Development of a spectral setup for determining the sexual dimorphism of the bird embryo

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Abstract. Humane culling of embryos before incubation and one-day-old chicks in the first days of incubation is the most important unsolved problem in the production of poultry products. A number of European countries have laws prohibiting the destruction of one-day-old male chicks and are developing restrictions to limit the destruction of live embryos in avian eggs after 6 days of incubation. A spectral setup for determining the sexual dimorphism of an embryo in a bird egg has been developed based on the MV1-D2048x1088-HS05-96-G2-10 hyperspectral camera with the possibility of illuminating the object of study with an adjustable radiation source. This installation has been tested for functionality. It has been established that the mass, area, volume and shape factor of the eggs do not correlate with the sex of the embryo of the egg before incubation. Informative wavelengths have been determined for further research to determine the sexual dimorphism of chicken egg embryos in the first days of their incubation: 640, 660 and 688 nm.

1 Introduction

Along with the continuous growth of the total number of people living on our planet, the food production of the population is also growing evenly. The predicted uniform demand for dietary poultry meat and eggs will grow by 1.77 and 1.11% per year, respectively, and will increase by 2.2 times by 2050 [1]. Such a need can be met by changing the strategy of poultry farming, in particular, the use of individual monitoring of living organisms in real time during incubation, based on modern methods and technical means. Destruction of one-day-old male chicks during incubation is one of the most ruthless procedures in the poultry industry. Every year, about ten billion male chickens die in incubators due to low efficiency and inexpediency of their further cultivation [2-3]. Therefore, for example, in Germany and France, from January 1, 2022, a law prohibiting the mass culling of day-old chicks is in force. Austria, Ireland, Luxembourg and Portugal supported these measures, and Switzerland plans to introduce a similar ban in 2026 [4]. Producers were required to use technology to determine the sex of a chick before it is born and to prevent males from hatching. In addition, from 2024, a ban will be implemented on the destruction of live embryos in a bird's egg after the 6th day of incubation [4]. This is due to the fact that already on the 3rd day of incubation, using an acoustic microphone, the heart contractions of the developing embryo were recorded

[5] and the gray zone of the pain threshold of the embryo was established on the 7th day of incubation of the chicken egg [6].

2 Choice of research direction

The first commercial egg sex determination technology was introduced into production only in 2018 [6]. It is based on the Selegt method.

The essence of the method is as follows. At the blunt end of the egg, a hole was burned into the shell with a laser. A small dose of the egg content solution was then withdrawn through the hole and used to measure the estrogen content. The basis for determining sexual dimorphism was the difference in the amount of estrone sulfate in female and male embryos. In this case, the hormone can be detected in the egg only on days 8–10 of incubation [7–8].

This technology is invasive and requires higher sanitary conditions during incubation. In this regard, for example, Germany allocated 9.7 million euros in 2022 to create new effective methods for assessing sexual dimorphism in eggs [4].

Computer vision systems are intensively used to control all technological processes in poultry farming [9-10]. The use of computer vision reduces the cost of labor-intensive processes associated with the increasing intensification of poultry production, the need to create acceptable conditions for the welfare of animal development. Computer vision systems can provide reliable, non-invasive and precise technology for sensing and monitoring various aspects of poultry production processes. They will ensure the formation of a large amount of various data on the assessment of vital activity and the prediction of sexual characteristics of egg embryos for subsequent analysis. Since computer vision uses mathematical tools to obtain images, it will be effective in developing methods for predicting the sex characteristics of embryos during their incubation.

For example, in [10], when using LED light sources in computer vision installations, images of two batches of chicken eggs were obtained on the 3–6th, 8th, and 10th day of incubation, respectively.

On the obtained images on the 4th day of incubation, blood vessels were visible. Several areas of features characterizing the sexual dimorphism of embryos were identified on the images. After processing these feature parameters representing the texture features of blood vessels representing a chicken embryo, a genetic algorithm was developed to optimize the initial weights and backpropagation thresholds of neural networks with different hidden layers.

But it is extremely difficult to make such a prediction, despite the fact that computer methods produce identification, image classification, semantic segmentation, detection and recognition of the structural elements of the objects under study using machine learning methods based on deep neural networks [10]. The reliability of the forecast will be affected by the color and quality of the shell. In addition, the implementation of the genetic algorithm described in the article will require obtaining a large amount of data obtained experimentally on batches of eggs obtained from various crosses of birds, the egg shell of which is not pure white, without inclusions.

In our opinion, hyperspectral imaging methods have a significant prospect of predicting sex before incubation. They are quite widespread in scientific research to assess the state of a living organism [11-13]. Basically, these systems fix the light transmission spectrum. This spectrum is located in a wide region of the wavelength range - from visible to short-wave infrared. Such methods do not destroy the structure of the hatching egg; they can be used for input quality control before incubation: the presence of microcracks, the viability of the embryo, etc. [12].

