Online Hyperspectral Line-Scan Fluorescence Imaging for Safety Inspection of Apples

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Abstract
A recently developed fast hyperspectral line-scan imaging system integrated with a commercial apple-sorting machine was evaluated for rapid detection of animal faeces matter on apples online. Golden Delicious apples obtained from a local orchard were artificially contaminated with thin smear of cow faeces. For the online trial, hyperspectral fluorescence images of 30 contiguous spectral channels from 400 to 700 nm were acquired from samples moving at a processing sorting-line speed of three apples per second. Based on fluorescence ratio as a multispectral image fusion method, a 100% detection rate (118 out of 118 faeces treated apples) with no false positives (0 out of 120 apples, 60 wholesome and 60 apples with defects acquired prior to the faeces treatment) were achieved.

INTRODUCTION
To minimize food-related illness, safe production of foods for consumption is a public concern (Mead et al., 1999). Fruits and vegetables contaminated with animal faecal matter are recognized as a major culprit for pathogenic E. coli O157:H7 (Armstrong et al., 1996; Cody et al., 1999). Optical imaging or machine vision techniques enable rapid quality and safety inspection of fruits and vegetables. Non-destructive sensing methods including visible/near-infrared (Vis/NIR) and fluorescence imaging techniques have been evaluated for quality and safety inspection of agricultural commodities (Chao et al., 2006; Chen et al., 1998; Kim et al., 2001; Liu et al., 2005). In particular, the efficacy of fluorescence imaging for post-harvest food safety inspection has been demonstrated using apples empirically contaminated with a range of diluted animal faeces (Kim et al., 2005; Lefcourt et al., 2003; Vargas et al., 2005).

Researchers at the Instrumentation and Sensing Laboratory, USDA, have recently developed a prototype hyperspectral line-scan imaging system capable of reflectance and fluorescence measurements to inspect apples online for quality and safety. In this paper, results obtained from a line-scan hyperspectral imaging system integrated with a commercial apple-sorting machine for detection of faecal contamination on apples, mainly based on the fluorescence method, are presented. Apples used in this investigation were artificially contaminated with cow faeces to demonstrate rapid online detection of animal faecal contamination on apples.

MATERIALS AND METHODS
Online Hyperspectral Imaging System
A prototype, online hyperspectral line-scan imaging system was developed to inspect apples for animal faecal contamination. The hyperspectral line-scan inspection system integrated with a commercial-grade apple-sorting machine (FMC Corp, Philadelphia, PA, USA) is shown in Fig. 1. Line speed of the apple-sorting machine was adjusted to run at approximately three apples per second.
The hyperspectral line-scan imaging system utilizes an electron-multiplying charge-coupled-device (EMCCD: PhotonMAX, Roper Scientific, Inc., Trenton, NJ, USA). It has 512×512 pixels and is thermoelectric cooled down to -70°C via a three-stage Peltier device. The imaging device is coupled with a 10 MHz (pixel-readout rate), 16-bit digitizer. An imaging spectrograph (ImSpector V10, Spectral Imaging Ltd., Oulu, Finland) and a C-mount lens (Rainbow CCTV S6X11, International Space Optics, S.A., Irvine, CA, USA) are attached to EMCCD, respectively. The Instantaneous field of view (IFOV) is limited to a thin line by the spectrograph aperture slit (50 µm). Through the slit, light from the scanned line is dispersed by a prism-grating-prism device and projected onto the EMCCD. Therefore, for each line-scan, a two-dimensional (spatial and spectral) image is created with spatial along the horizontal axis and spectral along the vertical axis of the EMCCD. Reflectance and fluorescence imaging required a passive light source, and each method used independent continuous wave (CW) light sources. The illumination sources are 150 w, quartz halogen lamps and a high intensity UV lamp (ML-3500, Spectronics Corp., Westbury, NY, USA) for reflectance and fluorescence, respectively.

Interface software (WinView/32 version 2.5.19.0) provided by the EMCCD manufacture was used for imaging system control and data acquisition. To increase imaging speed, the original image size, 512×512 pixels, was reduced by 6×6 binning to 85×85 pixels. The 6×6 binning and the apple-sorting machine speed resulted in the spatial pixel resolution of approximately 2 mm². Furthermore, it should be noted that not all pixels in the spectral dimension were utilized; the dispersed light by the spectrograph did not span the full vertical width of the EMCCD. Thus, it further reduced the effective spectral dimension to 60 pixels (channels) spanning from approximately 400 to 1000 nm with approximately 10 nm channel interval. A detailed description of spectral calibration is omitted for brevity. With UV-A, most of biological materials exhibit fluorescence emission from 400 to 700 nm. Thus, fluorescence spectra are presented only in that spectral range.

We developed image processing and analysis software on a MS Visual Basic (Version 6.0) platform operating in Windows. Using the downloaded hyperspectral image cube data, it allows automated visualization of individual apple images and detection of faeces-contaminated spots as the stream of hyperspectral image cube data are accessed. We are currently incorporating the system data acquisition function to the software to achieve real-time visualization/detection.

A preliminary test suggested that we could process over 50 apples per second using a PC with 2 GHz processor. With the current imaging configuration (e.g., hyperspectral), one of the limiting factors for processing more than three apples per second is the data transfer rate (i.e., 10 MHz pixel readout rate). The system can be configured to acquire only several spectral channels (multispectral mode) that will markedly increase the data transfer rate per line-scan. We are also in the process of updating the EMCCD with a greater than 30 MHz pixel readout rate.

