DETECTION OF ORGANIC RESIDUES ON POULTRY PROCESSING EQUIPMENT SURFACES BY LED-INDUCED FLUORESCENCE IMAGING

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ABSTRACT. Organic residues on equipment surfaces in poultry processing plants can generate cross contamination and increase the risk of unsafe food for consumers. This research was aimed to investigate the potential of LED-induced fluorescence imaging technique for rapid inspection of organic residues on poultry processing equipment surfaces. High-power blue LEDs with a spectral output at 400 nm were used as the excitation source for a line-scanning hyperspectral imaging system. Common chicken residue samples including fat, blood, and feces from ceca, colon, duodenum, and small intestine were prepared on stainless steel sheets. Fluorescence emission images were acquired from 120 samples (20 for each type of residue) in the wavelength range of 500 to 700 nm. LED-induced fluorescence characteristics of the tested samples were determined. PCA (principal component analysis) was performed to analyze fluorescence spectral data. Two SIMCA (soft independent modeling of class analogy) models were developed to differentiate organic residues and stainless steel samples. Classification accuracies using 2-class (‘stainless steel’ and ‘organic residue’) and 4-class (‘stainless steel,’ ‘fat,’ ‘blood,’ and ‘feces’) SIMCA models were 100% and 97.5%, respectively. An optimal single-band and a band-pair that are promising for rapid residue detection were identified by correlation analysis. The single-band approach using the selected wavelength of 666 nm could generate false negative errors for chicken blood inspection. Two-band ratio images using 503 and 666 nm (F503/F666) have great potential for detecting various chicken residues on stainless steel surfaces. This wavelength pair can be adopted for developing a LED-based hand-held fluorescence imaging device for inspecting poultry processing equipment surfaces.

Keywords. Food safety, Sanitation monitoring, Hyperspectral imaging, Fluorescence, LED, Poultry.

Various organic residues (e.g., chicken fat, blood, and feces) remaining attached to equipment surfaces during poultry processing operations can potentially generate cross-contamination and thus increase the risk of unsafe food for consumers. Current pre-operational sanitation monitoring mainly relies on human visual inspection, which is subjective, labor-intensive, and time-consuming. There is a need to develop a rapid, accurate, and non-invasive method for monitoring the processing line for the food industry. A hand-held sensitive detection device, for example, can facilitate detection of residues on equipment surfaces in poultry processing plant, especially for those residues that are not readily discerned by human eyes.

Reflectance and fluorescence techniques have been investigated for years in the area of optical sensing technologies for food quality and safety inspection. Both techniques can be implemented in either spectroscopy measurement or hyperspectral imaging. Broadband light sources (e.g., tungsten halogen lamps) are usually used to illuminate samples in reflectance measurements. The spectral constitution of the incident light is not changed after light-sample interactions. The measurement is performed based on intensity changes at different wavelengths. Reflectance methods have been used for detecting chicken fecal/ingesta contaminants as well as other organic residues (Windham et al., 2003; Cho et al., 2007; Chao et al., 2008a). Fluorescence techniques, on the other hand, rely on the measurement of light emitted at different wavelengths from samples when they are excited by a high-intensity narrowband light. The emission is generally in a broad spectral range towards longer wavelengths, and it carries composition information of the target. Fluorescence is considered to be a sensitive optical technique since it can detect subtle changes of biological materials (e.g., animal and plant tissues). Hyperspectral fluorescence imaging has been applied to the evaluation of quality and safety of food and agricultural products using excitation sources such as ultraviolet (UV) fluorescent lamps (Kim et al., 2002; Jun et al., 2009) and lasers with pulsed (Kim et al., 2003; Cho et al., 2009) or continuous output (Noh and Lu, 2007).

