Testing Wheat Grain Authenticity with Fast, Non-destructive Multispectral Image Analysis

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Multispectral imaging is a rapid and non-destructive approach to assess quality in a wide range of products and materials, including food, pharmaceutical products and raw ingredients. Compared to normal pictures multispectral images have vastly more data contained within, which can be interrogated to reveal information that's invisible to the naked human eye and provide feedback on which to make process decisions.

Traditional colour imaging uses three broad bands of colour (red, green and blue), known as RGB imaging and is designed for our human perception. However, RGB imaging has limited spectral resolution and so cannot differentiate between samples with a very similar colour. For example, chlorophyll a and chlorophyll b are difficult to separate using RGB data. They are both simply green.

Multispectral imaging uses precise reflectance data at multiple wavelength bands over a spectral range. They are a stack of many images showing the percentage light reflectance at many colours along a range that is wider than human visual perception. Multispectral images are far more data-rich than RGB images and we can apply multivariate statistical methods originally developed for satellite image analysis to reveal high-dimensional patterns that would not be 'seen' otherwise.

The VideometerLab 3 is a bench-top multispectral imaging system from the Danish company Videometer A/S. It uses selected wavelengths of precisely controlled illumination with high-intensity light emitting diodes (LEDs) at 19 intervals between 375-970 nm (ultraviolet, visual and infrared light). A high-resolution monochrome CCD camera (2056 x 2056 pixels, 45 µm x 45 µm area per pixel) records an image at each LED illumination wavelength. An optional filter wheel adds the ability to separate fluorescence emission (by excitation at each LED wavelength) from overall reflectance of a sample, though this option was not used in this study and models were built on calibrated reflectance data only.

Samples are simply placed in the target area (slightly larger than a petri dish) and image acquisition is started. The integrating sphere descends to enclose the sample and eliminate interference from ambient light. The LEDs strobe in sequence for precisely-controlled time periods and a monochrome reflectance image is captured at each of the 19 illumination wavelengths (plus up to 27 more fluorescence-only images if the filter wheel is used). Full control over the light conditions inside the sphere allows us to optimise signal to noise at every LED wavelength separately, unlike a panchromatic light source such as halogen bulb, and use the full dynamic range of the camera at each of those wavelengths.

Figure 1: VideometerLab 3: 19 LEDs at separate wavelengths are strobed successively to illuminate the sample with monochrome light. A CCD camera captures an image during each LED strobe period.

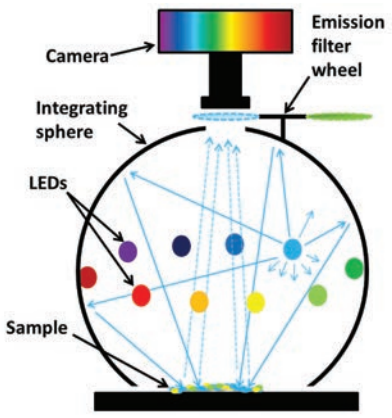

Figure 2: Schematic of the VL3: Internal diffuse reflection of the LEDs by the ultra-white inner surface of the sphere ensures diffuse homogeneous light for increased reproducibility, dynamic range, and low scatter/shadow effects.

The 19 monochrome images are combined into a single 5 megapixel multispectral image datacube; every pixel in the image has a calibrated 19 data-point UV-Vis-IR reflectance spectrum for a 45 µm x 45 µm area of the sample. Image acquisition takes about 5 seconds and analysis models can be run from a pre-saved menu, meaning analysis results are available within 10-15 seconds (including sample handling time).

Illumination settings and image analysis recipes for particular sample types can be saved and run as standard procedures by technicians for fast, semi-automated analysis. Multiple parameters can be checked simultaneously by running different analysis models on the same image datacube. Image data is easily stored for later use in developing new analysis models. High throughput non-destructive image analysis of products and ingredients can be made a routine part of a testing regime without sacrificing the ability to run further destructive testing on the same samples.

Multispectral imaging is well suited to grain and seed analysis compared to traditional spectroscopy techniques. Even closely related variants, like *Triticum aestivum* (common or bread wheat) and *Triticum durum* (durum or macaroni wheat) grains, will have differences in their spectral response signatures. But these differences are hidden if we measure the overall average spectrum of a grain mixture with conventional NIR – if a grain sample is adulterated at a relatively low level, the tell-tale signal of an adulterant may be missed.