The purpose of this study is to develop an experimental setup for hyperspectral imaging to determine the sex of an embryo in an egg and test its functioning.

3 Materials and methods

To test the possibility of predicting gender, the Hyperspectrum setup was used (Figure 1), using the MV1-D2048x1088-HS05-96-G2-10 hyperspectral camera with the IMEC CMV2K-LS150-VNIR sensor [14].

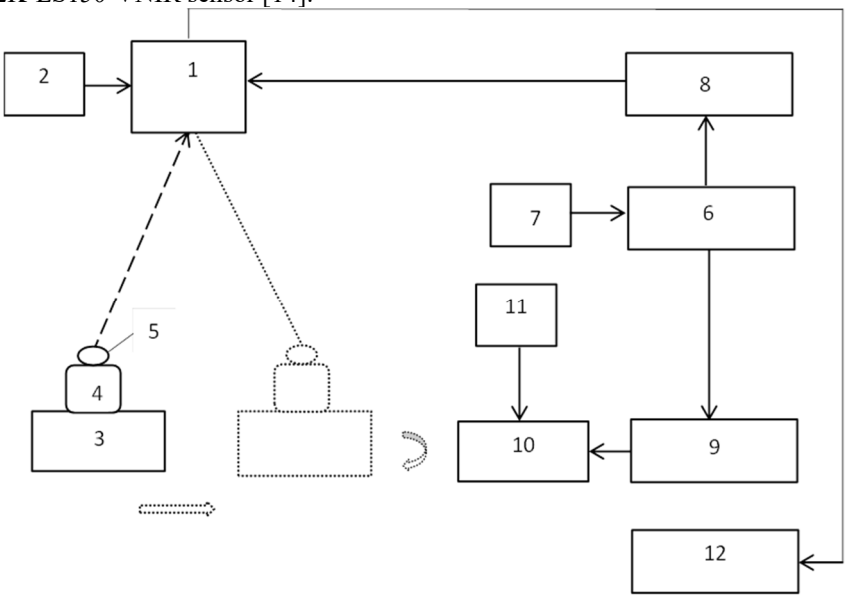

Fig. 1. Structural diagram of the Hyperspectrum installation.

The features of this camera include the presence of interference filters, consisting of a set of bands of different spectral transmission and a scanning mode of operation. The spectral bandwidth of the camera is divided into 149 channels, ranging from 475 to 900 nm with a spectral resolution of 3 nm. The spatial resolution of the camera is 2048×1088 pixels, the viewing angle is 14°×7°.

The setup includes: a hyperspectral camera 1 with a power source (12 V), fixed at a height corresponding to the size of the frame 20×10 cm; movable coordinate Table 3, on which the illuminator 4 and the object of study - egg 5 are placed [15].

The developed illuminator 4 is made in the form of a Falcon Eyes ML-09 RGB LED lamp controlled from a stand-alone infrared remote control. It is placed in a light-tight case, which is mounted on a coordinate table.

To control the operation of the installation, a special controller 6 has been developed that has a separate power supply 7 with a voltage of 9 V. The controller, through the generator 8, supplies external pulses to the camera 1 and controls the movement of the coordinate table as follows.

When the control signal arrives at the stepper motor driver 9, it shifts to a given angle, and performs micro-movement of the coordinate table 10, powered from the power source 11(12 V) by a step relative to the initial position. The controller moves the coordinate table over a specified distance at a specified speed (the speed was adjusted in advance based on the specified exposure time of the CCD camera matrix and the data transfer rate. In this case, a control signal is applied to the camera from the pulse generator board 8 and one frame of the image is taken. This is repeated until the object is completely scanned.

The resulting image files are written to a separate folder on laptop 12 and then assembled using custom Python software into a hypercube (a three-dimensional array of logged values in x, y, and λ coordinates) in the standard envi format.

The modular design of the unit available at the SFSC RAS was taken as a basis, which made it possible to combine the main components together, as well as provide easy access to replace or upgrade all components, including the camera, optics and lighting devices (Figure 2).

