VideometerLab 3 Multi-Spectral Imaging System:

Fast, non-destructive analysis of wheat grain adulteration

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Introduction

Multispectral imaging provides valuable information on the quality and safety of a vast array of materials from pharmaceuticals to raw meat and burned biscuits. The same techniques can be used to measure skin sensitivity to sticking plaster, detect counterfeit drugs and packaging and gain insight into historical artefacts such as medieval manuscripts and weapons.

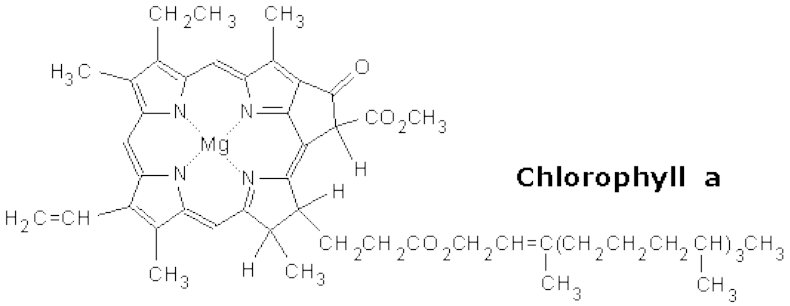
The non-destructive investigation of materials with non-uniform colour and texture can be difficult, tedious and expensive. Conventional techniques such as NIR spectroscopy only measure a single point or average over a fixed area and do not give an objective overall assessment of visual quality. Multispectral imaging can be described as a trade-off sacrificing spectral resolution to increase spatial information giving a 'snapshot' of the combined bulk properties of a sample, handling natural variation and heterogeneity.

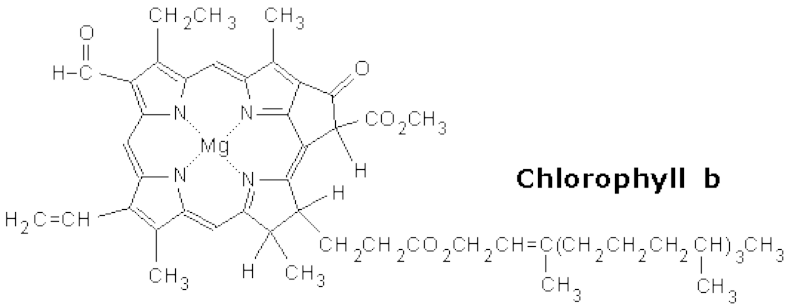
What is multispectral imaging?

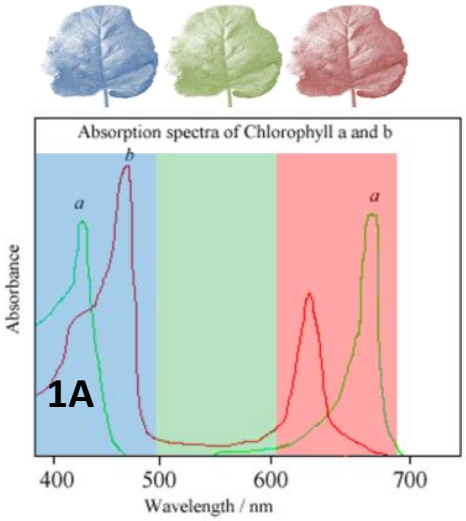
Traditional colour imaging uses three broad bands of colour (red, green and blue) and is known as RGB imaging. Realistic colour rendition is possible with just these three colours because our own eyes have a red, green and blue receptor system in the retina. As a consequence of the broad wavelength band ranges recorded by RGB cameras, RGB imaging has very limited spectral resolution and is unsuited to differentiating similar samples that only show separate spectral variations within a single broad band range. For example, chlorophyll a and chlorophyll b are very difficult to spectrally differentiate using just RGB data – they're both simply green in RGB images.

Multispectral images use precise, calibrated reflectance data at multiple discrete wavelength band ranges spaced over an extended spectral range. If you think of RGB images as a stack of three separate images showing overall red, green and blue light reflectance as we perceive them, multispectral images are a stack of

many images showing the exact percentage light reflectance at many discrete 'colours' along a range far wider than our human visual perception (appendix B). Multispectral images provide a detailed record of the spectral variation over a heterogenous sample, to which we can apply statistical methods originally developed for satellite image analysis to reveal differences and patterns that would not be 'seen' otherwise.







