doi: 10.1255/nirn.1583

Future trends in hyperspectral imaging[a]

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History of hyperspectral imaging technology

Hyperspectral imaging (HSI) is an imaging technique that combines aspects of conventional imaging with spectrophotometry and radiometry. This technique is capable of providing an absolute radiometric measurement over a contiguous spectral range for every pixel of an image. Thus, data from a hyperspectral image contains both spatial and spectral information. HSI data are considered as a three-dimensional hypercube (also referred to as a datacube) that can provide physical and/or chemical information on materials. This information includes physical and geometric observations of size, orientation, shape, colour and texture in addition to chemical or molecular information about constituents such as water, fat, proteins and other hydrogen-bonded molecules.

Typically, HSI obtains a spectrum, which has a wide range, fine resolution of wavelengths from each pixel in an image which can be used for finding objects, identifying materials or detecting processes. This ability enables us to develop hyperspectral sensors that look at objects using a vast portion of the electromagnetic spectrum from visible to near infrared (NIR) wavelengths, and processing systems for applications in astronomy, agriculture, biomedicine, geosciences, physics and surveillance.

An advantage of HSI is that no prior knowledge of the sample is needed and information can be obtained exclusively from the dataset. HSI can also take advantage of the spatial relationships between different spectra in neighbouring pixels, allowing more accurate segmentation and classification of the image with both spectral and spatial models.

In the early 1980s, HSI technologies were first introduced in airborne remote sensing (Figure 1). Since HSI has been developed as a powerful technique in earth remote sensing, it has been extended to other fields such as environmental, food, agricultural, forestry, medical, biological, geological and industrial analysis.

Current hyperspectral imaging technology

In the early applications stages, applications of HSI to industrial applications were limited due to the equipment cost and the large datasets which required to be processed. However, in recent years, advances in cost-effective fabrication have enabled the development of compact and inexpensive HSI systems with fast computing ability for commercial machine vision applications such as food sorting and pharmaceutical quality control.

Hyperspectral remote sensing monitors specific crops for issues such as detection of grape variety, early warning of disease, detection of water stress and nutrition in plants. Additionally, detection of animal proteins in compound feeds has been studied using HSI technology.

A recent report from the MicroMarket Monitor[1] (2015, Dallas, TX) predicted that the global market for HSI will grow at a compound annual rate of 12% until 2019. Driving that growth will be a fast shift toward inexpensive, compact systems that can speedily process large datacubes. In addition to enabling fast machine vision, these compact HSI sensors are available for precision agriculture on unmanned aerial vehicles (UAVs), military security and medical imaging applications.

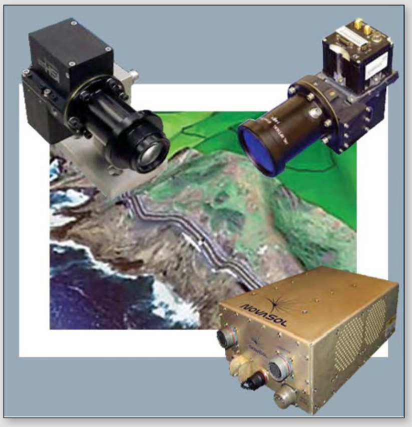

Figure 1. Airborne hyperspectral remote sensing (courtesy of Corning Inc.).

HSI for food research

Since HSI is a useful tool to thoroughly analyse the spectra of inhomogeneous food materials that contain a wide range of spectral and spatial information, researchers at the USDA ARS have developed a visible near infrared (VNIR) hyperspectral imaging method for detecting surface contaminants on poultry carcasses during processing.[2,3]

Although HSI technology has the potential for food safety inspection and quality control, several limitations to its wide adoption by the food industry currently exist. The most limiting factors are the relatively long time necessary for data acquisition and processing together with equipment cost. However, modern HSI or multispectral imaging technology[4] with wavelengths selected from HSI is an alternative and promising approach for the food processing industry. For food processing applications, HSI with real-time pattern recognition software communicating with a robotic manipulator can sort products by removing foreign materials from a conveyor belt based on their unique spectral signatures.