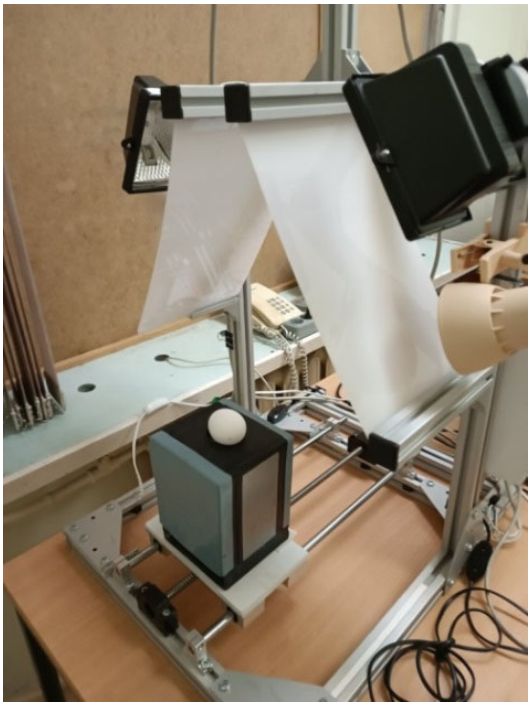

Fig. 2. A fragment of the design of the Hyperspectrum installation.

Previously, before incubation, an additional procedure was carried out for obtaining high-quality images of the eggs under study on a machine vision machine [16]. It uses a Canon EOS 2000D EF-S 18-55 III Kit digital camera with a modern CMOS-matrix (22.3 × 14.9 mm) and a powerful processor. The bird's egg was illuminated from below with the help of an illuminator. The digital device is paired with a laptop model ASUS VivoBook 17 K712EA-BX467W running the Windows 11 Home Single Language operating system, in which the received images were stored and processed.

This procedure is necessary to obtain the parameters of the research object, which can be correlated with gender characteristics [17]. The weight, area, volume of the egg and its shape index were chosen as parameters. The shape index was determined by the ratio of the transverse size of the egg to its longitudinal size [18].

In addition to the mass, all these parameters were determined from a digital image-twin of the egg using a special program. One pixel was chosen as the unit of measurement. The determination of the mass was carried out on an electronic balance OHAUS V31XH202 with a range from 0 to 200 g, resolution 0.01 g, serial number ARA530, inventory number 1468.

According to the results of shooting with a hyperspectral camera, data hypercubes were formed containing spectral and spatial information about the objects of study. In the center of each image, two rectangular regions of interest (ROI) sized 50x40 pixels were selected. One of the areas was used to form the training sample, the second for the test sample. The

resulting spectrum of eggs was calculated as the average value over the area for each wavelength.

Next, to improve the accuracy and reliability of the simulation, the spectrum was pre-processed to remove structural noise and the unwanted effects of light scattering from the data, as well as to highlight small differences between almost identical spectra. As suggested in [13], a 4-fold preprocessing was performed, including: (1) standard normal variation (SNV); (2) second-order Savitzky-Golay smoothing with a window of 9 wavebands (SG); (3) second order derivatives (2D); (4) medium centering (MC).

Data processing and analysis were performed using the ENVI 5.2 program (ITT Visual Information Solutions) and the R development environment.

4 Results and discussion

In the process of incubation of a batch of eggs of the Heisek white cross, 69 live chicks hatched, of which 4 chicks died in the brooder and 7 hatched with signs of rickets. Out of 58 healthy chicks, only 38 one-day-old chicks were reliably identified by sex.

In the course of research on digital twins of eggs, their basic features were determined (Table 1).

Analysis of the difference in the averages according to Student's t-test did not reveal statistically significant differences in features between the sexes (Table 2).

The average spectra of eggs before and after applying the filters are shown in Fig. 3. It can be seen from the graphs that there are no significant differences in the spectrum between the sexes.

However, to test the hypothesis about the possibility of determining the sex of eggs before incubation, classification models were built.

The data was further cropped to 478 to 830 nm to remove noise from the edges of the spectrum. Using the t-test, 3 wavelengths (640, 644 and 688 nm) were identified, at which there is a statistically significant ($p<0.05$) difference in the average values of the spectrum between the sexes (Figure 4).

5 Conclusions

A spectral setup for determining the sexual dimorphism of the avian embryo based on the MV1-D2048x1088-HS05-96-G2-10 hyperspectral camera with the possibility of local illumination of the research object from below by an adjustable radiation source has been developed. The installation was tested for its performance.

It has been established that the mass, area, volume and shape factor of the eggs do not correlate with the sex of the egg embryo.

Informative wavelengths have been determined for further research to determine the sexual dimorphism of chicken egg embryos in the first days of their incubation: 640, 644 and 688 nm.

Table 1. Experimental data on the object of study.