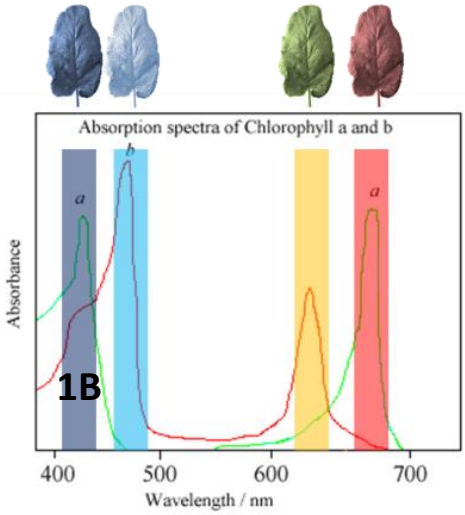


Figure 1A: RGB photographs have limited spectral resolution, so are not suited to spectral analysis; chlorophyll a and b give almost the same RGB signal and are not spectrally separated.

Figure 1B: Multispectral analysis using just 4 wavelength bands with tightly defined ranges; chlorophyll a and b can easily be distinguished by their differing absorption levels at each wavelength band. VideometerLab 3 uses 19 wavelength bands between 375-970nm, allowing much finer spectral discrimination.

VideometerLab 3 Multispectral Imaging System

The VideometerLab 3 is a bench-top, lab based multispectral imaging system from the Danish company Videometer A/S. It uses precisely controlled strobed illumination with high-intensity light emitting diodes (LEDs) at 19 intervals between 375-970nm (ultraviolet, visual and infrared light), and a calibrated, high-resolution CCD camera (2056x2056 pixels, 45µmx45µm resolution) to record an image at each LED illumination wavelength (Figures 2 and 3 below).

The LEDs are spaced around the inside circumference of an integrating sphere to ensure diffuse and even illumination of the sample from all directions; light emitted from each point-source LED is reflected multiple times in all directions off the internal high-reflectance barium sulphate coating to eliminate directional bias before hitting the sample. An optional filter wheel adds the ability to separate fluorescent emission (resulting from excitation at each LED wavelength) from simple diffuse reflectance of a sample, though this option was not used in this study.

Image acquisition is controlled via the VideometerLab software package, which includes automated procedures for LED strobe time settings and spatial/spectral calibrations (NIST-traceable). Samples are simply placed in the target area (slightly larger than a petri dish) and acquisition is started. The entire integrating sphere descends to completely enclose the sample and eliminate interference from ambient light. The LEDs strobe in sequence 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).

Figure 2: VideometerLab 3 - Up to 19 wavelengths via LEDs, uniformly spaced around the inside of the integrating sphere are strobed successively, each generating a monochrome image.

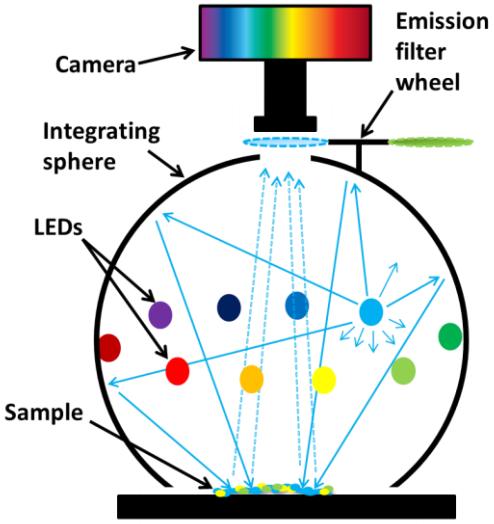


Figure 3: Schematic of the VL3 - Internal diffuse reflection of the LEDs by the ultra-white inner surface of the sphere ensures diffuse homogeneous light for increased reproducibility, dynamic range, and low scatter/shadow effects.

The 19 monochrome images are combined into a single high-resolution multispectral image datacube (two spatial image dimensions plus one spectral dimension make a cube of data points) with every pixel in the

combined image containing a 19 data-point UV-Vis-IR reflectance spectrum for that 45µmx45µm area of the sample. Datacube analysis and image processing is achieved with the VideometerLab software, to allow average users access to sophisticated multivariate analysis techniques without requiring a deep understanding of imaging, statistics or spectroscopy. Image acquisition takes roughly 5 seconds and user-prepared analysis models can be run from a saved menu, meaning analysis results are available within 10-15 seconds, including sample handling time.

Precise illumination settings and multi-step image analysis recipes for particular sample types can be saved and run as standard procedures by technicians to quickly run through samples in a semi-automated fashion. Full automation with petri-dish handling robotics or conveyor belts for bulk granular samples can make high-throughput multispectral analysis a viable solution to the need for fast, non-destructive testing of many samples (including online, real-time QA analysis). Multiple product quality parameters can be checked simultaneously by running separate analysis models on the same image datacube, and image data is easily stored for later use in developing new analysis models or to retrospectively apply novel analysis recipes developed later in a project.