Owing to the demands for cheap, powerful, robust, and reliable light sources, light emitting diode (LED) technology
has advanced rapidly during the past decade. LEDs are solid state sources that emit light when electricity is applied to a semiconductor. Depending on the materials used for the p-n junction inside the LEDs, they can generate narrowband light at different wavelengths (colors) in the visible range. As a new type of light source, LEDs have many advantages over traditional lighting (e.g., tungsten halogen lamps), such as long lifetime, low power consumption with same irradiance, low heat generation, small size, fast response, robustness, and non-sensitivity to vibration. They can be assembled in different arrangements (e.g., spot, line, and ring lights) to satisfy different illumination requirements. LEDs that can produce high intensity broadband light have recently been developed by mixing red, blue, and green monochromatic lights (Steigerwald et al., 2002). With these advantages, broadband LED lights have already been used as illumination sources for reflectance measurement in the area of food quality and safety inspection, such as inspecting for contaminants on and wholesomeness of poultry (Lawrence et al., 2007; Chao et al., 2008b). Narrowband LED lights now also serve as excitation sources for fluorescence measurement (Dasgupta et al., 2003; Buah-Bassuah et al., 2008). The use of LEDs as illumination and excitation sources for agricultural applications is likely to expand in the near future.

The objective of this research was to investigate the potential of LED-induced fluorescence imaging technique for rapid inspection of organic residues on poultry processing equipment surfaces. High-power blue LEDs were used as the excitation source for fluorescence measurement, and their feasibility for use in development of a hand-held fluorescence imaging device was explored. Specific objectives of this study were:

- to use a hyperspectral imaging system equipped with a high-power LED excitation source to measure fluorescence images from common organic poultry residues (i.e., chicken fat, blood, and feces) and stainless steel;
- to develop PCA (principal component analysis) and SIMCA (soft independent modeling of class analogy) models to analyze fluorescence spectra and differentiate organic residues and stainless steel samples; and
- to identify important wavelengths that can be adopted by a future hand-held fluorescence imaging device and develop corresponding image processing and classification algorithms for inspecting organic residues.

**MATERIALS AND METHODS**

**ORGANIC RESIDUE SAMPLES**

Chicken fat, blood, and feces are the primary organic residues that may remain attached to the equipment surfaces in chicken processing plants, and stainless steel is the most commonly used material in the manufacture of poultry processing equipment. Hence they were chosen to be tested in this study. The organic residue samples were collected from 20 fresh chicken carcasses at a chicken processing plant in Cordova, Maryland. Chicken fat and blood samples were obtained from the carcasses. Fecal samples were extracted from the digestive tracts (i.e., ceca, colon, duodenum, and small intestine) of the chickens, and they were stored in small cuvettes separately. The samples for imaging were prepared in the center area of stainless steel sheets (S30200, Allegheny Ludlum, Pittsburgh, Pa.). Chicken fat samples were cut using a scalpel and then were placed on the stainless steel sheets. Blood and fecal samples were dropped on the stainless steel sheets by pipette. The size of the steel sheets was 52 × 22 mm², and the diameter of each sample was in the range of 10 to 15 mm (fig. 1). Twenty samples for each type of organic residue (i.e., fat, blood, and four types of feces including ceca, colon, duodenum, and small intestine) were created, hence a total of 120 samples were tested in this investigation.

**HYPERSONTAL IMAGING SYSTEM**

A hyperspectral imaging system was assembled to acquire fluorescence images from samples on stainless steel sheets (fig. 2). It is a push broom, line-scanning imaging system utilizing LEDs as excitation source and an electron-multiplying charge-coupled-device (EMCCD) camera to collect fluorescence signals. The excitation source consists of two identical high-power blue LED units. Twenty 3-W LEDs (SAILUX Semiconductor Lighting, Gwangju, South Korea) with a spectral output peak at 400 nm and a bandwidth (full width at half maximum) of 20 nm are mounted on top of back mirrors in each lighting unit. The two LED lights are mounted at two sides of the camera along the sample moving direction. They illuminate the line of the instantaneous field of view (IFOV) of the imaging system at angles of approximately 35° from the vertical position. The EMCCD camera (Luca-R, Andor Technology Inc., South Windsor, Conn.) has 1004(H) × 1002(V) pixels and is thermoelectrically cooled to -20°C through a double-stage Peltier device during image acquisition. A reflection grating based imaging spectrograph working in the wavelength range of 400 to

