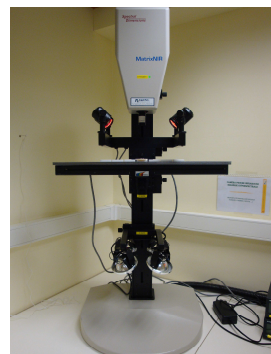
NIR imaging and chemometrics in support to the detection at the single kernel level of GMO

J. A. Fernández Pierna, G. Berben, Ph. Vermeulen, E. Janssen, P. Dardenne & V. Baeten

Quality Department of Agro-food Products, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium
e-mail: fernandez@cra.wallonie.be; Tel: +32 81620350; Fax: +32 81620388

CONTEXT

In the framework of the COEXTRA FP6 project (GM and non-GM supply chains: their CO-EXistence and TRAceability), the CRA-W is in charge to investigate the potential of NIR hyperspectral imaging together with chemometrics for GMO (Genetically modified organisms) detection.



Instrument parameters:

- Wavelength range: 900-1700 nm by step of 10 nm
 - Reflection mode
 - 16 coadds/wavelength
 - Average on 4 scans/image
 - 1 acquisition = 1 image = 240 * 320 pixels = 76800 spectra
 - 1 pixel = 80µm * 80 µm = 0.0064 mm²
 - Analysed surface = +/- 5cm²
 - Time of acquisition = 5 min/image
- 1 image = 10 - 100 kernels
1 kernel = 100 - 3500 pixels
Surface analysed : 1 - 25 mm²/kernel
1 pixel = 1 spectrum
1 kernel = 1 mean spectrum

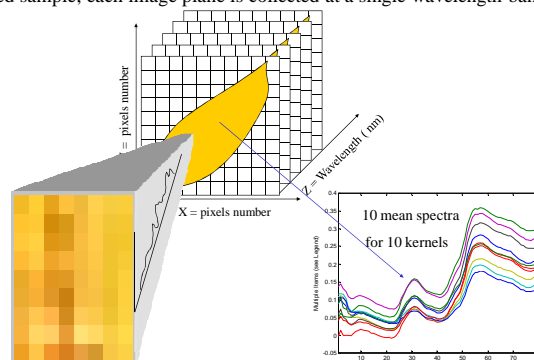
Each kernel used in this study has been measured twice (both sides of the kernel) in order to include a large part of information. A large number of spectra has been collected coming from different origins, different varieties and some of them being transgenic. Once the kernels have been measured with the camera, two approaches can be applied: 1) working with all the pixels for each kernel (each kernel contains +/- 3500 pixels); or 2) working only with the mean spectrum of each kernel considering this mean as representative of the characteristics of the kernel.

Two main studies have been performed:

- STUDY 1: *Detection of transgenic soybean by hyperspectral imaging*
STUDY 2: *Detection of transgenic barley by hyperspectral imaging*

HYPERSENSPECTRAL IMAGING

The MatrixNIR™ Chemical Imaging System (Malvern instruments Ltd) is a near infrared hyperspectral imaging spectrometer gathering spectral and spatial data (hypercube) simultaneously by recording sequential images of a pre-defined sample; each image plane is collected at a single wavelength band.



1 pixel = 1 spectrum

STUDY 1 - SOYBEAN

The dataset used in Study 1 contains 372 spectra coming from the following samples:

No transgenic					Transgenic
20070092	GMO 815-2	GMO 816-3	GMO 816-9	GMO 816-15	20070093
20070094	GMO 815-3	GMO 816-4	GMO 816-10		20070091
20070098	GMO 815-4	GMO 816-5	GMO 816-11		20070099
20070100	GMO 815-5	GMO 816-6	GMO 816-12		20070095
20070096	GMO 816-1	GMO 816-7	GMO 816-13		20070097
GMO 815-1	GMO 816-2	GMO 816-8	GMO 816-14		GMO 814

In order to determine the mean of each kernel a mask has been constructed by a process of erosion in order to determine the contour of each grain. This mask is based on the different intensities found in the image.