Multispectral imaging in grain analysis

Multi-spectral imaging is well suited to grain and seed analysis compared to traditional spectroscopy techniques. The heterogeneity of a sample of grain, with variations in species (if adulterated), disease state, size, shape and quality, rule out the use of NIR spectroscopy, which assumes a homogeneous sample to give meaningful data. Heterogeneous samples require us to capture spatial as well as spectral information, which is the strength of the VideometerLab 3 system and multispectral imaging in general. Even closely related species, like *Triticum aesitvum* (common or bread wheat) and *Triticum durum* (durum or macaroni wheat) grains, will have minor yet significant differences in their spectral response signatures, as well as size, shape and texture variations, so the extra spatial data inherent in an wide-area image (roughly 9cmx9cm) allows us to build models that account for the spatial separation of objects as well as their spectral characteristics.

In this trial, we assessed the ability of the VideometerLab 3 system to distinguish between specific varieties of *Triticum aesitvum* and *Triticum durum* wheat grains based on the spectral signature of each grain species for wheat authenticity applications. Once the software has 'learned' the unique spectral response characteristics of each grain species it can then distinguish between them, score a particular grain as being more likely to be *T. durum* or *T. aestivum* and return a results table for a sample of many grains with the number and proportion of each type in the sample. A more robust model would also incorporate size, shape, fluorescence and texture data, however the results of this model based solely on spectral reflectance characteristics already show an impressive ability to discriminate. Also, this model only scores the identity of each grain in order to return a count of each type in the sample; a more sophisticated model could return data on the average size and shape, disease state, hydration level etc.

Materials and Methods

A wheat test panel was 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). This comprised 100 seeds of 0%, 2%, 3%, 5%, 10%, and 100% (seed/seed) of a *T. aestivum* cultivar in the *T. durum* cultivar, and 200 seeds of 0.5% (seed/seed) *T. aestivum* in *T. durum.* Control samples for the two cultivars of *T. aestivum and T. durum* were also provided in the form of 300 seeds of each. Each sample of wheat grain was emptied into a standard Petri dish and a multi-spectral image data-cube was acquired with the VideometerLab 3 (Figure 2 and 3). The first two samples – 300 grain samples of just *T. durum* or *T. aestivum* – serve as the basis by which we will

'teach' the software the difference between the spectral signatures of *T. durum* and *T. aestivum* (Figure 4). The software builds a statistical transformation model that can score subsequent grains presented to the software on their likelihood of being *T. durum* or *T. aestivum*, based on the spectral similarity to the two extremes of the scale.

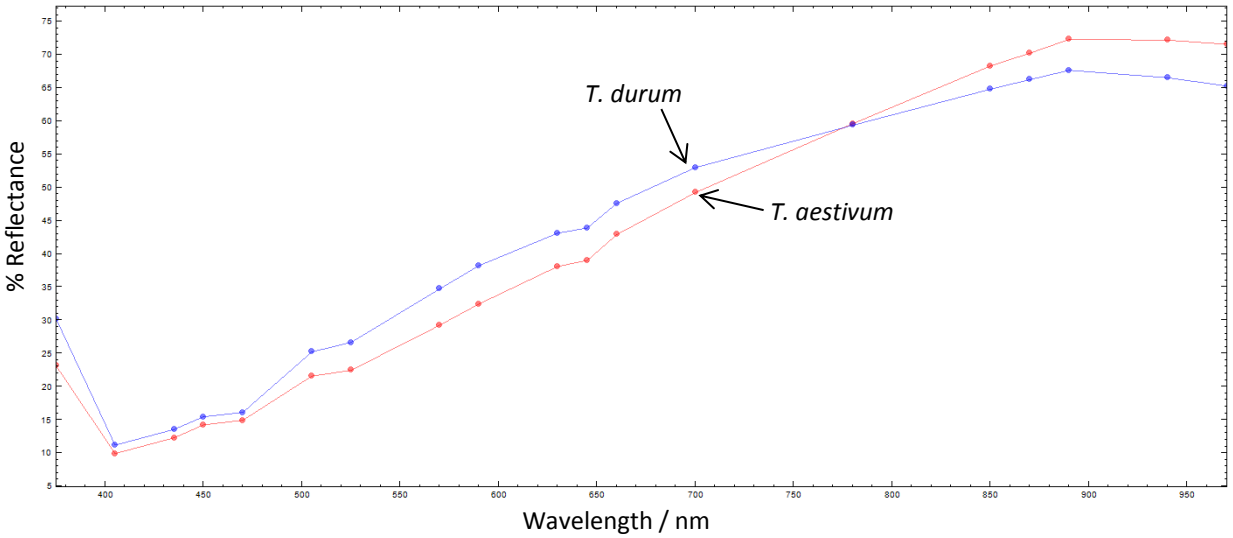


Figure 4: Representative reflectance spectra of *T. dur*um (blue data points) and *T. aestivum* (red data points). Illumination wavelength is on the x-axis and percentage reflectance is on the y-axis. Each plot has 19 data points, one for each LED illumination wavelength (Appendix B). Though similar in overall shape there is a clear difference between them, which allows the VideometerLab software to recognise and discriminate between the two.

