

## Article

# AI Classification of Eggs' Origin from *Mycoplasma synoviae*-Infected or Non-Infected Poultry via Analysis of the Spectral Response

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**Featured Application:** The proposed AI-supported classification method, together with the described portable multispectral fibre-optics reflectometer, is recommended for use as a fast-warning detection tool against eggshell changes caused by *Mycoplasma synoviae* in flocks of birds. Other application areas are eggs wholesalers and distributors, veterinarians, sanitary stations, border services, etc.

**Abstract:** Rapid detection of *Mycoplasma synoviae* (MS) in a flock is crucial from the perspective of animals' health and economic income. MS are highly contagious bacteria that can cause significant losses in commercial poultry populations when its prevalence is not limited. MS infections can cause losses associated with a range of clinical symptoms related to the respiratory, mobility and reproductive systems. Lesions related to the reproductive system and changes in the eggshell result in losses associated with infection or embryo death or complete destruction of the eggs. The authors propose using spectral measurements backed up by an AI data processing algorithm to classify eggs' origin: from healthy hens or MS-infected ones. The newest obtained classification factors are F-scores for white eggshells of 99% and scores for brown eggshells of 99%—all data used for classification were obtained using a portable multispectral fibre-optics reflectometer. The proposed method may be used directly on the farm by staff members with limited qualifications, as well as by veterinary doctors, assistants, or customs officers. Moreover, this method is scalable to mass production of eggs. Standard methods such as serological tests require either specialized staff or laboratory equipment. Implementation of a non-destructive optical measurement method, which is easily adapted for use on a production line, is economically reasonable.

**Keywords:** *Mycoplasma synoviae*; pathogen detection; optical measurements; spectral measurements; optical spectroscopy; machine learning; artificial intelligence AI; origin classification; food safety; food monitoring



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## 1. Introduction

Detecting contamination, the presence of parasites, or infection with viral or bacterial pathogens in animals, is crucial in today's food industry. Mass food production constantly encounters such problems. Therefore, the critical issue is an early response to the contamination, which is possible with the necessary measurement equipment. This article proposes advanced AI classification of eggshells' origin to determine whether they were obtained from a healthy hen or a *Mycoplasma synoviae* (MS)-infected one by means of spectral analysis.

*Mycoplasma synoviae* is a bacterium that can be transmitted both horizontally and vertically through an infected egg. Its presence in the oviducts of hens can lead to changes in the shell surface associated with eggshell apex abnormalities syndrome. Shell lesions include altered shell surface, thinning and increased transparency in various areas, which can lead to more cracks and fractures of the eggshell [1,2]. The occurrence of MS in flocks of laying hens results not only in a decrease in laying rates, but also in a significant decrease in shell quality associated with ultrastructural changes in the shell surface [3,4]. The occurrence of cracks due to changes caused by the presence of MS in the reproductive system can lead to damage, dehydration, and microbial infection of the embryo, leading to higher embryonic mortality [5,6]. Many methods have been developed to detect MS infection from serum, swabs, and tissue samples. Serological tests such as the serum plate agglutination test (SPA), enzyme-linked immunosorbent assays (ELISA) [7,8], and hemagglutination inhibition test (HI) are commonly used for diagnosis [9,10]. Culture methods using pleuropneumonia-like organisms (PPLO) broth can also be employed, but they are time-consuming, taking up to 28 days [11]. Molecular methods, including polymerase chain reaction (PCR) [12–14] and its variations like real-time PCR [15], multiplex PCR, loop-mediated isothermal amplification (LAMP) [13–19], and polymerase spiral reaction (PSR), are widely used for MS detection. PSR, for instance, is 100 times more sensitive than PCR and has a higher positive rate (69.9%) than ELISA (65.3%).

The authors propose different approaches to detecting MS infection to those mentioned above. The proposed method may be used directly on farms by staff members with limited qualifications, as well as by veterinary doctors, assistants, or customs officers. It involves spectral, rapid measurement with data post-processing and AI classification [20–24]. Classifying samples' biological origins through spectral data analysis is now a trend, i.e., honey-type classification [25] or whether an egg comes from an MS-infected chicken or healthy chicken [26–28].