HSI platforms

For the performance of HSI measurements, several different platforms are currently available; these include pushbroom, acousto-optic tuneable filter (AOTF), filter wheel and liquid crystal tuneable filter (LCTF) techniques. When selecting a platform for a specific application, several parameters,

[a]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

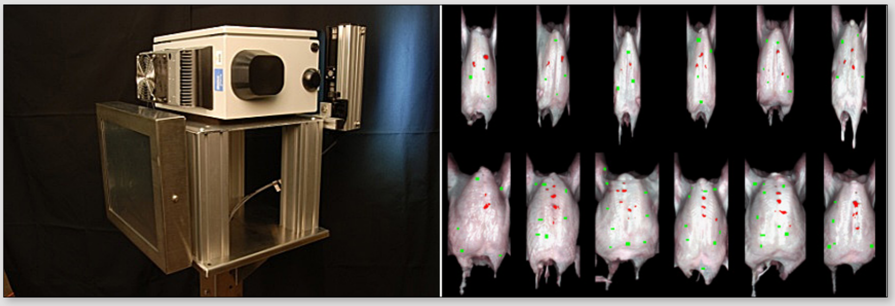

Figure 2. Real-time in-line hyperspectral imaging.

including imaging method, spectral and spatial resolution required, image capture, intensity transmission and switching speed, must be considered. A pushbroom-based platform uses dispersive and mechanical scanning with a grating–prism–grating method for wavelength generation. Pushbroom techniques have a high spectral resolution and relatively high transmission and variable switching speed. In contrast, AOTF uses a solid-state, non-linear crystal to generate hyperspectral images. It has dynamically variable spectral resolution and variable sequential band-pass with random access ability that is useful for multispectral applications involving discrete and variable wavelengths. An LCTF-based platform has a fixed, defined *a priori* spectral resolution and fixed sequential band-pass with a random access image capture; however, this platform possesses relatively lower transmission properties compared to the others. Also, like AOTF, switching speed is not fast. Thus, the selection of an appropriate HSI platform is fully dependent on applications. USDA ARS researchers have developed two platforms (including pushbroom and AOTF) for the detection of contaminants[5] and foodborne pathogens. They demonstrated the properties and potential for both different hyperspectral imaging systems, i.e. pushbroom for detection and measurement of bacterial colonies on agar plates[6,7] and AOTF for microscopic imaging of live bacterial cells from microcolonies on agar plates,[8–11] respectively.

Future hyperspectral imaging technology

Real-time hyperspectral imaging technology

A pushbroom, line-scan HSI system with appropriate image processing algorithms has the potential for real-time applications in the food processing industry. ARS researchers in Beltsville and Athens have developed a line-scan real-time HSI system for high-speed non-invasive wholesomeness inspection of broilers as well as for faecal contaminant detection for on-line poultry processing.[5] The real-time HSI system (Figure 2) consists of a line-scan hyperspectral camera, imaging spectrograph, camera sensor, objective lens, two pairs of light sources (tungsten–halogen and light-emitting diode or LED), power supplies and an industrial computer. The line-scan real-time HSI system is available with data binning utilising the unique capability of the electron-multiplying charge coupled device (EMCCD) sensor for random access to user-defined areas on the charge-coupled device (CCD) sensor, and multitasking software that is crucial for system customisation for various applications.

High-throughput hyperspectral imaging technology

Another future trend of HSI technology includes development of a bench-top HSI system for pathogen or other micro-scale sample detection. For example, since it is challenging to rapidly and accurately identify the non-0157 Shiga toxin-producing *Escherichia coli* (STEC) colonies by the naked eye due to phenotypic variability in STEC populations and the presence of background microflora, new objective and accurate optical methods are needed to reduce the time to identify presumptive-positive STEC colonies on agar plates for clinical laboratories as well as the food industry. With HSI techniques, researchers have developed methods to classify and identify STEC serogroups by acquiring both spatial and spectral information from every pixel in each STEC serogroup on selective agar plates.[6,7] Spectral fingerprints of bacterial colonies acquired by HSI can be used for identifying pathogens grown on agar media. In particular, a VNIR HSI technique with multivariate classification models has been shown to be useful for differentiating colonies of non-0157 STEC bacteria.