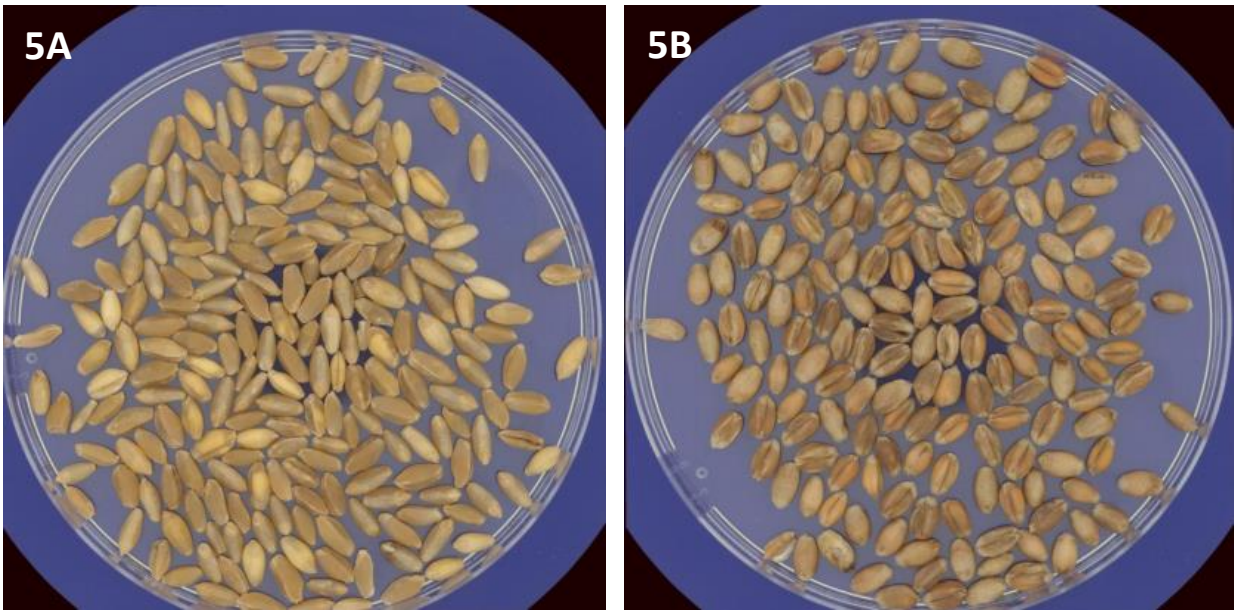


Figure 5: sRGB renderings of the pure samples of *T. dur*um (5A) and *T. aestivum* (5B) with 300 grains per sample.

Once an image of each pure sample was obtained, an nCDA (normalised Canonical Discrimination Analysis) model was built. Using the layer painting tool, the software is 'told' that the blue paint layer represents one class of object, and the yellow paint layer represents another class of object (Figures 5A.1 & 5B.1 below). Using

an nCDA transformation in the MSI (multi-spectral imaging) toolbox, the software analyses the spectral signature of all the pixels represented in each layer and builds a model to discriminate between the two classes.