Given the wide range of possible biological samples and their inherent variations, numerous approaches are employed for the analysis of obtained spectral data. Moreover, spectral data may also vary depending on what kind of spectral response is measured: transmittance, reflectance, absorption, scattering, fluorescence, etc. Principal Component Analysis (PCA) is one of the most popular data analysis methods [29–33]. However, due to factors like subraces, age of hens, egg colouring, diet, and climate, eggshells' diversity is so extensive that standard PCA algorithms prove ineffective. Alternative approaches, such as using classifiers like the Spanning tree combined with various data reduction techniques, can be successfully employed, as shown in [27]. Employing this classifier in a specific case leads to the analysis of multiple levels in a tree structure. In their research, the authors concluded that machine learning algorithms were the most efficient for differentiating whether eggs originated from healthy or MS-infected hens.

The presented paper is a fruitful follow-up of a previous work. Two optical system configurations, one with transmitted light and the other with reflected light, were created and tested for the analysis and classification of eggshells. In the case of transmitted light analysis on chicken eggs, they achieved an accuracy of 88.8%, specifically for white eggshells [26]. The measurements can be conducted without destroying the egg by utilising reflective light, making them more applicable in industrial settings. Eggshells from infected and non-infected hens exhibit distinct reflective properties. The study conducted by the authors [26–28,33] demonstrated that it is possible to detect changes caused by MS infection in a chicken flock by analysing back-reflected signals from eggshells at selected spectral wavelengths of a white light source. By employing machine learning algorithms, the researchers were able to differentiate tested samples of various origins with a reasonable probability. In the case of white eggshells, the F-scores reached 95.75%, while for brown eggshells, the F-scores reached 86.21% [31]. When using modified machine learning algorithms, the F-scores for white eggshells were 86% while for brown eggshells, they were 96% [27]. The last two results, reported in [28,33], were obtained using a portable multispectral fibre-optics reflectometer that uses selected single-colour LEDs instead of a broadband light source and an optical fibre bundle.

Deep learning methods are sometimes employed in more complex scenarios that require information about molecules. This approach was utilised by Gosh et al. in their work, in which the researchers used deep learning to predict molecular excitation spectra [19]. Their results demonstrated that this type of network could achieve up to 97% accuracy in learning spectra and infer spectra solely from molecular data. Joung et al. [34] presented a similar application of deep learning, in which they successfully predicted seven optical properties related to organic compounds. Additionally, this method has been proven effective in drug identification, as shown by Ting et al. [35]. This approach enables efficient work in this field. However, as demonstrated above, less complex machine learning methods are predominantly used to analyse the spectra distribution for any material.

## 2. Materials and Methods

### 2.1. Samples

The authors used the Portable Multispectral Fibre-Optics Reflectometer for further AI classification. A dataset comprising 2521 eggshell samples was prepared. This dataset consisted of brown and white eggshells originating from healthy or infected hens. The quantity of each subset of samples is presented in Table 1.

**Table 1.** Quantity in different measurement subsets of samples.

Origin	Eggshell Colouring	
	White	Brown
H—healthy	701	624
I—infected	572	624
Total	1273	1248
	2521	

The samples classified as healthy were sourced from the inner reference flock of the Department of Poultry Diseases, National Veterinary Research Institute (NVRI). On the other hand, the MS-infected eggs were obtained from commercial flocks under the veterinary supervision of the NVRI. The infection status of these eggs was confirmed using three techniques: specific MS PCR, LAMP, and sequencing of the *vlhA* gene.

