Detection of Chicken Egg Fertility and Early Embryo Development Using Hyperspectral Imaging

L. Liu and M. O. Ngadi*

Department of Bioresource Engineering, McGill University, Macdonald Campus. 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada. H9X 3V9.

Abstract

In this study, a non-destructive, rapid and accurate method was developed based on hyperspectral imaging technology to determine the fertility and early embryo development of eggs. A total of 170 white-shell chicken eggs including 152 fertile eggs and 18 infertile eggs were used in this study and all eggs were incubated in a commercial incubator for 4 days. A transmission-based hypercube with the wavelength range of 900-1700 nm was obtained for each egg on each day of incubation. After imaging on every day, randomly selected developing embryos were stopped by injecting sodium azide (NaNO₃). The region of interest (ROI) of each hypercube was segmented and the image texture information was extracted from the ROI. The $K$-means clustering algorithm was used on the spectral transmission characteristics to classify fertile eggs, infertile eggs and dead embryos. A perfect detection (100% accuracy) of fertility prior to incubation (Day 0) was obtained. The classification results for all eggs on each day of incubation were 65.29% at Day1, 61.18% at Day2, 72.94% at Day3 and 84.12% at Day4. The low classification results at Day 1 and Day 2 indicated that the embryo development is hard to be detected during the first 2 days of incubation.

1. Introduction

Hatchability of eggs is the critical economic factor for hatcheries and poultry breeding farms. Early detection of non-fertile and non-hatchable eggs would allow hatcheries to remove them before transfer to the incubator/hatcher, thus saving space, handling costs and contamination from exploder eggs. In poultry industry, candling is widely used to assess flock fertility to remove infertile eggs or dead embryos from the incubator. However, since candling is labour consuming, only few eggs in the incubator are candled and thereby most non-fertile and non-hatchable eggs will be remained in the incubator until transfer to the hatcher. Thus, the development of an efficient, non-destructive and accurate method for detecting the fertility and the embryo development of eggs would be advantageous to the hatchery industry.

Some researchers have attempted to detect fertility and embryo development with computer vision technology. Das and Evans (1992a, 1992b) developed machine vision systems to determine fertility of hatching eggs by combining image histogram characterization with neural network classifiers. Bamelis et al. (2002) applied the spectrophotometric method to detect embryo development of chicken eggs during the first 12 days of incubation by injecting sodium azide into the developing embryos at different times during incubation. Recently, the advanced hyperspectral imaging technique combining the conventional two-dimensional (2-D) digital imagery with spectroscopy has been applied to detect egg fertility and embryo development (Lawrence et al., 2006; Smith et al., 2008). A hyperspectral image contains not only spatial information but also spectral information for each pixel in the image, which means that it can be used to detect physical and geometric characteristics (such as color, size, shape and texture) and to extract some intrinsic chemical information (such as water, fat and protein). Lawrence et al. (2006) applied the hyperspectral imaging technique for detecting early embryo development of hatching eggs at the first three days of incubation and obtained extremely promising results. The same research group later introduced infertile eggs to evaluate the hyperspectral imaging system and a predictive modeling technique for determining fertility prior to incubation and embryo development during the first three days.
of incubation (Smith et al., 2008). However, the prediction model produced much lower classification results both on validation data (71% for Day 0, 63% for Day 1, 65% for Day 2 and 83 for Day3) and verification data (50.8% overall). The results in both studies indicated that development of proper HSI-based techniques for accurate prediction is necessary for detecting early embryo development and/or fertility prior to incubation.

When observing the contents of the egg in a dark room with candling, the living embryo appears as a dark spot in the large end of the egg surrounded by a faint outline of blood vessels, while dead embryos sometimes appear as a ring or smear of blood in the egg or a dark spot dried to the inside of the shell. The non-fertile egg brightly transmits light in comparison. These differences might be influenced in the image texture information. Thus, the possibility of using image texture feature for detecting fertility and embryo development should be considered as they could possibly bring improvement for the fertility and embryo detection.

The objective of this study was to develop an efficient and accurate hyperspectral imaging system based on image texture features to determine fertility of hatching eggs prior to incubation and embryo development during the first four days of incubation.

2. Materials and methods

2.1 Eggs and sample evolution

A total of 170 freshly laid white eggs including 152 fertile eggs and 18 infertile eggs were collected from McGill Farm in five batches over three months. On Day 0 (just prior to incubation), all eggs were first imaged by the hyperspectral imaging system and then a total of 20 fertile eggs over five batches were randomly selected for inducing dead embryo (stopping embryo development). Each of the 20 fertile eggs was injected with a 100 µl of a 5% sodium azide (NaN₃) solution using a sterile needle through a tiny hole drilled at the sharp end of egg (Bamelis et al., 2002). After imaging and injecting, all eggs were immediately incubated in a 2362N Circulated Air Hova-Bator incubator (G.Q.F. Manufacturing Co.) at 37.78°C (100°F) and 55% relative humidity, and were turned every hour. On Days 1, 2, 3 and 4 of incubation, eggs were removed for imaging in sequence within a period of 3 min. and then immediately returned to the incubator. After all eggs were imaged on each day (Days 1, 2 and 3), a set of 20 fertile eggs were randomly selected for injection. After 7 days of incubation, eggs were candled and broken out to determined fertility and embryo viability.