![Figure 1. Chicken fat samples prepared on stainless steel sheets.](image-url)
1000 nm (Hyperspec VNIR, Headwall Photonics, Fitchburg, Mass.) and a C-mount zoom lens (Xenoplan 1.4/23, Schneider Optics, Hauppauge, N.Y.) are mounted to the camera. The IFOV is limited to a thin line by the spectrograph aperture slit (60 µm wide and 18 mm long). Through the slit, light from the scanned IFOV line is dispersed by an Offner spectrograph (consisting of a pair of concentric spherical mirrors and a convex reflection grating) and projected onto the EMCCD. Therefore, for each scanned line, a two-dimensional (spatial and spectral) image is created with the spatial dimension along the horizontal axis and the spectral dimension along the vertical axis of the EMCCD. Spectral calibration of the system was performed using an Hg-Ne spectral calibration lamp (Oriel Instruments, Stratford, Conn.). Detailed procedures for hyperspectral imaging system calibrations can be found in Kim et al. (2001).

A programmable, motorized positioning table (BiSlide-MN10, Velmex, Bloomfield, N.Y.) moved the residue samples (20 for each run) transversely through the line of the IFOV. Eight hundred and fifty lines were scanned for 20 samples arranged in two rows, and 400 pixels covering the width of the stainless steel sheets in each scan were saved, generating a 3-D hyperspectral image cube with the spatial dimension of $850 \times 400$ for each band. Exposure time of the camera was set to 0.05 s for image acquisition. A gelatin long pass optical filter with a cutoff wavelength at 500 nm (minus blue filter, Wratten No. 12, Kodak, New York, N.Y.) was used to block the 400-nm output as well as the long-wavelength spectral tail from the blue LEDs. A gelatin long pass optical filter with a cutoff wavelength at 500 nm (minus blue filter, Wratten No. 12, Kodak, New York, N.Y.) was used to block the 400-nm output as well as the long-wavelength spectral tail from the blue LEDs. Also, only the spectral image data up to 700 nm were saved with the aim of avoiding second-order effects of excitation. Thus hyperspectral fluorescence images in the spectral region of 500 to 700 nm (totaling 42 bands with a spectral resolution of 4.8 nm) were used for further data analysis. The parameterization and data-transfer interface software for the hyperspectral imaging system were developed using a SDK (Software Development Kit) provided by the camera manufacturer on a Microsoft Visual Basic (Version 6.0) platform in the Windows operating system.

DATA ANALYSIS

Extraction of Fluorescence Spectra

Image masking operations were first performed using the original hyperspectral images to remove the background areas surrounding the stainless steel sheets. Fluorescence spectra of chicken fat, blood, four types of feces, and stainless steel were then extracted from region of interest (ROI) hyperspectral image data using ENVI software (ITT Visual Information Solutions, Boulder, Colo.). The ROI was manually selected in a zoom window using various ROI selection methods provided by ENVI (e.g., polygon, rectangle, ellipse, grow, and merge). Efforts were made to maintain the purity of each ROI in a way such that all the pixels in an ROI belonged to one desired sample type, and edge and other undesired pixels were excluded. All the samples were used for the ROI selections. One ‘organic residue’ ROI and one ‘stainless steel’ ROI were selected within each sample, respectively. The mean spectrum was computed for each ROI. Thus a total of 240 mean fluorescence spectra (120 for organic residue samples and 120 for stainless steel sheets) were obtained for further data analysis.

Multivariate Spectral Data Analysis and Modeling

Principal component analysis (PCA) is a useful tool for spectral data compression and information extraction. PCA generally finds far fewer principal components (PCs) than original variables (wavelengths) through orthogonal transformation to maximize representation of the original spectral data. The redundant data can thus be largely reduced by observing a few scores (weighted sums of the original variables) without significant loss of useful information. In this study, PCA was performed on all 240 spectra extracted from hyperspectral images for general characterization of the
fluorescence information from organic residue samples and stainless steel sheets. Fluorescence spectra were mean-centered and scaled to unit variance. Standard singular value decomposition (SVD) algorithm was used for PCA.