A multispectral image will reveal the spatially separated grain varieties as being different to one another. A grain sample with low level adulteration may be imaged to see where the adulterant grains are based on their separate spectral signature. This is not an average of many grains but is looking at each grain individually to decide if it is the correct variant or not, thus giving much more detailed information on the sample.

Developing a MSI Model for Wheat Authenticity Testing

The VideometerLab 3 system was used to distinguish between specific varieties of *Triticum aestivum* and *Triticum durum* wheat grains based on the spectral signature of each grain type. Once the software has 'learned' the unique spectral signature of each grain type, it can then score a particular wheat grain as being more likely to be *T. durum* or *T. aestivum*

Control samples of *T. aestivum and T. durum* were used to train a software model to distinguish *T. durum* from *T. aestivum*, which was then applied to blind samples to test the level of adulteration. The control samples and blind test panel were prepared using wheat grains from two authenticated wheat cultivars of *T. durum* and *T. aestivum* sourced and provided by Frontier Agriculture Ltd (Diss, Norfolk, UK). Full results for this test panel are available in the project whitepaper available from Analytik and LGC, and published in the Defra report FA0136 'Feasibility study for using rapid and automated spectral imaging for food authenticity testing'.

Pixels of *T. durum* and *T. aestivum* wheat grains are highlighted to teach the software the average spectral signature of each grain type (*Figure 3 and 4*). Statistical analysis of the spectral information collected from the few-thousand training set pixels gives us data on the mean and standard deviation of reflectance at 19 wavelengths for each training set; the combined pattern of results for each set can be called a spectral signature.

The software builds a statistical discrimination model to assess any other given pixel-spectrum on whether it is more like the *T. durum* spectral signature or the T. aestivum spectral signature and assigns a score between +2 and -2 to each pixel based on its degree of spectral similarity to the *T. durum* or the *T. aestivum* spectral signature.

A false-colour scheme is applied to visually highlight the spatial variation; red pixels have a positive score because their spectrum is like the *T. durum* spectral signature

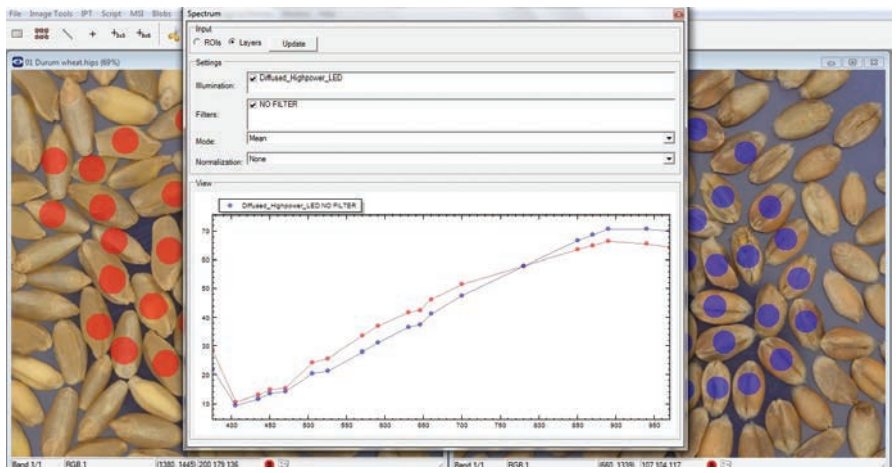

Figure 3: Spectral signatures of T. durum (left image, red highlight and plot) and T. aestivum (right image, blue highlight and plot). Illumination wavelength is on the x-axis and percentage reflectance is on the y-axis. Each plot has 19 data points, one for each LED illumination wavelength against the mean (average) reflectance of all pixels highlighted with the paint-brush tool. Though similar in overall shape there is a clear difference between them, which allows the VideometerLab software to recognise and discriminate between the two. Note the cross-over point at 780 nm, a tell-tale mark that these two spectra are distinct and a model can be built to distinguish them.