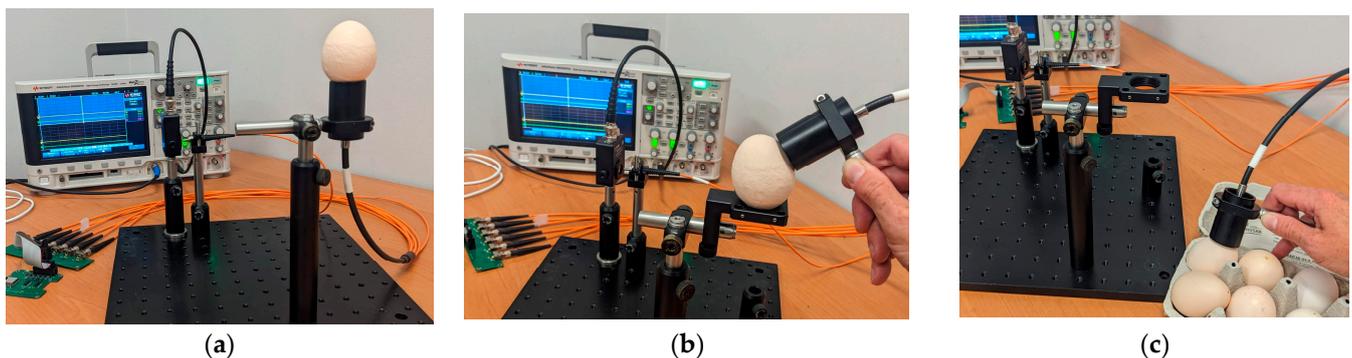
Obtaining samples infected with MS is quite a difficult issue. Farmers tend to keep an infection a secret due to the repercussions—extermination of the whole flock is the most drastic consequence. This approach limits the sample availability. Moreover, obtaining samples that are purely infected by a certain pathogen only is even more difficult. Due to that reason, this study was provided with a limited number of eggshells. Some of them were whole, some were broken—just pieces. The whole eggshell was tested several times in different areas (apex, bottom, on the equator, etc.)—due to the change in the thickness, surface profile, and structure of the eggshell, such measurements were treated as separate. Broken pieces of eggshell, depending on their sizes, were tested once or in 2–3 areas.

### 2.2. Portable Multispectral Fibre-Optic Reflectometer

All spectral measurements, the results of which are discussed above, were performed on Portable Multispectral Fibre-Optic Reflectometer [28]. The eggshell, the whole egg or part of it in question, is placed on the measurement head. The measurement head can also be manually oriented and positioned regarding the sample, and the spectral measurement is then performed. The eggshell is sequentially illuminated by the light emitted by six LEDs covering the visible electromagnetic wave region. The dominant wavelengths and spectral range (FWHM) of the used LEDs are shown in Table 2. The key issue of LEDs selection for this system is their spectral separation therefore, the FWHMs parameter is so important. The light is introduced to the measurement head by the 1 × 7 fun-out fibre-optic bundle, which makes it possible to flexibly reach the sample from the desired angle. The possible scenarios of head–egg measurement positions are shown in Figure 1.

**Table 2.** The dominant wavelengths and spectral range of used LEDs.

Light Source	Dominant Wavelength [nm]	Spectral Range—FWHM [nm]
LED 1	699	24
LED 2	664	20
LED 3	604	14
LED 4	533	34
LED 5	504	34
LED 6	413	18



**Figure 1.** The possible scenarios of measurement head–egg positions: (a) Fixed measurement head with an egg placed on top; (b) manually operated measurement head positioning with an egg placed on a platform; (c) manually operated measurement head positioning with egg stored in the standard egg tray.

The signal carrying the measurement information is gathered by means of back reflection from the sample. It travels through the central core of the  $1 \times 7$  fun-out fibre-optic bundle to the detector. The single measurement cycle lasts less than 1 s. The signal is preprocessed and then introduced to the AI algorithm. Details on the Portable Multispectral Fibre-Optic Reflectometer design, operation and signal processing have been widely discussed in [19].

### 2.3. AI Classification

The increasing accessibility of advanced measurement methods in biological sciences has led to a growing adoption of sophisticated data processing techniques to extract valuable information effectively. Machine learning approaches have become particularly advantageous in this context, with a rapid growth of solutions emerging in this field. These solutions are well suited for data classification or clustering in biological sciences, including DNA and spectroscopic data analysis. Recently, we embarked on analysing such data specifically to study the occurrence of MS, achieving a detection level of (F-score) up to 96% [28,33]. We employed the Support Vector Machine (SVM) method for data analysis, a commonly used approach [36]. The essence of SVM is to calculate the best hyperplane that separates different data classes while maintaining a maximum margin of confidence. Our algorithm was based on Radial Basis Functions (RBF) [37,38]. Despite the many advantages of SVM, it has a few drawbacks, one of which can significantly impact the prediction results for the data we obtain in our portable multispectral fibre-optics reflectometer. Specifically, SVM does not perform optimally when the input dataset consists of overlapping values assigned to different classes.