Table 1 lists the sample evolution during the first 4 days of incubation. A total of 6 groups were obtained for the whole period of incubation: fertile eggs (76), infertile eggs (18), eggs injected (i.e., stopped embryo development) on Day 0 (20), 1 (19), 2 (18) and 3 (19), respectively; while for each day of incubation, there were 3 classes: fertile eggs, infertile eggs and injected embryo. The numbers of eggs in the 3 classes varied with the time of incubation.
2.2 Hyperspectral imaging system and image preprocessing

A laboratory near-infrared (NIR) hyperspectral imaging system was built up for the study. This imaging system consisted of a line-scan spectrograph (Hyperspec™, Headwall Photonics Inc. USA), an InGaAs camera connected to the spectrograph, a conveyer (Donner 2200 series, Donner Mfg. Corp., USA) driven by a stepping motor (MDIP22314, Intelligent motion system Inc., USA) with a user-defined speed, a tungsten halogen lamp (50 W) providing back illumination to eggs, an enclosure supporting the system, a data acquisition and pre-processing software (Hyperspec, Headwall Photonics Inc. USA) and a PC. The system collected spectral images in a wavelength range between 900 and 1700 nm with a spectral resolution of 2.8 nm. Four egg samples vertically placed on a wood board were conveyed to the field of view of the camera with a pre-defined speed which was decided by trial-to-error to avoid image distortion.

Image pre-processing was conducted to calibrate the spectral images by percent transmission. A dark and a white image was obtained for calculating the percent transmission of the output spectral images by covering the lens with a cap and allowing the light to go through the lens, respectively. Thus, the pixel values of each plane of the output hypercube are between 0 and 1.

2.3 Image texture feature extraction

A hyperspectral image captured by the imaging system normally consists of four eggs. The selection of ROI of a sample image is to create a mask for each egg and then to segment each egg from the original image. In order to extract useful and helpful image texture information of eggs, Gabor filters were applied to the ROI of hyperspectral images. An oriented Gabor filter \( G \), which is a Gaussian function modulated by an oriented harmonic function, can be defined as follows:

\[
G(x, y; u, \sigma, \theta) = \frac{1}{2\pi\sigma^2} \exp\left(-\frac{x^2 + y^2}{2\sigma^2}\right) \cos[2\pi u(x \cos \theta + y \sin \theta)],
\]

where \((x, y)\) is the coordinate of point in 2-D space, \(u\) is the frequency of the sinusoidal wave, \(\sigma\) is the standard deviation of the Gaussian envelope, and \(\theta\) controls the orientation of the Gabor filter.

2.4 Spectral transmission characteristics

Two types of spectral transmission characteristics \( MS \) and \( MG \) were used for the further data analysis. \( MS \) is the mean spectra of ROI over the spectral range of 900-1700 nm, while \( MG \) is the mean spectra of the Gabor-filtered ROI over the same spectral range. In order to remove high- and low-frequency interferences, a 5x1 mean filter and a baseline filtering method were used to remove noise (high frequency) and baseline/background (low frequency) from the original spectral transmission characteristics, respectively.

2.5 Data analysis

Spectral transmission characteristics were first projected onto a lower dimensional linear space using Principal Component Analysis (PCA) to extract useful features. The extracted features were then classified by using a typical unsupervised classification method, i.e. \(K\)-means clustering.
3. Results and discussions

3.1 Spectral transmission characteristics

The region of interest (ROI) of each hyperspectral image, i.e. the egg areas, was segmented using a predefined mask and four regions of interest were obtained for each hyperspectral image. Four oriented Gabor filters, i.e. $G_0$, $G_1$, $G_2$, $G_3$, were used to convolve with each ROI along the directions of $0$, $\pi/4$, $\pi/2$, $3\pi/4$, respectively, and obtain four corresponding Gabor filtered ROI images. Typical curves of spectral transmission characteristics, $MS$ and $MG_{0-3}$, for different groups of eggs at Day 4 are shown in Fig. 1. The largest deviation between 6 groups is found at the wavelength producing the peak value for every spectral transmission characteristics, no matter $MS$ or $MG_{0-3}$ used, which indicates that the values of spectral transmission characteristics around the peak could be used to discriminate the fertile eggs, infertile eggs and dead embryos.

