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Mapping distributions of components in food through image analysis

Many foods and food materials have a non-uniform structure and composition. They may contain multiple components such as chocolate or caramel in a confectionery product, fruit pieces in yoghurt, structures such as marbling fat in meat, or oil and water in an emulsion, layers in packaging materials, or gradients of fat, water or salt. For measurement of average composition, methods such as grinding and blending are used to prepare representative subsamples for bulk analysis, or it may be possible to separate large components for individual analysis. However, the distribution of composition is also relevant in some cases, for example to identify specific components within the structure, to understand the function of particular components, to check that ingredients are uniformly dispersed or to study the migration of components such as water within a food product.

This white paper looks at the various ways in which this can be achieved. To discuss any of the techniques described or to find out more about how we can help you with any specific issue, contact:

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Introduction

A wide range of imaging and microscopy methods are used to study the structure and appearance of food products. Several methods are also available to identify the composition of features within such images and to map the distribution of particular compounds. These include staining and fluorescence methods to highlight the location of features of interest, and advanced spectroscopic methods that provide compositional information about each pixel.

Colour

Colour is a familiar basis for recognising different components, for example to discriminate lean regions from fat in meat for assessment of marbling. Chromameters and spectrophotometers are widely used in the food industry for accurate measurement of average colour over an area of a sample. A common measurement scale is the CIELAB system, which provides a standardised description of colour by three parameters *L**, *a** and *b**:

- *L** Lightness (0-100)
- *a** Variation from green (-100) to red (+100)
- *b** Variation from blue (-100) to yellow (+100)

Methods are available to calibrate colour imaging devices against such scales, enabling accurate colour measurements to be made for individual regions. Approaches include hyperspectral imaging using spectral cameras or a series of narrow-band illumination sources (e.g. Videometer), or calibration of a digital camera. At Campden BRI, we use a DigiEye imaging system (Verivide Ltd) for this purpose. The system includes an imaging cabinet with standardised lighting. Images are taken with a high resolution digital camera and the system is calibrated against a reference colour chart. The system can be used for colour measurement, and provides accurate images for documentation of process trials and production of specification images.

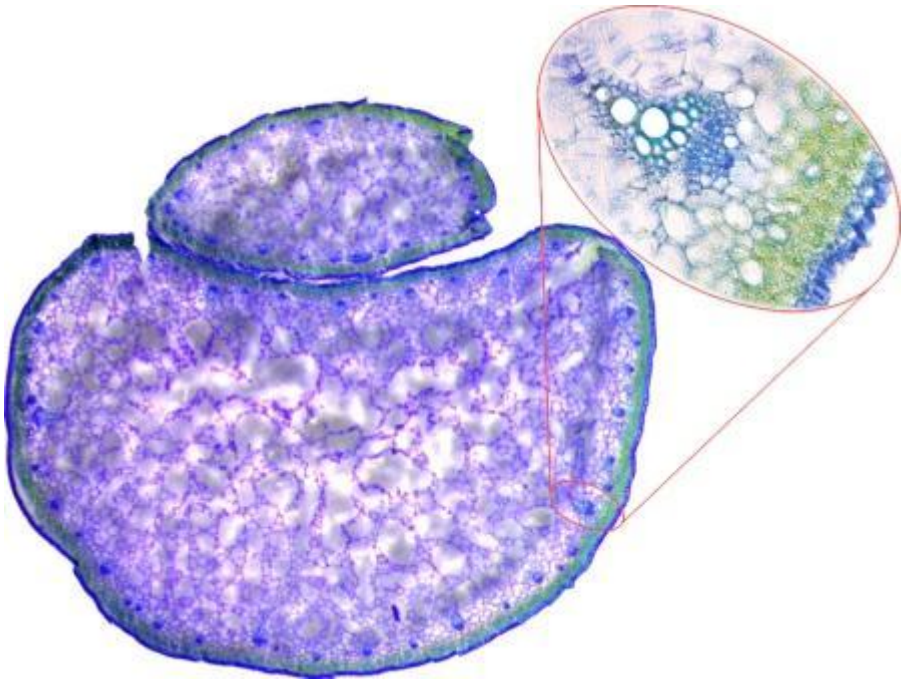
Microscopy

Optical microscopy is a useful tool for studying food structure, and can also provide information on composition. A range of methods are available to create contrast between materials of interest, including the use of polarised light, fluorescence and stains. Polarised light is useful for study of crystalline materials such as sugar crystals, ungelatinised starch granules and birefringent plastics. Methods such as Differential or Nomarski Interference Contrast can be used to provide contrast between materials of differing refractive index. Chemical stains can be added to reveal specific components and are widely used in microscopy. Table 1 lists some of the methods we use to identify particular food components. Figure 1 shows an example of a spring onion stained with Toluidine Blue, revealing cellulose and lignin.

