ANIMAL WELL-BEING AND BEHAVIOR

In-ovo sexing of 14-day-old chicken embryos by pattern analysis in hyperspectral images (VIS/NIR spectra): A non-destructive method for layer lines with gender-specific down feather color

Doreen Göhler,[1] Björn Fischer, and Sven Meissner

EVONTA-Technology GmbH, Maria-Reiche-Straße 1, 01109 Dresden, Germany

ABSTRACT Up to now there is no economically maintainable modality for chicken sexing in early embryonic stages (first 3 d) that is suitable for large-scale application in the commercial hatcheries. Hence, the culling of male day-old chicks of layer lines is still the normal procedure.

In this paper we present a non-destructive optical technique for gender determination in layer lines with gender-specific down feather color. This particular chicken strain presents a sexual dimorphism in feather color, where the female day-old chicks have brown down feathers and the males have yellow down feathers.

The eggs are candled with halogen lamps and a hyperspectral camera collects the transmitted light within the spectral range from 400 nm to 1,000 nm. For data analysis and classification, common methods like principal component analysis and linear discriminant analysis are used. The accuracy of gender determination was determined for 11- to 14-day-old embryos. At 14 d of incubation (7 d before hatch) the sex can be determined with an overall accuracy of approximately 97%.

Key words: in ovo sexing, hyperspectral imaging, sexing of chicken embryo

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INTRODUCTION

The culling of male day-old chicks of layer lines is still the normal procedure in commercial hatcheries. Due to the genetics of the layer chicken lines, it is not practical to keep the male chicks for meat production. Approximately 42 million male chicks are culled at 1 d of age in Germany per year. This produces ethical questions, but up to now there is no modality for chicken sexing in early embryonic stages that is economically suitable for large-scale application.

A method developed by Weissmann et al. (2013) determines the concentration of estrogen sulfate within the allantoic fluid after d 9 of incubation. The fluid is taken with a tiny needle through a small hole in the eggshell (hole diameter ca. 1 mm). The prediction accuracy is 98%, but the analysis of the fluid takes approximately 4 h, too long for large-scale application in hatcheries. Additionally, the procedure decreases the hatchability 3%.

Some spectroscopic approaches like Raman (Bartels et al., 2011) or infrared (Steiner et al., 2011) might also be suitable for sex determination, even earlier during incubation than the endocrine technique. These methods measure the difference of DNA content between females and males in embryonic tissues. These methods require the egg to be opened to allow direct optical access to the embryonic blood vessels. Opening of the eggshell can have a significant impact on the embryo, through contamination by bacteria, or other negative effects on embryo development and hatchability. Therefore, a non-invasive method would be better suited for embryonic development as well as simplifying the process for commercial scale hatcheries.

For a particular chicken strain that presents a sexual dimorphism in feather color, we found a non-invasive method as described in this paper. It has yet to be proven if the method is applicable to other strains of chicken.

MATERIAL AND METHODS

For the study, brown eggs from Lohmann Tierzucht GmbH are used. The eggs are from layer lines, where female day-old chicks have brown down feathers and the males have yellow down feathers. The eggs were incubated for 11 to 14 d.

Data Acquisition

A scheme of the measurement setup is shown in Figure 1. The egg is candled with a halogen lamp (230 V/35 W and color temperature: 2,800 K) and the transmitted light is collected by a hyperspectral

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[1]Corresponding author: doreen.goehler@evonta-technology.de

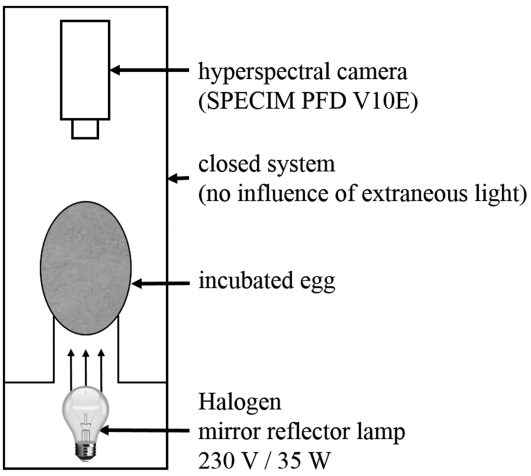

Figure 1. Scheme of the developed data acquisition system.