![Typical curves of spectral transmission characteristics](image)

Fig. 1. Typical curves of spectral transmission characteristics (a) $MS$ and (b)-(e) $MG_{0-3}$ for different groups of eggs at Day 4.

3.2 Evolution of transmission

The evolution of average peak values of spectral transmission characteristics for different groups is shown in Fig. 2, where the marker face presents the average value and error bars illustrate the standard error of mean (SEM). A big difference of the average peak transmission between fertile eggs and infertile eggs was observed at Day 0 (prior to incubation) and Day 4 (after 96 h of incubation), which indicated that fertile and infertile eggs could be totally classified on the two days.

The groups of treatments after different periods of incubation show various evolution profiles with different injection time. The developing embryos stopped at Day 0 and Day 1 produced similar evolution profiles with the infertile eggs, while the eggs injected at Day 3 had a similar profile as fertile eggs. The embryos injected at Day 2 showed a mixed profile.
which had similar evolution as fertile eggs during the first 3 days and no significant decrease happened at Day 4. For all spectral transmission characteristics, the later injected eggs produced the larger difference with the first injected group, which is same as the result in Bamelis et al. (2002). The average peak transmission became constant after 24 h of injection.

Fig. 2. Evolution of the peak transmission of (a) MS and (b)-(e) MG0-3 in different groups. Mean of each group at different time are presented, error bars show SEM.

### Table 2 Accuracy (%) of K-means clustering based on the first 3 bands of maximum responses and the first 3 PCs.

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>MS</th>
<th>MG0</th>
<th>MG1</th>
<th>MG2</th>
<th>MG3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bands*</td>
<td>PCs**</td>
<td>Bands</td>
<td>PCs</td>
<td>Bands</td>
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<td>Day0</td>
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<td>79.41</td>
<td>78.24</td>
<td>84.71</td>
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<tr>
<td>Day1</td>
<td>64.71</td>
<td>64.71</td>
<td>65.29</td>
<td>57.06</td>
<td>65.29</td>
</tr>
<tr>
<td>Day2</td>
<td>54.12</td>
<td>61.18</td>
<td>53.53</td>
<td>57.06</td>
<td>61.18</td>
</tr>
<tr>
<td>Day3</td>
<td>62.94</td>
<td>68.24</td>
<td>57.65</td>
<td>71.76</td>
<td>62.94</td>
</tr>
<tr>
<td>Day4</td>
<td>74.71</td>
<td>72.35</td>
<td>82.35</td>
<td>82.94</td>
<td>84.12</td>
</tr>
</tbody>
</table>

* Bands mean the first 3 bands of maximum responses.

** PCs mean the first 3 PCs.

#### 3.3 Classification results

An unsupervised classification method, K-means clustering, was employed to investigate the ability of spectral transmission characteristics and PCs to discriminate the three classes of egg samples, i.e. fertile eggs, infertile eggs and injected embryos (i.e. dead embryos). The first 3
bands with maximum responses of spectral transmission for each characteristic and the first 3 PCs of each spectral transmission characteristics were used for classification. The $K$-means clustering results, i.e. the total classification accuracy over three classes, are listed in Table 2 for different spectral transmission characteristics on different days using the first 3 bands of maximum responses and the first 3 PCs, respectively.

The best classification accuracies for all eggs are 100% at Day0, 65.29% at Day1, 61.18 at Day2, 72.94% at Day3, and 84.12% at Day4. The best total classification result on each day of incubation was obtained with MG used except Day 0, which suggested that the image texture information is suitable for detecting early embryo development of chicken eggs. The low classification results at Day 1 and Day 2 indicated that the embryo development is hard to be detected during the first 2 days of incubation, which is consistent with the fact that blood formation starts in the developing embryo from Day 2 (Romanoff, 1960; Bodemer, 1970).

4. Conclusions

A hyperspectral imaging system was developed to detect fertility and early embryo development of white-shell chicken eggs in the first 4 days of incubation. The evolution of average peak values of spectral transmission characteristics showed the later injected eggs produced the larger difference with the first injected group and the average peak transmission remained constant after 24 h of injection. The unsupervised classification results for all eggs were 100% at Day 0, 65.29% at Day 1, 61.18% at Day 2, 72.94% at Day 3 and 84.12% at Day 4. The 100% classification result at Day 0 was obtained using the first 3 bands of maximum responses, which means a perfect detection of fertility prior to incubation. The low classification results at Day 1 and Day 2 indicated that the embryo development is hard to be detected during the first 2 days of incubation, which is consistent with the period of blood formation in the developing embryo. Since the best classification results on each day of incubation was obtained when using MG except Day 0, the image texture information is useful to detect early embryo development of chicken eggs.

References