Table 1 – Optical microscopy techniques used to identify food components

Target material	Stain	Positive result
Cellulose cell walls	Toluidine Blue	Purple
Lignin	Phloroglucinol-HCl	Pink or Red
Cellulose	IKI-H_2SO_4	Blue
Cellulose	Zinc Chlor-iodide	Blue
Pectin	Ruthenium Red	Pink or Red
Starch	Iodine	Black
Starch	Congo Red	Red
Starch	Polarised light	Ungelatinised: Maltese cross Gelatinised: No Maltese cross
Polysaccharides	Periodic Acid – Schiff's (PAS)	Purplish Red
Fats	Sudan stains	Red Black
Fats	Osmium Tetroxide	Brown
Gelatinised starch	Trypan Blue	Blue
Protein	Eosin	Pink
Bone fragments	Alizarin Red S	Red
Lipids	Oil Red O	Lipids: Red

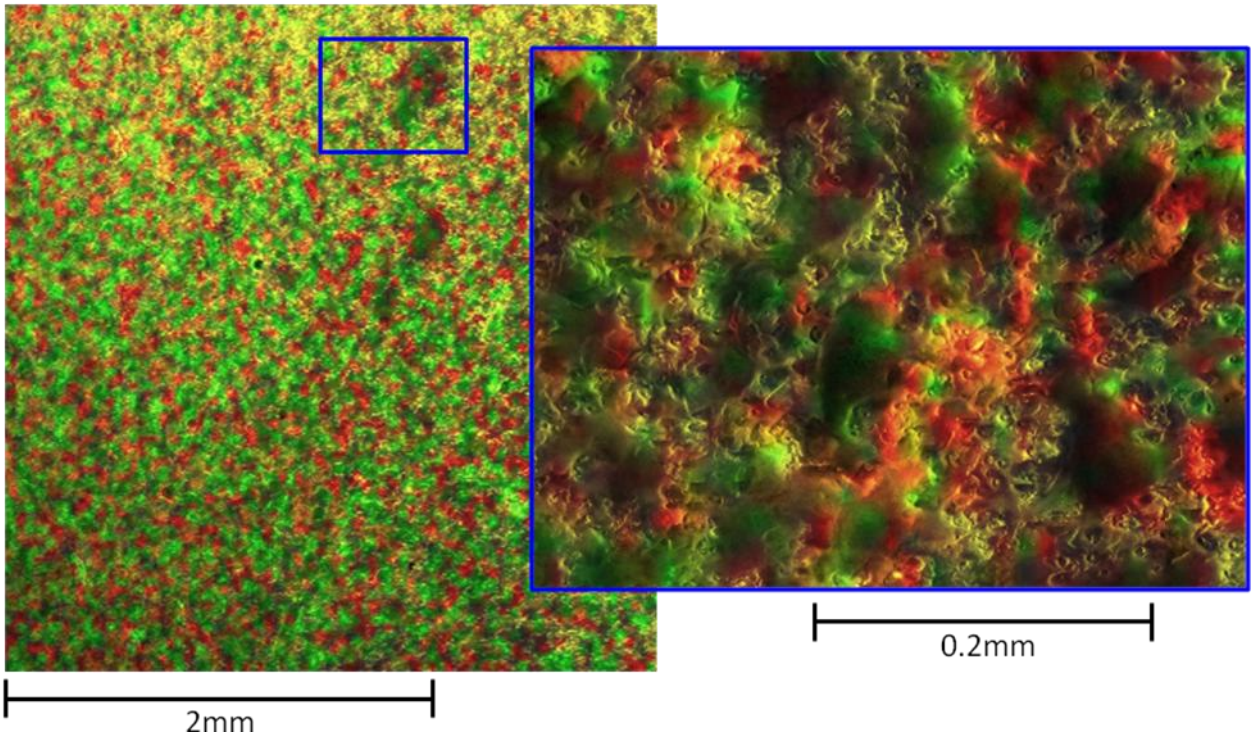
Figure 1 **Cross-section of a spring onion, stained with Toluidine Blue. The stain reveals cellulose in the outer layer and lignin in the underlying vascular bundles. The green colour is chlorophyll.**