PCA is an unsupervised method since it does not use class information when the model is created. Hence it is not optimal for spectral data classification. A SIMCA (soft independent modeling of class analogy) model, on the other hand, is a supervised method. The SIMCA model is essentially a combination of several PCA models, in which a sub-model is generated for each class of data. Each PCA sub-model in SIMCA has all the features of a regular PCA model. SIMCA incorporates multiple class information and therefore it is useful for data classification using properties of the PCA models. To evaluate the separability of the tested samples, two SIMCA models were developed for classification of fluorescence spectral data at two levels: a 2-class model (‘stainless steel’ and ‘organic residue’), and a 4-class model (‘stainless steel’, ‘fat’, ‘blood’, and ‘feces’). The class of ‘feces’ included fecal samples from ceca, colon, duodenum, and small intestine. Each SIMCA model validates the PCA model of each class using the leave-one-out cross-validation method. The cross-validation PRESS (Predictive Residual Error Sum of Squares) curves were used to determine the optimal number of principal components in building each PCA model. Two-thirds of each data set was used to create SIMCA models, and the remaining one-third was used as an independent set to test the classification accuracies, which resulted in 160 samples for the calibration set and 80 samples for the validation set.

Band Selection and Image Classification

This research aimed to identify one or two important wavelengths that can be adopted for developing a LED-based hand-held fluorescence imaging device for inspection of chicken processing equipment surfaces. Correlation analysis (CA) has been used for hyperspectral band selections in various agricultural applications (Thenkabail et al., 2000; Park et al., 2006; Lee et al., 2008). In this study, CA was used for selecting optimal single band and band pair at which fluorescence images (intensity and/or band ratio) can be used for detecting organic residues on stainless steel sheets. To facilitate the correlation analysis, organic residue samples including chicken fat, blood, and four types of feces were labeled with ‘1,’ and stainless steel sheets with ‘0.’ Correlation coefficients were calculated between the label values (i.e., 1 and 0) and single-band fluorescence intensities as well as two-band ratios of the ROI spectra in an exhaustive way (i.e., evaluating all possible two-band combinations from among 42 wavebands). The single-band and band pair that gave the highest correlation coefficients were considered as optimal for further image processing and classification. After key wavelengths were identified, two-band ratio images (i.e., $F_{\lambda_1}/F_{\lambda_2}$, where $F_{\lambda_n}$ denotes single-band fluorescence image at the wavelength of $\lambda_n$) were computed. The capability of LED-induced fluorescence imaging for contamination detection was demonstrated by applying simple thresholding and morphological filtering to the band ratio images in an attempt to segregate organic residues from the background of the stainless steel sheets.

The data analysis procedures described above (i.e., PCA, SIMCA, and CA) were executed using programs developed in MATLAB R2007b (MathWorks, Natick, Mass.) along with PLS_Toolbox 5.2 (Eigenvector Research, Wenatchee, Wash.).

RESULTS AND DISCUSSION

Characterization and Classification of Fluorescence Spectra

Fluorescence spectra of the tested samples are shown in figure 3 to demonstrate the general emission patterns produced from excitation by 400-nm blue LEDs. Each residue sample spectrum was an average of 20 samples within each category. The spectrum of stainless steel was an average of all 120 spectra. Fluorescence emission maxima were observed at 580 nm for all fecal samples. A spectral peak at 580 nm was also observed for the stainless steel sheets. This peak was not fluorescence emission although it appeared at the same wavelength position with one of the

![Figure 3. Representative fluorescence emission spectra of organic residues and stainless steel excited by 400-nm LEDs.](image-url)
peaks of the fecal samples. It is probably attributed to specular reflectance from direct illumination of two LED lights on the shining surfaces of the steel sheets. No specular reflectance was observed for the chicken residues because incident light was diffused on their surfaces. At 580 nm, the intensities of fecal samples from colon, duodenum, and small intestine were higher than those of ceca and stainless steel samples. The four types of feces shared a common spectral shoulder at 546 nm. Ceca showed three other emission maxima in the red spectral region at 627, 637, and 694 nm. The intensities at 627 and 637 nm were higher than those at 546, 580, and 694 nm. Two spectral peaks at 627 and 637 nm were also observed for colon and small intestine, respectively. The fluorescence emission peaks of feces observed above are attributed to chemical compounds in chicken feed as well as metabolites generated in the poultry digestive tract. Chicken fat, on the other hand, exhibited a broad emission peak between 570 and 610 nm, which is probably attributable to complex compounds in chicken skin, such as elastin and collagen. The spectrum of chicken blood was relatively flat in the green region. It showed an emission peak at 618 nm and a spectral tail towards the far-red region. Due to the strong light absorption by hemoglobin in blood, the emission intensity of the blood was the lowest among the tested residues. Except for the blood, stainless steel generally showed lower spectral intensity compared to the organic residue samples in the spectral region under investigation.