No egg	Sexing	Weight (g)	Area $(pel)^2$	Volume $(pel)^3$	Form Index
18	Male	58.12	669155.5	375388313.8	0.806400917
30	Male	60.66	704993.5	399285919.5	0.791373485
31	Male	52.18	627660.0	341736677.2	0.813135093
33	Male	55.01	667937.0	366093774.0	0.768738205
34	Male	56,01	663496.5	363378351.2	0.797215420
35	Male	58.63	690694.0	384657524.6	0.772872683
37	Male	58.66	697568.5	388562209.8	0,771898236

43	Male	56.62	668809.0	373764313.7	0.804256256
48	Male	60.11	716388.0	408709127.2	0.766195325
49	Male	64.87	729074.5	422436130.6	0.800984595
50	Male	63.07	727348.5	412359643.1	0.785310224
51	Male	55.26	660972.5	357698189.9	0.775947963
52	Male	54.54	657703.5	361423916.0	0.779978569
55	Male	60.43	720280.0	401328005.7	0.747938028
56	Male	54.11	640112.0	347046945.3	0.789794475
60	Male	54.83	645252.5	355402688.2	0.803596223
62	Male	56.93	653537.0	355529821.7	0.792485127
63	Male	57.97	683953.0	383669212.3	0.791394056
64	Male	57.86	671389.0	383584343.1	0.822117110
71	Male	57.92	678078.0	377925004.0	0.795762301
72	Male	53.59	640986.0	344664907.9	0.773992255
73	Male	58.29	683183.0	379979952.7	0.774101572
75	Male	58.40	684639.0	378030724.7	0.782527998
76	Male	60.46	708949.5	405205638.3	0.782333550
15	Female	53.82	610434.5	330865784.0	0.850390503
17	Female	62.00	710476.0	409323132.1	0.811351778
32	Female	60.61	711859.5	408153877.3	0.789486711
42	Female	55.84	670456.0	367294630.0	0.765349306
44	Female	59.79	689856.0	388016549.0	0.803825851
47	Female	59.67	702646.5	397082119.8	0.782238692
57	Female	52.79	627515.5	340454135.5	0.810908261
58	Female	57.48	692528.0	382416923.7	0.749747534
59	Female	61.21	712227.0	413800373.7	0.797736535
61	Female	56.04	668423.5	372505526.3	0.800935029
69	Female	54.25	654131.5	354014646.9	0.779040428
70	Female	54.28	660276.5	357791633.5	0.771883816
74	Female	60.75	712746.0	403027732.4	0.769512427
77	Female	58.59	702333.0	389310801.0	0.754826670

Table 2. Results of the analysis of the difference in the average values of features.

Sign	Average difference	Degrees of freedom	t- meaning	P- meaning
Weight	- 0.037	36	- 0.036341	0.9712
Area	1582.107	36	0.15527	0.8775
Volume	1748006.001	36	0.21697	0.8295
Form Index	0.00126131	36	0.17863	0.8592

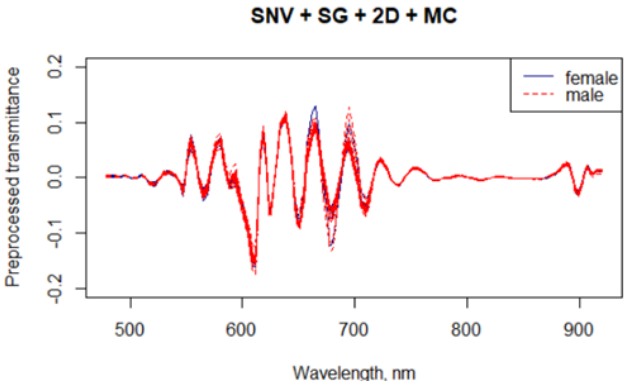

Fig. 3. The spectrum of the studied batch of eggs after applying filters.

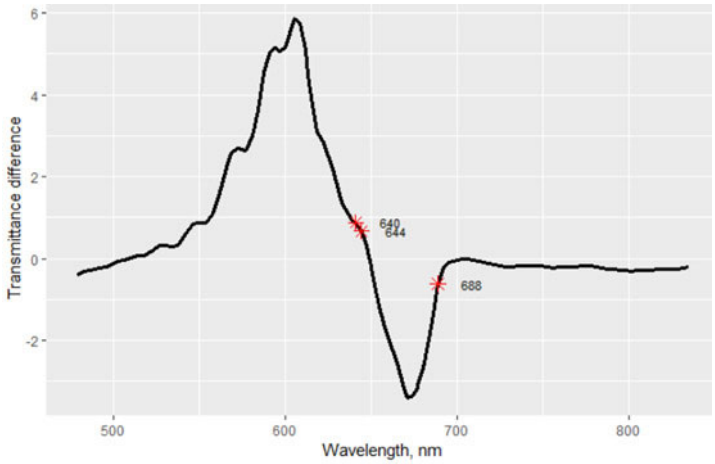

Fig. 4. The difference in the average values of the spectrum between the sexes (*informative wavelengths are marked).

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