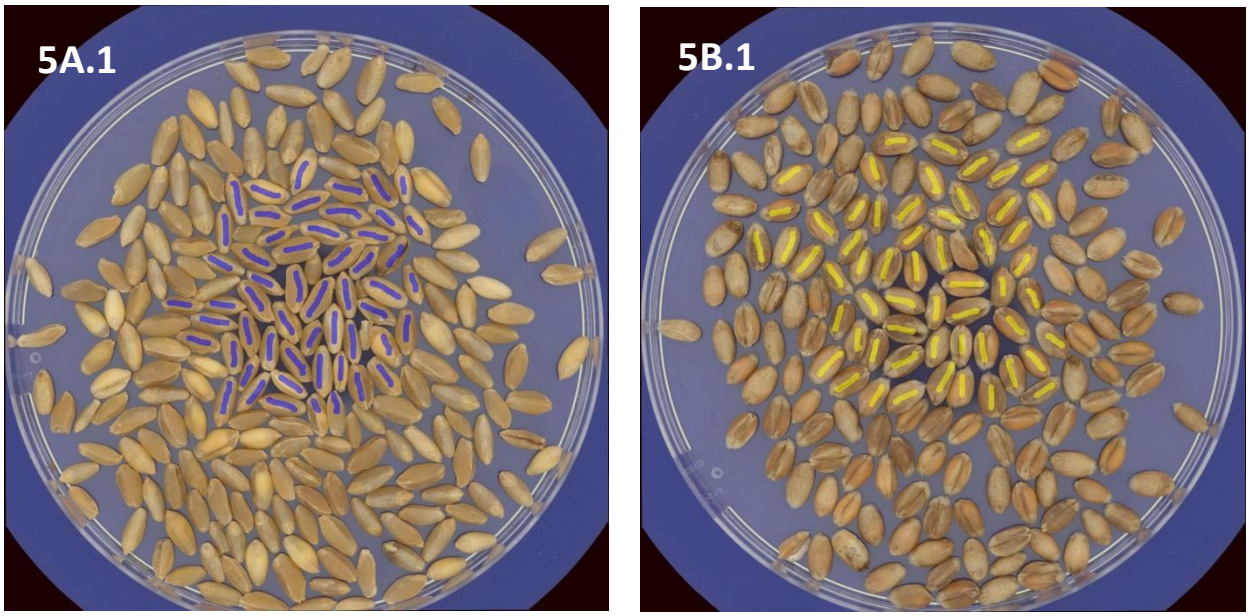
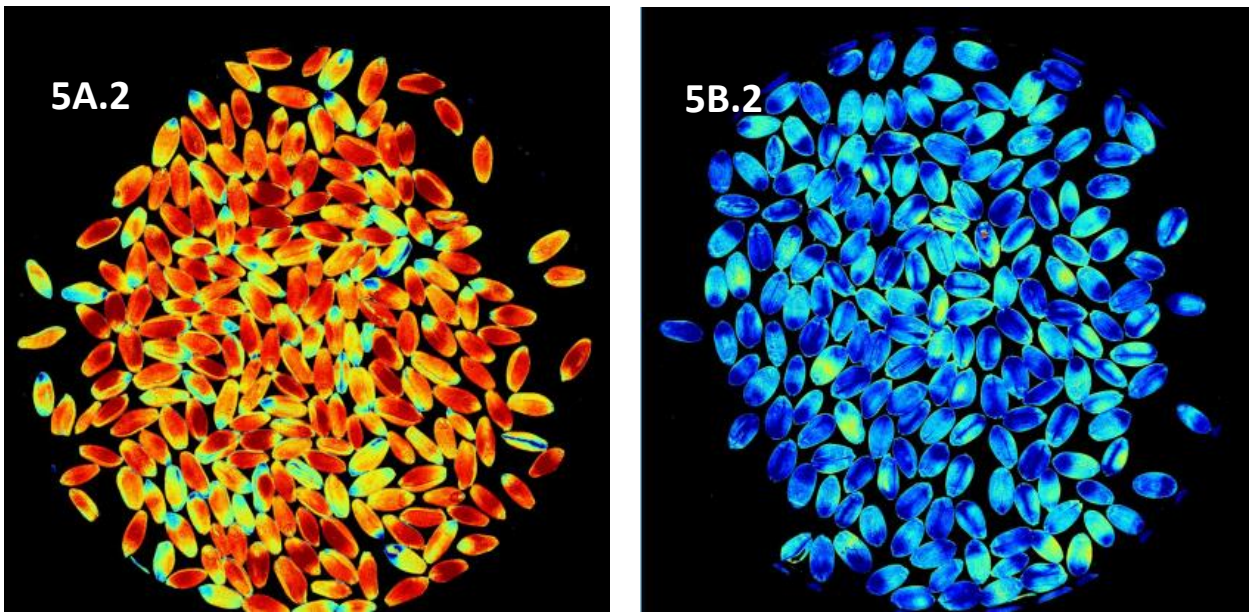


Figure 5.1: Representative *T. durum* (A) and *T. aestivum* (B) wheat grains in each image are 'painted' (5A.1, 5B.1) to indicate to the software that we would like to compare the differing spectral characteristics of the pixels highlighted by each separate paint layer. From this information, the VideometerLab software can build an nCDA discrimination model to score pixels on whether they are more 'blue-like' (5B.2) or more 'yellow-like' (5A.2). Below, the model has been applied to the original images and a false-colour scheme applied to visually highlight differences. As expected, the model has graded nearly all pixels in the 5A.2 image as *T. durum* and nearly all pixels in the 5B.2 image as *T. aestivum*.



When presented with a new image, the nCDA-based model is applied and scores each pixel in the new image on an arbitrary scale as to whether it matches the spectral signature of the blue painted layer or the yellow painted layer. If the original pure sample images are presented to the model, it correctly grades every grain in the image as *T. durum* (Figure 5A.2) or *T. aestivum* (Figure 5B.2) respectively. In a sample of unknown composition (a mixture of *T. durum* and *T. aestivum*), those grains with pixel spectra that are more like the *T. durum* spectrum will be scored as highly positive (false coloured red), and conversely those grains with pixel spectra that are more like the *T. aestivum* spectrum will be scored highly negative (false coloured blue) (Figure 6).