**Apples and Animal Faeces**

A total of 60 apples, ‘Golden Delicious’ destined for making unpasteurized apple cider were randomly selected from a batch of over 500 samples obtained from a local orchard located in Maryland. Apples used for making apple cider or juice can include those with black pox, sooty blotch, bruises, cuts, rots, insect bites, and physical damages. In addition, 60 wholesome apples (without visual defects) were used as control samples for this study.

Fresh cow faeces from animals fed feedstuffs containing green roughage were collected from USDA farm facilities in Beltsville, MD. A thin cow faeces spot (approximately 2 cm in diameter) was artificially created on each apple (destined for making apple cider) by smearing the cow faeces on the apple using a spatula. A total of 59 apples were treated with the cow faeces; one of the apples was left out while applying faeces smear. Note that the cow faeces smears created transparent film-like coatings on apples and. Visually, were not easily discernable by human eye.
For this investigation, online imaging of the apples with defects were acquired prior to the faeces treatment (60 apples), one day after the faeces treatment (59 apples), and after storing the faeces treated apples in a cold storage room for one month (59 apples). Thus, the total number of apple images acquired was 238 including the 60 wholesome apples.

RESULTS AND DISCUSSION

Representative fluorescence spectra from 400 to 700 nm extracted from hyper-spectral images of wholesome, rotted (defects) and faeces-contaminated portions of apples are shown in Fig. 2. Note that the individual spectra were obtained from a region of interest consisted of approximately 9 pixels (3×3 pixel intensity average per wavelength). With UV-A excitation, Golden Delicious apples exhibit a broad emission in the blue and green region of the spectrum with maxima located approximately at 460 and 530 nm, respectively. Chlorophyll \(a\) fluorescence with emission maximum at near 680 nm is also observed from Golden Delicious apples. When apples are coated with transparent smears of faeces, a blue shift in chlorophyll \(a\) emission peak is typically observed (Kim et al., 2002). The rotted spots showed a broad and relatively low blue-green fluorescence and a minimal chlorophyll \(a\) emission compared to the emission of the greenish Golden Delicious apples.

Fig. 3 illustrates fluorescence images of representative samples containing defects (e.g., bruises and cuts, diseased, fungal growths, such as sooty blotch) along with the artificially contaminated faeces spots at the emission maxima in the blue-green at 460 and 530 nm. In addition, emission maximum bands for the bovine-faeces at near 660 nm and chlorophyll \(a\) emission maximum at 680 nm are shown. Note that the imaging parameters used in this investigation, such as pixel readout rate and binning, and the sorting-line speed, resulted in approximately 900 pixels for an apple. The defect portions of the samples in the images were relatively darker than the surrounding wholesome apple surfaces. The advantage of utilizing a relatively fast data transfer board (i.e., pixel readout rate of 10 MHz) was being able to capture hyperspectral images of fast moving targets. Both spectral and spatial (imagery) responses were analogous to those data acquired in our previous studies using more stationary targets.

In previous studies, we found two-fluorescence band ratio as an efficient multi-spectral image fusion method for detection of faeces contamination on apples (Kim et al., 2002, 2005). Figs. 4a and 4b show the ratio images of 660 nm over 460 nm (F660/F460) and 660 nm over 530 nm, respectively. The faeces smear spots are brighter than surrounding apple surfaces regardless of the presence of defects and/or fungal spots. Below each ratio image in Fig. 4, a binary image highlighting the detection of faecal contamination is shown. The binary images were obtained by subjecting the ratio images to a simple thresholding method using a global threshold value (e.g., 1.29 for F660/F460 and 1.00 for F660/F530). On each apple, the region (pixels) identified as faeces contaminated spot was dependant upon the two-wavelength combinations. Nevertheless, based on the samples in this investigation, a 100% detection rate (118 faeces treated apples) with no false positives (0 out of 120 apples, 60 wholesome and the 60 apples with defects acquired prior to the faeces treatment) for the both ratio combinations were achieved.

CONCLUSIONS

A prototype hyperspectral line-scan imaging system integrated with a commercial apple-sorting machine was evaluated for detection of apples contaminated with animal faecal matter at the processing line speed of three apples per second. Results showed that fluorescence imaging (two-band ratio) allowed detection of faecal contaminated spots on apples with no false positives. With the current imaging configuration (e.g., hyperspectral), one of the limiting factors for acquiring more than three apples per second is the data transfer rate (i.e., 10 MHz pixel readout rate). The system can be configured to acquire only several spectral channels (multispectral mode) that will markedly increase
the data transfer rate per line scan. We are also in the process of updating the EMCCD with a greater than 30 MHz pixel readout rate. These modifications may allow us to inspect apples moving at much faster rates, over 10 apples per second.

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Literature Cited


Fig. 1. Critical components of the hyperspectral line-scan imaging system.

Fig. 2. Representative fluorescence spectra of extracted from hyperspectral apple images for wholesome, defect (rots), and faeces contaminated spots.
Fig. 3. Representative fluorescence images at 460, 530, 660, and 680 nm. Images were acquired with the apple-sorting machine line speed of three apples per second.

Fig. 4. Representative fluorescence ratio images of samples, a) 660 nm/440 nm, and b) 660 nm/530 nm. Binary images for faecal contamination were obtained by the application of a simple thresholding method with a global threshold value.