X-ray micro-analysis

This method is used to identify and map chemical elements at the microscopic scale. The method is used in conjunction with Scanning Electron Microscopy (SEM). Scanning Electron Microscopy is a useful tool to image food structures at a wide range of scales. An image is formed by scanning a focussed beam of electrons across a sample and measuring the resulting emissions from each point. The secondary electron signal is commonly used to generate an image of the sample structure, whilst the backscattered electron signal can be used to give "atomic number contrast" to identify areas of differing elemental composition. The incident electron beam also causes X-rays to be emitted at specific energies that depend on the chemical elements present in the sample. By measuring the X-ray spectrum, individual elements can be identified and mapped.

Figure 2 **SEM image of chocolate with elemental composition mapped using an X-ray microanalyser. Green: potassium, present in cocoa solids; Red: calcium, present in milk solids.**



We have a modern variable pressure SEM which enables food samples to be studied with minimal sample preparation. The system is equipped with an energy dispersive X-ray microanalyser, enabling us to identify the composition of features of interest. A common application of this method at is to aid in the identification of foreign bodies such as glass and metals. Different types of glass can be identified by comparison against a database. The method can also be applied to foods themselves. Most food materials contain large proportions of carbon, oxygen and hydrogen and, in proteins, nitrogen, typically relatively uniformly dispersed. However, other elements are also present. For example, Figure 2 shows potassium and calcium in chocolate, revealing the distribution of cocoa and milk solids.

Infra-red spectroscopy

Different chemical bonds or small molecular groups absorb electromagnetic radiation of specific energies, corresponding to specific frequencies or wavelengths. For organic compounds typical of food and plastic packaging, many of these absorptions fall in the infra-red region of the spectrum. The infra-red spectrum is typically subdivided into:

- Long wavelength infrared 8-15µm
- Mid infrared ..... 2.5-8µm (1250 – 4000 cm^{-1}*)
- Near or short wavelength infrared ... 0.7-2.5µm

** Wavenumber is commonly used in mid-IR spectroscopy as an alternative to wavelength.*

Long wavelength infrared is commonly used for remote temperature measurements, such as thermal imaging, based on the radiation emitted by objects, the spectrum of which depends on their temperature. We use this to validate cooking processes to ensure uniform temperatures for food safety and quality, and to test suitability of packaging materials. Compositional measurements more commonly use the mid- and near-infrared regions, based on measurement of the reflectance or transmittance spectrum.

The mid infrared (mid IR) region contains strong, well defined absorbances that provide a fingerprint to identify specific small molecules or molecular groups. The method can be used, for example, to identify plastics within packaging materials or the presence of proteins, phenolics and other molecules within food items.

The near infrared (NIR) or short wavelength infrared (SWIR) region contains overtone absorbances with broader, often overlapping bands, which provide less distinctive identification than the mid infrared, but simpler sample presentation, widely used in the food industry for rapid, quantitative analysis of components such as moisture, fat and protein, using calibrations developed against reference samples. NIR spectroscopy is most commonly used for analysis of bulk samples, for both laboratory and on-line measurements, but can also be used as an imaging method.