PCA was performed on all 240 fluorescence spectra. The first four principal components were found to account for 99.75% of the total variation to the entire data set, with 87.89% for PC1 and 9.86% for PC2. Figure 4 shows scores on the first two PCs of all the tested samples. The score plot reveals that the stainless steel sheets can be easily separated from the organic residues samples due to their negative score values on both PC1 and PC2. In addition, chicken fat and blood samples are also separable from other samples due to large positive PC1 scores and the combination of negative PC1 and positive PC2 scores, respectively. Stainless steel, fat, and blood samples generally congregate in groups owing to their relatively small score variations on the first two PCs. Fecal samples, on the other hand, spread across a wide area because of their relatively large score variations. Feces from colon, duodenum, and small intestine are mixed and difficult to discriminate from each other. Feces from ceca can be differentiated from other three types of feces due to their unique fluorescence emission spectra.

Classification results using two SIMCA models are shown in figure 5. Based on the cross-validation PRESS curves, the optimal 2-class SIMCA model was found to use two PCs and four PCs for the ‘stainless steel’ class and the ‘organic residue’ class, respectively. The 2-class SIMCA model correctly identified 100% for both stainless steel samples and organic residue samples in the test data set. Similarly, the optimal number of factors for each PCA model in the 4-class SIMCA model was determined as two, two, three, and four for the classes of ‘stainless steel,’ ‘fat,’ ‘blood,’ and ‘feces,’ respectively. Only two among 80 samples in the test data set were misclassified by the 4-class SIMCA model. One stainless steel sample and one chicken fat sample were misclassified to the class of ‘feces,’ which resulted in an accuracy of 97.5% for the 4-class spectral classification. The classification results obtained using the SIMCA models suggest that LED-induced fluorescence spectra could provide sufficient useful information to discriminate various organic residues from stainless steel surfaces.

**SELECTION OF OPTIMAL WAVELENGTHS AND IMAGE CLASSIFICATION**

Figure 6(a) shows correlation coefficients (r) between single-band fluorescence intensities and sample classes in the wavelength range of 500 to 700 nm. Due to the weak emissions of blood and ceca samples in the spectral region of 500 to 610 nm (fig. 3), the correlation coefficients were low (0.37-0.60) below 610 nm. Beyond 610 nm, the correlations increased since the fluorescence intensities of organic residues were generally higher than those of stainless steel samples. In the range of 610 to 700 nm, the correlation coefficients were greater than 0.66, with the maximum r value of 0.85 at 666 nm. Fluorescence intensity values at 666 nm of all 240 samples are plotted in figure 6(b). Owing to the high emission intensities, chicken fat samples and four types of fecal samples can be completely separated from the stainless steel samples. Fluorescence intensities of the blood

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Figure 4. Scores on first two principal components for fluorescence spectra of organic residues and stainless steel.
Figure 5. Classifications of stainless steel and organic residue samples using (a) 2-class SIMCA model and (b) 4-class SIMCA model.

Figure 6. Correlation analysis for selection of single band: (a) correlation coefficients between single band fluorescence intensities and sample classes, and (b) intensity values of all the samples at the wavelength with highest correlation (666 nm).

Samples were close to those of the stainless steel samples. Using the highest intensity of stainless steel (F666 = 3496) as a threshold, one blood sample (F666 = 3392) was misclassified as stainless steel. These results suggest that fluorescence emissions at 666 nm could be used to detect chicken fat and various fecal residues on stainless steel equipment surfaces. False negative errors could occur when the single-band inspection is performed on the chicken blood residues.