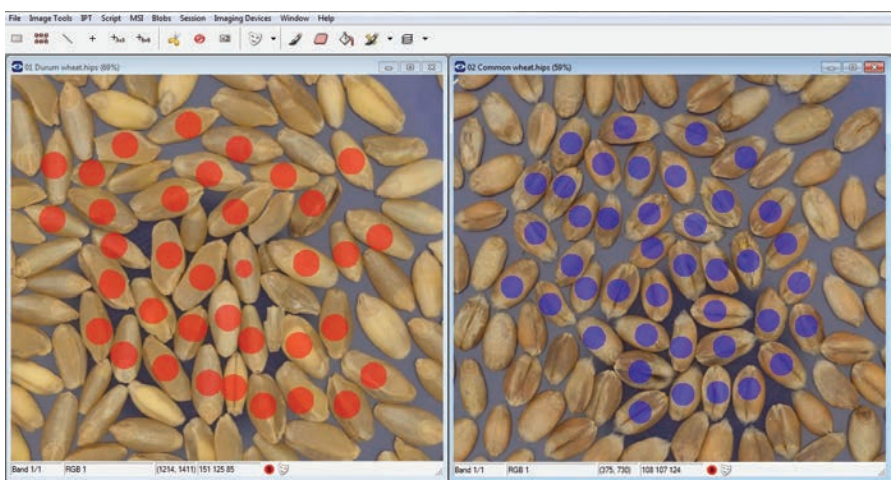

Figure 4: To train the statistical model, control samples of T. durum (left) and T. aestivum (right) wheat grains in each image are highlighted to provide sets of training pixels to discriminate. These training sets were used in Figure 3 to plot the average (mean) reflectance value for each wavelength, as our sample from the total population of grain pixels.

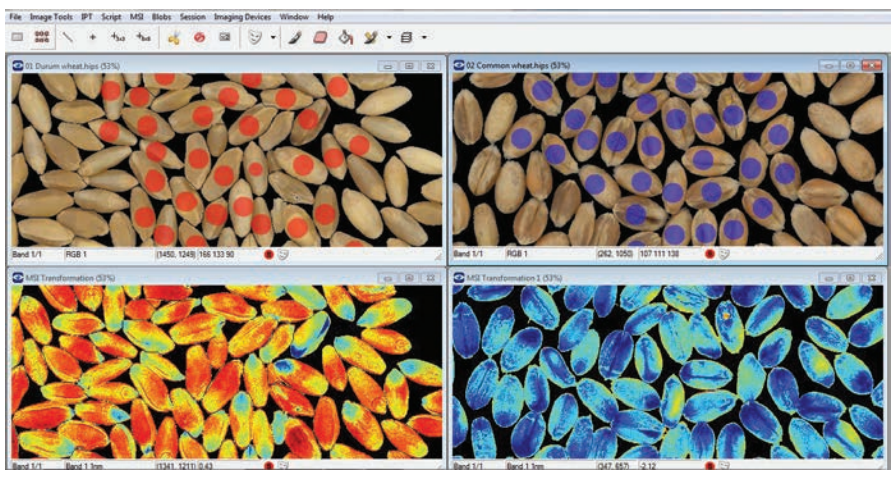

Figure 5: Original (top images) and statistically transformed images (bottom images) of pure samples of T. durum (left) and T. aestivum (right). The discrimination model has scored every pixel in both images from the trained model. If a pixel has a spectrum more like T. durum it is coloured red, if it is more like T. aestivum it is coloured blue.

and blue pixels score negatively as they are more like the *T. aestivum* spectral signature. When the model is run on the control samples, it scores nearly all pixels in the left image as T. durum and nearly all pixels in the right image as T. aestivum, as would be expected for a pure sample (*Figure 5*).

For an image of a sample of unknown composition - a mixture of T. durum and T. aestivum (*Figure 6A*) – those grain pixels with a spectrum that are more like the T. durum spectrum will be scored as highly positive (false coloured red), and grain pixels with spectra that are more like the T. aestivum spectrum will be scored highly negative (false coloured blue) (*Figure 6B*).

It is immediately clear that this sample is a mixture of two different types because each grain is false-coloured based on an objective score of its similarity to the known control sample spectra.

Figure 6A: Application of the model to a blind sample of mixed wheat grains. The software automatically removes pixels it recognises as either the blue background plate or the petri dish, leaving just the grains (6A). Some grains are touching each other, so the software separates them with a thin one-pixel wide line.