Imaging spectrometers

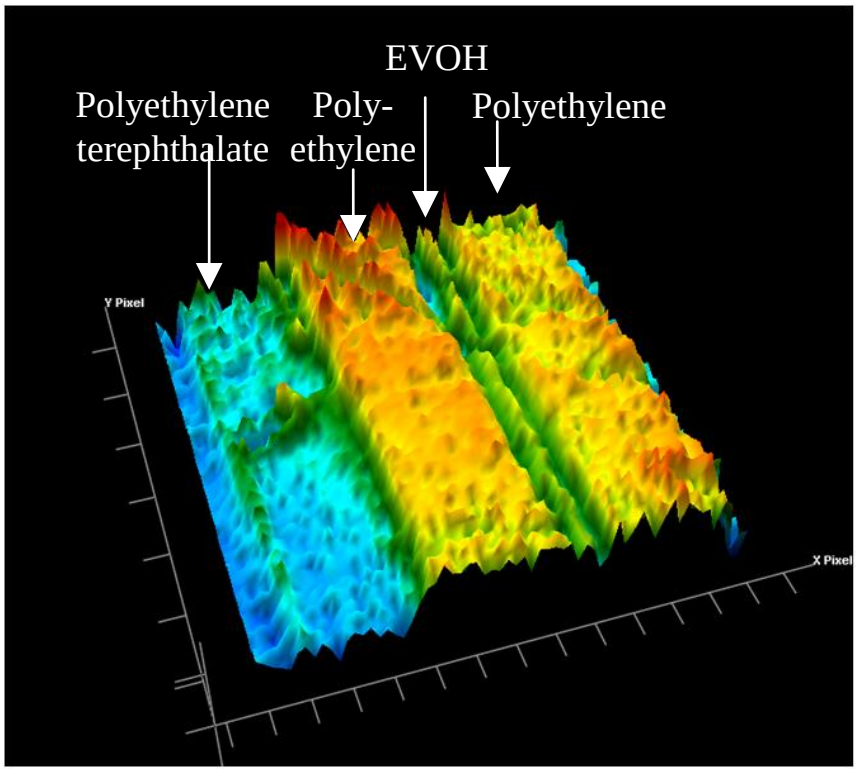
Imaging spectrometers enable a spectrum to be measured for each pixel in an image. By assessing characteristic spectral features at each pixel or by using calibrations to quantify individual components, the distribution of food components can be determined. Spectral data are acquired either by imaging the full sample area and using a scanning method to accumulate spectral data ('staring' or 'area-scan' imaging), or by using a spectrograph to view spectra for a line across the sample and then moving the imaging system relative to the sample to accumulate data for the full sample area ('pushbroom' imaging). The former method requires no moving parts and is well suited to microscopy applications. The latter is typically faster and is well suited to on-line applications to image samples as they move past the imaging system.

Fourier Transform Infrared (FTIR) microspectroscopy

We use mid infrared spectroscopy as a microscopy method to study the composition of small structures. An optical microscope is first used to select a region of interest. Mid infrared spectra can

then be collected for a selected field of view of 70µm to 2.6mm, with a resolution of down to 2-3µm, using an area-scan approach. FTIR is an approach used to measure spectral information, by passing the light for each pixel through an interferometer and measuring how the signal varies as the path length of the interferometer is adjusted. The process requires just a few minutes to acquire a full spectral image. This can be used to map the distribution of a specific bond or compound across a sample, such as different layers in packaging (Figure 3), or fats and oils in chocolate. A spectrum can be extracted from any of the 4096 pixels in the chemical image, which can then be searched against reference libraries to identify a specific component within the sample.

Figure 3 FTIR microscopy of layers in plastic packaging. A map of absorbance at 2914cm^{-1} is shown, corresponding to polyethylene layers.



Near infra-red hyperspectral imaging

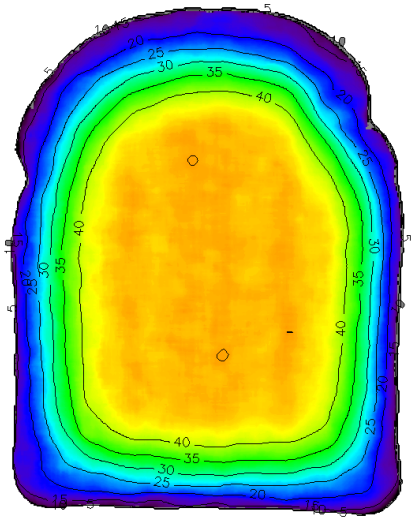
We use a hyperspectral NIR imaging system to measure distributions of composition in a range of food samples. Applications have included (Whitworth, 2012, Whitworth *et al.*, 2010):

- Moisture distribution in bread (Figure 4a), biscuits (Figure 4b) and multi-component products such as custard tarts.
- Fat distribution in fried chips (Figure 5) and doughnuts;
- Detection of crystalline sucrose in biscuits;
- Measurement of components in powders, for example to detect particles of added dried gluten in wheat flour (Figure 6);
- Detection of damage in fruit;
- Measurement of fish (Chau *et al.*, 2009) and beef (Moss *et al.*, 2010) quality.