Contour plot of the correlation coefficients between two-band ratios (F_{503}/F_{666}) and sample classes is illustrated in figure 7(a). Relatively high correlation coefficients appeared in a long and narrow area in the lower triangle of the plot with a horizontal spectral range of 620 to 700 nm and a vertical spectral range of 500 to 510 nm. Within this region, the two-band ratio between 503 and 666 nm (F_{503}/F_{666}) gave the maximum absolute correlation value of 0.94, indicating the potential of this wavelength pair for detection of organic...
residues. Note that 666 nm was also selected by the single-band correlation analysis. Figure 7(b) shows two-band ratio values (F503/F666) of all 240 samples. It can be seen that the ratio values of the stainless steel samples were clearly higher than those of the organic residue samples due to their relatively low fluorescence intensities at 666 nm. Although no particular spectral features were observed at the selected 503-nm waveband, the introduction of this band to the ratio calculations effectively reduced the variations of single-band intensities at 666 nm among different spectra. Consequently it is reasonable to apply a simple thresholding method to segregate organic residue samples from stainless steel samples. For all the samples tested in this study, a threshold of 0.165 for F503/F666 gave 100% accuracy for the 2-class classification. Compared to the single-band method, the band ratio method could enhance the detection accuracy by utilizing the spectral information from the two selected bands. Also, the ratio method is invariant to illumination scaling, which is a significant advantage for practical applications under uncontrolled lighting conditions (e.g., poultry processing plant).

Figure 8 shows the major steps of the two-band ratio-based image processing and classification method for detecting organic residues on stainless steel sheets. Images of individual samples were acquired separately and a mosaic including six sheets was created to demonstrate typical images of different chicken residues. Background areas other than stainless steel sheets were already removed by image masking operations. It was found that the blood samples appeared darker than the other five residues in both single-band images at 503 and 666 nm. The two-band ratio image (F503/F666) was computed using the single-band images at 503 and 666 nm. All six organic residue areas appeared dark in the ratio image due to their relatively low spectral ratio values. The stainless steel sheets appeared brighter than the residues due to their higher ratio values [see fig. 7(b)]. Binary images for classification were obtained after simple thresholding and morphological filtering were applied to the ratio image. Image pixels were converted to zero (i.e., displayed as black) if their values were greater than the threshold (i.e., 0.165). The morphological filter helped to remove undesired small size features such as tiny fluorescent spots on the sheets. As a result, the stainless steel sheets were converted to black. Only organic residues remained as white spots in the final binary image. Similar results were obtained for other samples tested in this study. The results above indicate that the band ratio approach using 400-nm LED-induced fluorescence imaging at 503 and 666 nm can be used to inspect for various chicken residues on stainless steel equipment surfaces.
CONCLUSIONS

This study demonstrated that LED-induced fluorescence imaging technique is capable of detecting organic residues on poultry processing equipment surfaces. High-power blue LEDs can excite organic residues attached on stainless steel surfaces and generate fluorescence emission spectra. Chicken fat and four types of feces extracted from chicken digestive tracts generally showed higher emission intensities than stainless steel and chicken blood in the spectral region of 500 to 700 nm. High classification accuracies obtained by 2-class and 4-class SIMCA models suggest that fluorescence spectra excited by 400-nm LEDs could carry sufficient useful information for differentiating various organic residues from stainless steel surfaces. Single-band fluorescence images at 666 nm can be used to inspect chicken fat and feces on stainless steel surfaces. Errors could occur when the single-band detection is carried out on blood residues since the emission intensity of blood is close to that of stainless steel at this selected wavelength. The band ratio method improved the classification accuracy by using the information from two selected bands at 503 and 666 nm. Two-band ratio images (F503/F666) correctly detected all types of chicken residues tested in this study. Future work will be conducted to implement the band-ratio-based image processing and classification methods in a LED-based hand-held fluorescence imaging device that can facilitate equipment sanitation inspection in poultry processing plants.

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REFERENCES