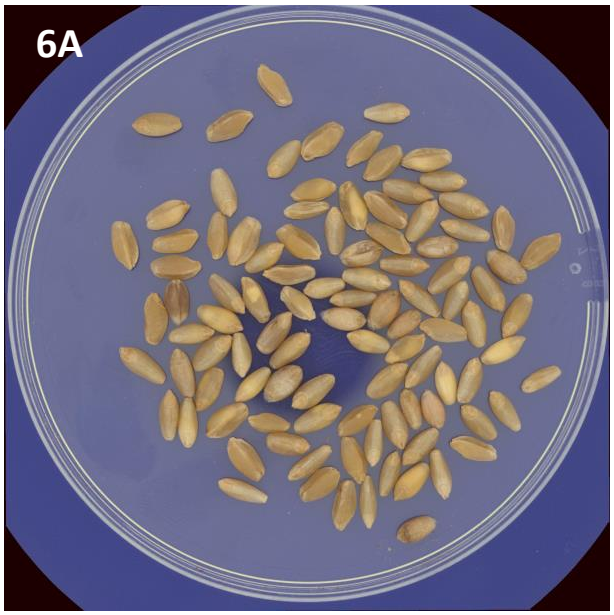
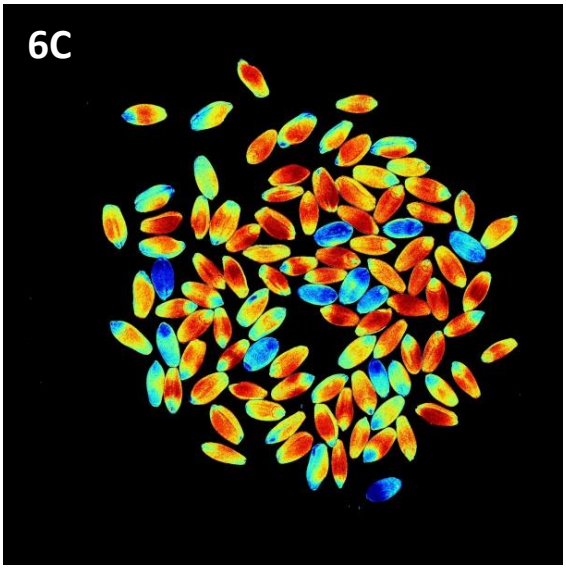


Figure 6: Application of the model to an unknown sample, specifically Sample 3. These three images represent 'behind the scenes' stages in the VideometerLab software processing.

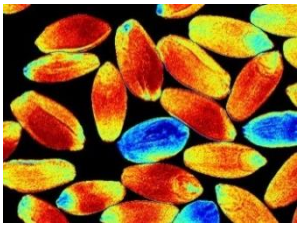
6A: A raw image data-cube, viewed as an sRGB rendering. The image includes *T. durum* grains, *T. aestivum* grains, the blue background plate and the clear petri dish holding the grain sample.

6B: The software automatically removes pixels it recognises as either the blue background plate or the petri dish, leaving just the grains. Some grains are touching each other, so the software separates them with a thin one-pixel wide line for further analysis with the blob software toolbox.

Enlarged image showing object separation of individual grains



6C: The nCDA discrimination model is applied to every pixel left in the image. If a pixel's spectrum is more like the characteristic spectrum 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). Here we can see that the model has graded 11 of the grains as more likely to be *T. aestivum*.



Enlarged image showing nCDA model scoring of individual grain

When combined with the object separation and analysis capabilities of the software (blob toolbox), the software can automatically separate touching grains, score each one as being more like *T. durum* or more like *T. aestivum*, and return a table of results indicating the number and percentage of each different type of grain in an image (Figure 7).