Samples are presented on a moving translation stage and imaged with a SWIR spectral camera (Specim Ltd.) using a pushbroom approach. A HgCdTe detector provides a wavelength range of 970 to 2500nm and a selection of lenses provide a field of view of 10 to 300mm with a minimum pixel size of 30µm. The method is rapid, requiring a few seconds to scan a sample. The system at Campden BRI is designed for laboratory and at-line use. The same approach can also be adapted for on-line measurement of samples on moving production lines.

Figure 4 Distribution of moisture content

(a) Bread



(b) Biscuits at multiple stages of baking

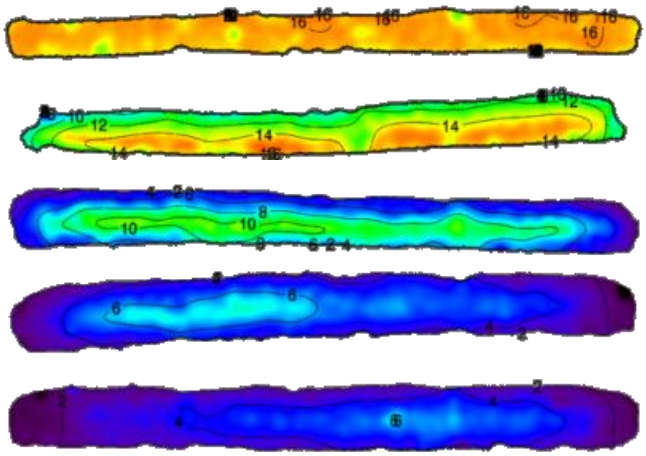


Figure 5 Distribution of fat in chips with increasing frying time

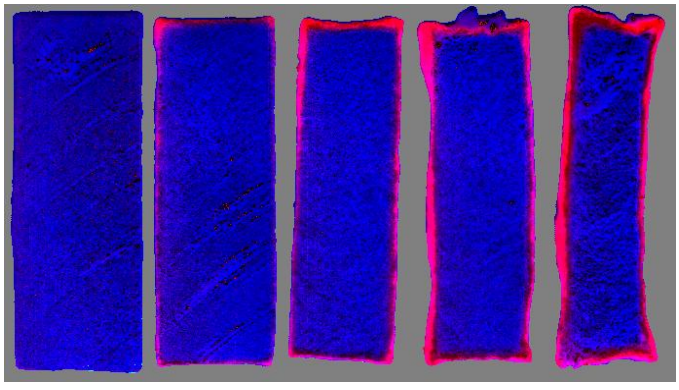
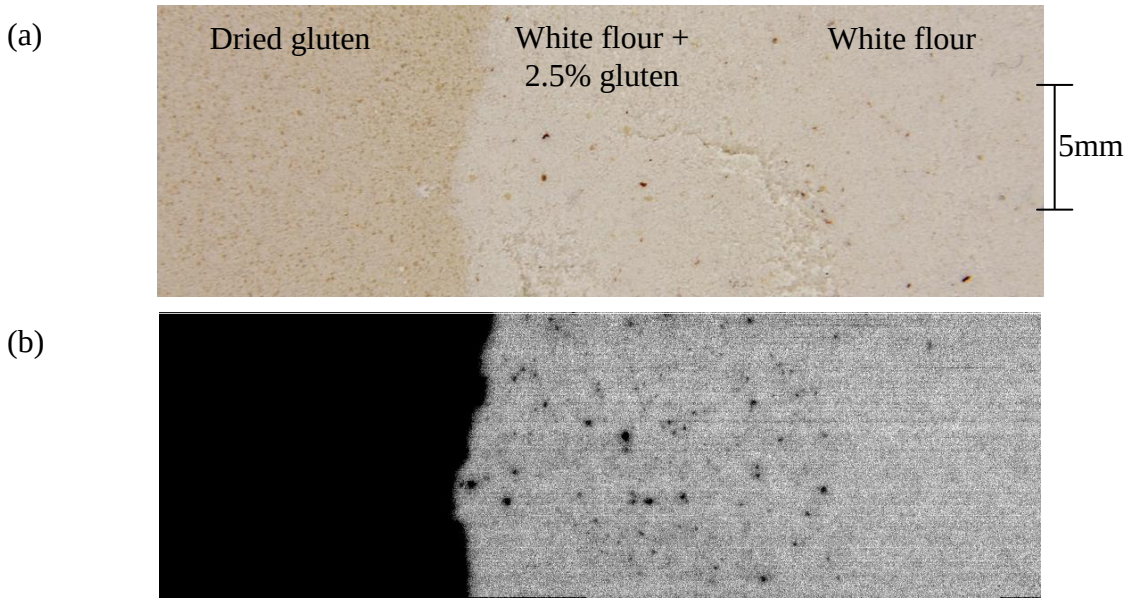