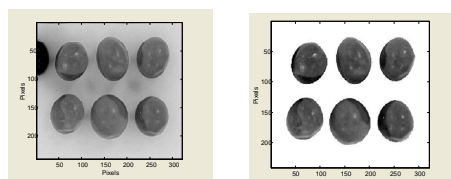
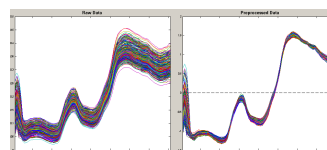


Image of soybean grains obtained with the NIR camera and mask from the previous image



Spectra before and after SNV correction

Different chemometric tools (PLS-DA, ANN and SVM) have been used in order to discriminate between GMO and not GMO. The results, expressed as the percentage of correctly classified samples, are shown in the following tables for both calibration and test (independent) sets.

Belonging to...	Loosev (internal valid)		Test (external valid)	
	% classified as...		% classified as...	
GMO	85.7	14.3	100	0
Non GMO	27	73	23.8	76.2

PLS-DA results

Belonging to...	Loosev (internal valid)		Test (external valid)	
	% classified as...		% classified as...	
GMO	100	0	77.8	22.2
Non GMO	0	100	6.3	93.7

SVM results

Belonging to...	Loosev (internal valid)		Test (external valid)	
	% classified as...		% classified as...	
GMO	100	0	77.8	22.2
Non GMO	0	100	0	100

ANN results

In this case, the best results for the test set in average are obtained with PLS-DA (88.1%) and ANN (88.9%).

This study consists on NIR imaging analysis of barley samples collected in Belgium (Gembloux) and United Kingdom. In total 642 spectra have been obtained measured at 81 wavelengths (900:10:1700nm) including samples with different varieties and samples being transgenic and not transgenic.

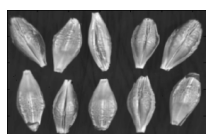
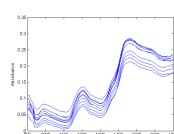


Image of 10 barley kernels obtained with the NIR camera



Mask of the image of 10 barley kernels used to calculate the mean spectrum of each barley kernel



Mean spectra obtained for each of the 10 grains represented in the previous figure

When working with the whole data set, the PCA results do not show conclusive results concerning discrimination between GMO and not GMO. Then PCA models have been performed on individual varieties.

CONCLUSION & REFERENCES

In all data sets the results have shown that a good discrimination could be performed according to the variety and the presence of GM material.

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

- Fernández Pierna, J.A., Vermeulen, P., Buhigiro, T., Baeten, V. & Dardenne, P. (2006). *Les graines sous l'œil de l'imagerie hyperspectrale proche infrarouge*. Chimimétrie 2006, Paris, 30/11-01/12/2006.
- Baeten, V., Fernández Pierna, J.A. & Dardenne, P. (2007). Hyperspectral imaging techniques: an attractive solution for the analysis of biological and agricultural materials. In: *Techniques and Applications of Hyperspectral Image Analysis*, Editors, Hans F. Grunh & Paul Geladi.
- Fernández Pierna, J.A., Baeten, V., Vermeulen, P., Buhigiro, T., Berben, G., Janssen, E. & Dardenne, P. (2007). *GMO detection using NIR and chemometrics*. Poster in: 13th International Conference on Near Infrared Spectroscopy, Umea - Sweden, 15-21 June 2007.
- Vermeulen, P., Fernández Pierna, J.A., Boudinot A., Berben G., Janssen E., Sinnave G., Stilmant D., Tossens A., Dardenne P. & Baeten V. (2009). *Near Infrared Hyperspectral Imaging for single kernel analysis*. Poster in EASIM-09 Hyperspectral imaging, Gembloux, Belgium, 3-4 March 2009.