The bench-top HSI system (Figure 3) consists of a pushbroom line-scan VNIR hyperspectral imager including a CCD camera, spectrograph, objective lens with

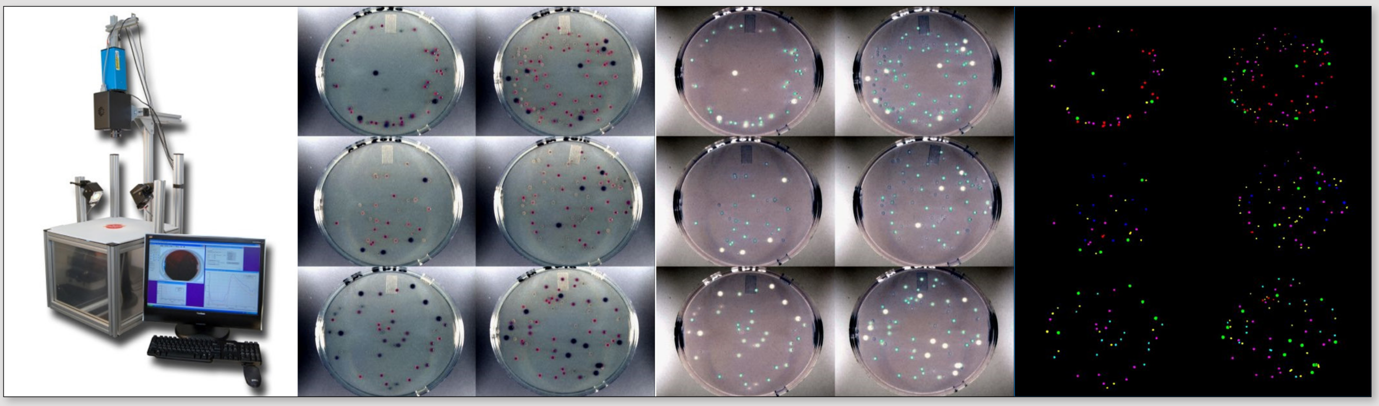

Figure 3. Hyperspectral imaging for identification of bacterial colonies on agar plate.

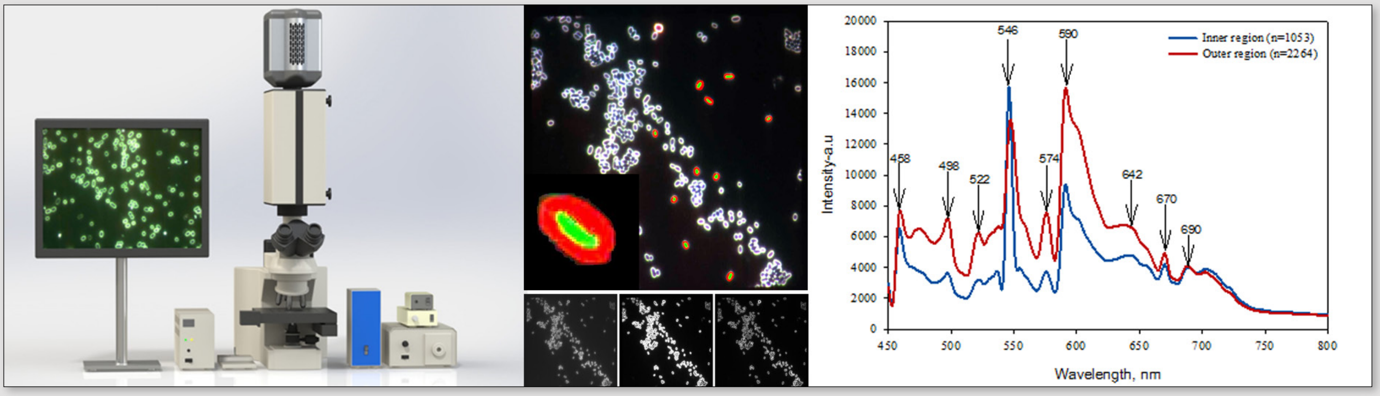

Figure 4. Hyperspectral microscope imaging for bacteria identification.

motion controller, custom sample holder and a computer. The system acquires reflectance spectral images from 368 nm to 1024 nm with a 1.27-nm interval. Two tungsten–halogen lamps are used with a 2 × 2 binning and 30 ms integration time. Similar to other HSI techniques, this method is able to collect data hypercubes from colonies of the non-0157 STEC serogroups on Rainbow agar plates inoculated with mixed cultures. Both spatial and spectral data analysis has demonstrated that differences in appearance of the STEC colonies are mainly due to the differences in specific absorption bands and colour tones, which are both collectable simultaneously with a HSI system.