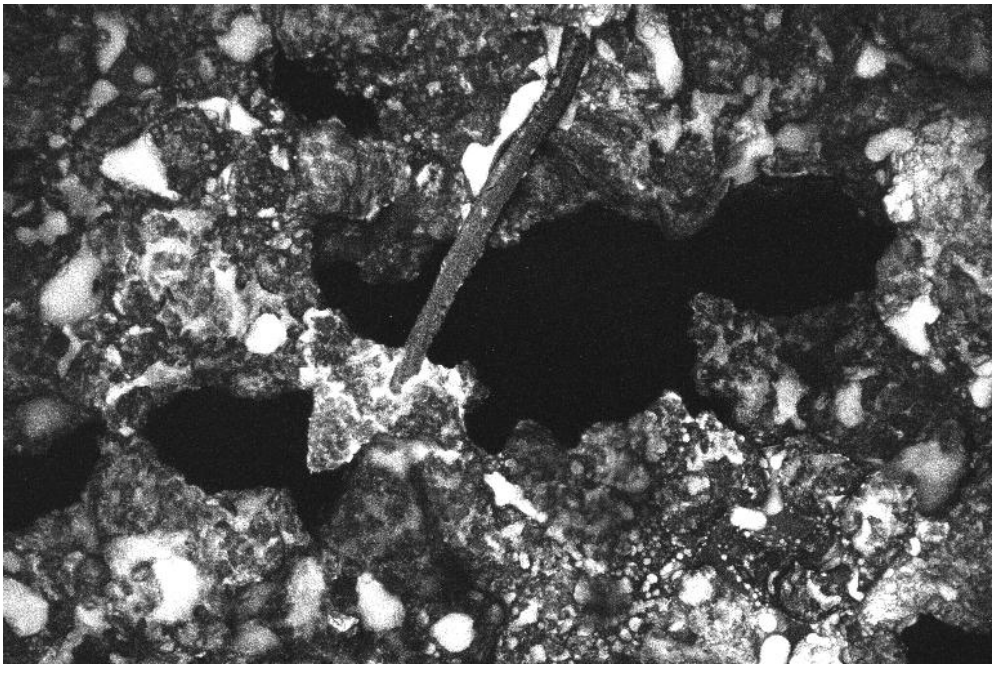
Figure 6 **Detection of dried gluten in flour. (a) Visible light image; (b) NIR image showing the presence of dried gluten (dark shading)**



Fluorescence

Some stains and food components fluoresce when illuminated with light of a specific excitation wavelength, emitting light of a longer wavelength. Special fluorescence microscope facilities provide the required illumination and imaging conditions. A related technique is confocal laser scanning microscopy (CLSM), in which an image is formed by scanning a fluorescent stained sample with a focussed laser beam at the excitation wavelength and mapping the emission from each point. Figure 7 shows an example of biscuit structure, using a fluorescent stain for fat.

Figure 7 **CLSM image of biscuit structure showing the location of fat (bright regions), stained with a fluorescent stain**



Some food materials are naturally autofluorescent, without the requirement for staining, which has been exploited in several practical food applications:

Some types of fungi have a greenish-yellow fluorescence, which is used to inspect commodities such as maize for potential aflatoxin contamination.

Fluorescence of ferulic acid in the aleurone layer of wheat under ultraviolet light enables this colourless material to be detected and measured in flour. This has been exploited in the Fluoroscan instrument (Branscan Ltd), based on imaging methods originally developed by Campden BRI, and is used by mills and flour testing laboratories to measure the concentration of bran and aleurone particles in white flour.

Chlorophyll fluoresces at an excitation wavelength of 430nm. We demonstrated the feasibility of using a commercial hand-held fluorescence imager to detect chlorophyll as a marker of faecal contamination in meat carcasses (Burfoot *et al.*, 2011), and this approach is used by some slaughterhouses as part of their control procedures.

Contact us

Image analysis provides a versatile suite of techniques for mapping the distribution of components within a food product. Different methods will be applicable, depending on what you need to know. Please contact us if you think we can help you.

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