Table 1. Exposure times for data acquisition (required time for one frame that contains the transmission spectra of up to 10 eggs).

Incubation Time	Exposure Time in ms
11 d	250
12 d	350
13 d	400
14 d	500

camera (PFD V10E manufactured by SPECIM) within the spectral range from 400 nm to 1,000 nm. Up to 10 eggs can be analyzed simultaneously within the camera's field of view. Extraneous light would mask the important signal. Therefore, the measurement setup requires a dark environment, which is ensured by a light-proof case.

Halogen lamps produce significant amounts of heat that might have a negative impact to the embryo if the candling time is too long. We used illumination only during image acquisition (max. 500 ms) and we have observed no negative effects on the developing embryo.

The camera provides a spectral sampling of 0.78 nm/pixel without binning of sensor elements. With binning, it is possible to collect the charge of 2, 4, or 8 pixels in spatial and/or spectral dimension. For constant exposure time, binning increases signal amplitude, but reduces the spatial and/or spectral resolution. We decided to use a spectral binning of 2 and a spatial binning of 8. To get a signal with sufficient amplitude but without saturation, an exposure time between 250 ms and 500 ms was used, depending on the age of the embryo (Table 1).

Figure 2 shows a frame acquired by the camera for 10 eggs. Each column of the frame represents the spectral profile of a spatial position in line scan. The example of a single spectrum shows that the incubated egg