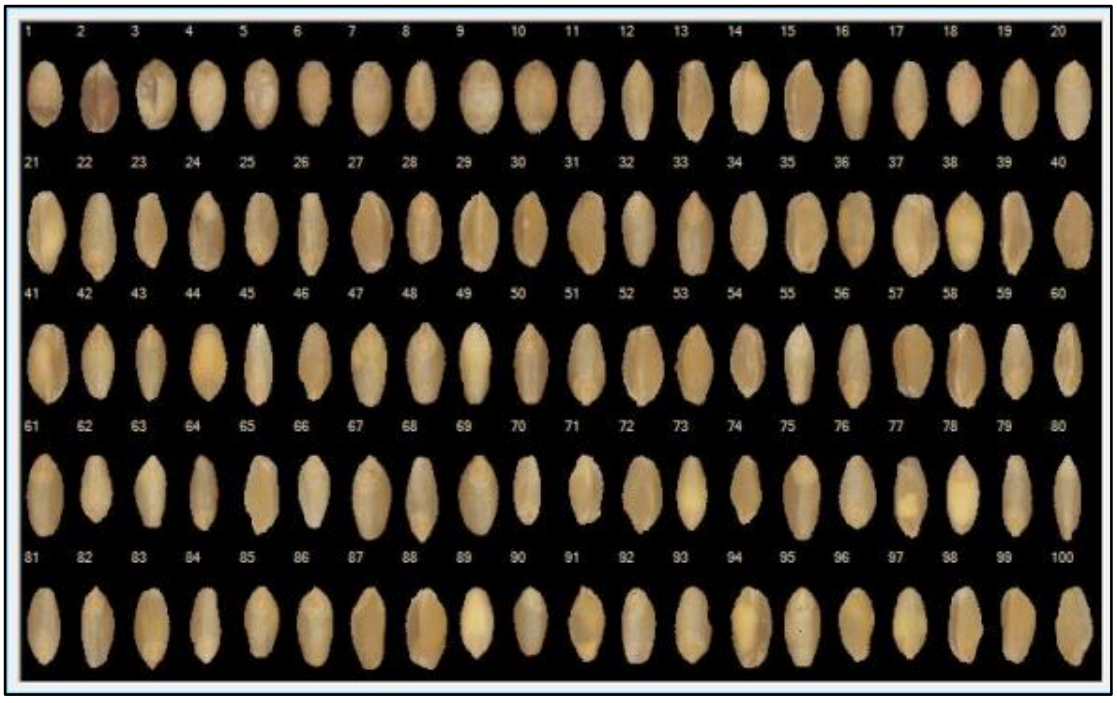


Figure 7: Blob toolbox software module automatically segments and sorts the grains in sample 3 in the order of likelihood of being *T. durum* grains. The sorting score is based on our nCDA model, which was built for spectral discrimination only. A more robust, sophisticated model would incorporate further 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.

Results

The study investigated a wheat adulteration test panel of 23 samples of wheat grains comprising control samples containing three hundred seed samples of a particular cultivar for each of the two wheat species to be analysed (*T. durum* and *T. aestivum*), plus 21 blind samples to be analysed and scored by the VideometerLab 3.

A spectral discrimination model, based on an nCDA statistical data transformation, was built using the two known wheat control samples. The model was applied to subsequent unknown samples, and results were returned based on the model's scoring of how closely the spectral signature of each grain in an image matched either the *T. durum* or *T. aestivum*.

Sample ID	Number of grains sampled	Estimated number *of T.durum* grains	Estimated number of *T.aestivum* grains	Estimated % *T. aestivum* grains	Actual % *T. aestivum* grains
1	300	300	0	0.00	*T.durum* Control
2	300	0	300	100.00	*T. aestivum* Control
3	100	89	11	11.00	10% adulterated
4	100	95	5	5.00	3% adulterated
5	100	0	100	100.00	100% adulterated
6	100	95	5	5.00	3% adulterated
7	101	98	3	2.97	2% adulterated
8	100	94	6	6.00	5% adulterated
9	101	90	11	10.89	10% adulterated
10	199	195	4	2.01	0.5% adulterated
11	100	98	2	2.00	0% adulterated
12	200	198	2	1.00	0.5% adulterated
13	100	95	5	5.00	5% adulterated
14	100	95	5	5.00	5% adulterated
15	90	87	3	3.33	2% adulterated
16	100	91	9	9.00	10% adulterated
17	100	99	1	1.00	0% adulterated
18	100	0	100	100.00	100% adulterated

19	197	197	0	0.00	0.5% adulterated
20	100	98	2	2.00	2% adulterated
21	100	0	100	100.00	100% adulterated
22	100	100	0	0.00	0% adulterated
23	100	96	4	4.00	3% adulterated

Table 1. Results obtained for the blind test of 23 samples of *T.durum* for which a specific number had been adulterated with *T.aestivum*

From the results presented in Table 1 it can be seen that the VideometerLab 3 was capable of reliably distinguishing between the two wheat types and assigning percentage adulteration levels that agreed well with the assigned values.

Conclusion

The current method of choice for determination of pasta adulteration is to use molecular biology methods, in particular real-time PCR. However, whilst molecular biology approaches are effective they could be criticised in terms of the need for specialist laboratory equipment and consumables, costly reagents and a requirement for specialist training. Additionally, most molecular biology approaches for food authenticity testing are destructive as DNA is often extracted from the sample itself.

The VideometerLab 3 instrument has the capability to differentiate between surface colour, texture and chemical composition for a range of materials, and is applicable to both grain and seed analysis compared to traditional spectroscopy techniques. Even closely related species such as *T. durum* (durum wheat) and *T. aestivum* (common wheat) will have significantly different spectral response signatures which can be used to build a discriminatory model for identification and quantification purposes. The results presented here demonstrate that the VideometerLab 3 can reliably distinguish between the two wheat cultivars used to represent *T. durum* and *T. aestivum*, and estimated levels of adulteration in blind test samples appears to agree well with expectations.