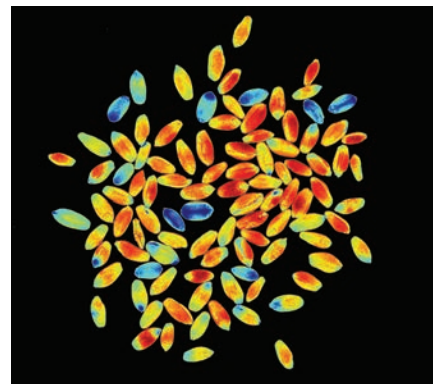

Figure 6B: The nCDA discrimination model is applied to every pixel left in the image. If a pixel's spectrum is more like the spectral signature of a T. durum grain it is graded on an arbitrary scale as positive (false-coloured red) and if it is more like T. aestivum it is graded as negative (false-coloured blue). Adulterant grains are immediately obvious in the image.

Object separation and analysis (blob toolbox) automatically separates touching grains, scores each one as being more like *T. durum* or more like *T. aestivum*, and returns a table of results indicating the number and percentage of each different type of grain in an image (*Figure 6*). The process can very quickly image and analyse samples to give an objective assessment of whether and to what degree a sample of grain is adulterated with other types of grain, and save this data for further analysis.

It is easy to update models to take account of seasonal and geographic variation in wheat phenotypes, so users can always be sure they have a reliable, fast and objective way to quickly detect adulteration and contamination. The Spectraseed program (developed by Videometer and Aarhus University) aims to provide an ISTA-certified database of seed and grain spectral characteristics providing a trusted resource to develop and update models for seed/grain discrimination, disease and more.

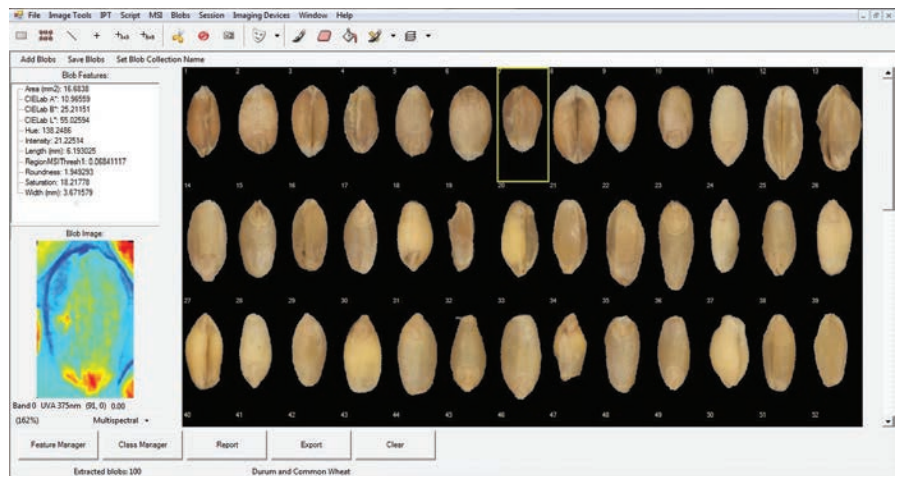

Figure 7: Blob toolbox software module automatically segments and sorts the grains in the order of likelihood of being T. durum grains (least likely first i.e. the adulterant T. aestivum grains). CIELab colour, size and shape information is given for each blob by default, and more features can be calculated if desired. The sorting score is based on a nCDA model that was built for spectral discrimination only. A more robust, sophisticated model would incorporate further stages of size, shape and texture analysis to improve the ability of the model to discriminate T. durum grains from T. aestivum grains based on multiple correlated factors.

Conclusion - Multispectral Imaging as a QA tool

Current methods of choice for determination of adulteration involve time-consuming and expensive molecular biology methods, in particular real-time PCR. Whilst molecular biology approaches are effective, they need specialist laboratory equipment and consumables, costly reagents and a requirement for specialist training. Most molecular biology approaches for food authenticity testing are also destructive as the sample must be ground down so that DNA can be extracted.

The VideometerLab 3 instrument can differentiate between surface colour, texture and chemical composition for a range of materials. It is more applicable to grain and seed analysis compared to traditional spectroscopy techniques because spatial information on a sample reveals contamination, disease and adulteration that would be missed. Even closely related varieties such as *T. durum* (durum wheat) and *T. aestivum* (common wheat) have significantly different spectral response signatures which can be used to build a model for identification and quantification purposes in suspected cases of fraud.

Acknowledgements

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