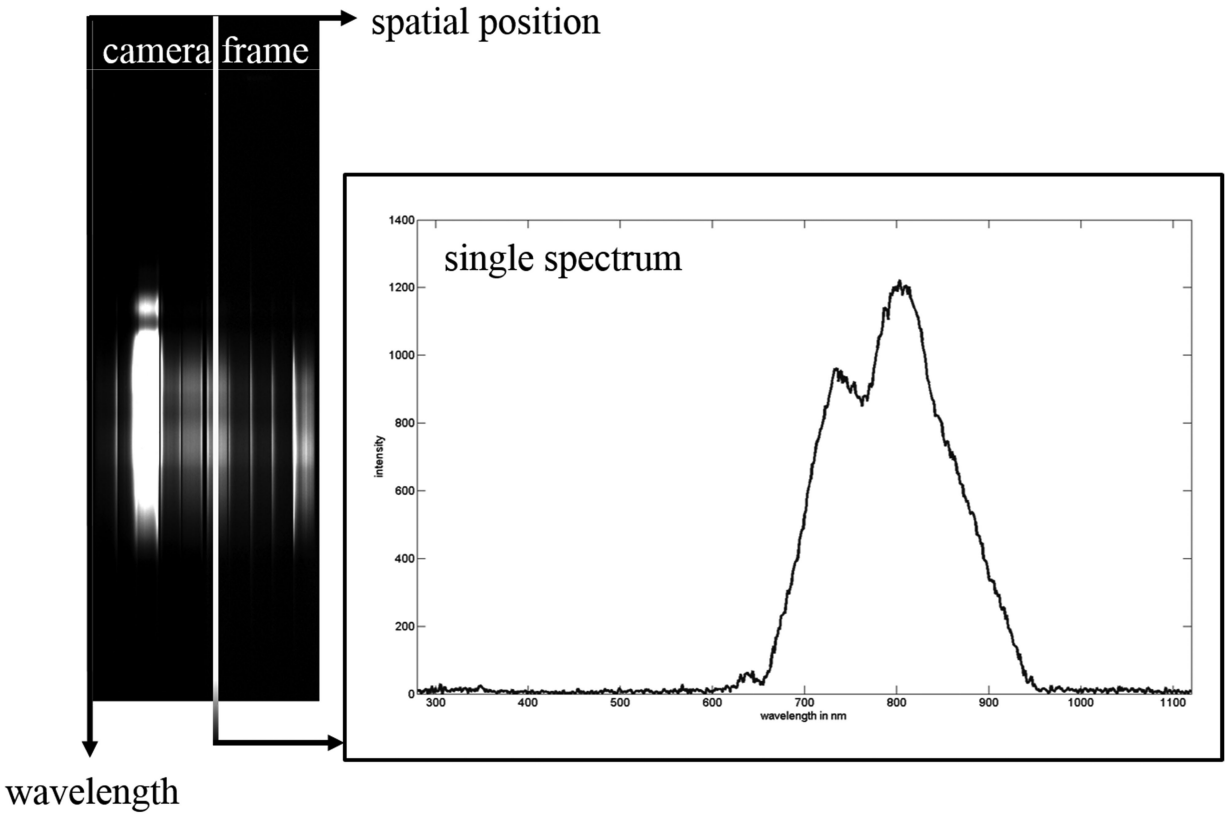

Figure 2. Left: Spectral image of 10 eggs (10 visible 'bands' in spatial direction) after 14 d of incubation acquired by the hyperspectral camera. For acquisition an exposure time of 500 ms, a spatial binning of 8 and a spectral binning of 2 was used. Right: One single spectrum (one column of the acquired frame, marked by the vertical line) of the sixth egg. Each egg is represented by approximately 20 single spectra and the 5 central spectra are averaged to get one spectral fingerprint per egg, which is used for further analysis.

Table 2. Amount of female and male spectral fingerprints (is equal to the number of eggs) for incubation times of 11 to 14 d.

Incubation Time	female	male
11 d	548	553
12 d	613	607
13 d	778	753
14 d	244	237

transmits light within the spectral range from 600 nm to 950 nm.

Because of the low level of signal, increasing the signal-noise-ratio is required. Three different approaches have been investigated. The first one is embedded in acquisition process by averaging several frames. The advantage of this method is that embryo movement, which might affect the signal, is balanced. On the other hand, it is a time-consuming approach. Time for acquisition $T_{acquistion}$ can be calculated to

$$T_{acquisition} = N_{frames} \cdot T_{exposure} \quad (1)$$

with number of frames N_{frames} and exposure time $T_{exposure}$. Therefore it is not the first choice for fast measurements.

After frame acquisition, there are 2 more possibilities. One option is the smoothing in spatial dimension by the averaging of columns in acquired frames. Differences in intensity, caused by pigment structure of the eggshell, can be balanced with this averaging method. This is applicable, if more than one column in the frame represents the signal of one egg. The third option is the smoothing in the spectral dimension using a moving average filter, binomial filter, or polynomial filter.

We applied smoothing in spatial as well as in the spectral dimension. In our tests, approximately 20 columns represent one egg and we averaged the 5 central spectra to get one spectral fingerprint per egg. In the spectral dimension, we used a binomial filter with 3 points of support.

The following analysis is based on 4,333 spectral fingerprints of 11- to 14-day-old male and female embryos, which were captured within a hatchery of Lohmann Tierzucht GmbH. Based on the embryos' age and sex, these spectra are grouped into 8 datasets according to Table 2.

Data Analysis

The spectra are cropped to spectral range from 600 nm to 950 nm. Afterwards the absolute value of the vector is normalized to 1.

The normalized spectra are parted randomly in training and validation datasets. One half of the data samples are used for training, the other half is used for model validation. The amount of samples for each group is summarized in Table 3.

Table 3. Amount of female and male spectral fingerprints in training and validation datasets.

	female		male	
Incubation Time	*Training*	*Validation*	*Training*	*Validation*
11 d	274	274	277	276
12 d	307	306	304	303
13 d	389	389	377	376
14 d	122	122	119	118

Table 4. Overview of classification success in %.

	female		male	
Incubation Time	*Training*	*Validation*	*Training*	*Validation*
11 d	66.35	61.16	62.60	58.33
12 d	75.55	72.29	71.71	70.43
13 d	87.81	86.89	90.72	90.32
14 d	97.21	96.72	97.48	97.46

Due to the high collinearity between variables of spectral data, a principal component analysis (as described in Alpaydin, 2010) is applied to the training data to obtain independent variables. Subsequently a linear discriminant is calculated which is suitable to separate the training data in class "female" and "male". Additionally, for each principal component, the discriminant strength is determined.

To validate the calculated discriminant, the validation data is also transformed to principal component space and classified within it.

RESULTS AND DISCUSSION

Classification results are summarized in Table 4. It could be observed that the success of classification increases with advanced incubation time (Figure 3), even if the signal intensity decreases with incubation time. As the down feathers start to develop around 11 d of incubation, it is obvious that the spectral difference between male and female embryo is mainly caused by the color and stage of down feathers. At 11 d of incubation, the classification accuracy is quite low, potentially due to the lack of down feathers at this stage. Only very few of the embryos were recognized as slightly brown or white.