Consequently, we decided to employ a different classification algorithm in our subsequent study. We chose the Self-Organizing Tree Algorithm (SOTA), an unsupervised neural network with a binary tree topology. It was developed in 1997 by Dopazo and Carazo [39]. SOTA combines hierarchical clustering and a Self-Organizing Map (SOM)

based on a single-layer neural network [40]. In SOTA, the processing time is approximately directly proportional to the number of elements to be classified. This presents a clear advantage over SVM, which is perceived as slow when dealing with large datasets. The processing in SOTA begins with the node exhibiting the highest diversity, which is then divided into two nodes called cells. The splitting process can be stopped at any node.

The data processing was performed using KNIME version 4.5.0. KNIME is an open-source platform that offers various components suitable for data exploration. One of these components includes the implementation of the SOTA algorithm. However, the SVM algorithm is not available in the set of KNIME components. This is not a problem, since the creators of this environment have provided a feature that enables running Python code, through which access to the SVM algorithm can be achieved. Unfortunately, we could not find an implementation of the SOTA algorithm in any of the Python libraries.

### 3. Results

The data collected using the portable multispectral fibre-optics reflectometer were divided into two independent groups in the analysis, one representing white eggshells and the other representing brown eggshells. Within each of these groups, there were two subgroups: one consisted of eggshells from healthy hens and one from diseased hens. The data from each group were processed separately. In the first step, the data were randomly divided into training data and validation data at a ratio of 7:3. The training data were normalised to unity, and the normalisation parameters were recorded. In the second step, the SOTA network was trained. After completing the training, the third step involved normalising the test data using the normalisation parameters calculated from the training data. The final stage was prediction, and the results were recorded in the output dataset. This processing path is illustrated by the diagram Figure 2. All these steps were repeated one hundred times to mitigate the influence of random data arrangement. The final result was calculated as the average for this set.

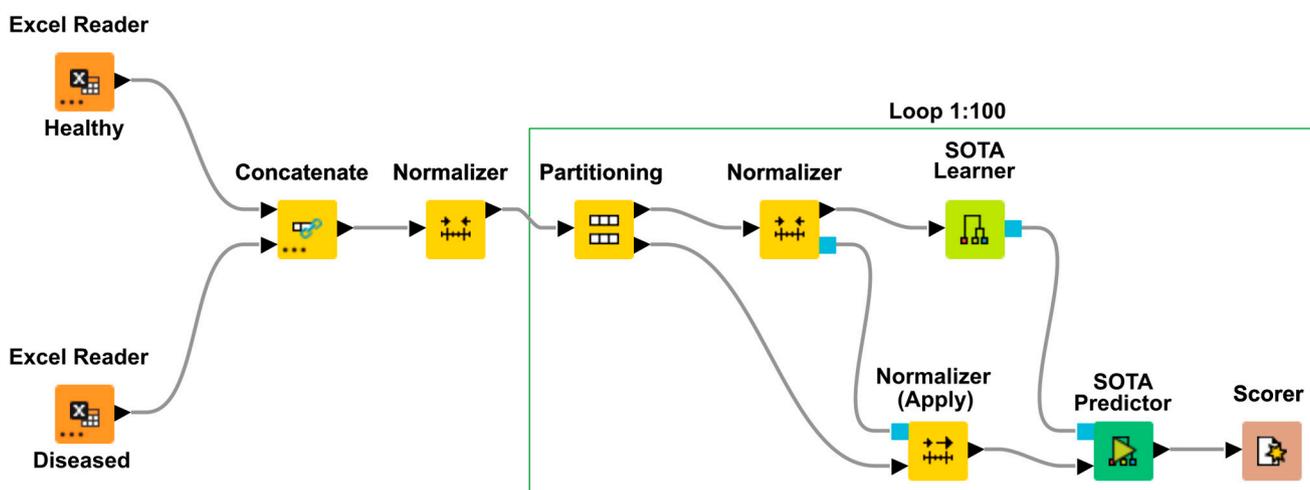


Figure 2. The data processing method used for the evaluation of the SOTA algorithm.

The machine learning algorithm’s performance was evaluated based on F-score, Precision, and Recall metrics. These metrics are based on the values of TP (true positives), TN (true negatives), FP (false positives), and FN (false negatives) [32].

The Precision is calculated as  $Precision = TP / (TP + FP)$  and indicates how well the algorithm correctly classifies instances relative to all the data identified as correct.