Appendix A: VideometerLab 3 Technical Specifications

Light sources	19 high power strobed LED sources with a range from 375 to 970 nm. Optional bright-field, dark-field and front-light sources are available.
Image size	2056 x 2056 pixels
Resolution	~45µm x 45µm per pixel
Dynamic range	Optimized according to the application using autolight setup
Calibration	NIST traceable reflectance calibration using 2 reflectance calibration targets and one geometric calibration target. Simple calibration wizard procedure that takes 3 minutes. Drift correction calibration areas are included in every image.
Sample size	Height max. 90 mm, diameter of inspection opening 110 mm
Time of analysis	5-10 seconds per sample (optimisation for specialised applications, e.g. in-line machine vision quality grading, can reduce this time to under a second)
Dimensions	490-585 mm(h) x 420 mm(w) x 590 mm(d)
Weight	14.1 kg (Net), 26.6 kg (Gross)
Power supply	90 – 260 VAC, 47 – 63 Hz
Ambient temperature	Operation: 5 - 40 °C, Storage; -5 – 50 °C
Ambient humidity	20-90 % RH non-condensing
PC requirements	Minimum configuration: Intel i7 or better, 16GB RAM, USB2 port, Gigabit Ethernet card
Software	Microsoft Windows 7 Professional 64 bit, full windows update
Hardware Options	Various hardware options are available, including dark-field and bright-field lighting for microbiology applications, a vibratory feeder for presentation of bulk granular samples and bar code reader for automatic registration of sample ID to the VideometerLab software

Appendix B: LED illumination in spectral imaging

Multispectral imaging systems far surpass the abilities of our own visual perception; using light in the UV or NIR region adds information simply not visible to the human eye and calibrated CCD sensors can easily detect minute changes in light intensity that are imperceptible to us. Like all modern imaging techniques, even minor variations in the exact setup of light source, physical geometry, sensor sensitivity and imaging method can affect the data quality and hence our ability to apply robust models that give us information on our sample.

The VideometerLab 3 is carefully designed to ensure maximum repeatability of illumination and sensor conditions, both across time and between separate instruments. LEDs have very stable emission characteristics, the strobe controller microprocessor regulates emission periods with µs precision, the integrating sphere ensures emitted light is evenly distributed from all angles over the sample surface, and the CCD camera is calibrated with automatic background procedures and by the user at regular intervals. High quality design certainly makes our data reliable, but the choice of LEDs as an illumination source has another advantage too.

Using strobed LED illumination at multiple specific wavelengths allows the illumination conditions for each different sample type to be optimised, as we are not restricted to the fixed emission characteristics of a single broadband light source, like a halogen bulb. For example, if our sample reflects very little UV light we will struggle with low signal to noise ratio in that wavelength region. The VL3 can simply increase the strobe time of the UV LED to increase the amount of UV light reaching the sample, compensate for the sample's low UV reflectance and gain better signal.

This specificity isn't possible with a broadband illumination source, as increasing the UV output of a halogen bulb (normally very low) entails increasing output in the whole emission spectrum (by making the bulb brighter) and will saturate the detector at higher wavelengths. We cannot optimise the illumination for any one wavelength without affecting the signal to noise ratio at other wavelengths, forcing a compromise to get the best data we can. Separated narrowband LED illumination allows specific illumination optimisation at each point in our spectrum, so we get optimal signal to noise and hence the best data possible.

Once the exact strobe time of each LED is optimised for a certain sample type using the VideometerLab software's light setup wizard, the illumination profile settings are saved for later use on similar samples and we can be sure that the data will be comparable. We can run the same analysis model on each image datacube and be confident that the results are comparing like for like, whether the images were acquired a year apart or from two separate VL3 instruments.

Appendix C: Table of LED emission wavelengths

The VideometerLab 3 is equipped with the following LEDs. Each LED emits light of a given centre wavelength. The table below shows the centre wavelengths together with colour names and application/compound examples (spectral peaks or shoulders) for each wavelength.

Band	Wavelength/nm	Spectral region	Application/compound example
1	375	UVA	Fluorescence, GFP excitation
2	405	Violet	Melanins
3	435	Indigo	Chlorophyll A (absorption and excitation)
4	450	Blue	Riboflavin, Chlorophyll B, β-caroten
5	470	Blue	RGB Blue
6	505	Cyan	RGB green, Metmyoglobin
7	525	Green	Myoglobin
8	570	Yellow	Oxymyoglobin
9	590	Amber	Oxymyoglobin
10	630	Red	RGB red, Metmyoglobin (weak)
11	645	Red	Chlorophyll B
12	660	Red	Oxidation, Chlorophyll A
13	700	Red	Oxidation
14	780	Deep red	Oxidation
15	850	NIR	Baseline
16	870	NIR	Baseline
17	890	NIR	Fat shoulder
18	940	NIR	Fat
19	970	NIR	Water
Optional	Bright-field/Dark-field/Back-light illumination	Visual	Translucency, diffuse dark-field scattering, surface topography
Optional	Longpass Emission Filter wheel	Longpass cut-off at 400nm, 500nm, 600nm and 700nm (other filter sets available)	Fluorescence emission stimulated at each LED excitation wavelength, provides 27 extra images to add to datacube analysis

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