Between 12 and 13 d of incubation, most embryos have down feathers on most parts of their bodies. In the population there exist embryos that are clearly white and clearly brown as well as embryos with mixed down feather colors. This high color variation might explain the misclassifications in these stages of development.

After 14 d of incubation, the difference between spectra of female and male samples is large enough to separate the groups with an accuracy of nearly 97%. Caused by the low signal, the data acquisition takes 500 ms per frame. Exposure time is the bottleneck of our method, because the pre-processing and classification take no significant time. Decreasing exposure might be possible when using a higher binning in spectral dimension, e.g., 4 or 8, instead of 2, because this will reduce exposure

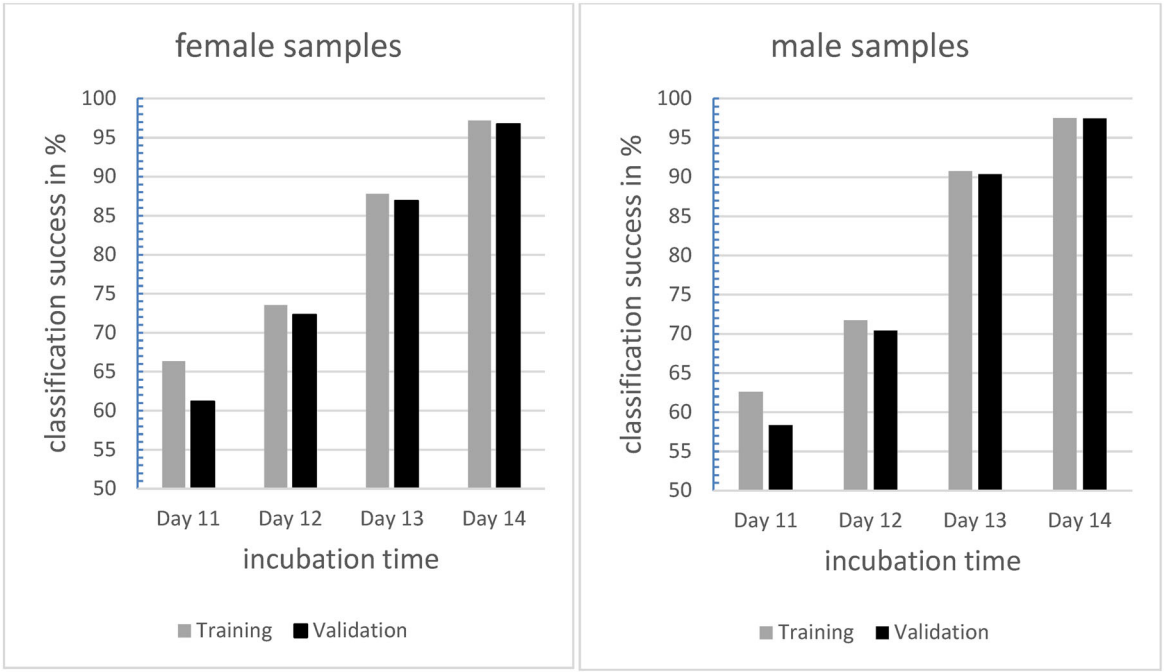

Figure 3. Graphs, showing the classification accuracy in training and validation dataset for female (left) and male (right) embryos. The accuracy increases with the age of the embryos.

time by factor 2 or 4. Nevertheless, it has to be investigated first, whether the lower spectral resolution has negative effects on classification accuracy.

Our method is a non-invasive optical method and the results to date show a high degree of accuracy. We have observed that the hatchability is not influenced in a negative way, a requirement for any method to be commercially acceptable. In future experiments we plan to gather more data in strains with sexually dimorphic feather color to prove the predictivity of the classifier and we want to test our method in other chicken strains that provide no sexual dimorphism in feather color.

With respect to classification accuracy, performance and operating costs it can be resumed that our method might be suitable for large-scale application for layer lines with gender-specific down feather color.

The major drawback of the method is the stage of embryo development at which the accuracy becomes acceptable. Close et al. (1997) determined that bird embryos are capable of perceiving pain in the second half of gestation, after 10.5 d of incubation. Due to the development of the down feathers later in incubation, we cannot accurately determine the sex of these embryos before d 14. While our method is an improvement over day-old chick culling, it still does not meet the political and potential regulatory requirements of determining sex before d 10.5 of incubation.

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