The Recall is calculated similarly as  $Recall = TP / (TP + FN)$ , but it refers to all the elements that should have been identified as correct.

F-score is calculated as  $F\text{-score} = 2 \times (Precision \times Recall) / (Precision + Recall)$ . It represents their harmonic mean. This metric helps identify whether either Precision or Recall are too low. It is used in statistics and machine learning when it is desirous to

have both high Precision and high Recall at the same time. When one or both of these parameters are low, then the F-score calculated for them is low. Only when both are high is the F-score high.

The results of the eggshell origin classifications quality for white and brown eggs obtained using the portable multispectral fibre-optic reflectometer and the SOTA algorithm are shown in Table 3.

**Table 3.** Quality of the origin classifications of white and brown eggs obtained using the portable multispectral fibre-optic reflectometer and the SOTA algorithm. I—eggshells obtained from infected hens, H—eggshells obtained from healthy hens.

Eggshell Colouring	Origin	Precision	Recall	F-Score
White	I	0.99	0.99	0.99
	H	1	1	1
Brown	I	0.98	0.99	0.99
	H	0.99	0.99	0.99

The use of the SOTA algorithm proved to be justified in the case of the analysed data. Compared to the SVM algorithm, the average Precision increased by 0.08 across all groups, with the maximum increase observed in the case of diseased white eggs at 0.18. Regarding Recall, the increases were 0.08 and 0.17, respectively, with the maximum difference visible in the case of healthy white eggs. For the F-score parameter, the overall result also improved by an average of 0.08. The maximum improvement was observed for both diseased and healthy white eggs cases, and it was equal to 0.13.

#### 4. Discussion and Conclusions

*Mycoplasmas* are the smallest and simplest self-replicating bacterial pathogens which do not have cell walls and have lost many biochemical pathways, making them obligate parasites highly dependent on their host. Some strains of *Mycoplasmas* can be extremely cytotoxic to their hosts, which may be related to the presence of variable surface antigens and lipoproteins [41,42].

Infections with *M. synoviae* can be subclinical. However, clinical signs can be associated with birds' respiratory and musculoskeletal systems, especially hens and turkeys, and their reproductive systems [11]. This pathogen is responsible for a condition called infectious synovitis, which is characterised by inflammation of the synovial membrane in the joints. Birds infected with MS may exhibit lameness, swollen joints, and reduced mobility [43]. In commercial poultry flocks, the developed infection can lead to severely reduced growth rates, decreased egg production, and poor overall performance. In addition to its impact on the musculoskeletal system, MS can also cause respiratory problems [11,43]. Infected birds may show signs such as nasal discharge, sneezing, coughing, and difficulty breathing. These respiratory symptoms can further compromise the overall health of the birds and make them more susceptible to secondary infections. *Mycoplasma synoviae* is highly contagious and can spread rapidly through direct contact with infected birds and through contaminated equipment, feed, and water sources. The bacterium can survive in the environment for several weeks, making it a persistent threat to poultry farms [44–47].

Controlling MS requires strict biosecurity measures, such as isolating infected birds, maintaining clean facilities, and disinfecting equipment [47–49]. Maintaining healthy flocks requires rigorous biosecurity practices. Vaccination is also an important tool in preventing and managing the disease. However, it is worth noting that the bacterium can develop resistance to certain antibiotics over time, complicating treatment efforts [50–53]. Overall, *Mycoplasma synoviae* poses a significant risk to poultry health and productivity. Poultry producers need to remain vigilant and take proactive measures to prevent and control its spread within their flocks. Regular monitoring, proper biosecurity protocols, and timely intervention can help mitigate the negative impact of this pathogen on poultry populations. Using spectral measurements strongly supported by AI data processing algorithms will

allow the classification of eggs from healthy hens or MS-infected hens. The proposed method can be applied directly on the farm. The use of a portable multispectral fibre-optic reflectometer allowed the classification rate to F-scores for white eggshells 99% and for brown eggshells 99%.

Based on the analysis of the eggshells, we found that the proposed solution detects the presence of *Mycoplasma* in the flock with an average Precision, Recall, and F-score level of 0.99. Our next goal is to conduct tests on a significantly larger number of samples in real-world conditions. If these tests confirm the laboratory findings, will consider our work a complete success, and we plan to start working on a product that can be implemented on the market.

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