Hyperspectral microscope imaging technology

Hyperspectral microscope imaging (HMI) for bacteria detection at the cell level is another emerging area of HSI applications. The current pathogenic bacteria detection method takes at least four days from enrichment of samples, multiplex real-time PCR tests, immunomagnetic separation (IMS) beads with antibodies, and plating onto selective agar media before genetic and biochemical identification. The HMI method enables identification of bacteria using microcolony samples grown on selective agar media in less than 8 h. Researchers have developed a HMI technique to enhance the limit of detection (a few fully grown cells) to identify spectral signatures of bacteria from microcolonies.

The HMI system (Figure 4) consists of a Nikon upright microscope, acousto-optic tuneable filters (AOTFs), a high-performance cooled EMCCD camera and dark-field illumination lighting sources for scattering image acquisition from a single cell. For image acquisition from live cells, immobilisation of cells and image acquisition time is crucial for quality spectral images. The AOTF-based HMI has advantages in terms of high-speed, high-throughput, a random-access optical filter with high rejected light levels and an area scan ability compared to the line scan feature of a pushbroom-based HMI system. AOTF delivers diffraction limited image quality with variable bandwidth resolution down to within 2 nm over the spectral range 450–800 nm. AOTF-based HMI uses scanning spectrophotometers employing an instrumental technology with no moving parts, capable of high scan speed and random access to any number of preselected wavelengths.

With HMI techniques, foodborne pathogenic bacteria such as STEC serogroups or *Salmonella* serotypes can be identified with high detection accuracy using chemometric data analysis (Figure 5). With this technique, gram-positive and gram-negative bacteria from chicken carcass rinses can be identified with a reported 99% classification accuracy.[10] Currently, ARS researchers are developing a spectral database by collecting spectral signatures from many bacterial samples extracted from food matrices to generate a spectral library "fingerprint" which may be used to identify unknown samples inspected with a HMI technique.

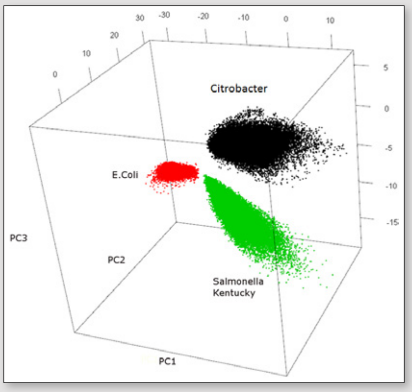

Figure 5. Classification of bacteria (*E. coli*, *Salmonella* and *Citrobacter*) with HMI

Future NIR HMI research

ARS researchers continue developing the NIR HMI system for further study of the biochemical constituents in food-borne bacteria to understand the basis for genotyping and serotyping. Also, HMI will be frequently used for characterising nanoscale substrates for further research on food-borne or water-borne bacterial detection with high sensitivity. Further HMI research will be conducted to detect multiple bacteria simultaneously with multiplex fluorescence *in situ* hybridisation (m-FISH). In addition, prototype hyperspectral sensors with 32 bands of tiled spectral filters, 100 bands line-scan and 25 bands in a 5 × 5 mosaic are available for real-time video and snapshot applications.

HSI research group

ARS Researchers in Athens, Georgia who are working on hyperspectral imaging technologies are Drs Gary Gamble, Kurt Lawrence, Bosoon Park and Seung-Chul Yoon.

Acknowledgement

The author would like to thank Dr Nasreen Bano, Dr Sun Choi and Dr Youngwook Seo for their contributions for HSI research at Quality and Safety Assessment Research Unit.

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